Thyroid endocrine system disruption by pentachlorophenol: An 
in vitro and in vivo assay

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A B S T R A C T
The present study aimed to evaluate the disruption caused to the thyroid endocrine system by pentachlorophenol (PCP) using in vitro and in vivo assays. In the in vitro assay, rat pituitary GH3 cells were exposed to 0, 0.1, 0.3, and 1.0 µM PCP. PCP exposure significantly downregulated basal and triiodothyronine (T3)-induced Dio 1 transcription, indicating the antagonistic activity of PCP in vitro. In the in vivo assay, zebrafish embryos were exposed to 0, 1, 3, and 10 µg/L of PCP until 14 days post-fertilization. PCP exposure resulted in decreased thyroxine (T4) levels, but elevated contents of whole-body T3. PCP exposure significantly upregulated the mRNA expression of genes along hypothalamic–pituitary–thyroid (HPT) axis, including those encoding thyroid-stimulating hormone, sodium/iodide symporter, thyroglobulin, Dio 1 and Dio 2, alpha and beta thyroid hormone receptor, and uridine triphosphate-glucuronosyl-transferase. PCP exposure did not influence the transcription of the transthyretin (TTR) gene. The results indicate that PCP potentially disrupts the thyroid endocrine system both in vitro and in vivo.

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1. Introduction
PCP is mainly used as pesticide and wood preservative worldwide. Its widespread use for these purposes has led to PCP becoming a ubiquitous environmental contaminant (reviewed by Zheng et al., 2012). Although many countries have banned or controlled the use of PCP, it is still used as a wood preservative, and may be detected in the aquatic environment, wild animals and human samples (Farhadi et al., 2009; Zheng et al., 2011, 2012; Montaño et al., 2013). Levels of PCP in freshwater bodies in European countries have decreased and are typically at the lowest 0.2 µg/L (Muir and Eduljee, 1999). China restricted the use of PCP in 1997. However, PCP has been used as a molluscicide to control the re-emergence of schistosomiasis in some areas (Zheng et al., 2000; Tan and Zhang, 2008). A high concentration of PCP has been detected in Dongting lake (up to 103.7 µg/L), due to its application in the control of snail-borne schistosomiasis (Zheng et al., 2000). The use of large amounts of PCP has resulted in widespread environmental contamination. PCP has been detected in surface waters in China (up to 1.15 µg/L) (Gao et al., 2008; Zheng et al., 2012). These data indicated that PCP usage caused significant PCP pollution in the local water environment and may cause adverse effects on aquatic organisms and public health.

The impact of environmental chemicals on the thyroid endocrine system has received much attention in recent years, especially because thyroid hormones (THs) are particularly important in fetal development: the brain is dependent on normal levels of THs (reviewed by Boas et al., 2012). Endocrine system disrupting chemicals can have a direct impact on TH synthesis, transport, binding, catabolism and clearance of circulating THs (Kloas and Lutz, 2006). Limited information showed that PCP might disrupt thyroid endocrine functions. For example, in Xenopus laevis, PCP was shown to have T3-antagonist activity by in vitro and in vivo assays (Sugiyama et al., 2005). Developmental exposure to PCP in rats caused lowered total T4 concentrations in plasma (Kawaguchi et al., 2008). In humans, a negative association between maternal plasma PCP levels and cord plasma free T4 (fT4) concentrations in neonates was reported (Dallaire et al., 2009). Given the important roles of THs in the growth and development of fetuses, the potential disruption caused by PCP to the thyroid endocrine system has raised great concern over its adverse environmental health risks.

To evaluate TH endocrine system disruption, an in vitro model using the rat pituitary tumor cell line GH3 (T-Screen) has been developed. GH3 cells can synthesize and secrete growth hormone (GH) and prolactin (PRL) and T3 can induce production of the two hormones as well as induce gene transcription (Spindler et al., 1982; Stanley, 1988). This T-Screen is based on the T3-dependent cell growth, mediated by specific, high-affinity thyroid receptor (TR), where THs bind to thyroid hormone responsive elements (TREs) and ultimately lead to gene expression. Thus, the T-Screen assay can be used for in vitro detection of agonistic and antagonistic.
properties of compounds at the level of the TR in the absence and in the presence of T3 (Gutieé et al., 2005; Schriks et al., 2006). In addition, gene transcription can be measured based on the modulation of basal GH and PRL secretion in GH3 cells, which is related directly to regulation of GH and PRL mRNA expressions (Tamura et al., 2000). Furthermore, GH3 cells possess both deiodinase 1 (Dio 1) and 2 (Dio 2) activity (St Germain, 1985; Mori et al., 2007), and are responsive to T3 (Baur et al., 1997). Both deiodinases have an important role for maintaining local T3 levels in the brain and pituitary in vivo. The T-Screen assay successfully predicted the effects of certain thyroid hormone disrupting chemicals, such as plasticizers, alkylphenols, pesticides, polychlorinated biphenyls, brominated flame-retardants and personal care products (Ghisari and Bonefeld-Jørgensen, 2005; Hamers et al., 2006; Schriks et al., 2006; Hansen et al., 2009; Taxvig et al., 2011; Hinther et al., 2011).

In fish and amphibians, the thyroid endocrine system is controlled primarily by the hypothalamic–pituitary–thyroid (HPT) axis, which is responsible for regulating TH dynamics by coordinating their synthesis, secretion, transport and metabolism (Carr and Patiño, 2011). Several studies have demonstrated the sensitivity of the HPT axis in Xenopus laevis to a variety of known thyroid disrupting chemicals, including perchlorate, 6-propylthiouracil, methimazole, and ethylenethiourea (Degitz et al., 2005; Tietge et al., 2005, 2010; Opitz et al., 2009). Zebrafish thyroid system is similar to the mammalian or the amphibian thyroid system, and zebrafish has become very popular vertebrate model to screen for thyroid-disrupting chemical pollutants, and evaluating their risk to animals and humans (Raldau and Babin, 2009; Schmidt and Braunbeck, 2011; Heijlen et al., 2013). Recently, an in vivo model for testing endocrine disruption of THs was developed using zebrafish larvae (Yu et al., 2010; Schmidt and Braunbeck, 2011). This assay indicated that the zebrafish HPT axis can be used to determine thyroid endocrine system disruption by TH endocrine disruptors, and also, to some extent, to indicate potential mechanisms of action, e.g., polybrominated diphenyl ethers (PBDEs) (Yu et al., 2010; Chan and Chan, 2012), herbicide isoxylon (Campinho and Power, 2013), fungicides hexaconazole and tebuconazole (Yu et al., 2013), and triclosan (Pinto et al., 2013). However, the disruption of the thyroid endocrine system by PCP and the environmental risk to fish remains unknown.

The objectives of the present study were to investigate the disruption to the thyroid endocrine system by PCP using in vitro and in vivo assays. In the in vitro study, disruption of the TH-endocrine system was assessed using the T-Screen assay. TH-responsive gene transcription was examined upon PCP exposure. We further examined the impact of PCP on THs levels and mRNA expression of genes in the HPT axis and the potential mechanisms of disruption of the thyroid endocrine system in the developing zebrafish larvae.

2. Materials and methods

2.1. Chemicals

PCP (99%, CAS No. 87-86-5) and T3 (95%, CAS No. 6893-02-3) were purchased from Sigma–Aldrich (St. Louis, MO, USA). PCP was dissolved in dimethyl sulfoxide (DMSO), and stored at 4 °C. 4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma. All other chemicals, including cell culture medium and antibiotics (Gibco) used in the present study were of analytical grade.

2.2. Cell culture

Rat pituitary GH3 cells were obtained from the Cell Center of the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China). The cells were cultured in phenol red-free DMEM/F12 medium (Sigma), supplemented with 10% fetal calf serum (FCS, Gibco), 2.5% 100 U/mL penicillin, and 100 μg/mL of streptomycin. The cells were maintained at 37 °C in an atmosphere of 5% CO2.

2.3. Cell viability assay and chemical exposure

Cell viability was measured by monitoring the colorimetric conversion of MTT to formazan. Briefly, GH3 cells were seeded into 96-well culture plates (Falcon, Franklin Lakes, NJ, USA) at a density of 5 x 10^4 cells/well. After 24 h of culture, the cultured medium was removed and serum-starved culture medium was added for another 24 h. PCP was diluted with serum-free culture medium (0, 0.1, 0.3, and 1.0 μM) and added to the wells for 44 h. Subsequently, 20 μL of MTT solution (5 mg/mL MTT in PBS) was added to each well and the plates were incubated for an additional 4 h. The culture medium was removed and 150 μL of DMSO added to each well. After incubation for another 20 min, the extent to which MTT was reduced to the formazan product was determined using a microplate reader (M2, Molecular Devices, Union City, CA, USA) at 490 nm (Yu et al., 2008). Cell viability was expressed as the percentage of cell survival compared with the control. There were three experimental replicates and each treatment contained a blank and solvent control (0.1% DMSO).

For the thyroid endocrine system disruption assay, GH3 cells at a density of 2 x 10^5 per well were seeded into 12-well plates for 24 h. The culture medium was removed and the cells were rinsed with serum-free medium. The cells were then exposed to PCP (0.1, 0.3, and 1.0 μM) in serum- and phenol red-free DMEM/F12 for 48 h. There were three replicates for each treatment and control.

2.4. Zebrafish maintenance and embryo exposure

The maintenance of adult zebrafish (Danio rerio) (AB strain) and embryo exposure to PCP were carried out previously described (Yu et al., 2010). Briefly, normally developing embryos that had reached the blastula stage (2 h post-fertilization, hpf) were selected for experiments. Approximately 400 embryos were randomly distributed into glass beakers containing 500 mL of PCP solution (0, 1, 3, and 10 μg/L, equivalent to 0.0037, 0.011, 0.033 μM). There were three replicates in each exposure group and control group for the gene transcription assay, and both the exposure and control group received 0.01% (v/v) DMSO. During the experimental period, the exposure solution was renewed daily, and zebrafish larvae were fed cultured live paramecia and Artemia twice daily. At 14 days post-fertilization (dpf), the larvae were randomly sampled, immediately frozen in liquid nitrogen, and stored at −80 °C for subsequent analysis of gene transcriptions and TH assays. The hatching, malformation, growth and survival were also recorded.

2.5. RNA isolation and quantitative real-time polymerase chain reaction (qRT-PCR)

For GH3 cells, the procedures for RNA extraction and mRNA expression pattern analysis were performed as described previously (Ma et al., 2012). For zebrafish larvae, total RNA was extracted from 30 homogenized zebrafish larvae (Yu et al., 2011) using the Trizol Reagent (Takara, Dalian, China), according to the manufacturer’s instructions. Total RNA was digested and purified with RNase-free DNase (Promega, Madison, WI, USA) to remove genomic DNA contamination. Total RNA concentration was assayed at 260 and 280 nm using a spectrophotometer (M2, Molecular Devices, CA, USA). The purity of the RNA in each sample was verified by determining the A260/A280 ratio and by confirming 1.0 μg of RNA using 1% agarose-formaldehyde gel electrophoresis with
ethidium bromide staining. The purified RNA was used immediately for reverse transcription (RT) or stored at −80 °C until analysis.

First-strand cDNA synthesis was performed using a PrimeScript® RT reagent Kit (Takara, Dalian, China) with a 1 μg aliquot of total RNA, according to the manufacturer’s instructions. Q-RT-PCR was performed on a ABI 7300 real time PCR system (Perkin-Elmer Applied Biosystems, Foster City, CA, USA) using SYBR® Green PCR master mix (Toyobo, Osaka, Japan). The primer sequences of the selected genes were obtained by using the online Primer 3 program (http://frodo.wi.mit.edu/) and are shown in Table 1. The transcription of the housekeeping genes glyceraldehyde 3-phosphate dehydrogenase (gapdh) and ribosomal protein L8 (rpl8) did not vary under the experimental conditions of the present study (data not shown) and were used as internal controls in the GH3 cells and zebrafish larvae, respectively. For each selected gene, qRT-PCR reactions were performed in three replicate samples and repeated three times. The mRNA expression level of each target gene was normalized to the mRNA content of its reference gene (gapdh or rpl8, respectively) and the change in the mRNA expression of the relevant genes was analyzed by the 2−ΔΔCT method.

2.6. TH extraction and measurement

Extraction of THs from zebrafish larvae was based on a previous method (Yu et al., 2011). Briefly, 200 zebrafish larvae were homogenized in 2 mL ice-cold methanol:ammonia (99:1, vol/vol) containing 1 mM 6-n-propyl-2-thiouracil (PTU, Sigma, St. Louis, MO, USA), sonicated, vortexed and centrifuged. The supernatants were dried by evaporation under a gentle nitrogen stream. All extracts were resuspended in a mixture of 0.125 mL barbital buffer (pH 8.6) containing 2.5 mg/mL 8-anilino-1-naphthalene sulfonic acid (ANS), 0.125 mL ethanol and 0.5 mL chloroform. The samples were vortexed for solubilization and then centrifuged to separate the top aqueous phase containing THs. Total T4 (TT4) and T3 (TT3) concentrations were measured in duplicate using commercial enzyme-linked immunosorbent assay (ELISA) kits (Uscn Life Science Inc, Wuhan, China), according to the manufacturer’s instructions. The detection limits, intra-assay and inter-assay variations reported by the manufacturer are 1.2 ng/mL, 4.3%, and 7.5% for TT4, and 0.1 ng/mL, 4.5%, and 7.2% for TT3, respectively.

To determine the extraction efficiencies, 100 μL 125I radioiodinated T4 and T3 (Beijing North Institute of Biotechnology, Beijing, China) were added to larvae (n = 6) and treated as described above. The mean recoveries were 71.6% for T4 (70.4–72.8%) and 62.3% for T3 (61.0–63.0%). The measured extract efficiencies were used to correct for the predicted extraction efficiencies of the experimental samples.

2.7. Statistical analysis

The normality of the data was analyzed using the Kolmogorov–Smirnov test, and if necessary, data were log-transformed to approximate normality. Homogeneity of variances was verified by Levene’s test. All data were shown as mean ± standard error (SEM) and analyzed by one-way analysis of variance (ANOVA), followed by Tukey’s test. All the analyses were conducted using SPSS statistical software version 13.0 (SPSS, Inc., Chicago, IL, USA). A P value <0.05 was considered statistically significant.

3. Results

3.1. In vitro effects of PCP on GH3 cells

3.1.1. Cell viability

There was no significant difference in MTT reduction in the PCP exposure groups compared with the control (data not shown).
3.1.2. Gene transcription profiles

In our study, GH3 cells were treated with 0.25 nM T3 at a physiological level for 48 h. The most sensitive response to T3 treatment was by the Dio 1 gene, which was 45-fold upregulated transcriptionally. The mRNA expressions of prolactin gene (Prl) and Dio 2 were not significantly different, compared with the solvent control, while a small but significant transcriptional upregulation of growth hormone (GH) was observed (data not shown). Thus, in the following experiments, Dio 1 was selected to examine the effects of PCP on TH-responsive genes. A time-course study also indicated that Dio 1 gene transcription was the most stable and sensitive gene measured upon exposure to 0.25 nM T3 (Fig. 1). In this regard, Dio 1 gene transcription could be recommended as a suitable candidate for evaluating the disruption caused by chemicals on the TH endocrine system in the T-Screen assay. A previous study also showed that T3 can induce Dio 1 activity in GH3 cells, but not Dio 2 (Baur et al., 1997).

Upon exposing GH3 cells to 0.1, 0.3 and 1.0 μM of PCP, Dio 1 mRNA expression was downregulated by 1.42-, 2.02- and 2.45-fold, respectively (Fig. 2). Co-exposure to PCP (0.1, 0.3 and 1.0 μM) and T3 (0.25 nM) caused a marked downregulation of Dio 1 mRNA expression (by −11.69-, −8.85- and −5.19-fold, respectively) relative to T3 treatment alone (45-fold) (Fig. 3).

3.2. In vivo effects on zebrafish larvae

3.2.1. Developmental toxicity

There were no significant effects on hatching, survival rates and body weight after exposure to 1.0, 3.0 and 10.0 μg/L of PCP compared with those in the control until 14 dpf (Table 2). However, a small, but significant, increase in malformation rate was recorded in the 10.0 μg/L exposure group relative to the control (Table 2).

3.2.2. Gene transcription in the HPT axis in zebrafish larvae

Transcription of the thyroid stimulating hormone (TSHβ) gene was upregulated in the 3, and 10 μg/L of PCP (Fig. 4). The mRNA expression of the sodium/iodide symporter (NIS) and the thyroglobulin (TG) genes was significantly upregulated upon
Table 2
Development index of zebrafish larvae after exposure to PCP (0, 1, 3 and 10 μg/L) for 14 days.ª

<table>
<thead>
<tr>
<th>PCP (μg/L)</th>
<th>0</th>
<th>1</th>
<th>3</th>
<th>10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hatching (%)</td>
<td>95.6 ± 1.1</td>
<td>96.4 ± 1.2</td>
<td>97.7 ± 0.4</td>
<td>95.8 ± 0.7</td>
</tr>
<tr>
<td>Malformation (%)</td>
<td>2.67 ± 0.44</td>
<td>2.75 ± 0.38</td>
<td>3.08 ± 0.54</td>
<td>4.17 ± 0.25</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>71.4 ± 1.9</td>
<td>70.4 ± 1.2</td>
<td>71.9 ± 1.2</td>
<td>66.9 ± 1.8</td>
</tr>
<tr>
<td>Weight (mg)</td>
<td>0.56 ± 0.03</td>
<td>0.57 ± 0.03</td>
<td>0.63 ± 0.01</td>
<td>0.55 ± 0.01</td>
</tr>
</tbody>
</table>

ª The values represent mean ± standard error (SEM) of 3 replicate groups.

ª P<0.05 indicates significant difference between exposure groups and the control group.

Treatment with 1.0, 3.0 and 10.0 μg/L of PCP, respectively (Fig. 4). Significant transcriptional upregulation of Dio 1 and Dio 2 was observed at 1 and 3 μg/L of PCP treatment (Fig. 4). The mRNA expressions of the thyroid hormone nuclear receptor genes (TRα and TRβ) were significantly upregulated in a concentration-dependent manner (Fig. 4). There was no significant difference in the mRNA expression of the transthyretin (TRT) gene among the exposure groups (Fig. 4). The mRNA expression of the uridine diphosphate glucuronosyltransferases gene (UGT1ab) was significantly increased after exposure to 1, 3, and 10 μg/L of PCP, respectively (Fig. 4).

3.2.3. TH contents

After 14 days of exposure, the whole-body TH levels were measured in zebrafish larvae. The TT4 levels were reduced (by 12.7%, 9.1% and 15.8%), and showed significant difference in 10 μg/L exposure group relative to the control (Fig. 5A). However, we observed an increase of TT3 levels in the exposure groups, which was significant in the 10 μg/L PCP group (46.9%) (Fig. 5B). Meanwhile, a significant increase in the T3–T4 ratios was observed in the group exposed to 10 μg/L of PCP relative to the control (1.72-fold) (Fig. 5C).

4. Discussion

Our results showed that the T-Screen assay could rapidly evaluate the thyroid endocrine disruption caused by PCP. PCP altered whole-body T4 and T3 concentrations and our study clearly demonstrated that PCP disrupts the thyroid endocrine system in fish.

In the T-Screen assay, compounds are tested both in the absence and in the presence of TH to test for agonistic and antagonistic activities (Gutleb et al., 2005). PCP treatment of GH3 cells caused a concentration-dependent downregulation of Dio 1 mRNA expression. Co-administration of PCP with T3 showed a marked transcriptional downregulation of Dio 1 relative to the 0.25 nM T3 treatment; both results revealed anti-TH activities of PCP. PCP has also been shown to exhibit T3-antagonist activity on TRβ-mediated transcription using in vitro and in vivo assays in Xenopus laevis tadpoles (Sugiyama et al., 2005). Thus, the T-screen assay indicated that PCP exhibited antagonistic activity.

In the in vivo assay, T4 levels were decreased, while T3 levels were significantly elevated, which indicated thyroid endocrine disruption. PCP decreased the plasma T4 levels in rats (Brucker-Davis, 1998; Kawaguchi et al., 2008). Decreased T4 levels are frequently observed in mammals, amphibian and fish models exposed to TH endocrine disruptors, e.g., PBDEs (Tomy et al., 2004; Ellis-Hutchings et al., 2006; Lema et al., 2008; Yu et al., 2010), PCBs (Donahue et al., 2004; Martin and Klaassen, 2010; Schnitzler et al., 2011), and perchlorates (Mukhi and Patino, 2007; Opitz et al., 2009; Schmidt et al., 2012). Decreased TT4 and increased TT3 levels and the consequent increased ratio of T3/T4 are frequently observed in fish exposed to TH endocrine disruptors (Chen et al., 2012; Wang et al., 2013). An increased T3/T4 ratio associated with a decreased T4 level is usually present in primary hypothyroidism (Wilkin and Isles, 1984). Although the mechanisms for disturbing THs in fish are not well known, THs are particularly important.

![Fig. 5. Levels of: (A) T4, (B) T3 and (C) T3/T4 ratio in zebrafish larvae after exposure to PCP (0, 1, 3 and 10 μg/L) for 14 days. Values are expressed as means ± SEM of six replicate samples (n = 6). **P<0.01 indicates significant differences between exposure groups and the control group.](image-url)
in fetal brain development; therefore, substances that interfere with TH activity may compromise normal central nervous system development. Thus, the impact of lower concentrations of PCB on the nervous system may be needed further investigation.

The thyroid endocrine system is controlled primarily by the HPT axis (Carr and Patiño, 2011). Thus, it is conceivable that endocrine disruptors could act on any sites along the HPT axis, and if the action overwhelms the regulation capacity of the axis, TH endocrine disruption could occur. Elevated transcription of TSHβ genes was observed. Previous studies found that changes in TSHβ mRNA levels may be related to alterations of T4 levels in fish (Lema et al., 2009; Yu et al., 2010). In addition, enhanced TSHβ mRNA expression related to reduced levels of T4 was also found in fish after exposure to PBDEs (Chen et al., 2012). Consistent with these results, our study reveal that elevated transcription of TSH genes could be attributed to a negative feedback effect on the regulation of the disrupted hormonal equilibrium.

In the present study, we also observed significantly induced mRNA expression of the sodium-iodide symporter (NIS) and thyroglobulin (TG) genes. In zebrafish, the NIS gene is expressed in the thyroid and its product is responsible for iodine uptake from the bloodstream (Alt et al., 2006; Porazzi et al., 2009). The products of NIS and TG genes are known to be involved in TH synthesis. The induced transcription of NIS and TG may be a compensatory response to the decreased levels of T4. A significant upregulation of NIS and TG gene transcriptions has also been reported in zebrafish larvae treated with PBDEs, where T4 levels were significantly reduced (Yu et al., 2010; Chen et al., 2012).

THs mediate their effects through interaction with different isoforms of the TRs, which play crucial roles in embryogenesis and larval development and are encoded by the TRa and TRβ genes, respectively (Liu and Chan, 2002). Both TRα and TRβ mRNA were found to be present in zebrafish larvae and to respond to exogenous TH (Essner et al., 1997; Liu and Chan, 2002). In our study, PCB exposure resulted in the upregulation of TRα and TRβ expression. Previous studies have indicated that administration of T3 resulted in a significant increase in TRα and TRβ mRNA expression (Crumpl et al., 2008). Thus, transcriptional upregulation of TRs genes could result from the increased T3 levels. However, TRα overexpression could disrupt hindbrain development during zebrafish embryogenesis (Essner et al., 1999).

TRs has been proposed to be an important transport protein for THs to various target tissues, and play an important role in the thyroid axis in fish (Power et al., 2000). In the present study, TRβ mRNA expression did not change. It has been generally accepted that structural resemblance of xenobiotics to THs causes displacement of T4 from TRβ (Meerts et al., 2000). Several environmental contaminants (e.g., PBDEs and their hydroxylated metabolites and phenol compounds) were shown to compete for their binding sites on transport proteins and to interfere with TH homeostasis by binding to TR (Boas et al., 2006; Morgado et al., 2009). PBDE exposure causes downregulation of the TTR mRNA or protein expressions in rodents, birds and fish (Zhou et al., 2002; Crumpl et al., 2008; Yu et al., 2010; Chen et al., 2012). In our study, PCB exposure did not affect TTR mRNA expression, which, provided that the transcriptional changes are reflected in protein expression, indicates that variations in TTR expression are not responsible for the disruption of the TH endocrine system caused by PCB.

Deiodinases are important regulators of circulating and peripheral TH levels in vertebrates, and are sensitive to changes in TH levels. In zebrafish, it has been demonstrated that Dio 2 plays a pivotal role in producing active T3, allowing an adequate availability of local and systemic T3 (Walpita et al., 2007). In the present study, both Dio 1 and Dio 2 mRNAs were significantly increased in larvae. Our results are consistent with previous studies demonstrating that hypothyroidism increases Dio 1 and Dio 2 mRNA expression (Orozco and Valverde, 2005; Van der Geyten et al., 2005). A plausible explanation is that modulations of the expressions of the two deiodinases upon PCB treatment are, to some extent, responsible for the increased T3 levels. The upregulation of Dio 2 could result in a reduction in the levels of circulating T4 and have a significant impact on TH homeostasis in tissues. In addition, it has been suggested that the levels of circulating THs may be reduced by the induction of uridine diphosphate glucuronosyltransferases (UDPGT) (Hood and Klaassen, 2000). The enzyme catalyses the glucuronidation of T4, thereby increasing the biliary excretion of conjugated hormone, which could partly explain the reduced T4 levels. In the present study, we observed a significant upregulation of UGT1ab mRNA expression, which, again provided that this transcriptional change is reflected at the protein level, may lead to faster metabolism of TH. In this regard, several previous studies have shown that thyroid endocrine disruptors (e.g., PBDEs) increased the activity of hepatic UDPGT-T4 and the mRNA expression of gene encoding a UDPGT isozyme, as well as decreasing the T4 production in male rat pups (Szabo et al., 2009) and zebrafish (Yu et al., 2010).

In summary, the in vitro screening assay showed that PCB could disrupt the thyroid endocrine system and elicit T3-antagonist activity in GH3 cells. This in vitro assay provided rapid and cost-effective for testing disruption of the TH endocrine system by toxics and could, to some extent, reveal their mechanisms of action. It should also be noted that in vivo assays do not take account of absorption, distribution, metabolism or excretion, and thus would not provide the potential intracellular sites for thyroid disruption by endocrine disruptors. On the other hand, endocrine disruptors may interfere with multiple endocrine functions in vivo. For example, a study showed the antiestrogenic/antiandrogenic activity of PCB in cultured Xenopus oocytes and inhibition of ovarian steroidogenesis, accompanied by decreased production of both progesterone and T (Orton et al., 2009). Furthermore, there is accumulating evidence of cross-talk between the thyroid and estrogen signaling pathways, and the impact of xenohormones cannot be assessed exploring only a single pathway (Ghisari and Bonefeld-Jorgensen, 2005; Liu et al., 2011). Hence, in vitro tests and further in vivo studies will be needed to investigate the impact of endocrine disruptors on certain endocrine systems at the whole-organism level. Despite these limitations, GH3 cells could be used for screening the disruption of the TH endocrine system by PCB. This activity was further confirmed in developing zebrafish larvae, validating the reliability of using developing zebrafish larvae for testing disruption of the TH endocrine system by PCB.

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