Effects of tris(1,3-dichloro-2-propyl) phosphate and triphenyl phosphate on receptor-associated mRNA expression in zebrafish embryos/larvae

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ABSTRACT

Tris(1,3-dichloro-2-propyl) phosphate (TDCPP) and triphenyl phosphate (TPP) are frequently detected in biota, including fish. However, knowledge of the toxicological and molecular effects of these currently used flame retardants is limited. In the present study, an *in vivo* screening approach was developed to evaluate effects of TDCPP and TPP on developmental endpoints and receptor-associated expression of mRNA in zebrafish embryos/larvae. Exposure to TDCPP or TPP resulted in significantly smaller rates of hatching and survival, in dose- and time-dependent manners. The median lethal concentration (LC₅₀) was 7.0 mg/L for TDCPP and 29.6 mg/L for TPP at 120 hour post-fertilization (hpf). Real-time PCR revealed alterations in expression of mRNAs involved in aryl hydrocarbon receptors (AhRs)-, peroxisome proliferator-activated receptor alpha (PPARα)-, estrogenic receptors (ERs)-, thyroid hormone receptor alpha (TRα)-, glucocorticoid receptor (GR)-, and mineralocorticoid receptor (MR)-centered gene networks. Exposure to positive control chemicals significantly altered abundances of mRNA in corresponding receptor-centered gene networks, a result that suggests that it is feasible to use zebrafish embryos/larvae to evaluate effects of chemicals on mRNA expression in these gene networks. Exposure to TDCPP altered transcriptional profiles in all six receptor-centered gene networks, thus exerting multiple toxic effects. TPP was easily metabolized and its potency to change expression of mRNA involved in receptor-centered gene networks was weaker than that of TDCPP. The PPARα- and TRα-centered gene networks might be the primary pathways affected by TPP. Taken together, these results demonstrated that TDCPP and TPP could alter mRNA expression of genes involved in the six receptor-centered gene networks in zebrafish embryos/larvae, and TDCPP seemed to have higher potency in changing the mRNA expression of these genes.

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1. Introduction

Organophosphate esters (OP esters) are a group of synthetic chemicals that were widely used as flame retardants and plasticizers in various products, such as plastics, foams, textiles, varnishes, electronics equipment and furniture with high-production volume worldwide (van der Veen and de Boer, 2012). Recently, the use of OP esters has been increasing gradually as other flame retardants such as penta- and octabrominated diphenyl ethers (PBDEs) have

been phased out of use (Reemtsma et al., 2008; van der Veen and de Boer, 2012).

Among OP esters, tris(1,3-dichloro-2-propyl) phosphate (TDCPP) and triphenyl phosphate (TPP) are commonly used with annual production in the United States been estimated to be between 4.5×10^5 and 4.5×10^6 kg from 1986 to 1990, and increased to between 4.5×10^6 and 2.3×10^7 kg in 1994, 1998, 2002 and 2006 (U.S. Environmental Protection Agency Inventory Update Reporting, <http://www.epa.gov/iur>). Environmental monitoring has demonstrated that TDCPP and TPP were distributed in indoor air, surface water, drinking water, sediment, wildlife and human tissues (Andresen et al., 2004; Bacaloni et al., 2007; Cao et al., 2012; Chen et al., 2011; Hartmann et al., 2004; Marklund et al., 2005; Stackelberg et al., 2004; Sundkvist et al., 2010; van

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der Veen and de Boer, 2012). For example, in Germany, TDCPP has been detected in surface water at a maximum concentration of 50 ng/L in the River Ruhr (Andresen et al., 2004). Concentrations of 11 OP esters were measured in fishes and mussels from Swedish lakes and coastal areas, and TPP was one of the most frequently detected compounds, with concentrations ranging from 21 to 180 ng/g lipid weight (Sundkvist et al., 2010).

Limited information is currently available on the thresholds and mechanisms of toxicity of TDCPP and TPP. Results of epidemiology and controlled laboratory studies with animals suggest that TDCPP or/and TPP cause developmental toxicity and endocrine-disruption (Dishaw et al., 2011; C. Liu et al., 2012; X. Liu et al., 2012; McGee et al., 2012; Meeker and Stapleton, 2010). For example, McGee et al. (2012) reported that exposure to TDCPP (0.05–50 μ M) from 5.25 hpf to 96 hpf in zebrafish embryos/larvae caused developmental toxicity by altering embryogenesis, with a LC_{50} of 8.5 μ M (3.7 mg/L). C. Liu et al. (2012) and X. Liu et al. (2012) reported that exposure to TDCPP or TPP significantly up-regulated expression of mRNA of genes involved in steroidogenesis and resulted in greater concentrations of both testosterone (T) and 17 β -estradiol (E2) in culture medium of H295R cells and in blood plasma of zebrafish. Using primary cultured avian hepatocytes, Crump et al. (2012) demonstrated that TDCPP could alter abundances of mRNA of genes associated with phase I and II metabolism, lipid regulation, and growth.

Coordinated expression of gene networks in various metabolic, physiological, and developmental processes were, in large part, mediated by a superfamily of receptors, mostly nuclear receptors (McKenna and O'Malley, 2002). Results of multiple studies have demonstrated that several environmental pollutants, such as dioxin-like chemicals and estrogens, can bind to one or several receptors and alter related expression of mRNA that can cause adverse effects on health (Ankley et al., 2010; Mandal, 2005; Mortensen and Arukwe, 2007). In the present study, 48 genes involved in six receptor-centered gene networks were collected from SABioscience Gene Network Central (<http://www.sabiosciences.com/genenetwork/genenetworkcentral.php>) and previous published data in fishes (Pellegrini et al., 2005; Zhang et al., 2008), and were integrated as “associations” and visualized as networks as described in SABioscience Gene Network Central. Furthermore, quantitative real-time polymerase chain reaction (qRT-PCR) was developed to evaluate effects of six positive control chemicals and two OP esters (TDCPP and TPP) on expression of mRNA of receptors and selected associated genes in zebrafish embryos/larvae.

2. Materials and methods

2.1. Materials and reagents

Perfluorooctanesulfonic acid potassium salt (PFOS) was obtained from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan). Benzo[a]pyrene (B[a]P), 17 β -estradiol (E2), 3,3',5-triiodo-L-thyronine (T3), dexamethasone (DEX), fludrocortisone acetate (FCA), tris(1,3-dichloro-2-propyl) phosphate (TDCPP), triphenyl phosphate (TPP) and 3-amino-benzoic acid ethyl ester, methane-sulfonate salt (MS-222) were purchased from Sigma (St. Louis, MO, USA). All chemicals were dissolved in dimethyl sulfoxide (DMSO) as stock solutions and stored in -20°C . RNAlater RNA Stabilization Reagents were obtained from QIAGEN (Tokyo, Japan). GeneJETTM RNA Purification and Maxima[®] First Strand cDNA Synthesis Kits were purchased from Fermentas (St Leon-Rot, Germany). SYBR[®] Realtime PCR Master Mix -Plus- Kits were purchased from Toyobo (Tokyo, Japan). Standards of TDCPP and TPP were purchased from Dr. Ehrenstorfer GmbH (Germany). Tributyl-D₂₇-phosphate

(TnBP-D₂₇) was obtained from Cambridge Isotope Laboratories (UK).

2.2. Animals and chemical exposure protocol

Fertilization of eggs and culture of adult zebrafish (AB strain, 6-month old) were performed as previously described (Liu et al., 2009; Shi et al., 2009). Eggs were examined under a stereo microscope and those exhibiting normal development were used for subsequent experiments. Briefly, eggs were randomly distributed into six-well culture plates. Each well contained 20 eggs, with 10 mL of exposure solution. Eggs were exposed from 4 to 120 hour post-fertilization (hpf), by which time they had developed into free-swimming larvae and most organs had completed development (Amsterdam et al., 2004). Each exposure concentration was replicated in four separate wells. Control groups received 0.01% DMSO (v/v). At 48 hpf exposure solutions were replaced by fresh water, or exposed to appropriate concentration and exposure continued until 120 hpf. For positive control chemicals, exposure concentrations were 10 μ g B[a]P/L, 5 mg PFOS/L, 200 μ g E2/L, 30 μ g T3/L, 25 mg DEX/L, and 25 mg FCA/L. These concentrations were selected based on published data or range-finding studies to determine concentrations that significantly altered expression of corresponding receptor-associated mRNAs (Duarte-Guterman et al., 2010; Kazeto et al., 2004; Krøvel et al., 2008; J. Wang et al., 2011; X. Wang et al., 2011; Tseng et al., 2005). For TDCPP and TPP, exposure experiments included two parts. First, zebrafish embryos were exposed to 0, 0.8, 4, 20, 100 or 500 mg/L TDCPP or TPP from 4 to 120 hpf, and rates of hatching and survival monitored. Second, based on the results of the acute toxicity test, a different set of zebrafish embryos was exposed to 0.02, 0.2 or 2 mg/L TDCPP or TPP from 4 to 120 hpf, and effects on expression of mRNA of genes in six receptor-centered networks were determined. After exposure, larvae were anesthetized with MS-222, and preserved in RNAlater RNA Stabilization Reagents for subsequent total RNA isolation.

2.3. Quantification of TDCPP or TPP in exposure solutions

In acute toxicity test, exposure solutions were collected at 4, 48(1) (prior to renewing of exposure solutions), 48(2) (after renewing of exposure solutions) and 120 hpf in 0.8-mg/L treatment group for TDCPP or TPP measurement. Concentrations of TDCPP or TPP in exposure solutions were also determined at 48(2) (after renewing of exposure solutions) and 120 hpf when zebrafish embryos were exposed to 0.02, 0.2 and 2 mg/L TDCPP or TPP. Quantification of TDCPP or TPP was performed using a published protocol (J. Wang et al., 2011; X. Wang et al., 2011). Briefly, exposure solutions were filtered through 0.22 μ m nylon membrane filters (Jiuding High-Tech Filtration, China), and the filtered samples were immediately used for LC-MS/MS analysis. The calibration and quantification were performed by using an internal standard method with TDCPP or TPP standard prepared in water/acetonitrile (60/40), with TnBP-D₂₇ internal standard.

2.4. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from larvae using GeneJETTM RNA Purification kits following the manufacturer's instructions. Concentration of total mRNA was determined and quality was verified by use of a previously reported protocol (C. Liu et al., 2012; X. Liu et al., 2012). Syntheses of first-strand cDNA and qRT-PCR were performed by use of Maxima[®] First Strand cDNA Synthesis and SYBR[®] Realtime PCR Master Mix -Plus- Kits according to the manufacturer's instructions (C. Liu et al., 2012; X. Liu et al., 2012). Sequences for primers were designed using Primer 3 software (<http://frodo.wi.mit.edu/as>) (Table S1). Housekeeping

genes glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and 18S small subunit ribosomal RNA (18S rRNA) did not vary upon chemical exposure and were used as internal controls. The thermal cycle was set at 95 °C for 2 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The mRNA expression level of gene was normalized to housekeeping mRNA contents using the $2^{-\Delta\Delta C_t}$ method. The average cycle threshold (C_t) value of GAPDH and 18S rRNA was used for the expression calculation of target genes. Each concentration included four biological replicates and each replicate included 15 larvae.

2.5. Statistical analyses

Normality and homogeneity of data were evaluated by use of the Kolmogorov–Smirnow and Levene's tests, respectively. One-way analysis of variance (ANOVA) and Tukey's multiple range test were used to determine the significant differences between the control and each exposure group. A level of significance for type I error (α) was set at P -value < 0.05. All data were expressed as mean $\pm$ SEM. The statistical analyses were conducted using Kyplot Demo 3.0 software (Tokyo, Japan)

3. Results

3.1. Quantification of TDCPP or TPP in exposure solutions

In time-course study, mean concentrations of TDCPP in 0.8-mg/L treatment group were 0.80, 0.74, 0.84 and 0.80 mg/L at 4, 48(1) (prior to renewing of exposure solutions), 48(2) (after renewing of exposure solutions), and 120 hpf, respectively. Mean concentrations of TPP in 0.8-mg/L treatment group were 0.83, 0.79, 0.80 and 0.00 mg/L at 4, 48(1) (prior to renewing of exposure solutions), 48(2) (after renewing of exposure solutions), and 120 hpf, respectively. No TDCPP or TPP were detected in control groups.

Based on results above, we measured concentrations of TDCPP or TPP in 0.02, 0.2 and 2 mg/L treatment groups at 48(2) (after renewing of exposure solutions) and 120 hpf. Mean concentrations of TDCPP in 0.02, 0.2 and 2 mg/L treatment groups were 0.02, 0.19 and 1.96 mg/L at 48(2) hpf and 0.02, 0.17 and 1.89 mg/L at 120 hpf, respectively. Mean concentrations of TPP in 0.02, 0.2 and 2 mg/L treatment groups were 0.02, 0.19 and 1.80 mg/L at 48(2) hpf and 0.00, 0.00 and 1.39 mg/L at 120 hpf, respectively.

3.2. Acute toxicity

No significant effects on rates of hatching of eggs or survival and malformation (spinal curvature, pericardial edema and yolk sac edema) of larvae were observed after exposure to 0 μ g B[a]P/L, 5 mg PFOS/L, 200 μ g E2/L, 30 μ g T3/L, 25 mg DEX/L, or 25 mg FCA/L. Rates of hatching and survival were >90% and rate of malformation was <5% in all exposure groups including controls.

Exposure to TDCPP caused dose- and time-dependent decrease in the rate of hatching and survival (Fig. 1). No significant effects on survival were observed in embryos/larvae exposed to 0.8 or 4 mg/L 12, 24, 48, 72, 96 and 120 hpf. However, exposure to 20 mg/L TDCPP resulted in significantly lesser survival by 18.2%, 20.1%, 20.1%, 100% and 100% at 24, 48, 72, 96 and 120 hpf, respectively, relative to the control. Exposure to greater concentrations (100 or 500 mg/L) caused 100% mortality by 12 hpf. The 120-hpf median lethal concentration (LC_{50}) was 7.0 mg TDCPP/L. Treatment with 0.8 or 4 mg/L TDCPP did not significantly alter rates of hatching at 48, 54, 60 or 72 hpf. However, significantly lower rates of hatching were observed at 54, 60 and 72 hpf when exposed to 20, 100 or 500 mg/L. No significant effects on hatching, survival or malformation (spinal curvature, pericardial edema and yolk sac edema) were observed when exposed to 0.02, 0.2 or 2 mg/L TDCPP. Rates

Fig. 1. Cumulative survival (A and B) and hatching (C) of zebrafish embryos/larvae exposed to 0, 0.8, 4, 20, 100 or 500 mg/L tris(1,3-dichloro-2-propyl) phosphate (TDCPP). Values represent mean $\pm$ SEM ($n = 4$ samples). Significant difference from the control group is indicated by * $P < 0.05$.

of hatching and survival were >90% and rate of malformation was <5% in all exposure groups including controls.

TPP caused dose- and time-dependent decrease in the rate of hatching and survival (Fig. 2). At 12, 24 and 48 hpf exposure to 0.8, 4, 20, 100 or 500 mg TPP/L caused no significant effects on rates of hatching and survival. However, at 72, 96 and 120 hpf, exposure to 100 or 500 mg TPP/L resulted in 100% mortality. The LC_{50} was 29.6 mg TPP/L at 120 hpf. Rates of hatching at 48 hpf were not significantly different from that of the control, when exposed to 0.8, 4, 20, 100 or 500 mg TPP/L. However, treatment with 100 or 500 mg TPP/L resulted in significantly lesser rates of hatching that was 100% at 54 hpf. No significant effects on rates of survival, hatching or malformation (spinal curvature, pericardial edema and yolk sac edema) were observed when exposed to 0.02, 0.2 or 2 mg TPP/L. Rates of

Fig. 2. Cumulative survival (A and B) and hatching (C) of zebrafish embryos/larvae exposed to 0, 0.8, 4, 20, 100 or 500 mg/L tris(1,3-dichloro-2-propyl) phosphate (TPP). Values represent mean $\pm$ SEM ($n = 4$ samples). Significant difference from the control group is indicated by * $P < 0.05$.

hatching and survival were >90% and rate of malformation was <5% in all exposure groups including controls.

3.3. Transcriptional responses to positive control chemicals

Exposure to positive control chemicals significantly altered expression of mRNA of genes in the six receptor-centered networks (Table S2 and Fig. 3). Exposure to 10 μ g B[a]P/L significantly up-regulated mRNA expression of aryl hydrocarbon receptor 1b (AhR1b), aryl hydrocarbon receptor 2 (AhR2), AhR repressor a (AhRRA), aryl hydrocarbon receptor interacting protein (AIP), cytochrome P450 1A1 (CYP1A1), nuclear receptor co-repressor 2 (NCOR2), cytochrome P450 1B1 (CYP1B1) and sp1 transcriptional

factor (SP1) genes by 2.3-, 2.2-, 1.4-, 1.2-, 2.5-, 1.3-, 1.6- and 1.3-fold, respectively. Exposure to 5 mg PFOS/L significantly up-regulated the expression of mRNA of peroxisome proliferator-activated receptor alpha (PPAR α) (1.7-fold), lipoprotein lipase (LPL) (2.0-fold), interleukin 8 (IL8) (1.4-fold) and interleukin 6 (IL6) (5.3-fold) genes, while mRNA expression of dUTP pyrophosphatase (DUT) gene was significantly down-regulated by 0.6-fold. Expression of mRNA of other genes, such as peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PPARGC1a) and peroxisome proliferator-activated receptor gamma (PPARG) gene were not altered by exposure to PFOS. Significant up-regulation of expression of mRNA of estrogen receptor 1 (ER1) (2.1-fold), estrogen receptor 2b (ER2b) (1.7-fold), vitellogenin 1 (VTG1) (9.8-fold), vitellogenin 2 (VTG2) (6.1-fold), vitellogenin 4 (VTG4) (40.8-fold), vitellogenin 5 (VTG5) (9.0-fold), and progesterone receptor (PGR) (2.1-fold) genes were observed upon exposure to 200 μ g E2/L, while expressions of mRNA of estrogen receptor 2a (ER2a) and cyclin D1 (CCND1) genes were significantly down-regulated by 0.6- and 0.7-fold, respectively. Treatment with thyroid hormone at a concentration of 30 μ g T3/L significantly up-regulated expression of mRNA of thyroid hormone receptor alpha (TR α) (4.9-fold), PPARGC1a (1.3-fold), NCOR1 (1.3-fold), C1D nuclear receptor corepressor (C1D) (1.3-fold), NCOR2 (1.4-fold) and histone deacetylase 3 (HDAC3) (1.3-fold) genes, while mRNA expression of fusion (FUS) gene was not significantly altered. Exposure to 25 mg/L of the glucocorticoid receptor (GR) agonist DEX caused significant up-regulation of mRNA expression of GR (2.1-fold), heparanase (HPSE) (1.9-fold), P65 transcriptional factor (RelA) (1.6-fold), and heat shock protein alpha, class 1 member 1 (HSP90aa1) (1.2-fold) genes, while mRNA expression of death-associated protein 3 (DAP3) and transforming growth factor beta 1 (TGFB1) genes was not significantly changed. Exposure to 25 mg/L of the mineralocorticoid receptor (MR) agonist FCA significantly up-regulated expression of mRNA of MR (2.7-fold), HPSE (2.2-fold), adrenergic receptor beta 2a (ADRB2a) (1.7-fold) and epidermal growth factor receptor (EGFR) (1.6-fold) genes, while expression of mRNA of 11 beta hydroxysteroid dehydrogenase (11 β HSD), adrenergic receptor beta 2b (ADRB2b) and ubiquitin-conjugating enzyme 2I (UBE2I) genes were not changed.

3.4. Transcriptional responses to TDCPP

Exposure to TDCPP caused dose-dependent alterations in expression of mRNA related to the following receptor-centered gene networks: AhRs, PPAR α , ERs, TR α , GR and MR (Table S2 and Fig. 4). Exposure to the two concentrations of TDCPP caused significant up-regulation in expression of mRNA of AhR1b (1.2- and 1.2-fold), AIP (1.2- and 1.5-fold), aryl hydrocarbon receptor nuclear translocator 2 (ARNT2) (1.2- and 1.7-fold), aryl hydrocarbon receptor nuclear translocator-like 1a (ARNT1a) (1.5- and 2.0-fold), aryl hydrocarbon receptor nuclear translocator-like 1b (ARNT1b) (1.5- and 2.1-fold), NCOR2 (1.3- and 1.5-fold), CYP1B1 (1.3- and 1.4-fold) and SP1 (1.3- and 1.4-fold) genes in 0.2 and 2 mg/L exposure groups. TDCPP significantly up-regulated mRNA expression of all of the genes selected for the PPAR α -centered gene network, in a dose-dependent manner. PPARGC1a and LPL mRNA expressions were significantly up-regulated by 1.4- and 2.1-fold, and 1.3- and 2.0-fold when exposed to 0.2 or 2 mg TDCPP/L, respectively. Exposure to 0.02 mg TDCPP/L significantly up-regulated expression of mRNAs of ER1 (1.8-fold), ER2b (1.5-fold), VTG1 (1.8-fold), VTG2 (1.9-fold), VTG4 (2.0-fold), VTG5 (2.2-fold) and PGR (1.8-fold) genes. No significant effects were observed at the two greatest concentrations (0.2 and 2 mg/L). TDCPP caused significant up-regulation of expression of mRNA of ER2a, NCOA1, NCOA2, NCOA3, and CCND1 genes relative to the control in larvae exposed to 0.2 or 2 mg/L. TDCPP significantly up-regulated expression of mRNA of genes in the

Fig. 3. Receptor-centered gene networks modified from SABioscience Gene Network Central (<http://www.sabiosciences.com/genenetwork/genenetworkcentral.php>) and effects of (A) 10 µg B[a]P/L (benzo[a]pyrene), (B) 5 mg PFOS/L (heptadecafluorooctanesulfonic acid potassium salt), (C) 200 µg E2/L (17β-estradiol), (D) 30 µg T3/L (3,3',5-triiodo-L-thyronine), (E) 25 mg DEX/L (dexamethasone), and (F) 25 mg FCA/L (fludrocortisone acetate) on expression of mRNA of selected genes in embryos/larvae of zebrafish. Physical interaction: a relationship where two gene products are interacting with each other physically; up-regulation: activating types of functional and transcriptional regulations; down-regulation: inhibiting types of functional and transcriptional regulations; other: relationships where the type has not been yet classified; regulation: functional and transcriptional regulations where the regulation has not been identified as inhibiting or activating; co-expression: genes whose expressions have been shown to be positively or negatively correlated in microarray experiments; chemical modification: phosphorylations, acetylations, ubiquinations, and other types of post-translational modification; predicted protein interaction: protein interactions that have been predicted through computational algorithms; predict TF regulation: transcriptional regulations where *cis*-regulatory element for a given transcription factor (TF) have been found in the promoter of a target gene.

Fig. 3. (Continued)

TR α -centered gene network. The following effects were observed at different concentrations of TDCPP: TR α (1.2- and 1.6-fold), PPARgc1a (1.4- and 2.1-fold), NCOR (1.2- and 1.4-fold), C1D (1.2- and 1.4-fold), NCOR2 (1.3- and 1.5-fold), HDAC3 (1.2- and 1.4-fold) and FUS (1.5- and 1.8-fold) in 0.2 or 2 mg/L. In the GR-centered gene network, a significant up-regulation in the mRNA expression

of GR (1.3- and 1.3-fold), DAP3 (1.3- and 1.8-fold), RelA (1.3- and 1.5-fold), TGFb1 (1.3- and 1.5-fold), and HSP90aa1 (1.2- and 1.9-fold) genes was observed upon exposure to 0.2 or 2 mg/L TDCPP. Exposure to 2 mg TDCPP/L significantly up-regulated expression of mRNAs of MR, 11 β HSD, ADRB2a and UBE2I genes by 1.2-, 1.4-, 1.3- and 1.6-fold, respectively.

Fig. 3. (Continued)

3.5. Transcriptional responses to TPP

TPP caused transcriptional responses mainly in AhRs-, PPAR α -, TR α -, GR- and MR-centered gene networks in the present study (Table S2 and Fig. 4). Exposure to 2 mg TPP/L resulted in significant up-regulation of expression of mRNA of CYP1A1, NCOR2 and CYP1B1 genes by 1.7-, 1.5- and 1.4-fold, respectively. In the

PPAR α - and TR α -centered gene networks, expressions of mRNA for PPAR α , PPAR γ 1a, LPL, IL6, PPAR γ , TR α , and NCOR2 genes were up-regulated by 1.2-, 1.3-, 1.8-, 3.9-, 1.2-, 1.4- and 1.5-fold, respectively, after exposure to 2 mg TPP/L. Exposure to 2 mg TPP/L also up-regulated expression of mRNA of RelA, TGFb1, HSP90aa1, 11 β HSD and EGFR genes, while expression of MR and HPSE genes was down-regulated.

Fig. 4. Dose-dependent effects of (A) tris(1,3-dichloro-2-propyl) phosphate (TDCPP) and (B) triphenyl phosphate (TPP) on expression of mRNA for genes in six receptor-centered gene networks in zebrafish embryos/larvae.

4. Discussion

Results of previous studies have suggested that zebrafish embryos/larvae could be used as a model to investigate endocrine-disrupting effects of chemicals (Chen et al., 2012; Jin et al., 2009; Raldúa and Babin, 2009; Yu et al., 2010). In this study, 48 genes involved in six receptor-centered gene networks were collected, and integrated as “associations” and visualized as networks. Furthermore, to evaluate the effects of six positive control chemicals and two organophosphate esters (OP esters: TDCPP and TPP) on mRNA expression along these constructed gene networks, a PCR array was developed for zebrafish embryos/larvae.

Exposure to positive control chemicals B[a]P, PFOS, E2, T3, DEX or FCA altered expression of mRNA in AhRs-, PPAR α -, ERs-, TR α -, GR- and MR-centered gene networks, respectively. These results suggested that 5-day old zebrafish larvae could be used to evaluate effects of chemicals on transcription of genes in these receptor-centered gene networks. AhR is a ligand-activated transcription factor that mediates responses to numerous chemicals, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). In zebrafish, there are three AhR genes, AhR1a, AhR1b and AhR2. The AhR1b and AhR2 proteins were capable of high-affinity binding of TCDD and caused associated transcriptional responses, while AhR1a does not appear to be capable of binding TCDD (Andreassen et al., 2002; Karchner

and Franks, 2005). Therefore, in the present study, AhR1b and AhR2 were selected and the AhRs-centered gene network was constructed. Exposure to AhR agonists, such as TCDD and B[a]P up-regulated expression of AhR, AhRR, AIP, CYP1A1, NCOR2, SP1 and CYP1B1 (Hakkola et al., 1997; Huang et al., 2002; Jin et al., 2012; Ma, 1997; Tsuchiya et al., 2003; Zhang et al., 2006). Those results are consistent with the findings of the study reported upon here. Exposure to B[a]P can significantly up-regulate expression of mRNA for ARNT in mouse hepatoma Hepa1c1c7 cells (Ko et al., 2004). However, no significant alterations in expression of ARNT11a, ARNT11b or ARNT2 mRNAs were observed in the present study. The reasons for this are not known. There are complicated regulation/interaction relationships between AhRs and ARNTs according to the constructed gene network. Therefore, additional studies might be needed to clarify the relationship between AhRs and ARNTs in zebrafish embryos/larvae after B[a]P exposure in future. Furthermore, exposure to TPP did not alter expression of mRNA of AhR1b or AhR2, but expressions of mRNA of CYP1A1, NCOR2 and CYP1B1 were significantly up-regulated in 2-mg/L exposure group. Therefore, time-course experiments with mRNA and protein might be needed to understand mechanisms. Exposure to TDCPP significantly up-regulated expression of mRNA for AhR1b and all associated genes: AhRRa, AhRRb, AIP, ARNT2, ARNT11a, ARNT11b, CYP1A1, NCOR2, CYP1B1 and SP1 in a dose-dependent manner.

These results suggested that, at least, TDCPP could up-regulate expression of mRNA of AhR1b gene and increase abundances of transcriptions of associated genes.

PPAR α belongs to the nuclear receptor super-family and is involved in lipid homeostasis, inflammation, adipogenesis, reproduction, wound healing and carcinogenesis (Abbott, 2009). In the present study, six genes that were demonstrated to have interaction/regulation relationships with PPAR α were selected. IL6 and IL8 are involved in immune responses and LPL is involved in metabolism of lipids (Moyes et al., 2009). In the present study, exposure to the PPAR α agonist PFOS resulted in significantly greater abundances of mRNA of PPAR α , LPL, IL6 and IL8 genes. The results of previous studies have also demonstrated that treatment with PFOS could up-regulate expression of mRNA for IL6 and LPL genes both *in vivo* and *in vitro*, which resulted in immunotoxicity (DeWitt et al., 2009; Krøvel et al., 2008; Liao et al., 2012; Wan et al., 2012). DUT catalyses hydrolysis of dUTP to dUMP in the presence of magnesium ions, and is responsible for maintaining balance of pools of free nucleotides in cells (McCarthy et al., 2009). In the present study exposure to PFOS significantly down-regulated expression of mRNA of DUT gene, a result that is opposite to the effect on mRNA expression of PPAR α gene. Therefore, further study might be needed to clarify the relationship between PPAR α and DUT after PFOS exposure. In the present study exposure to TDCPP or TPP significantly up-regulated expression of mRNA of PPAR α and several other genes involved in the PPAR α -centered gene network.

ERs are transcriptional factors that can regulate target genes involved in reproduction, development, metabolism and homeostasis in vertebrates (Shibata et al., 1997; McKenna et al., 1999). In zebrafish, there are three ER genes, encoding ER1, ER2a and ER2b. Each protein can bind E2 and transactivate reporter genes *in vitro* (Menuet et al., 2002). However, specific functions and mechanisms of action of these three ERs remain unknown. In the present study, three ER and nine associated genes, those encoding VTG1, VTG2, VTG4, VTG5, NCOA1, NCOA2, NCOA3, PGR and CCND1, were selected based on the information from Gene Network Central and previous published results of studies with fishes and were integrated as “associations”. Exposure to E2 significantly up-regulated mRNA expressions of ER1 (ER α), ER2b (ER β 2), VTG1, VTG2, VTG4, VTG5 and PGR, and down-regulated mRNA expressions of ER2a (ER β 1) and CCND1 in 5-day zebrafish larvae. mRNA expressions of NCOAs (NCOA1, NCOA2 and NCOA3) were not changed. These results suggest that different ERs might have different functions. The results of a previous study demonstrated that E2 exposure up-regulated ER α expression and down-regulated ER β 1 expression, while expression of ER β 2 remained unchanged in liver of zebrafish (Menuet et al., 2004). In the present study when embryos were exposed to TPP, mRNA expression of only ER2b was slightly up-regulated, while mRNA expression of other genes was not significantly altered. However, exposure to TDCPP caused significant up-regulation of mRNA expression of all the genes selected, a result that suggests that TDCPP might have estrogenic activity. The results of a previous study have also demonstrated that TDCPP can increase the concentration of E2 in blood plasma and up-regulate expression of VTG in zebrafish (C. Liu et al., 2012; X. Liu et al., 2012). Our results suggest, according to integrated “associations” between these genes in this study, that up-regulation of expression of VTGs and PGR might have resulted from up-regulation of ER1 and ER2b, and up-regulation of ER2a might be the result from up-regulation of expression of NCOAs. When exposed to E2 down-regulation of mRNA expression of ER2a gene was accompanied by down-regulation of mRNA expression of CCND1 gene, while mRNA expression of NCOA genes was not affected. This result suggests that down-regulation of expression of CCND1 might be responsible for the observed down-regulation of expression of ER2a

after exposure to E2. Collectively, the results demonstrated that TDCPP could up-regulate mRNA expressions of ER1, ER2b and other associated genes, such as VTGs, and thus might have estrogenic activity.

Thyroid hormones, thyroxine (T4) and triiodothyronine (T3) are produced by the thyroid gland, and their roles in development and metabolism have been well established in vertebrates (Power et al., 2001). The two hormones (T4 and T3) exert their major effects by binding to their receptors (TRs), such as TR α in zebrafish (Power et al., 2001). In this study, six genes were selected and integrated as “associations”. Exposure to T3 up-regulated mRNA expression of all the genes selected except for FUS, which suggests that the TR α -associated pathway was responsible for exposure to TR agonists in 5-day zebrafish larvae. Treatment with TPP or TDCPP caused transcriptional responses in the TR α -associated pathway, where TDCPP had greater potency. The results of an epidemiology study suggested that TDCPP and TPP might have thyroid system-disrupting activity, where TDCPP and TPP concentrations in dust were correlated with the concentrations of free T4 in human plasma (Meeker and Stapleton, 2010). The results of the correlation analysis suggested that TDCPP and TPP could modulate functions of the thyroid system by interacting with TR α . Steroid hormones, including glucocorticoids and mineralocorticoids, are essential for regulation of diverse physiological functions, such as glucose metabolism, mineral balance, and behavior (Greenwood et al., 2003). Glucocorticoids and mineralocorticoids exert their major effects by binding to their receptors (GR and MR, respectively), thus resulting in alterations in down-stream transcription (Greenwood et al., 2003). In the present study, exposure to GR or MR agonist significantly up-regulated mRNA expression of several genes involved in GR- or MR-centered gene networks, which suggests that it is feasible to use 5-day old zebrafish larvae to evaluate effects of chemical on GR- and MR-associated mRNA expression. Treatment with TDCPP or TPP significantly up-regulated expression of GR and MR and several other genes involved in the two gene networks.

In summary, the results of this study confirmed that 5-day old zebrafish larvae could be used to evaluate the effects of chemicals on mRNA expression in AhR-, PPAR α -, ER-, TR α -, GR- and MR-centered gene networks. Exposure to TDCPP altered profiles or relative abundances of transcripts of all the receptor-centered gene networks studied. TPP was less potent compared with TDCPP, and the PPAR α - and TR α -centered gene networks were the primary targets for TPP. Furthermore, TPP could be easily metabolized by zebrafish larvae, which might be primary reason for weaker potency in altering expression of mRNAs involved in receptor-centered gene networks compared with TDCPP. In addition, concentrations of TDCPP or TPP that we determined to be effective in expression of mRNAs involved in six receptor-centered gene networks in this study were several orders of magnitude greater than those reported in the water environment (Andresen et al., 2004; van der Veen and de Boer, 2012). Therefore, direct effects of TDCPP or TPP on expression of mRNA involved in these receptor-centered networks might not be expected at their current levels of occurrence.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.aquatox.2012.12.010>.

References

- Abbott, B.D., 2009. Review of the expression of peroxisome proliferator-activated receptors alpha (PPAR α), beta (PPAR β), and gamma (PPAR γ) in rodent and human development. *Reproductive Toxicology* 27, 246–257.
- Amsterdam, A., Nissen, R.M., Sun, Z., Swindell, E.C., Farrington, S., Hopkins, N., 2004. Identification of 315 genes essential for early zebrafish development. *Proceedings of the National Academy of Sciences of the United States of America* 101, 12792–12797.
- Andreasen, E.A., Spitsbergen, J.M., Tanguay, R.L., Stegeman, J.J., Heideman, W., Peterson, R.E., 2002. Tissue-specific expression of AHR2, ARNT2, and CYP1A in zebrafish embryos and larvae: effects of developmental stage and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin exposure. *Toxicological Sciences* 68, 403–419.
- Andresen, J.A., Grundmann, A., Bester, K., 2004. Organophosphorus flame retardants and plasticizers in surface waters. *Science of the Total Environment* 332, 155–166.
- Ankley, G.T., Bennett, R.S., Erickson, R.J., Hoff, D.J., Hornung, M.W., Johnson, R.D., Mount, D.R., Nichols, J.W., Russom, C.L., Schmieder, P.K., Serrano, J.A., Tietge, J.E., Villeneuve, D.L., 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. *Environmental Toxicology and Chemistry* 29, 730–741.
- Bacaloni, A., Cavaliere, C., Foglia, P., Nazzari, M., Samperi, R., Lagana, A., 2007. Liquid chromatography/tandem mass spectrometry determination of organophosphorus flame retardants and plasticizers in drinking and surface waters. *Rapid Communications in Mass Spectrometry* 21, 1123–1130.
- Cao, S., Zeng, X., Song, H., Li, H., Yu, Z., Sheng, G., Fu, J., 2012. Levels and distributions of organophosphate flame retardants and plasticizers in sediment from Taihu Lake, China. *Environmental Toxicology and Chemistry* 31, 1478–1484.
- Chen, D., Letcher, R.J., Chu, S., 2011. Determination of non-halogenated, chlorinated and brominated organophosphate flame retardants in herring gull eggs based on liquid chromatography–tandem quadrupole mass spectrometry. *Journal of Chromatography A* 1220, 169–174.
- Chen, Q., Yu, L., Yang, L., Zhou, B., 2012. Bioconcentration and metabolism of decarboxymodiphenyl ether (BDE-209) result in thyroid endocrine disruption in zebrafish larvae. *Aquatic Toxicology* 110, 141–148.
- Crump, D., Chiu, S., Kennedy, S.W., 2012. Effects of tris(1,3-dichloro-2-propyl) phosphate and tris(1-chloropropyl) phosphate on cytotoxicity and mRNA expression in primary cultures of avian hepatocytes and neuronal cells. *Toxicological Sciences* 126, 140–148.
- DeWitt, J.C., Shnyra, A., Badr, M.Z., Loveless, S.E., Hoban, D., Frame, S.R., Cunard, R., Anderson, S.E., Meade, B.J., Peden-Adams, M.M., Luebke, R.W., Luster, M.I., 2009. Immunotoxicity of perfluorooctanoic acid and perfluorooctane sulfonate and the role of peroxisome proliferator-activated receptor alpha. *Critical Reviews in Toxicology* 39, 76–94.
- Dishaw, L.V., Powers, C.M., Ryde, I.T., Roberts, S.C., Seidler, F.J., Slotkin, T.A., Stapleton, H.M., 2011. Is the pentaBDE replacement, tris(1,3-dichloro-2-propyl) phosphate (TDCPP), a developmental neurotoxicant? Studies in PC12 cells. *Toxicology and Applied Pharmacology* 256, 281–289.
- Duarte-Guterman, P., Langlois, V.S., Pauli, B.D., Trudear, V.L., 2010. Expression and T3 regulation of thyroid hormone- and sex steroid-related genes during silurana (*Xenopus*) tropicalis early development. *General and Comparative Endocrinology* 166, 428–435.
- Greenwood, A.K., Butler, P.C., White, R.B., Demarco, U., Pearce, D., Fernald, A.D., 2003. Multiple corticosteroid receptors in a teleost fish: distinct sequences, expression patterns, and transcriptional activities. *Endocrinology* 144, 4226–4236.
- Hakkola, J., Pasanen, M., Pelkonen, O., Hukkanen, J., Evisalmi, S., Anttila, S., Rane, A., Mäntylä, M., Purkunen, R., Saarikoski, S., Tooming, M., Raunio, H., 1997. Expression of CYP1B1 in human adult and fetal tissues and differential inducibility of CYP1B1 and CYP1A1 by Ah receptor ligands in human placenta and cultured cells. *Carcinogenesis* 18, 391–397.
- Hartmann, P.C., Burgi, D., Giger, W., 2004. Organophosphate flame retardants and plasticizers in indoor air. *Chemosphere* 57, 781–787.
- Huang, P., Ceccatelli, S., Håkansson, H., Grandison, L., Rannug, A., 2002. Constitutive and TCDD-induced expression of Ah receptor-responsive genes in the pituitary. *Neurotoxicology* 23, 783–793.
- Jin, K., Park, C.M., Lee, Y., 2012. Identification of differentially expressed genes by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in human bronchial epithelial cells. *Human and Experimental Toxicology* 31, 107–112.
- Jin, Y., Chen, R., Sun, L., Qian, H., Liu, W., Fu, Z., 2009. Induction of estrogen-responsive gene transcription in the embryo, larval, juvenile and adult life stages of zebrafish as biomarkers of short-term exposure to endocrine disrupting chemicals. *Comparative Biochemistry and Physiology* 150C, 414–420.
- Karchner, S.I., Franks, D.G., hahn, M.E., 2005. AHR1B, a new functional aryl hydrocarbon receptor in zebrafish: tandem arrangement of ahr1b and ahr2 genes. *Biochemistry Journal* 15, 153–161.
- Kazeto, Y., Place, A.R., Trant, J.M., 2004. Effects of endocrine disrupting chemicals on the expression of CYP19 genes in zebrafish (*Danio rerio*) juveniles. *Aquatic Toxicology* 69, 25–34.
- Ko, C., Kim, S., Park, C., Kim, B., Shin, C., Choi, S., Chung, S., Noh, J., Jeun, J., Kim, N., Park, R., 2004. Benzo(a)pyrene-induced apoptotic death of mouse hepatoma Hepa1c17 cells via activation of intrinsic caspase cascade and mitochondrial dysfunction. *Toxicology* 199, 35–46.
- Krovel, A.V., Söfteland, L., Torstensen, B., Olsvik, P.A., 2008. Transcriptional effects of PFOS in isolated hepatocytes from Atlantic salmon *Salmo salar* L. *Comparative Biochemistry and Physiology* 148C, 14–22.
- Liao, Y., Wang, J., Huang, Q., Fang, C., Kiyama, R., Shen, H., Dong, S., 2012. Evaluation of cellular response to perfluorooctane sulfonate in human umbilical vein endothelial cells. *Toxicology In Vitro* 26, 421–428.
- Liu, C., Yu, L., Deng, J., Lam, P.K.S., Wu, R.S.S., Zhou, B., 2009. Waterborne exposure to fluorotelomer alcohol 6:2 FTOH alters plasma sex hormone and gene transcription in the hypothalamic-pituitary-gonadal (HPG) axis of zebrafish. *Aquatic Toxicology* 93, 131–137.
- Liu, C., Yan, W., Zhou, B., Guo, Y., Liu, H., Yu, H., Giesy, J.P., Wang, J., Li, G., Zhang, X., 2012. Characterization of a bystander effect induced by the endocrine-disrupting chemical 6-propyl-2-thiouracil in zebrafish embryos. *Aquatic Toxicology* 118, 108–115.
- Liu, X., Ji, K., Choi, K., 2012. Endocrine disruption potentials of organophosphate flame retardants and related mechanisms in H295R and MVLN cell lines and in zebrafish. *Aquatic Toxicology* 114, 173–181.
- Ma Jr., Q.J.P.W., 1997. A novel cytoplasmic protein that interacts with the Ah receptor, contains tetratricopeptide repeat motifs, and augments the transcriptional response to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Journal of Biological Chemistry* 272, 8878–8884.
- Mandal, P.K., 2005. Dioxin: a review of its environmental effects and its aryl hydrocarbon receptor biology. *Journal of Comparative Physiology* 175B, 221–230.
- Marklund, A., Andersson, B., Haglund, P., 2005. Organophosphorus flame retardants and plasticizers in air from various indoor environment. *Journal of Environmental Monitoring* 7, 814–819.
- McCarthy, O., Musso-Buendia, A., Kaiser, M., Brun, R., Ruiz-Perez, L.M., Johansson, N.G., Pacanowska, D.G., Gilbert, I.H., 2009. Design, synthesis and evaluation of novel uracil acetamide derivatives as potential inhibitors of *Plasmodium falciparum* dUTP nucleotidohydrolase. *European Journal of Medical Chemistry* 44, 678–688.
- McGee, S.P., Cooper, E.M., Stapleton, H.M., Volz, D.C., 2012. Early zebrafish embryogenesis is susceptible to developmental TDCPP exposure. *Environmental Health Perspectives* 120, 1585–1591.
- McKenna, N.J., Lanz, R.B., O'Malley, B.W., 1999. Nuclear receptor co-regulators: cellular and molecular biology. *Endocrine Reviews* 20, 321–344.
- McKenna, N.J., O'Malley, B.W., 2002. Combinatorial control of gene expression by nuclear receptors and coregulators. *Cell* 108, 465–474.
- Meeker, J.D., Stapleton, H.M., 2010. House dust concentrations of organophosphate flame retardants in relation to hormone levels and semen quality parameters. *Environmental Health Perspectives* 118, 318–323.
- Menuet, A., Pellegrini, E., Anglade, I., Blaise, O., Laudet, V., Kah, O., Pakdel, F., 2002. Molecular characterization of three estrogen receptor forms in zebrafish: binding characteristics, transactivation properties, and tissue distributions. *Biology of Reproduction* 66, 1881–1892.
- Menuet, A., Page, Y.L., Torres, O., Kern, L., Kah, O., Pakde, F., 2004. Analysis of the estrogen receptor (ER) reveals distinct effects of ERalpha, ERbeta1 and ERbeta2. *Journal of Molecular Endocrinology* 32, 975–986.
- Mortensen, A.S., Arukwe, A., 2007. Effects of 17 α -ethynylestradiol on hormonal responses and xenobiotic biotransformation system of atlantic salmon (*Salmo salar*). *Aquatic Toxicology* 85, 113–123.
- Moyes, K.M., Drackley, J.K., Morin, D.E., Bionaz, M., Rodriguez-Zas, S.L., Everts, R.E., Lewin, H.A., Loo, J.J., 2009. Gene network and pathway analysis of bovine mammary tissue challenged with *Streptococcus uberis* reveals induction of cell proliferation and inhibition of PPAR γ signaling as potential mechanism for the negative relationships between immune response and lipid metabolism. *BMC Genomics* 10, 542.
- Pellegrini, E., Menuet, A., Lethimonier, C., Adrio, F., Gueguen, M.M., Tascon, C., Anglade, I., Pakdel, F., Kah, O., 2005. Relationships between aromatase and estrogen receptors in the brain of teleost fish. *General and Comparative Endocrinology* 142, 60–66.
- Power, D.M., Llewellyn, L., Faustino, M., Nowell, M.A., Bjornsson, B.T., Einarsdottir, I.E., Canario, A.V.M., Sweeney, G.E., 2001. Thyroid hormones in growth and development of fish. *Comparative Biochemistry and Physiology* 130C, 447–459.
- Raldúa, D., Babin, P.J., 2009. Simple, rapid zebrafish larva bioassay for assessing the potential of chemical pollutants and drugs to disrupt thyroid gland function. *Environmental Science and Technology* 43, 6844–6850.
- Reemtsma, J., Quintana, J., Rodil, R., García-López, M., Rodríguez, I., 2008. Organophosphorus flame retardants and plasticizers in water and air. I. Occurrence and fate. *Trends in Analytical Chemistry* 27, 727–737.
- Shi, X., Liu, C., Wu, G., Zhou, B., 2009. Waterborne exposure to PFOS causes disruption of the hypothalamic-pituitary-thyroid axis in zebrafish larvae. *Chemosphere* 77, 1010–1018.
- Shibata, H., Spencer, T.E., Onate, S.A., Jenster, G., Tsai, S.Y., Tsai, M., O'Malley, B.W., 1997. Role of co-activators and co-repressors in the mechanism of steroid/thyroid receptor action. *Recent Progress in Hormone Research* 52, 141–149.
- Stackelberg, P.E., Furlong, E.T., Meyer, M.T., Zaugg, S.D., Henderson, A.K., Reissman, D.B., 2004. Persistence of pharmaceutical compounds and other organic wastewater contaminants in a conventional drinking-water-treatment plant. *Science of the Total Environment* 329, 99–113.

- Sundkvist, A.M., Olofsson, U., Haglund, P., 2010. Organophosphorus flame retardants and plasticizers in marine and fresh water biota and in human milk. *Journal of Environmental Monitoring* 12, 943–951.
- Tseng, H., Hseu, T., Buhler, D.R., Wang, W., Hu, C., 2005. Constitutive and xenobiotics-induced expression of a novel CYP3A gene from zebrafish larva. *Toxicology and Applied Pharmacology* 205, 247–258.
- Tsuchiya, Y., Nakajima, M., Yokoi, T., 2003. Critical enhancer region to which AhR/ARNT and Sp1 bind in the human *CYP1B1* gene. *Journal of Biochemistry* 133, 583–592.
- van der Veen, I., de Boer, J., 2012. Phosphorus flame retardants: properties, production, environmental occurrence, toxicity and analysis. *Chemosphere* 88, 1119–1153.
- Wan, H.T., Zhao, Y.G., Wei, X., Hui, K.Y., Giesy, J.P., Wong, C.K.C., 2012. PFOS-induced hepatic steatosis, the mechanistic actions on β -oxidation and lipid transport. *Biochimica et Biophysica Acta* 1820, 1092–1101.
- Wang, J., Shi, X., Du, Y., Zhou, B., 2011. Effects of xenoestrogens on the expression of vitellogenin (vtg) and cytochrome P450 aromatase (cyp19a and b) genes. *Journal of Environmental Science and Health* 40A, 960–967.
- Wang, X., Liu, J., Yin, Y., 2011. Development of an ultra-high-performance liquid chromatography–tandem mass spectrometry method for high throughput determination of organophosphorus flame retardants in environmental water. *Journal of Chromatography A* 1218, 6705–6711.
- Yu, L., Deng, J., Shi, X., Liu, C., Yu, K., Zhou, B., 2010. Exposure to DE-71 alters thyroid hormone levels and gene transcription in the hypothalamic-pituitary-thyroid axis of zebrafish larvae. *Aquatic Toxicology* 97, 226–233.
- Zhang, X., Hecker, M., Jones, P.D., Newsted, J., Au, D., Kong, R., Wu, R.S.S., Giesy, J.P., 2008. Responses of the medaka HPG axis PCR array and reproduction to prochloraz and ketoconazole. *Environmental Science and Technology* 42, 6762–6769.
- Zhang, Z., Pelletier, R.D., Wong, Y.N., Sugawara, M., Zhao, N., Littlefield, B.A., 2006. Preferential inducibility of CYP1A1 and CYP1A2 by TCDD: differential regulation in primary human hepatocytes versus transformed human cells. *Biochemical and Biophysical Research Communications* 341, 399–407.