

ORIGINAL ARTICLE

Effects of long-term exposure to TDCPP in zebrafish (*Danio rerio*) – Alternations of hormone balance and gene transcriptions along hypothalamus-pituitary axes

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Abstract

Background: TDCPP is one of the major chemical of organophosphate flame retardants (OPFRs) that has been detected ubiquitously in both the environment and biota. Previously we observed that it influenced the concentrations of sex and thyroid hormones in a sex-dependent pattern, leading to reproductive impairments after short-term exposure in zebrafish. Here we investigate the consequences of longer-term exposure to TDCPP on the hypothalamic-pituitary-gonad (HPG), hypothalamic-pituitary-interrenal (HPI), and hypothalamic-pituitary-thyroid (HPT) axes of zebrafish (*Danio rerio*).

Methods: A 120-day exposure test to 0.005, 0.05 and 0.5 mg/L TDCPP was initiated with fertilized eggs. Sex steroid hormones in the treated fishes were measured and transcriptional changes were analyzed.

Results: In female fish, exposure to TDCPP resulted in increases in plasma cortisol, follicle stimulating hormone (FSH), luteinizing hormone (LH), 17 β -estradiol (E2), cortisol, thyroxine (T4), and triiodothyronine (T3). Transcription of most target genes along HPG, HPI and HPT axes were increased by the exposure. While in male fish the exposure led to decreases in cortisol, FSH, LH, T4, T3, testosterone (T), and 11-ketotestosterone (11-KT). Transcription of genes along HPG, HPI and HPT axes, especially steroidogenic genes, were inhibited in male zebrafish. While, E2/T or E2/11-KT ratio was increased in both female and females. The sex-dependent changes in hormones might be due to differential responses to TDCPP induced stresses. An increase in cortisol level coincided with increases in E2 and THs in female fish, while in males decreases in cortisol as well as T, 11-KT and THs were observed. Long-term exposure to TDCPP at very low (μ g/L) concentrations could disrupt hormone balances in a sex dependent way.

Conclusion: This study revealed that TDCPP could affect endocrine axes – HPG, HPI and HPT – in zebrafish, and impair zebrafish development.

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KEYWORDS

hypothalamus-pituitary-gonad, hypothalamus-pituitary-interrenal, hypothalamus-pituitary-thyroid, organophosphate flame retardants, Zebrafish

1 | INTRODUCTION

In vertebrates, the hypothalamic-pituitary-gonad (HPG), hypothalamic-pituitary-thyroid (HPT), and hypothalamic-pituitary-adrenal (HPA) axes are important regulators of the reproduction, thyroid and adrenal endocrine systems, respectively. Each axis is responsible for regulating the synthesis, secretion, transport, and metabolism of different hormones.¹⁻³ In fish, the reproduction process is mainly regulated by the HPG axis and gonadal steroidogenic pathway.^{4,5} Thyroid hormones (THs) play an important role in the development, growth and metabolism of vertebrates. Thyroid function is dependent on iodine uptake, synthesis, transport, tissue-specific deiodination, and binding to thyroid hormone nuclear receptor (TRs), processes that are mainly regulated by the HPT axis.⁶ The function of the HPA axis is important in responses to stressor exposures in teleosts,⁷ but the adrenal gland has been rather neglected in regulatory endocrine disruption screening or test schemes.⁸ The HPG, HPT, and HPA axes interact with one another rather than functioning independently.⁹ Previous studies showed that exposure to E2 could increase plasma cortisol concentrations in juvenile Atlantic Salmon,¹⁰ and thyroid hormones have been associated with the modulation of the expression of some genes along the HPG axis in teleosts.¹¹ It is likely that chemical-induced changes along one endocrine axis lead to changes in other endocrine axes. For example, fadrozole, an aromatase inhibitor, decreases plasma 17 β -estradiol (E2) concentrations in the fathead minnow (*Pimephales promelas*) or Japanese medaka (*Oryzias latipes*), and also modulates thyroid hormones in a frog (*Silurana tropicalis*).^{12,13} Exposure to propylthiouracil resulted in lower concentrations of T4 and T3 and a higher concentration of E2 in plasma of zebrafish,¹⁴ while exposure to polychlorinated biphenyl (PCB) 126 decreased plasma thyroxine (T4) and triiodothyronine (T3), but increased plasma concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH), and E2 in rats.^{15,16} However, the cross-talk among the axes has received little attention.

Tris(1,3-dichloro-2-propyl) phosphate (TDCPP) has been widely used as an organophosphate flame retardants (OPFRs) in polyurethane foams, which are commonly found in sofas, chairs, car upholstery, and related products.¹⁷⁻¹⁹ It has been widely detected in air and water environments.^{20,21} TDCPP is not easily degraded in water and up to 6.18 $\mu\text{g/L}$ could be detected in the raw water from a solid waste disposal site.²¹ Toxicological studies of TDCPP have exploded during recent decades, and available reports suggest that the ecological risk of TDCPP as a developmental toxin should not be neglected.²² An study has shown that the presence of TDCPP in house dust showed a positive association with increased prolactin and decreased thyroxine (T4) and testosterone (T) concentrations in men.²³ The presence of TDCPP in house dust has also been associated with decreased semen quality, suggesting a link with infertility among human adult males. Using mammalian or human cell lines, studies

have shown that the toxicity of TDCPP to human beings deserves further investigation.²⁴

We previously observed that TDCPP and triphenyl phosphate (TPhP) could influence the synthesis, metabolism and activation of E2 in human adrenal H295R cells.²⁵ Both compounds could also damage reproductive performance and alter the transcription of genes along the HPG axis in zebrafish after 21 days of exposure, and could change thyroid hormone levels and related gene expression along the HPT axis in adult zebrafish.^{26,27} Wang et al²⁸ also found that short term exposure of zebrafish embryos to TDCPP could alter the transcription of genes involved in the HPT axis and change thyroid hormone levels in zebrafish larvae. Liu et al²⁷ suggested that TDCPP could alter mRNA expression of genes involved in six receptor-centered gene networks. However, the exposure duration was rather short, less than 21 days, and the endocrine disrupting effects were investigated separately.

The zebrafish (*Danio rerio*) is a globally accepted experimental animal model.²⁹ Due to their small size, quick development, high reproducibility and transparent embryo, zebrafish are becoming popular in toxicity testing.³⁰ In addition, the whole gene map of the zebrafish is known, and transgenic or knockdown experiments can be performed for mechanism assay.³¹ Using zebrafish, we previously found that triphenyl phosphate (TPhP) could influence the HPG, HPI and HPT axes at environmentally relevant concentrations.³² Considering that TDCPP shows similar toxicity to TPhP, the endocrine disrupting effect of TDCPP at environmental concentrations deserves further study.³³

The aim of the present study was to investigate the effects of long-term exposure to TDCPP on the development of zebrafish and a series of events along the different endocrine regulating axes in zebrafish. Together, these results enable a more comprehensive understanding of the toxicity of TDCPP.

2 | METHODS

2.1 | Chemicals

TDCPP (CAS No. 13674-87-8) was purchased from Chem Service (West Chester) and Sigma-Aldrich. Dimethyl sulfoxide (DMSO) was used as solvent, and the final concentrations of solvent in the exposure media were less than 0.005% (v/v).

2.2 | Zebrafish exposure and sampling

2.2.1 | Embryo phase

The embryos were collected at 8 hours post-fertilization (hpf) and examined under a stereomicroscope. Embryos that had developed

normally and reached the blastula stage were selected for subsequent experiments. Approximately 100 normal embryos were randomly selected and distributed into glass beakers containing 300 mL of test solutions. The TDCPP concentrations tested – 0, 0.005, 0.05, and 0.5 mg/L – were determined by preliminary range finding tests. Deformation and mortality were monitored during the exposure duration. Developmental parameters such as conditional factor (CF) were calculated ($n = 14$).

2.2.2 | Larval-juvenile phase

Newly hatched fries were transferred into 3 L glass beakers. At 5–30 days post-fertilization (dpf), they were then moved to 15 L tanks where they remained until the end of the experiment at 120 dpf. At 40 dpf, CF was calculated ($n = 5$). During the exposure, approximately half of the exposure solution was renewed every 2 days. The solvent control and exposure embryos received 0.005% DMSO.

2.2.3 | Adult phase

All the surviving fishes were sacrificed at 120 dpf, and blood samples were collected from the caudal vein using a heparinized capillary tube (5–20 fishes were pooled as one sample and prepared for plasma hormone assay, $n = 3$ per sex). Whole body, gonad and liver weights and lengths were measured, and CF, gonadosomatic index (GSI) and hepatosomatic index (LSI) were calculated ($n = 5$ per sex). Brain, interrenal, gonad, and thyroid tissues were dissected and preserved in RNAlater[®] for subsequent RNA isolation ($n = 3$ per sex).

2.3 | Chemical analysis

Following previous methods,^{25,26} the actual concentrations of TDCPP in the exposure media were measured using a gas chromatograph interfaced with a mass spectrometer (GC/MS).

2.4 | Hormone measurements

Sex steroid hormones including testosterone (T), 17 β -estradiol (E2) and 11-ketotestosterone (11-KT) were measured by competitive enzyme-linked immunosorbent assay (ELISA) using commercially available kits (Cayman Chemical; T [Cat # 582701], E2 [Cat # 582251], 11-KT [Cat # 582751]). In zebrafish, plasma sex hormones were measured in male and female fish separately. Follicle stimulating hormone (FSH), leuteinizing hormone (LH) and cortisol were also measured by ELISA using commercially available kits (Cayman Chemical, FSH [Cat # 500710], LH [Cat

500720] and cortisol [Cat # 500360]). The intra- and inter-assay coefficients of variation (CV) were <30% (detection limits: 19 pg/mL for E2, 6 pg/mL for T and 1.3 pg/mL for 11-KT). Triiodothyronine (T3) and thyroxine (T4) levels were measured using an ELISA kit (Uscnlife, Wuhan, China), following the manufacturer's instructions. ELISAs for T3 and T4 were validated for the use of zebrafish samples by demonstrating parallelism between a series of diluted and spiked samples in relation to the standard curve.

2.5 | Quantitative real-time PCR

Total RNA was extracted from brain, interrenal, gonad and thyroid tissue using an RNeasy minikit (QIAGEN). The complementary DNAs were synthesized from the purified RNA samples using an iScript[™] cDNA Synthesis kit (BioRad, Hercules). Quantitative real-time PCR was performed using a SYBR Green PCR kit (Toyobo) and ABI 7300 system (PerkinElmer Applied Biosystems). The PCR reaction comprised an initial denaturation step at 50°C for 2 minutes, 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds, and 60°C for 1 minute. The amount of PCR product obtained was quantified using the threshold cycle (Ct) number, which corresponds to the cycle in which an increase in the signal associated with the exponential growth of PCR product is detected. For each selected gene, real-time PCR reactions were performed for three replicate samples and repeated twice. The expression level of each target mRNA was normalized to that of a reference gene (β -actin gene) using the delta delta Ct method (for primer information see Table S1).

2.6 | Statistical assay

Statistical analysis was performed using SPSS 18.0 for Windows[®] (SPSS Inc.). Levels of gene transcription in tissues were expressed as fold change relative to the average value of the solvent control. The Shapiro–Wilk test and Levene's test were used to evaluate normal distribution and homogeneity of variances, respectively. Differences between groups were tested by Dunnett's test following one-way analysis of variance (ANOVA) or Kruskal–Wallis test. Statistical significance was determined at $P < .05$.

3 | RESULTS

3.1 | Measured concentrations of TDCPP in media

Consistent with previous chemical analysis, the measured concentrations of TDCPP were persistent over the renewal interval, i.e. 48 hours (Table 1).^{25,26} For simplicity, nominal concentrations are used when presenting results in this paper.

TABLE 1 Nominal and measured concentrations of TDCPP in exposure media during 120 days' exposure

Nominal	Measured	
	0 hour	48 hours
TDCPP		
0.00	0.00	0.00
0.005	0.005	0.005
0.05	0.05	0.05
0.5	0.436	0.405

3.2 | Developmental toxicity of TDCPP

The CFs of larval and juvenile fish were not affected even at the highest experimental concentration of 0.5 mg/L TDCPP (Table 2). However, the CFs, GSI, and LSI in adult female fish were significantly reduced after exposure to 0.5 mg/L TDCPP. The CFs and GSI were also significantly decreased in adult male fish exposed to TDCPP.

3.3 | Hormone response to TDCPP

There were statistically significant differences among the treatment groups and the controls for most of the hormone endpoints measured. In females, concentrations of plasma cortisol, FSH and LH were significantly increased after exposure to TDCPP at the lowest concentration of 0.005 mg/L (Figure 1). The concentration of E2 was also significantly increased, but T and 11-KT showed no statistically significant difference, and the E2/T and E2/11-KT ratios were significantly increased (Figure 2). Concentrations of T4 and T3 were also significantly increased after exposure to TDCPP (Figure 3). In contrast to female fish, in males, the concentrations of cortisol, FSH and LH were significantly decreased at 0.05 mg/L TDCPP. No significant effect was observed in E2, but significantly decreased levels of T and 11-KT, and increased E2/T and E2/11-KT ratios were observed. Plasma levels of T4 and T3 were significantly decreased. Most of these endpoints measured in female or male showed no dose-response relationships.

3.4 | Transcriptional response to TDCPP

Changes in transcriptional profiles in zebrafish exposed to TDCPP were dose- and sex-dependent (Figure 4; see Table S2). In females, along the HPG axis, gene transcription of *GnRH3*, *GnRHR2*, *GnRHR3*, *ER α* , *FSH β* and *LH β* was significantly up-regulated in brain, while expression of other genes including *GnRH2*, *GnRHR1*, *GnRHR4*, *CYP19B*, *ER2 β 1* and *AR* showed no significant change. In gonad, expression of *FSHR*, *LHR* and *HMGRA* was significantly up-regulated, while among six steroidogenic genes, only expression of *StAR*, *CYP11A* and *CYP17* was up-regulated. Along the HPI axis, expression of *POMC* and *MR* in brain, and expression of *CYP11A*, *CYP17*, *17 β HSD* and *CYP11B* in

interrenal tissue was significantly up-regulated. Along the HPT axis, expression of *TRHR2* in brain and *TSHR*, *TPO*, *Pax8* and *DIO2* in thyroid was significantly up-regulated. In contrast to females, in males, expression of *POMC* and *CYP11B* along the HPI axis was significantly up-regulated, while expression of *GnRH3*, *FSH β* , *LH β* , *StAR*, *HMGRA* and *TRHR2* along the HPG or HPT axes was significantly down-regulated; expression of other genes along the HPG, HPI and HPT axes was not significantly changed.

4 | DISCUSSION

A recent study suggested that TDCPP at microgram per liter concentrations has the potential to disrupt the whole endocrine system, impair zebrafish development and influence endocrine hormone levels. In the present study, the observation of disruption of hormonal balance generally corresponds well with data from the previous study.²⁶ In the previous study, concentrations of E2 increased in females, and T concentrations decreased in males, and even though the pattern of change of E2 and T/11-KT was sex dependent, the E2/T and E2/11-KT ratios were increased in both females and males, which showed similar sex hormone alternation patterns after 14 days exposure to TDCPP in adult zebrafish.³³ This study demonstrated that TDCPP could up-regulate several steroidogenic genes (including *CYP11A1*, *CYP11B2*, *CYP19A* and *3 β HSD*) in H295R cells, or up-regulated *CYP17* and *CYP19A* in gonad of adult zebrafish. In the present study, in female fish, after exposure to TDCPP, GnRH/gonadotropin and steroidogenesis were activated, which might be responsible for the increased plasma E2 levels, while in male fish, exposure to TDCPP resulted in inhibition of GnRH/gonadotropin and steroidogenesis, which might be responsible for the decreased plasma T and 11-KT levels.

Changes in plasma cortisol suggested the activation or inhibition of the HPI axis in fish. In teleosts, cortisol is the major corticosteroid secreted by the interrenal tissue (analogous to the adrenal cortex in tetrapods) and plays an important role in the physiological adjustments essential to cope with general stress. Proopiomelanocortin (*POMC*) is one of the major components of the HPI axis; it is the common precursor protein of several biologically active peptides, including pituitary adrenocorticotropin hormone (*ACTH*), which promotes cortisol production in adrenal cells.³⁴ The up-regulation of *POMC*, *CYP11A* and *CYP11B* in females and down-regulation of *POMC* in males was accompanied by increases in cortisol in females and decreases in male fish; the same phenomenon was also found in a study by Palermo et al (2012).³⁵ *GR* and *MR* are ligand activated transcription factors that play an essential role in translating the cortisol signal, and cortisol binding to *MR* may provide key signaling for zebrafish development.³⁶ The up-regulation of *MR* in female fish after exposure to TDCPP would be related to the developmental toxicity of this chemical.

For THs, as reported by Liu et al,²⁶ on exposure to TDCPP, concentrations of T4 and T3 significantly increased in female fish, but decreased in male fish. These significant changes in TH concentrations

TABLE 2 Growth endpoints determined in zebrafish exposed to TDCPP

Chemicals	concentration (mg/L)	Larvae (n = 10)		Juvenile (n = 5)		Adult (male) (n = 5)		Adult (female) (n = 5)	
		K	GSI	K	GSI	K	GSI	K	GSI
TDCPP	SC	1.32 ± 0.26	1.67 ± 0.07	0.58 ± 0.07	1.67 ± 0.08	1.51 ± 0.15	1.67 ± 0.08	1.79 ± 0.07	16.57 ± 2.15
	0.005	1.17 ± 0.11	0.64 ± 0.06	0.64 ± 0.06	1.51 ± 0.36	1.46 ± 0.13	1.51 ± 0.36	1.74 ± 0.12	14.41 ± 1.43
	0.05	1.22 ± 0.17	0.62 ± 0.04	0.62 ± 0.04	1.36 ± 0.17	1.50 ± 0.14	1.36 ± 0.17	1.83 ± 0.15	15.65 ± 3.83
	0.5	1.39 ± 0.34	0.66 ± 0.08	0.66 ± 0.08	1.52 ± 0.27*	1.29 ± 0.18*	1.52 ± 0.27*	1.50 ± 0.06*	9.53 ± 0.98*

Note: Numbers represent mean ± standard deviation.

Abbreviations: GSI: gonadosomatic index; HSI: hepatosomatic index; K: conditional factor.

*Statistically different from solvent control (SC).

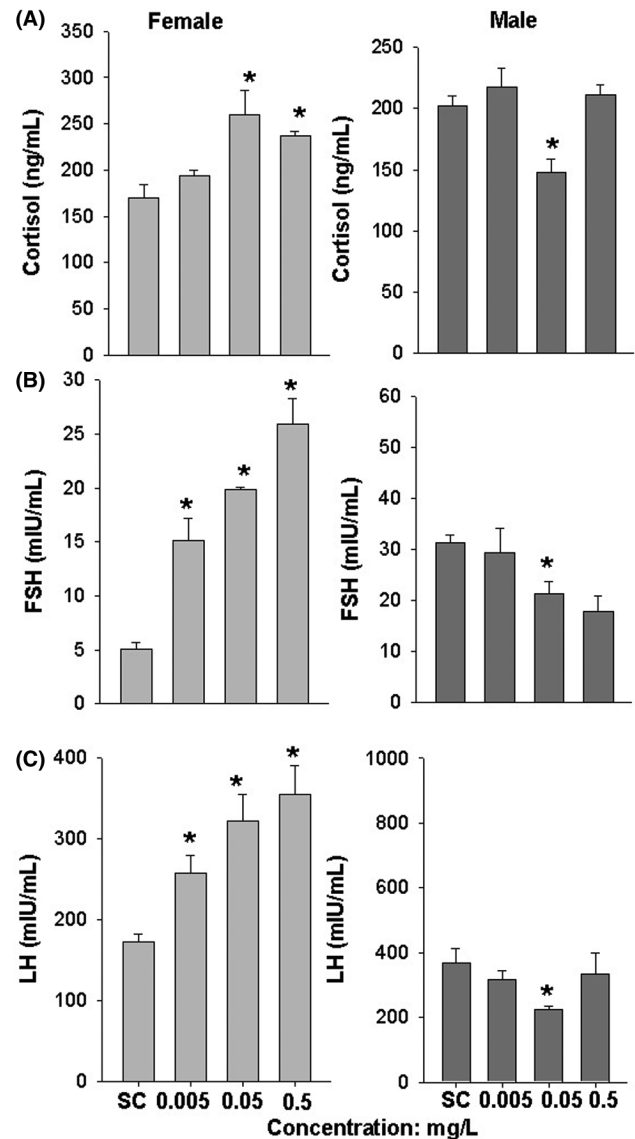


FIGURE 1 Effects of TDCPP on plasma cortisol (A), follicle stimulating hormone (FSH) (B), and leutinizing hormone (LH) (C) levels in female and male fish after 120 days' exposure. Data are expressed as mean ± SD of six replicates. Asterisk indicates significant difference from solvent control (SC, treated with 0.005% DMSO). The *P* value was determined based on ANOVA analysis

in zebrafish suggested that TDCPP disrupts thyroid function. In fish, THs are secreted from the thyroid gland and play major roles in development and growth. It was reported that exposure to different concentrations of polybrominated diphenyl ethers (PBDEs) could significantly alter T4 and T3 levels in zebrafish larvae accompanied by body weight reduction.^{37,38} In the present study, we observed that the CF, GSI and LSI of zebrafish were reduced by exposure to TDCPP, which highlights the interference of OPFRs in the growth of fish. Corticotropin-releasing hormone (CRH) has been demonstrated to have a similar activity to thyrotropin-releasing hormone (TRH) in nonmammalian vertebrates. In fish, CRH is a more potent gene factor than TRH for stimulating thyroid-stimulating hormone (TSH) release. Along the HPT axis that regulates the production of

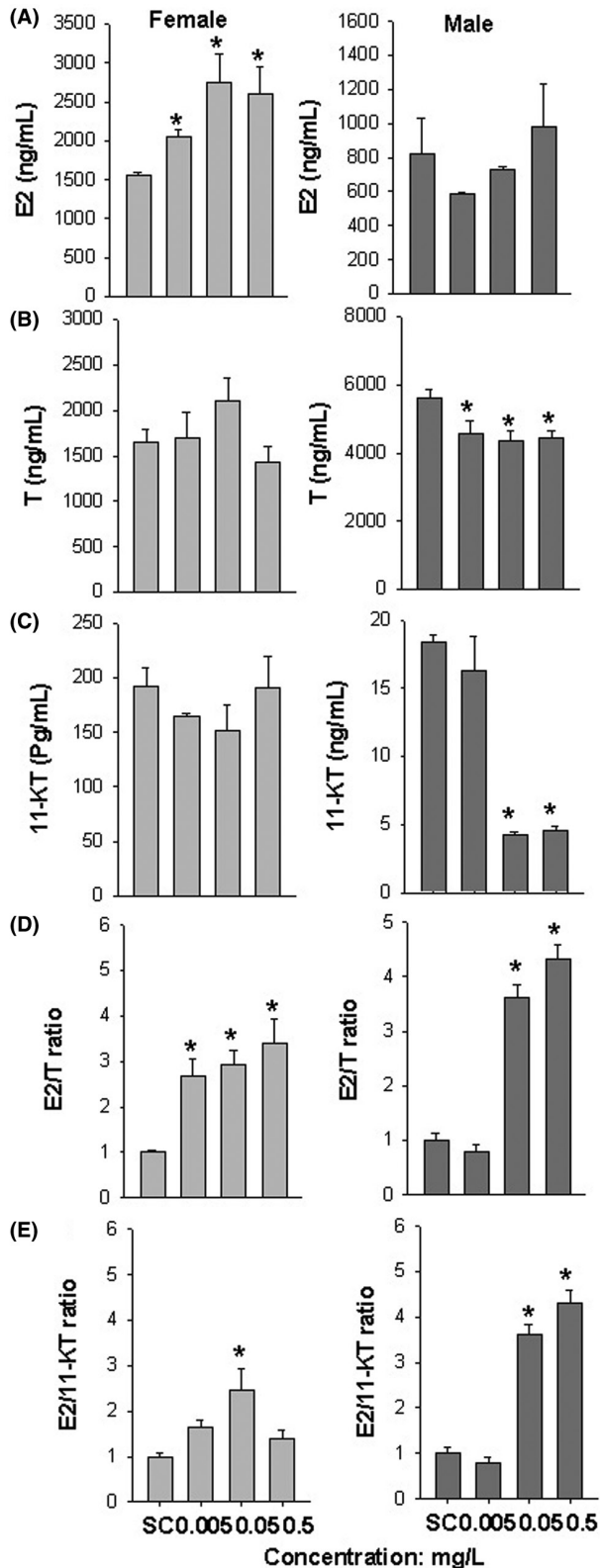


FIGURE 2 Effects of TDCPP on plasma sex hormone levels of 17-estradiol (E2) (A), testosterone (T) (B), 11-ketotestosterone (11-KT) (C), E2/T ratio (D), E2/11-KT ratio (E) in females and males after 120 days' exposure. Data are expressed as mean \pm SD of five replicates. Asterisk indicates significant difference from solvent control (SC, treated with 0.005% DMSO). The *P* value was determined based on ANOVA analysis

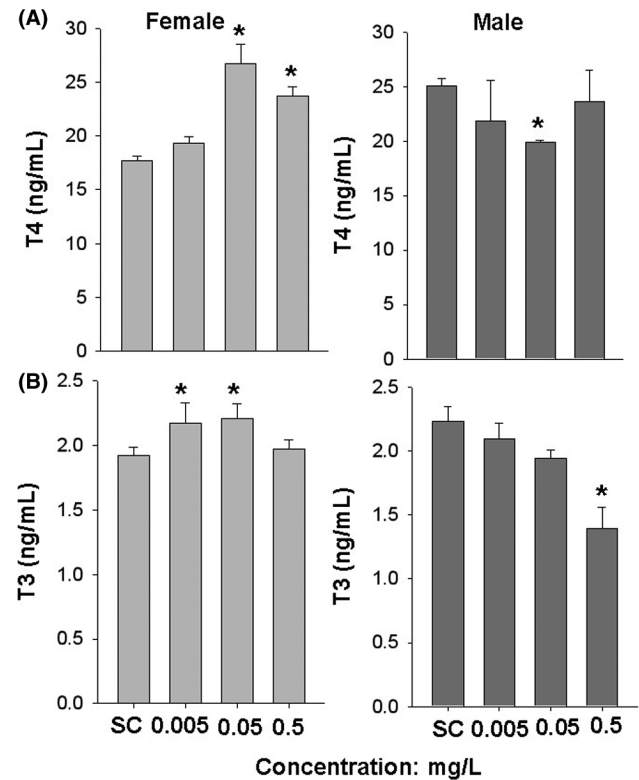


FIGURE 3 Effects of TDCPP on plasma thyroxine (T4) (A) and triiodothyronine (T3) (B) levels in female and male fish after 120 days' exposure. Data are expressed as mean \pm SD of three replicates. Asterisk indicates significant difference from solvent control (SC, treated with 0.005% DMSO). The *P* value was determined based on ANOVA analysis

THs, the expression of *CRH* or *TSH β* could be modulated by THs via negative feedback.³⁹ The expression of sodium/iodide symporter (*NIS*), *TG* and *TPO* genes is known to be involved in TH synthesis. The up-regulation of *TPO* suggested that TDCPP could induce gene expression, which might be responsible for the up-regulation of TH levels in females. On the other hand, a study by Wang et al (2013)²⁸ found that exposure of zebrafish embryos to TDCPP from 2 to 144 hpf significantly decreased T4 but increased T3 in fish larvae, and up-regulated the transcription of genes related to TH metabolism, synthesis or thyroid development. While Xu et al (2015)⁴⁰ exposed zebrafish from embryo to adult for six months and found that TDCPP significantly decreased T4 and T3 levels in females. These differences might be due to different exposure durations and suggest that varying responses of zebrafish to the same chemical may be phase dependent.

Interactions between HPI and HPG axes have been observed in previous studies.⁴¹ Animesh et al (2014)⁴² found that exposure to the sex steroid E2 resulted in an increase of cortisol levels in female. Poursaeid et al (2012)⁴¹ also showed that long-term stimulation of cortisol could suppress sex steroid levels and gonadal development in the cultured great sturgeon *Huso huso*. Liu et al (2011)¹⁴ suggested that the inhibition of E2 by prochloraz in zebrafish could responsible for the decreased concentration of plasma cortisol. It has also

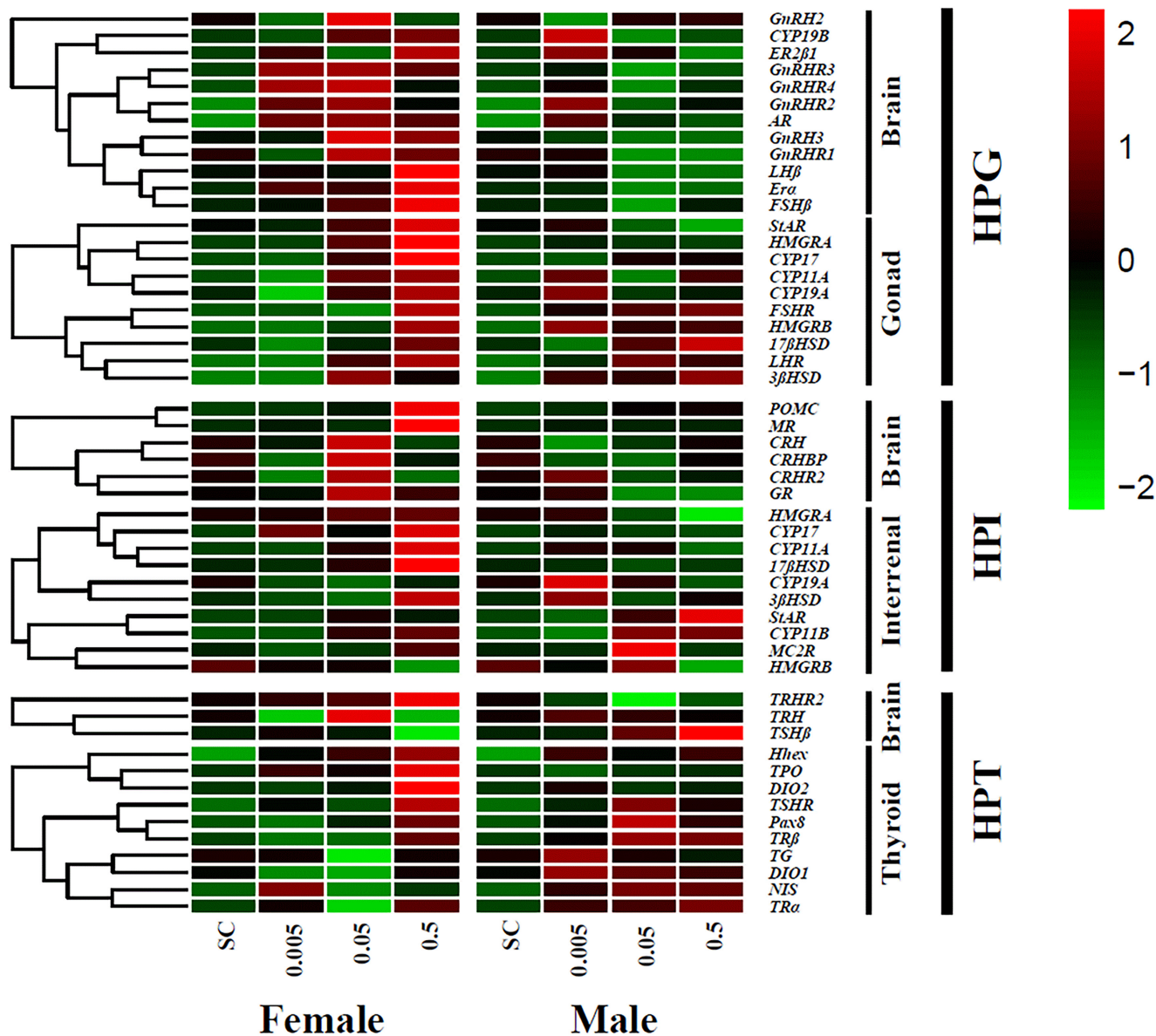


FIGURE 4 Sex-dependent response profile in adult zebrafish after exposure to TDCPP. Gene expression data from zebrafish treated with 0.005, 0.05 and 0.5 mg TDCPP/L are colour coded for the selected endocrine pathways along the zebrafish HPG, HPI, and HPT axes. Red: significantly up-regulated; Green: significantly down-regulated

been demonstrated that several putative transcription regulation elements, including two ER and several activation protein 1 (AP-1) and glucocorticoid receptor (GR) regulation elements, are found in the proximal promoter of zebrafish CRH.⁴³ Conversely, cortisol may also influence sex steroids, suggesting their reciprocal interaction along the reproductive cycle. Cortisol affected the vitellogenin production controlled by estrogen in Arctic charr,⁴⁴ and also inhibited the production of estrogen in rainbow trout.^{45,46} There are also reports of the effects of cortisol on GnRH and gonadotropins production. Marceau et al. (2015)⁴⁷ showed that cortisol treatments induced elevation of pituitary LH in rainbow trout. Even though cortisol mainly displays direct deleterious effects on female gametogenesis, it can also exhibit a spectrum of direct positive activities during oocyte maturation or ovulation.⁹ In female rhesus monkeys, androgens are

reported to be involved in the regulation of the final stages of synchronization and secretion of GnRHs and gonadotropins.⁴⁸ Many studies have indicated that cortisol inhibits male reproductive physiology over the whole reproductive cycle. Cortisol could directly inhibit androgen or inhibit gonadotropins.⁴⁹ It can also influence male reproduction: under stress or cortisol treatment, a delay in testicular development has been reported along with a few positive effects on spermatogenesis.⁵⁰ In the present study, in female fish, TDCPP exposure increased plasma cortisol levels, which in turn, might activate GnRHs and gonadotropin, and then activate steroidogenesis and increase sex hormones. In the male fish, on the other hand, decreased plasma cortisol was accompanied by the inhibition of GnRH/gonadotropin and steroidogenesis, which could be responsible for the decreased levels of T or 11-KT.

Cortisol has been also reported to influence thyroid activity and thyroid hormones in fish.⁵¹ Cortisol treatment led to decreases in plasma T3 levels in Coho salmon and in rainbow trout,⁵² but also to increased conversion of T4 to T3 in brook charr.⁵³ Poursaeid et al.⁴¹ suggested that the effects of cortisol on the thyroid hormones are species- and phase/season-dependent. Besides, cortisol and T4 exhibit synergistic interactions during larval development in fish.⁵⁴ T4 also promotes the secretion of cortisol by interrenal tissue.⁵⁵ Their plasma levels follow similar patterns during physiological events or chronic exposure to contaminants.^{56,57} Consistent with that, in the present study, after chronic exposure to TDCPP, cortisol and THs showed a similar increase in plasma in females, and a decrease in males. The decreased plasma cortisol in males suggested cortisol impairment, which would reduce the physiological competence of the fish and might be responsible for decreased THs. Sex-dependent patterns of change in the HPG and HPT axes might be associated with cortisol; however, the mechanisms underlying the cortisol and endocrine systems, and sex-dependent responses to stress caused by TDCPP warrant further studies.

In conclusion, the present study demonstrates that TDCPP can affect all three HPG, HPI and HPT endocrine axes, and impair zebrafish development, suggesting that effects of the chemical on one endocrine axis pathway may also indirectly affect the other endocrine axes in zebrafish. This finding may be helpful in describing the basic physiology of the three axes in organisms exposed to other chemicals.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

X.S. Liu performed the experiments, analyzed the data and draft the manuscript. J.B. Hong, J. Zhang, J.T. Lin, M.Z. Jiang and Q. Lin helped with the experiment and data analysis. J.J. Zhang and K. Choi initiated this study, designed the experiments, and finalized the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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