Published in final edited form as:

Neurotoxicol Teratol. 2016 ; 53: 1–10. doi:10.1016/j.ntt.2015.11.002.

Effects of embryonic exposure to polychlorinated biphenyls (PCBs) on larval zebrafish behavior

Ava K. Lovato¹, Robbert Creton², and Ruth M. Colwill¹

¹Department of Cognitive, Linguistic & Psychological Sciences, Brown University, Providence, Rhode Island ²Department of Molecular Biology, Cell Biology and Biochemistry, Brown University, Providence, Rhode Island

Abstract

Developmental disorders such as anxiety, autism, and attention deficit hyperactivity disorders have been linked to exposure to polychlorinated biphenyls (PCBs), a ubiquitous anthropogenic pollutant. The zebrafish is widely recognized as an excellent model system for assessing the effects of toxicant exposure on behavior and neurodevelopment. In the present study, we examined the effect of sub-chronic embryonic exposure to the PCB mixture, Aroclor (A) 1254 on anxiety-related behaviors in zebrafish larvae at 7 days post-fertilization (dpf). We found that exposure to low concentrations of A1254, from 2 to 26 hours post-fertilization (hpf) induced specific behavioral defects in two assays. In one assay with intermittent presentations of a moving visual stimulus, 5 ppm and 10 ppm PCB-exposed larvae displayed decreased avoidance behavior but no significant differences in thigmotaxis or freezing relative to controls. In the other assay with intermittent presentations of a moving visual stimulus and a stationary visual stimulus, 5 ppm and 10 ppm PCB-exposed larvae had elevated baseline levels of thigmotaxis but no significant differences in avoidance behavior relative to controls. The 5 ppm larvae also displayed higher terminal levels of freezing relative to controls. Collectively, our results show that exposure to ecologically valid PCB concentrations during embryonic development can induce functional deficits and alter behavioral responses to a visual threat.

Keywords

avoidance behavior; freezing; larvae; polychlorinated biphenyls; thigmotaxis; zebrafish

Address for correspondence: Ruth M. Colwill, Department of Cognitive, Linguistic & Psychological Sciences, Brown University, Box 1821, Providence, RI 02912, ruth_colwill@brown.edu, Tel: 401-863-2547, Fax: 401-863-1300.

Conflict of interest

The authors declare that there are no conflicts of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Polychlorinated biphenyls (PCBs) are lipophilic, chemically stable, chlorinated compounds or congeners that persist in the environment and bioaccumulate in humans and other animals (Herrick et al., 2007; Li et al., 2009; Ling et al., 2008; Safe, 1994; Tiernan et al., 1985). As a result, PCB levels are frequently found at substantially higher concentrations in fish and mammals than in the external environment. Humans are predominantly exposed to PCBs through ingestion of contaminated food, although recent work has identified inhalation of PCB-contaminated dust from deteriorating building products as another exposure risk (Herrick et al., 2007). PCBs remain in the body for prolonged periods of time, providing a means of exposure long after ingestion of the PCB contaminants.

Exposure to PCBs may also occur during the embryological period through maternal consumption of contaminated food. This kind of neurotoxic insult may lead to irreversible alterations of the developing fetal brain. Several large scale epidemiological studies suggest that PCBs can alter endocrine, immune and nervous system function, particularly during embryological and early post-natal growth when rapid changes are taking place (Aoki, 2001; Feeley and Brouwer, 2000; Gladen and Rogan, 1991; Gladen et al., 1988; Huisman et al., 1995a,b; Jacobson et al., 1990; Koopman-Esseboom et al., 1996). Furthermore, embryonic exposure to PCBs can produce detrimental effects on neurocognitive function in children from infancy to adolescence (Darvill et al., 2000; Grandjean and Landrigan, 2006; Jacobson and Jacobson, 1996; Winneke, 2011). Mood disorders such as depression and anxiety have also been documented in humans exposed to PCBs as a result of environmental pollution (Fitzgerald et al., 2008; Plusquellec et al., 2010; Winneke, 2011).

Animal studies of perinatal PCB exposure have found many of the same effects reported in humans (Tilson et al., 1990). These effects, largely documented in rodents, include spatial learning deficits, hyperactivity, impaired learning and an increase in anxiety-related behaviors including increased thigmotaxis and social avoidance (Corey et al., 1996; Eriksson and Fredriksson, 1996; Rice, 1999; Rice and Hayward, 1997; Schantz et al., 1995; Sugawara et al., 2006). For example, Orito et al. (2007) fed pregnant rats PCB 126 at a dosage of 30µg/kg in corn oil on gestational day 15. When the offspring were tested at 4–5 weeks of age, they exhibited a significantly greater preference for the edge of an open field than control pups whose mothers were given only the vehicle, corn oil. Further, when placed in an environment with a novel partner, the PCB-exposed pups displayed significantly less time engaged in social interaction than vehicle controls.

The zebrafish has emerged as an excellent model system for large scale studies of vertebrate neurodevelopment and behavior (Ali et al., 2011; Bailey et al., 2013; Bruni et al., 2014; Colwill and Creton, 2011a; He et al., 2014; Kalueff et al., 2014). Zebrafish embryos develop externally, are nearly transparent, and are accessible to genetic, chemical and experimental manipulations. Their development is also extremely rapid. Larvae hatch from their chorion at 2–3 days post fertilization (dpf) and acquire a complex repertoire of swimming, hunting, escape, and avoidance behaviors within the first week of development (Colwill and Creton, 2011b; Fero et al., 2011; Spence et al., 2008; Wolman and Granato, 2012). We have recently developed a novel automated imaging system for monitoring developing larvae in multiwell

plates and a behavioral assay to assess avoidance behavior in 7 dpf zebrafish larvae to a moving visual stimulus (Colwill and Creton, 2010; Creton, 2009; Pelkowski et al., 2011). Consistent with our view that this stimulus simulates predatory movement and thus constitutes a potential threat, we have found that larvae swim away from the stimulus (avoidance) towards the edge of the well (thigmotaxis) (Pelkowski et al., 2011). We have also confirmed that these behavioral indices of anxiety are appropriately modulated by anxiolytic and anxiogenic pharmaceuticals (Richendrfer et al., 2012).

In the present study, we investigated the effects of exposure to the commercial PCB mixture, Aroclor (A) 1254, during early-stage embryonic development (2 to 26 hours post-fertilization, hpf) on visual avoidance behavior in zebrafish larvae at 7 dpf. A1254 is suitable for testing PCB exposure effects in a new model system for several reasons. Exposure to PCB mixtures, not isolated congeners, is common in the environment (Kreiling et al., 2007; Lee et al., 2012); the congener profile of A1254 is similar to that found in human tissues (Angulo et al., 1999; Hansen, 1999; Kodavanti et al., 2011; Yang and Lein, 2010); and A1254 has been used extensively in research studies providing a wealth of information on PCB-exposure effects and mechanisms. To examine visual avoidance behavior, larvae were tested with 5-min periods of no visual stimulus alternating with 5-min periods of either a red 'bouncing' disk (BD assay) or a red 'bouncing' disk and a red stationary disk on the side opposite to the 'bouncing' disk (BD/SD assay) for two hours. We measured avoidance of the moving disk, preference for the edge of the well, and immobility (freezing). Embryonic exposure to 5 and 10 parts-per-million (ppm) but not 2 ppm of A1254 induced specific behavioral defects including reduced avoidance behavior on the BD assay and increased thigmotaxis on the BD/SD assay. These results are consistent with the literature suggesting that PCB-exposure in early development disrupts cognitive processing and increases anxiety-related behaviors.

2. Materials and methods

2.1 Embryo collection

Embryos were collected from a breeding population of adult male and female wild type zebrafish originally obtained from Carolina Biological Supply Co. (Burlington, NC) and maintained at Brown University as a genetically diverse outbred strain. Breeders 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 two hours 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

A stock solution of 50 mg/ml of Aroclor (A) 1254 (Ultra Scientific, Kingston, RI) in dimethyl sulfoxide (DMSO) was diluted 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). A1254 is a commercial PCB mixture consisting mainly of non-coplanar, ortho-substituted congeners.

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. The PCB concentrations selected for study have been shown to be environmentally relevant (Li et al., 2009; Wickizer et al., 1981). Egg water (EW) and DMSO at a final concentration of 0.1% in EW were used as the two controls.

Following treatment exposures, all embryos were transferred to microfuge tubes and triple-rinsed in EW. They were then placed in 1L plastic breeding tanks (Aquatic Habitats) containing approximately 500mL of EW and incubated at 28.5°C until they reached 7dpf. During this period, any dead larvae and other particles were removed and EW in each tank was replaced to maintain water quality. The developing larvae were examined for pericardial and yolk sac edemas, curved body axis and sidewise position. Any larvae exhibiting these visible malformations were not used in the behavioral assays. We observed no PCB-related delays in swim bladder inflation or increases in mortality. 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 have been described previously (Pelkowski et al., 2011). Flat-bottom 6-well plates (Corning Costar no. 3506) were prepared for behavioral testing by filling each well with 5 ml of agarose (0.5% w/v in deionized water). Agarose was allowed to set and then a center portion was punched out to create a 27mm diameter x 5mm deep swimming area surrounded by an agarose ring. 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 a camera (Canon EOS Rebel T1i digital camera with a Canon EF-S 55–250mm f/4.0–5.6 IS Zoom Lens) 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 with alternating 5 min periods of a white background with no visual stimuli (Fig. 1A) and 5 min periods of either a red 'bouncing' disk (BD assay, Fig. 1B) or a red 'bouncing' disk and a red stationary disk (BD/SD assay, Fig. 1C). Images were captured every 6 s for 125 minutes. The visual stimuli were created in Microsoft PowerPoint. For the BD and BD/SD assays, a red disc (RGB values were 255, 0, 0) with a 1.35 cm diameter was programmed to travel back and forth in a straight line across the upper half of each well at a rate of 1.65 cm/s. For the BD/SD assay, a red stationary disk also appeared in the lower half of each well throughout the time that the 'bouncing' disk was present in the upper half. Over three replications, a total of thirty larvae per group were tested using the BD assay; forty-eight larvae per group were tested using the BD/SD assay. Data from one of the 48 EW controls were discarded from analysis because the larva did not move during behavioral testing.

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(s), subtract the background, apply a threshold, and identify larvae on the basis of particle size. 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 behavior) and (2) in the center of the well or on the edge of the well (thigmotaxis). The X,Y coordinates of the larvae's centroids in pairs of consecutive images were used to quantify instances of inactivity (freezing) 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 behavioral data for each assay were analyzed separately using mixed factors analysis of variance (ANOVA) with Stimulus (2 levels) and Blocks (12 levels) as repeated measures and PCB (4 levels) as the between subjects factor (SPSS v. 22.0). The data from the two control groups (EW and DMSO) were combined for each assay because there were no statistically significant differences between these groups on the behavioral measures. There were no significant three way (Stimulus x Blocks x PCB) interactions (all p values $>.10$) but there were several significant two-way interactions. To explore the source of these significant two-way interactions, we followed up with one-way ANOVAs. Dunnett's tests (two-tailed) were used for post-hoc comparisons between the combined control group and the PCB-treated groups.

3. Results

3.1 Effects of PCB-exposure on avoidance

Embryonic PCB-exposure reduced percent avoidance in the BD assay (Fig. 2A–2D). There was a significant Stimulus x PCB interaction, $F(3,146) = 4.3$, $p < .01$. For all groups, mean percent avoidance was greater with visual stimuli than without visual stimuli ($ps < .01$). However, the magnitude of this difference was significantly larger in the control group relative to the 5 ppm ($p < .05$) and the 10 ppm ($p < .01$) but not the 2 ppm ($p > .10$) PCB-treated groups (Fig 3A; means used to calculate difference scores are shown in Table 1A). For the BD/SD assay (Fig. 2E–2H), the mean percent avoidance was also significantly greater with visual stimuli than without visual stimuli for all groups ($ps < .01$). However, although the Stimulus x PCB interaction approached significance, $F(3,235) = 2.5$, $p < .06$, analysis of the differences between percent avoidance with and without the visual stimuli revealed no statistically significant differences between the control group and any of the PCB groups ($ps > .10$, overall means shown in Table 1A).

The only other significant interaction was Stimulus x Blocks; for the BD assay, $F(11,1606) = 1.9$, $p < .05$, and for the BD/SD assay, $F(11, 2585) = 2.9$, $p = .001$, indicating that the decline in mean percent avoidance was greater across blocks with visual stimuli than without visual stimuli. The main effect of Blocks with visual stimuli was significant for the BD assay,

$F(11,1639) = 5.84, p < .001$, and for the BD/SD assay, $F(11, 2618) = 5.12, p < .001$. The main effect of Blocks with no visual stimuli was also significant for the BD assay, $F(11, 1639) = 2.12, p < .05$, but not for the BD/SD assay, $F(11, 2618) = 1.46, p > .10$.

3.2 Effects of PCB-exposure on thigmotaxis

PCB-exposure had no statistically significant effect on thigmotaxis in the BD assay (Fig. 4A–4D). There were significant main effects of Stimulus, $F(1, 146) = 19.5, p < .001$, and Blocks, $F(11, 1606) = 2.3, p < .01$, and a marginally nonsignificant Stimulus $\times$ Blocks interaction, $F(11, 1606) = 1.8, p = .054$. Mean percent edge collapsed across blocks was numerically higher with visual stimuli than without for all groups but these differences were only significant for controls and the 5 ppm group ($ps < .05$, means shown in Table 1B). No other main effects or interactions were significant for the BD assay ($ps > .10$).

Embryonic PCB-exposure did, however, have an effect on thigmotaxis in the BD/SD assay (Fig. 4E–4H). There was a significant Stimulus $\times$ PCB interaction, $F(3, 235) = 4.2, p < .01$. Mean percent edge overall was significantly higher with visual stimuli than without for all groups ($ps < .001$, means shown in Table 1B). The mean percent edge preference was significantly stronger for the 5 ppm and 10 ppm PCB-exposure groups relative to the control larvae when no visual stimuli were present ($ps = .013$, Fig 3B). There were no significant differences in mean edge preference between the control and PCB-treated larvae when the visual stimuli were present ($ps > .09$). Thigmotaxis also increased over blocks, $F(11, 2585) = 3.9, p < .001$.

3.3 Effects of PCB-exposure on freezing

Embryonic PCB-exposure had no statistically significant effect on freezing in the BD assay (Fig. 5A–5D). Mean percent freezing was significantly greater with the visual stimulus present than absent, $F(1,146) = 97.5, p < .001$, and mean percent freezing increased over blocks, $F(11,1606) = 8.7, p < .001$. Individual analyses revealed that mean percent freezing overall was significantly higher with visual stimuli than without visual stimuli for all groups ($ps < .001$, means shown in Table 1C). No other main effects or interactions were significant for the BD assay ($ps > .10$).

For the BD/SD assay (Fig. 5E–5H), there was a marginally nonsignificant Stimulus $\times$ PCB interaction, $F(3,235) = 2.5, p = .057$, a significant Blocks $\times$ PCB interaction, $F(11, 2585) = 1.5, p < .05$, and a significant Stimulus $\times$ Blocks interaction, $F(11, 2585) = 5.7, p < .001$. Individual analyses revealed that mean percent freezing was significantly higher with visual stimuli than without visual stimuli for all groups ($ps < .001$, means shown in Table 1C). However, analysis of the difference between mean percent freezing with and without the visual stimuli revealed no statistically significant differences between the control group and any of the PCB groups ($ps > .10$). In addition, for all groups, the magnitude of the difference between mean percent freezing with and without the visual stimuli decreased over blocks.

PCB exposure, however, was not entirely without effect on mean percent freezing in the BD/SD assay. Exploration of the significant Blocks $\times$ PCB interaction revealed that terminal

levels of percent freezing were significantly higher in the 5 ppm group relative to the control larvae, (Blocks 9, 11 and 12, $p < .05$).

3.4 Comparison of control larvae on the two assays

Inspection of Table 1 and Figs 2, 4 and 5 indicates apparent differences in the performance of the control larvae on the two assays. Specifically, the magnitude of avoidance behavior was attenuated in the control larvae tested on the BD/SD assay (mean difference score was $8.6\% \pm 1.1$) relative to the control larvae tested on the BD assay (mean difference score was $13.2\% \pm 1.6$) whereas thigmotaxis and, to a lesser extent, freezing were potentiated. Statistical analyses confirmed that the magnitude of the avoidance response was significantly smaller on the BD/SD assay compared to the BD assay, $F(1,153) = 6.2$, $p = .014$ and that the overall mean percent edge preference with visual stimuli present was significantly greater on the BD/SD assay ($89.4\% \pm 0.7$) than on the BD assay ($84.6\% \pm 1.5$), $F(1,153) = 10.0$, $p = .002$. No other differences were statistically significant, $F_s(1,153) < 1.8$, $p > .10$.

4. Discussion

Embryonic PCB-exposure altered the behavioral phenotype of 7 dpf zebrafish larvae on two visual avoidance assays. Specifically, avoidance behavior on the BD assay was reduced in the 5 ppm and 10 ppm PCB-exposed larvae relative to controls. On the BD/SD assay, relative to controls, the 5 ppm and 10 ppm PCB-exposed larvae did not differ in mean percent avoidance but did have elevated levels of thigmotaxis in the absence of visual stimuli and the 5 ppm larvae had higher terminal levels of freezing.

4.1 Performance of the control larvae on the two assays

The behavior of the control larvae was consistent with our hypothesis and previous findings that the red 'bouncing' disk is perceived as a visual threat and elicits anxiety-related responses. Avoidance, thigmotaxis and freezing, were all significantly greater with visual stimuli present than in their absence on both assays. The results from the BD/SD assay confirm the conclusion drawn by Pelkowski et al. (2011) that it is the movement of the disk that is avoided rather than the disk per se.

The behavior of the control larvae, however, was not identical on the two assays. The magnitude of avoidance behavior was attenuated in the control larvae tested on the BD/SD assay relative to the control larvae tested on the BD assay whereas thigmotaxis was potentiated in the presence of the visual stimuli. This pattern of differential responding is important because it demonstrates that the stationary disk in the BD/SD assay was in fact detected by the control larvae. Confirmation of these novel results using untreated larvae grown from the same set of egg collections and randomly assigned to the two assays would be valuable for two reasons. First, it would rule out any potential differences in maturation or nutrition that could account for the present observations (Clift et al., 2014; O'Neale et al., 2014). Second, it would support our assertion that the BD/SD assay has greater threat potential than the BD assay because larvae tested on the BD/SD assay may learn that the seemingly benign stationary disk actually has the potential to move.

The gradual decline in mean percent avoidance of the red ‘bouncing’ disk we observed on both assays over the course of repeated stimulus presentations is in line with other reports documenting the decremental impact on behavior of iterative stimulus presentations in larval zebrafish (Best et al., 2008; Burgess and Granato 2007; Roberts et al., 2011; Wolman et al., 2011). Elsewhere, we have discussed the pitfalls associated with interpreting such within-session decrements in behavior as evidence of learning, explained the need for an appropriate control treatment, and stressed the importance of a common test (O’Neale et al., 2014). Thus, further studies are needed to determine if the decrease in avoidance behavior seen here is related to a learning process like habituation or is the consequence of non-learning processes like sensory adaptation and motor fatigue. Additional research would also be useful to determine if the increase in percent freezing and percent thigmotaxis over blocks reflects a sensitization process induced by repeated exposure to the ‘bouncing’ disk. Such evidence might then favor a performance-based response competition account of the decline in percent avoidance.

4.2 Effects of embryonic PCB-exposure on behavior

On the BD assay, the PCB-exposed larvae differed from the controls on one of the three behavioral measures, showing attenuated avoidance of the ‘bouncing’ disk with the two highest A1254 exposure concentrations. Importantly, two of the three measures were significantly elevated when the ‘bouncing’ disk was present compared to its absence, suggesting that the ability to perceive the moving disk was not substantively affected in the PCB-exposed larvae despite the propensity for PCB exposure to target retinal development in zebrafish (Wang et al., 2012). Rather, it seems more likely that the avoidance defect on the BD assay resulted from an effect of PCB exposure on motor system development or on central processes involved in threat avoidance.

Chronic exposures to A1254 at concentrations lower than those used in the present study and to individual PCB congeners have been found to induce major structural deformities in embryonic and early stage larval zebrafish including cardiac, craniofacial and skeletal abnormalities (Billsson et al., 1998; Grimes et al., 2008; Ju et al., 2012; Li et al., 2014; Sisman et al., 2007; Wang et al., 2012). Although the PCB-exposed larvae we tested at 7 dpf did not display any visible malformations, the sub-chronic 2–26 hpf A1254 exposure window we used may have induced more subtle structural or functional defects in movement. Indeed, with the two higher exposure concentrations used here, we have observed a relatively minor defect in swim initiation in some 7 dpf larvae. Briefly, the affected larvae move with a ‘stutter’ as they transition from a resting state, perhaps indicative of a PCB-exposure effect on the number, arrangement or differentiation of trunk muscle fibers or on sarcomere length (Buss and Drapeau, 2000; Dou et al., 2008). Aberrations of this kind may have contributed to the attenuated avoidance we found on the BD assay and the behavioral changes we observed on the BD/SD assay.

Successful avoidance depends not only on executing a motor response but on detecting and assessing the potential threat. PCB-exposure has been associated with mild deficits in attention in children and animals possibly through its action on thyroid hormone (Eubig et al., 2010). Thus, the avoidance deficit we observed on the BD assay may stem from slower

detection of the ‘bouncing’ disk in the PCB-exposed larvae relative to the controls. It is also possible that, having detected the moving disk, the PCB-exposed larvae were more prone to panic, swimming haphazardly or directly to the nearest edge of the well. Adult zebrafish that had been fed a spiked diet containing an eco-relevant PCB mixture of 13 congeners for 8 months were hyper-reactive to a novel environment relative to solvent and untreated controls. Their behavioral profile, faster entry into the deep end, more time in the deep end and fewer exits from the deep end into the shallow end, is consistent with an exaggerated fear response (Péan et al., 2013). Although their offspring were found to be hyperactive at 5 dpf, they did not display a heightened fear response in a light-dark assay. A ceiling effect may have prevented detection of exaggerated startle to the light-dark transition, and thigmotaxis, a commonly used index of anxiety (Colwill and Creton, 2011a), was not measured. More research is therefore needed to determine if embryonic PCB exposure potentiates anxiety-related responses in zebrafish larvae. Likely targets mediating such an effect would include the habenula, a region involved in fear and anxiety (Agetsuma et al., 2010; Lee et al., 2010), its neurochemical pathways, particularly dopamine and serotonin, neurotransmitters which are both decreased following PCB-exposure, and the hypothalamus-pituitary-interrenal axis which is homologous to the human hypothalamus-pituitary-adrenal axis (Cachat et al., 2011).

In contrast to the control larvae, avoidance responding did not appear to differ between the BD and the BD/SD assay in the PCB-exposed larvae. Reduced visual acuity may have impaired the ability of the PCB-exposed larvae to detect the stationary disk thus rendering the two assays virtually identical. It has recently been shown that continuous exposure to A1254 from immediately after fertilization until testing at 7dpf affected performance on an optomotor assay in a manner consistent with poor visual acuity (Zhang et al., 2015). Alternatively, the PCB-exposed larvae may have a cognitive deficit that impaired their ability to generalize between the ‘bouncing’ and the stationary disks. This comparison of performance of the PCB-exposed larvae on the two assays would be more meaningful if the larvae had been drawn from the same egg collections and shared PCB exposure treatments, particularly since there were differences in thigmotaxis between the 5 ppm and 10 ppm PCB-exposed larvae on the two assays even before presentation of the visual stimuli.

4.3 Conclusion

Exposure to the PCB mixture A1254 from 2 to 26 hpf affected the performance of 7 dpf zebrafish larvae on two visual avoidance assays. Visual, motor and brain defects may have contributed to the pattern of impairments in the PCB-exposed larvae which included attenuated avoidance on the BD assay and elevated freezing and thigmotaxis on the BD/SD assay. One strategy that could prove useful in identifying the source of these functional deficits would be to vary the timing of the PCB-exposure window (Clift et al., 2015). Overall, these findings highlight the potential risks of PCB-exposure to human fetal and neonatal development and the importance of continued monitoring of PCB levels in the environment.

Acknowledgments

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

References

- Agetsuma M, Aizawa H, Tazu A, Nakayama R, Takahoko M, Goto M, et al. The habenula is crucial for experience-dependent modification of fear responses in zebrafish. *Nat Neurosci*. 2010; 13:1354–6. <http://dx.doi.org/10.1038/nn.2654>. [PubMed: 20935642]
- Ali S, Champagne DL, Spaink HP, Richardson MK. Zebrafish embryos and larvae: a new generation of disease models and drug screens. *Birth Defects Res C Embryo Today*. 2011; 93:115–33. <http://dx.doi.org/10.1002/bdrc.20206>. [PubMed: 21671352]
- Angulo R, Martinez P, Jodral ML. PCB congeners transferred by human milk, with an estimate of their daily intake. *Food Chem Toxicol*. 1999; 37:1081–8. [http://dx.doi.org/10.1016/S0278-6915\(99\)00101-5](http://dx.doi.org/10.1016/S0278-6915(99)00101-5). [PubMed: 10566879]
- Aoki Y. Polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins, and polychlorinated dibenzofurans as endocrine disrupters – what we have learned from Yusho disease. *Environ Res*. 2001; 86:2–11. <http://dx.doi.org/10.1006/enrs.2001.4244>. [PubMed: 11386736]
- Bailey J, Oliveri A, Levin ED. Zebrafish model systems for developmental neurobehavioral toxicology. *Birth Defects Res C Embryo Today*. 2013; 99:14–23. <http://dx.doi.org/10.1002/bdrc.21027>. [PubMed: 23723169]
- Best JD, Berghmans S, Hunt JJ, Clarke SC, Fleming A, Goldsmith P, Roach AG. Non-associative learning in larval zebrafish. *Neuropsychopharmacology*. 2008; 33:1206–15. <http://dx.doi.org/10.1038/sj.npp.1301489>. [PubMed: 17581529]
- Billsson K, Westerlund L, Tysklind M, Olsson P-E. Developmental disturbances caused by polychlorinated biphenyls in zebrafish (*Brachydanio rerio*). *Marine Environ Res*. 1998; 46:461–4. [http://dx.doi.org/10.1016/S0141-1136\(97\)00041-X](http://dx.doi.org/10.1016/S0141-1136(97)00041-X).
- Bruni G, Lakhani P, Kokel D. Discovering novel neuroactive drugs through high-throughput behavior-based chemical screening in the zebrafish. *Front Pharmacol*. 2014; 24:153. <http://dx.doi.org/10.3389/fphar.2014.00153>. [PubMed: 25104936]
- Burgess HA, Granato M. Sensorimotor gating in larval zebrafish. *J Neurosci*. 2007; 27:4984–94. <http://dx.doi.org/10.1523/JNEUROSCI.0615-07.2007>. [PubMed: 17475807]
- Buss RR, Drapeau P. Physiological properties of zebrafish embryonic red and white muscle fibers during early development. *J Neurophysiol*. 2000; 84:1545–57. [PubMed: 10980026]
- Cachat, JM.; Canavello, PR.; Elegante, MF.; Bartels, BK.; Elkhayat, SI.; Hart, PC., et al. Modeling stress and anxiety in zebrafish. In: Kalueff, AV.; Cachat, JM., editors. *Zebrafish Models in Neurobehavioral Research*. New York: Humana Press; 2011. p. 73-88.
- Clift DE, Richendrfer H, Thorn RJ, Colwill RM, Creton R. High-throughput analysis of behavior in zebrafish larvae: Effects of feeding. *Zebrafish*. 2014; 11:455–61. <http://dx.doi.org/10.1089/zeb.2014.0989>. [PubMed: 25153037]
- Clift DE, Thorn RJ, Passarelli EA, Kapoor M, LoPiccolo MK, Richendrfer HA, et al. Effects of embryonic cyclosporine exposures on brain development and behavior. *Behav Brain Res*. 2015; 282:117–24. <http://dx.doi.org/10.1016/j.bbr.2015.01.006>. [PubMed: 25591474]
- Colwill, RM.; Creton, R. Automated imaging of avoidance behavior in larval zebrafish. In: Kalueff, AV.; Cachat, JM., editors. *Neuromethods*. 1. Vol. 51. *Zebrafish Neurobehavioral Protocols* Humana Press, Springer Protocols; 2010. p. 35-48.
- Colwill RM, Creton R. Imaging escape and avoidance behavior in zebrafish larvae. *Rev Neurosci*. 2011a; 22:63–73. <http://dx.doi.org/10.1515/RNS.2011.008>. [PubMed: 21572576]
- Colwill RM, Creton R. Locomotor behaviors in zebrafish (*Danio rerio*) larvae. *Behav Processes*. 2011b; 86:222–9. <http://dx.doi.org/10.1016/j.beproc.2010.12.003>. [PubMed: 21147203]

- Corey DA, Juárez de Ku LM, Bingman VP, Meserve LA. Effects of exposure to polychlorinated biphenyl (PCB) from conception on growth, and development of endocrine, neurochemical, and cognitive measures in 60 day old rats. *Growth Dev Aging*. 1996; 60:131–43. [PubMed: 9007564]
- Creton R. Automated analysis of behavior in zebrafish larvae. *Behav Brain Res*. 2009; 203:127–36. <http://dx.doi.org/10.1016/j.bbr.2009.04.030>. [PubMed: 19409932]
- Darvill T, Lonky E, Reihman J, Stewart P, Pagano J. Prenatal exposure to PCBs and infant performance on the fagan test of infant intelligence. *Neurotoxicology*. 2000; 21:1029–38. [http://dx.doi.org/10.1016/S0892-0362\(99\)00056-2](http://dx.doi.org/10.1016/S0892-0362(99)00056-2). [PubMed: 11233749]
- Dou Y, Andersson-Lendahl M, Arner A. Structure and function of skeletal muscle in zebrafish early larvae. *J Gen Physiol*. 2008; 131:445–53. <http://dx.doi.org/10.1085/jgp.200809982>. [PubMed: 18443359]
- Eriksson P, Fredriksson A. Developmental neurotoxicity of four ortho-substituted polychlorinated biphenyls in the neonatal mouse. *Environ Toxicol Pharmacol*. 1996; 1:155–65. [http://dx.doi.org/10.1016/1382-6689\(96\)00015-4](http://dx.doi.org/10.1016/1382-6689(96)00015-4). [PubMed: 21781676]
- Eubig PA, Aguiar A, Schantz SL. Lead and PCBs as risk factors for attention deficit/hyperactivity disorder. *Environ Health Perspect*. 2010; 118:1654–67. <http://dx.doi.org/10.1289/ehp.0901852>. [PubMed: 20829149]
- Feeley M, Brouwer A. Health risks to infants from exposure to PCBs, PCDDs and PCDFs. *Food Addit Contam*. 2000; 17:325–33. <http://dx.doi.org/10.1080/026520300283397>. [PubMed: 10912246]
- Fero, K.; Yokogawa, T.; Burgess, HA. The behavioral repertoire of larval zebrafish. In: Kalueff, AV.; Cachat, JM., editors. *Zebrafish Models in Neurobehavioral Research*. New York: Springer; 2011. p. 249–91.
- Fitzgerald EF, Belanger EE, Gomez MI, Cayo M, McCaffrey RJ, Seegal RF, et al. Polychlorinated biphenyl exposure and neuropsychological status among older residents of upper Hudson River communities. *Environ Health Perspect*. 2008; 116:209–15. <http://dx.doi.org/10.1289/ehp.10432>. [PubMed: 18288320]
- Gladen BC, Rogan WJ. Effects of perinatal polychlorinated biphenyls and dichlorodiphenyl dichloroethene on later development. *J Pediatr*. 1991; 119:58–63. [http://dx.doi.org/10.1016/S0022-3476\(05\)81039-X](http://dx.doi.org/10.1016/S0022-3476(05)81039-X). [PubMed: 1906100]
- Gladen BC, Rogan WJ, Hardy P, Thullen J, Tingelstad J, Tully M. Development after exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene transplacentally and through human milk. *J Pediatr*. 1988; 113:991–5. [http://dx.doi.org/10.1016/S0022-3476\(88\)80569-9](http://dx.doi.org/10.1016/S0022-3476(88)80569-9). [PubMed: 3142988]
- Grandjean P, Landrigan PJ. Developmental neurotoxicity of industrial chemicals. *Lancet*. 2006; 368:2167–78. [http://dx.doi.org/10.1016/S0140-6736\(06\)69665-7](http://dx.doi.org/10.1016/S0140-6736(06)69665-7). [PubMed: 17174709]
- Grimes AC, Erwin KN, Stadt HA, Hunter GL, Gefroh HA, Tsai HJ, et al. PCB126 exposure disrupts zebrafish ventricular and branchial but not early neural crest development. *Toxicol Sci*. 2008; 106:193–205. <http://dx.doi.org/10.1093/toxsci/kfn154>. [PubMed: 18660518]
- Hansen, LG. *The ortho side of PCBs: Occurrence and disposition*. Boston, MA: Kluwer Academic Publishers; 1999. p. 269
- He J-H, Gao J-M, Huang C-J, Li C-Q. Zebrafish models for assessing developmental and reproductive toxicity. *Neurotoxicol Teratol*. 2014; 42:35–42. <http://dx.doi.org/10.1016/j.ntt.2014.01.006>. [PubMed: 24503215]
- Herrick RF, Lefkowitz DJ, Weymouth GA. Soil contamination from PCB-containing buildings. *Environ Health Perspect*. 2007; 115:173–5. <http://dx.doi.org/10.1289/ehp.9646>. [PubMed: 17384760]
- Huisman M, Koopman-Esseboom C, Fidler V, Hadders-Algra M, van der Paauw CG, Tuinstra LG, et al. Perinatal exposure to polychlorinated biphenyls and dioxins and its effect on neonatal neurological development. *Early Hum Dev*. 1995a; 41:111–27. [http://dx.doi.org/10.1016/0378-3782\(94\)01611-R](http://dx.doi.org/10.1016/0378-3782(94)01611-R). [PubMed: 7601016]
- Huisman M, Koopman-Esseboom C, Lanting CI, Van der Paauw CG, Tuinstra LGMTh, Fidler V. Neurological condition in 18-month-old children perinatally exposed to polychlorinated biphenyls and dioxins. *Early Hum Dev*. 1995b; 43:165–76. [http://dx.doi.org/10.1016/0378-3782\(95\)01674-0](http://dx.doi.org/10.1016/0378-3782(95)01674-0). [PubMed: 8903761]

- Jacobson JL, Jacobson SW. Intellectual impairment in children exposed to polychlorinated biphenyls *in utero*. *N Engl J Med*. 1996; 335:783–9. <http://dx.doi.org/10.1056/NEJM199609123351104>. [PubMed: 8703183]
- Jacobson JL, Jacobson SW, Humphrey HE. Effects of exposure to PCBs and related compounds on growth and activity in children. *Neurotoxicol Teratol*. 1990; 12:319–26. [http://dx.doi.org/10.1016/0892-0362\(90\)90050-M](http://dx.doi.org/10.1016/0892-0362(90)90050-M). [PubMed: 2118230]
- Jardine D, Litvak MK. Direct yolk sac volume manipulation of zebrafish embryos and the relationship between offspring size and yolk sac volume. *J Fish Biol*. 2003; 63:388–97. <http://dx.doi.org/10.1046/j.1095-8649.2003.00161.x>.
- Ju L, Tang K, Guo XR, Yang Y, Zhu GZ, Lou Y. Effects of embryonic exposure to polychlorinated biphenyls on zebra sh skeletal development. *Mol Med Rep*. 2012; 5:1227–31. <http://dx.doi.org/10.3892/mmr.2012.823>. [PubMed: 22395424]
- Kalueff AV, Stewart AM, Gerlai R. Zebrafish as an emerging model for studying complex brain disorders. *Trends Pharmacol Sci*. 2014; 35:63–75. <http://dx.doi.org/revproxy.brown.edu/10.1016/j.tips.2013.12.002>. [PubMed: 24412421]
- Kodavanti PRS, Osorio C, Royland JE, Ramabhadran R, Alzate O. Aroclor 1254, a developmental neurotoxicant, alters energy metabolism- and intracellular signaling-associated protein networks in rat cerebellum and hippocampus. *Toxicol Appl Pharmacol*. 2011; 256:290–9. <http://dx.doi.org/10.1016/j.taap.2011.07.005>. [PubMed: 21791222]
- Koopman-Esseboom C, Weisglas-Kuperus N, de Ridder MAJ, Van der Paauw CG, Tuinstra LGMTh, Sauer PJJ. Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infants' mental and psychomotor development. *Pediatr*. 1996; 97:700–6.
- Kreiling JA, Creton R, Reinisch C. Early embryonic exposure to polychlorinated biphenyls disrupts heat-shock protein 70 cognate expression in zebrafish. *J Toxicol Environ Health Part A*. 2007; 70:1005–13. <http://dx.doi.org/10.1080/15287390601171868>. [PubMed: 17497411]
- Lee A, Mathuru AS, Teh C, Kibat C, Korzh V, Penney TB, et al. The habenula prevents helpless behavior in larval zebrafish. *Curr Biol*. 2010; 20:2211–6. <http://dx.doi.org/10.1016/j.cub.2010.11.025>. [PubMed: 21145744]
- Lee DW, Notter SA, Thiruchelvam M, Dever DP, Fitzpatrick R, Kostyniak PJ, et al. Subchronic polychlorinated biphenyl (Aroclor 1254) exposure produces oxidative damage and neuronal death of ventral midbrain dopaminergic systems. *Toxicol Sci*. 2012; 125:496–508. <http://doi.org/10.1093/toxsci/kfr313>. [PubMed: 22094459]
- Li A, Rockne KJ, Sturchio N, Song W, Ford JC, Wei H. PCBs in sediments of the Great Lakes—distribution and trends, homolog and chlorine patterns, and in situ degradation. *Environ Pollut*. 2009; 157:141–7. <http://dx.doi.org/10.1016/j.envpol.2008.07.014>. [PubMed: 18771832]
- Li M, Wang X, Zhu J, Zhu S, Hu X, Zhu C, et al. Toxic effects of polychlorinated biphenyls on cardiac development in zebrafish. *Mol Biol Rep*. 2014; 41:7973–83. <http://dx.doi.org/10.1007/s11033-014-3692-6>. [PubMed: 25163633]
- Ling B, Han G, Xu Y. PCB levels in humans in an area of PCB transformer recycling. *Ann N Y Acad Sci*. 2008; 1140:135–42. <http://dx.doi.org/10.1196/annals.1454.030>. [PubMed: 18991912]
- O'Neale A, Ellis J, Creton R, Colwill RM. Single stimulus learning in zebrafish larvae. *Neurobiol Learn Mem*. 2014; 108:145–54. <http://dx.doi.org/10.1016/j.nlm.2013.08.016>. [PubMed: 24012906]
- Orito K, Gotanda N, Murakami M, Ikeda T, Egashira N, Mishima K, et al. Prenatal exposure to 3,3', 4,4'-pentachlorobiphenyl (PCB126) promotes anxiogenic behavior in rats. *Tohoku J Exp Med*. 2007; 212:151–7. <http://dx.doi.org/10.1620/tjem.212.151>. [PubMed: 17548959]
- Péan S, Daouk T, Vignet C, Lyphout L, Leguay D, Loizeau V, et al. Long-term dietary-exposure to non-coplanar PCBs induces behavioral disruptions in adult zebrafish and their offspring. *Neurotoxicol Teratol*. 2013; 39:45–56. <http://dx.doi.org/10.1016/j.ntt.2013.07.001>. [PubMed: 23851001]
- Pelkowski SD, Kapoor M, Richendrfer HA, Wang X, Colwill RM, Créton R. A novel high-throughput imaging system for automated analyses of avoidance behavior in zebrafish larvae. *Behav Brain Res*. 2011; 223:135–44. <http://dx.doi.org/10.1016/j.bbr.2011.04.033>. [PubMed: 21549762]

- Plusquellec P, Muckle G, Dewailly E, Ayotte P, Begin G, Desrosiers C, et al. The relation of environmental contaminants exposure to behavioral indicators in Inuit Preschoolers in Arctic Quebec. *Neurotoxicology*. 2010; 31:17–25. <http://dx.doi.org/10.1016/j.neuro.2009.10.008>. [PubMed: 19854214]
- Rice DC. Effect of exposure to 3,3',4,4',5-pentachlorobiphenyl (PCB 126) throughout gestation and lactation on development and spatial delayed alternation performance in rats. *Neurotoxicol Teratol*. 1999; 21:59–69. [http://dx.doi.org/10.1016/S0892-0362\(98\)00031-2](http://dx.doi.org/10.1016/S0892-0362(98)00031-2). [PubMed: 10023802]
- Rice DC, Hayward S. Effects of postnatal exposure to a PCB mixture in monkeys on nonspatial discrimination reversal and delayed alternation performance. *Neurotoxicology*. 1997; 18:479–94. [PubMed: 9291496]
- Richendrfer H, Pelkowski S, Colwill RM, Creton R. On the edge: pharmacological evidence for anxiety-related behavior in zebrafish larvae. *Behav Brain Res*. 2012; 228:99–106. <http://dx.doi.org/10.1016/j.bbr.2011.11.041>. [PubMed: 22155488]
- Roberts AC, Reichl J, Song MY, Dearing AD, Moridzadeh N, Lu ED, et al. Habituation of the C-start response in larval zebrafish exhibits several distinct phases and sensitivity to NMDA receptor blockade. *PLoS One*. 2011; 6:e29132. <http://dx.doi.org/10.1371/journal.pone.0029132>. [PubMed: 22216183]
- Safe SH. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. *Crit Rev Toxicol*. 1994; 24:87–149. <http://dx.doi.org/10.3109/10408449409049308>. [PubMed: 8037844]
- Schantz SL, Moshtaghian J, Ness DK. Spatial learning deficits in adult rats exposed to ortho-substituted PCB congeners during gestation and lactation. *Fundam Appl Toxicol*. 1995; 26:117–26. <http://dx.doi.org/10.1006/faat.1995.1081>. [PubMed: 7657055]
- Sisman T, Geyikoglu F, Atamanalp M. Early life-stage toxicity in zebrafish (*Danio rerio*) following embryonal exposure to selected polychlorinated biphenyls. *Toxicol Ind Health*. 2007; 23:529–36. <http://dx.doi.org/10.1177/0748233708089042>. [PubMed: 18681238]
- Spence R, Gerlach G, Lawrence C, Smith C. The behaviour and ecology of the zebrafish, *Danio rerio*. *Biol Rev*. 2008; 83:13–34. <http://dx.doi.org/10.1111/j.1469-185X.2007.00030.x>. [PubMed: 18093234]
- Sugawara N, Nakai K, Nakamura T, Ohba T, Suzuki K, Kameo S, et al. Developmental and neurobehavioral effects of perinatal exposure to polychlorinated biphenyls in mice. *Arch Toxicol*. 2006; 80:286–92. <http://dx.doi.org/10.1007/s00204-005-0042-4>. [PubMed: 16244857]
- Tieman TO, Taylor ML, Garrett JH, VanNess GF, Solch JG, Wagel DJ, et al. Sources and fate of polychlorinated dibenzodioxins, dibenzofurans and related compounds in human environments. *Environ Health Perspect*. 1985; 59:145–58. <http://dx.doi.org/10.2307/3429887>. [PubMed: 3921357]
- Tilson HA, Jacobson JL, Rogan WJ. Polychlorinated biphenyls and the developing nervous system: cross-species comparisons. *Neurotoxicol Teratol*. 1990; 12:239–48. [http://dx.doi.org/10.1016/0892-0362\(90\)90095-T](http://dx.doi.org/10.1016/0892-0362(90)90095-T). [PubMed: 2115098]
- Wang YP, Hong Q, Qin DN, Kou CZ, Zhang CM, Guo M, et al. Effects of embryonic exposure to polychlorinated biphenyls on zebrafish (*Danio rerio*) retinal development. *J Appl Toxicol*. 2012; 32:186–93. <http://dx.doi.org/10.1002/jat.1650>. [PubMed: 21381056]
- Wickizer TM, Brilliant LB, Copeland R, Tilden R. Polychlorinated biphenyl contamination of nursing mothers' milk in Michigan. *Am J Public Health*. 1981; 71:132–7. [PubMed: 6779648]
- Winneke G. Developmental aspects of environmental neurotoxicology: lessons from lead and polychlorinated biphenyls. *J Neurol Sci*. 2011; 308:9–15. <http://dx.doi.org/10.1016/j.jns.2011.05.020>. [PubMed: 21679971]
- Wolman M, Granato M. Behavioral genetics in larval zebrafish: Learning from the young. *Devel Neurobiol*. 2012; 72:366–72. <http://dx.doi.org/10.1002/dneu.20872>.
- Wolman MA, Jain RA, Liss L, Granato M. Chemical modulation of memory formation in larval zebrafish. *Proc Natl Acad Sci U S A*. 2011; 108:15468–73. <http://dx.doi.org/10.1073/pnas.1107156108>. [PubMed: 21876167]
- Yang D, Lein PJ. Polychlorinated biphenyls increase apoptosis in the developing rat brain. *Curr Neurobiol*. 2010; 1:70–6. [PubMed: 24052688]

Zhang X, Hong Q, Yang L, Zhang M, Guo X, Chi X, et al. PCB1254 exposure contributes to the abnormalities of optomotor responses and influence of the photoreceptor cell development in zebrafish larvae. *Ecotoxicology and Environmental Safety*. 2015; 118:133–8. <http://dx.doi.org/10.1016/j.ecoenv.2015.04.026>. [PubMed: 25938693]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Highlights

The effect of Aroclor 1254 on anxiety-related behaviors was evaluated in zebrafish larvae.

PCB-exposure from 2–26 hours post-fertilization induced behavioral defects at one week.

Defects included decreased avoidance of a moving visual stimulus and elevated thigmotaxis.

Continued monitoring of PCB levels in the environment is essential.

Fig. 1.

Automated analysis of visual avoidance behavior. Seven-day-old larvae were imaged for 5 minute periods without visual stimuli (A) alternating with 5 minute periods of either a red ‘bouncing’ disk for the BD assay (B) or a red ‘bouncing’ disk and a red stationary disk for the BD/SD assay (C). An automatic macro was used to split color channels to remove the red disk, subtract the background, apply a threshold, and identify the larva based on particle size (D–G).

Fig. 2.

Avoidance behavior on the two assays. Mean ($\pm$ SEM) percent avoidance with the red ‘bouncing’ disk (red squares) and without (black diamonds) for the BD assay (left column, A–D) and the BD/SD assay (right column, E–H). C denotes control larvae (EW and DMSO combined); 2, 5 and 10 denote exposure concentrations of 2, 5 and 10 ppm A1254, respectively; W denotes no visual stimuli; BD denotes red ‘bouncing’ disk; BD/SD denotes red ‘bouncing’ disk and red stationary disk. Data are shown separately for each of the twelve 5-min periods with and without the visual stimuli.

Fig. 3. PCB effects on behavior. A) Mean ($\pm$ SEM) percent avoidance difference scores on the BD assay. Each bar represents the difference between overall mean percent avoidance with and without the 'bouncing' disk. B) Mean ($\pm$ SEM) percent thigmotaxis on the BD/SD assay. White bars denote no visual stimulus; red bars denote 'bouncing' and stationary disks. For both graphs, C denotes control larvae (EW and DMSO combined); 2 ppm, 5 ppm and 10 ppm denote exposure concentrations of A1254. Asterisk above a bar indicates a significant difference from the control mean.

Fig. 4.

Thigmotaxis on the two assays. Mean ($\pm$ SEM) percent edge (thigmotaxis) with the red ‘bouncing’ disk (red squares) and without (black diamonds) for the BD assay (left column, A–D) and the BD/SD assay (right column, E–H). C denotes control larvae (EW and DMSO combined); 2, 5 and 10 denote exposure concentrations of 2, 5 and 10 ppm A1254, respectively; W denotes no visual stimuli; BD denotes red ‘bouncing’ disk; BD/SD denotes red ‘bouncing’ disk and red stationary disk. Data are shown separately for each of the twelve 5-min periods with and without visual stimuli.

Fig. 5. Freezing behavior on the two assays. Mean ($\pm$ SEM) percent freezing with the red 'bouncing' disk (red squares) and without (black diamonds) for the BD assay (left column, A–D) and the BD/SD assay (right column, E–H). C denotes control larvae (EW and DMSO combined); 2, 5 and 10 denote exposure concentrations of 2, 5 and 10 ppm A1254, respectively; W denotes no visual stimuli; BD denotes red 'bouncing' disk; BD/SD denotes red 'bouncing' disk and red stationary disk. Data are shown separately for each of the twelve 5-min periods with and without visual stimuli.

Table 1

Summary of behavioral measures on the visual avoidance assays. Columns show mean percent responses $\pm$ SEMs with and without visual stimuli on the BD assay (left) and the BD/SD assay (right) for the combined controls (EW and DMSO) and the 2, 5 and 10 ppm PCB-exposure groups. BD denotes the red ‘bouncing’ disk; BD/SD denotes the red ‘bouncing’ disk and the red stationary disk; W denotes white background with no visual stimuli. A) Avoidance signifies percent observations on side away from moving disk; B) Thigmotaxis signifies percent observations at edge of well; C) Freezing signifies percent observations of no movement between pairs of consecutive images.

	BD	W	BD/SD	W
A. Avoidance				
Controls	68.0% $\pm$ 3.1	54.8% $\pm$ 2.4	61.1% $\pm$ 2.6	52.5% $\pm$ 2.3
2 ppm	62.9% $\pm$ 4.9	54.0% $\pm$ 4.1	59.9% $\pm$ 2.8	53.9% $\pm$ 2.5
5 ppm	63.2% $\pm$ 4.0	56.7% $\pm$ 3.7	63.0% $\pm$ 3.8	52.0% $\pm$ 3.4
10 ppm	58.5% $\pm$ 4.4	52.8% $\pm$ 4.0	57.9% $\pm$ 3.7	51.7% $\pm$ 3.2
B. Thigmotaxis				
Controls	84.6% $\pm$ 1.5	81.3% $\pm$ 1.6	89.4% $\pm$ 0.7	81.2% $\pm$ 1.2
2 ppm	89.1% $\pm$ 1.8	87.0% $\pm$ 1.9	89.3% $\pm$ 1.5	82.4% $\pm$ 1.8
5 ppm	84.1% $\pm$ 2.6	80.8% $\pm$ 2.6	90.7% $\pm$ 1.2	87.2% $\pm$ 1.5
10 ppm	86.1% $\pm$ 1.5	83.9% $\pm$ 1.7	92.5% $\pm$ 1.0	87.2% $\pm$ 1.6
C. Freezing				
Controls	29.5% $\pm$ 3.0	18.7% $\pm$ 2.4	32.7% $\pm$ 2.2	22.8% $\pm$ 1.9
2 ppm	40.3% $\pm$ 4.4	26.3% $\pm$ 3.3	30.7% $\pm$ 2.7	23.2% $\pm$ 2.4
5 ppm	35.9% $\pm$ 4.5	23.1% $\pm$ 3.5	40.8% $\pm$ 3.1	29.3% $\pm$ 2.7
10 ppm	35.5% $\pm$ 4.1	23.7% $\pm$ 3.2	33.3% $\pm$ 2.7	27.1% $\pm$ 2.8