Effects of PCB126 on Early Life Stage Development of Zebrafish (*Danio rerio*): A RNA-Sequencing Approach

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Introduction. New international guidelines and ethical concerns about the use of living animals for chemical risk assessments have led to efforts to shift focus to alternative testing methods for elucidating adverse effects of chemicals. The Acute Fish Embryo Toxicity Test (FET) is an example of an alternative testing method because early life stages of fishes (larvae) are not protected according to European law (EU-directive 2010/63/EU). Although the FET is embedded in a 2013 OECD guideline (no. 236) the assay still lacks full acceptance by industry and regulators because of uncertainties regarding the potential impacts of early developmental processes on the results of chemical testing. To address this issue, elucidation of molecular level alterations such as changes in gene expression caused by toxicants is a useful approach to determine their toxic modes of action. Furthermore the findings can help to establish complete "*adverse outcome pathways*" (OECD no. 184) that can be used to predict biological effects of chemicals prospectively without any animals testing. In this study, embryos of the zebrafish (*Danio rerio*) were exposed to PCB126 from fertilization until 96 hours post fertilization (hpf). PCB126 is a potent agonist of the aryl-hydrocarbon receptor (AhR), a ligand activated transcription factor that mediates all known toxic effects of dioxin-like compounds to organisms. Transcriptional responses in three different embryonic stages of embryos exposed to PCB126 were determined by use of RNAseq in order to identify life-stage specific molecular and biochemical key pathways and processes by which PCB126 can cause adverse effects during early development.

Material and Methods. Newly fertilized eggs of zebrafish were exposed to either 0.075% DMSO or 7.5 µg/L PCB126 in medium (294 mg/L CaCl2·2 H2O, 123.3 mg/L MgSO4·7 H2O, 63 mg/L NaHCO3, 5.5 mg/L KCl). Embryos were sampled at 12 hpf, 48 hpf and 96 hpf, euthanized, and total RNA was extracted. Samples were sequenced by use of Illumina TruSeq technology on a Miseq sequencing platform. Differentially expressed genes were determined by dispersion analysis. For pathway analsyis Cytoscape and the ClueGO plugin were used.

Results & Discussion. Compared to controls, there was an increasing number of genes that was significantly altered as developmental time of embryos exposed to PCB126 increased. At 12 hpf, abundances of transcripts of 9 genes were significantly increased in embryos exposed to PCB126, whereas abundances of 46 transcripts were decreased. After 48 and 96 hpf, abundances of transcripts of 60 and 180 genes, respectively, were decreased, and 67 and 163 genes, respectively, were increased in embryos exposed to PCB126.
Genes regulated by the AhR signalling pathway were not differentially expressed at 12 hpf. However, expression of several genes regulated by the AhR signaling pathway, including the phase I biotransformation enzymes cytochrome P450 1A1 (*cyp1a)* and *cyp1b,* and the AhR repressor (*ahrr),* were up-regulated in embryos exposed to PCB126 until 48 hpf and 96 hpf. This suggests that expression of the AhR protein and the capacity to biotransform dioxin-like compounds does not occur until after 12 hpf. Expression of 9 genes important for muscle development were suppressed at 12 hpf. At 96 hpf the expression of genes primarily encoding structural proteins was down-regulated, whereas expression of genes encoding proteins of membrane attack complex proteins were up-regulated. These results are consistent with severe morphological malformations that were microscopically observed (like edema, curved spine etc.) in the embryos at 96 hpf.
Opn1sw1 was the only transcript that was found to be decreased after PCB126 exposure at all three timepoints. This gene encodes for the opsin1 protein that is involved in photo receptor development. Expression of several genes involved in photo receptor development were downregulated at 12 hpf suggesting that disruption of molecular processes by dioxin-like compounds that lead to impairment of vision is initiated early in development. Overall, these findings provide a first comprehensive insight into the effects of PCB126 on the embryonic development of zebrafish.

The authors acknowledge the German Academic Exchange Service (DAAD) and the German Federal Environmental Foundation (DBU) for personal scholarschips granted to the first author.