Supporting Information

Environmentally Relevant Concentrations of the Flame Retardant Tris(1,3-dichloro-2-propyl) Phosphate (TDCIPP) Inhibits Growth of Female Zebrafish and Decreases Fecundity

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Supporting Text 1: Protocol of analysis of TDCIPP and BDCIPP in exposure solutions

Triplicates of beakers were conducted for each concentration and concentrations of TDCIPP and BDCIPP were measured. Concentrations of TDCIPP in exposure solutions were quantified on the last day of exposure. Detailed protocols for identification and quantification of these two residues can be found elsewhere [1, 2]. In brief, analyses were conducted using a Waters ACQUITY UPLC® I-Class system (UHPLC) coupled to Waters® Xevos™ TQ-S mass spectrometer (TQ-S/MS) (Milford, MA, USA) using electrospray ionization (ESI(+)) in multiple reaction monitoring (MRM) mode. Due to minimal ionization in the ESI source which resulted in a poor limit of detection for BDCIPP, decamethonium hydroxide was used as a dicationic derivatization reagent which was mixed with mobile phase post-LC separation at a constant rate of 10 μL/min with a "T" connector. LC separation was carried out on a Cortecs™ UHPLC C18 column (2.1 mm × 50 mm, 1.6 μm particle size) (Waters, Mississauga, ON, Canada). Mobile phases for LC were water (A) and methanol (B), and both contained 2 mM of ammonium acetate. The flow rate of the mobile phase was 0.5 mL/min and the gradient was as follows: 0 min, 5% B; 0−5 min, 95% B (linear); hold for 1 min; 6−6.1 min, 5% B (linear) and hold for 4.9 min. The capillary voltage was 0.5 KV. The source and desolvation temperatures were 150 and 600 °C, respectively. The desolvation and cone gas flow rates were 800 and 150 L/h, respectively. TDCIPP and BDCIPP were quantified by use of transitions of 430.9 > 99 and 577.2 > 243.3, respectively. A 6-point calibration curve was run with each batch of samples to ensure linearity of the instrument's response. Method limits of quantification were 0.01 ng/mL and 0.015 ng/mL water for TDCIPP and BDCIPP, respectively.

Supporting Text 2: Protocol for quantification of TDCIPP in zebrafish organs

Detailed methods have been published previosuly [3]. In brief, organs collected from zebrafish were homogenized and transferred into a glass disposable culture tube (16×125 mm). A volume of 4 mL of 50/50 (v/v) DCM/HEX extraction solvent was added to the tube. After vortexing, 10 ng of the internal standard, d_{15}-TDCIPP, was spiked into the sample and mixed. After that, 0.2 g sodium chloride (NaCl) and 1.2 g anhydrous magnesium sulfate (MgSO4) were added into the sample. The sample tube was mixed with vortex for 1 min. The sample was then ultrasonicated in an ultrasonic-cleaner (1.9 L, 35 kHz, 140 W from VWR, Mississauga, Canada) for 10 min at room temperature. After centrifugation the extract was transferred into a disposable plain conical centrifuge tube. The ultrasonic extraction process (with DCM/HEX extraction solvent) was repeated two more times and the extracts were combined.

After solvents in the extract were evaporated with a steam of nitrogen to dryness, 1 mL MeOH was added into the sample. The sample was well mixed by vortex and ultrasonically extracted from 10 min. After centrifugation the supernatant (MeOH phase) was transferred into another disposable plain conical centrifuge tube. A 300 mg aliquant of PSA bonded silica was added into the sample solution.

The sample was mixed well by vortex mixing for 1 min, and then centrifuged. The supernatant was carefully transferred into a LC vial. The sample was ready for UHPLC-MS/MS analysis.

Supporting Text 3: Protocol of analysis of BDCIPP in zebrafish homogenates

After spiking with 10 ng of deuterated d_{10}-BDCIPP, samples were subjected to accelerated solvent extraction (ASE; Dionex ASE 200, Sunnyvale, CA, USA) with 50:50 acetone(ACE) /HEX with 1% acetic acid at 100 ^{o}C and 1500 psi for 3 cycles. The extract was reduced to a volume of approximately 1 ml under a gentle flow of nitrogen. This concentrated fraction was then further cleaned-up on a 1 g ISOLUTE® aminopropyl silica gel SPE column (Biotage, Charlotte, NC, USA) packed into a 6 mL Superclean^{TM} glass cartridge (Sigma-Aldrich). The SPE column was pre-washed with 10 mL of 5:95 water/methanol (MeOH) containing 0.1 M ammonium acetate, and then 3 mL each of MeOH and ACE, to clean and condition the silica gel absorbent. After loading the concentrated fraction onto the column with 3 mL ACE, the column was further rinsed with 6 mL ACE, 3 mL MeOH, 4 mL 5:95 water/MeOH containing 0.005 M ammonium acetate, and finally 1.5 mL 5:95 water/MeOH containing 0.1 M ammonium acetate. All of these eluates were discarded, and the target OP diesters were eluted in a final 4 mL of 5:95 water/MeOH containing 0.1 M ammonium acetate. After evaporation of MeOH using a constant flow of nitrogen the remaining water was diluted with 4 mL ACE and 1.5 g of sodium sulfate was added to remove moisture and ammonium acetate. The acetone portion of the solution was transferred into a new glass tube and evaporated to dryness under a flow of nitrogen. The residue was re-dissolved with 1000 μL of MeOH, and filtered through a centrifugal filter (0.2 μm Nylon membrane, 500 μL; VWR, Mississauga, ON, Canada). The resulting 1000 μL of filtrate was transferred to a vial for quantification by LC-MS/MS.

References:

1. Chen, D.; Letcher, R. J.; Chu, S. Determination of non-halogenated, chlorinated and brominated organophosphate flame retardants in herring gull eggs based on liquid chromatography–tandem quadrupole mass spectrometry. *J. Chromatogr. A* **2012,** *1220* (2), 169-174.

2. Su, G.; Greaves, A. K.; Gauthier, L.; Letcher, R. J. Liquid chromatography-electrospray–tandem mass spectrometry method for determination of organophosphate diesters in biotic samples including Great Lakes herring gull plasma. *J. Chromatogr. A* **2014,** *1374*, 85-92.

3. Chu, S.; Letcher, R. J. Determination of organophosphate flame retardants and plasticizers in lipid-rich matrices using dispersive solid-phase extraction as a sample cleanup step and ultra-high performance liquid chromatography with atmospheric pressure chemical ionization mass spectrometry. *Anal. Chim. Acta* **2015,** *885*, 183-190.

Table S1. Sequences of primers for the genes tested.

Target gene	Accession no.	Primer sequences (from 5' to 3')
ghrh	NM_001080092.1	F: TGGAAGACATGCTGATGCCA
		R: TCCACATCTTGCTTGTAGGTGT
gh	NM_001020492.2	F: TCGTTCTGCAACTCTGACTCC
		R: CCGATGGTCAGGCTGTTTGA
igf1	NM_131825.2	F: CAACGACACACAGGTCTTCCCAGG
		R: TCGGCTGTCCAACGGTTTCTCTT
igf2a	NM_131433	F: CGCCTGCCATGGATGATTAC
		R: TCAGTGAGCGCATCGTTGTT
igf2b	NM_001001815	F: AACCTGCCAAGTCAGAGAGGG
		R: GGACCTCCTGTTTTAATGCGG
fshβ	NM_205624.1	F: ACAGCACACCCAGAAGGTCT
		R: AGCTCCCCAGTCTGTTGTGT
lhβ	NM_205622.2	F: GAGACGGTATCGGTGGAAAA
		R: AACAGTCGGGCAGGTTAATG
vtg1	NM_001044897.2	F: CTGCGTGAAGTTGTCATGCT
		R: GACCAGCATTGCCCATAACT
cyp19a	AF226620.1	F: CCGTTCTTATGGCAGGTGAT
		R: TTGTGTGGTCGATGGTGTCT
3βhsd	AY279108.1	F: TGCCAGTCTTCATCTACACCAG
		R: TTCCCAGAGGCTCTTCTTCGTG
Activin-βA2	AJ238980	F: GCTGCTCATAACTCCAACTGTT
		R: TCCAACAACCAGTCCTGTTGGA
rpl8	NM_200713	F: TTGTTGGTGTTGTTGCTGGT
		R: GGATGCTCAACAGGGTTCAT

Table S2 Effects of different concentrations of TDCIPP on the relative expression of genes involved in HPGL axis of female and male zebrafish.

	Females				Males			
TDCIPP (ng/L)	0 ± 0	29 ± 2.1	600 ± 21	6300 ± 130	0 ± 0	29 ± 2.1	600 ± 21	6300 ± 130
Brain								
fshβ	1.07 ± 0.17	1.36 ± 0.38	1.48 ± 0.62	1.29 ± 0.36	1.19 ± 0.28	1.20 ± 0.23	0.75 ± 0.12	0.94 ± 0.19
lhβ	1.45 ± 0.67	1.74 ± 0.38	1.21 ± 0.19	1.79 ± 0.23	1.20 ± 0.43	1.18 ± 0.48	0.31 ± 0.08	0.36 ± 0.10
Liver								
vtg1	1.12 ± 0.21	0.60 ± 0.36	0.76 ± 0.23	1.78 ± 0.22	1.08 ± 0.26	0.52 ± 0.16	1.69 ± 0.28	0.97 ± 0.29
Gonad								
cyp19a	1.10 ± 0.21	0.77 ± 0.24	1.48 ± 0.45	1.56 ± 0.32	1.02 ± 0.10	1.66 ± 0.40	1.46 ± 0.45	1.82 ± 0.50
Activin-βA2	1.08 ± 0.18	0.82 ± 0.13	0.67 ± 0.10	0.99 ± 0.15	1.06 ± 0.18	1.30 ± 0.32	1.68 ± 0.27	1.67 ± 0.07
3β-hsd	1.05 ± 0.14	1.05 ± 0.07	1.15 ± 0.15	1.00 ± 0.15	1.02 ± 0.10	1.19 ± 0.26	1.34 ± 0.22	1.18 ± 0.16

Values are calculated using $2^{-\Delta\Delta C_T}$ method and represent mean±SE (n=6).

Figure S1

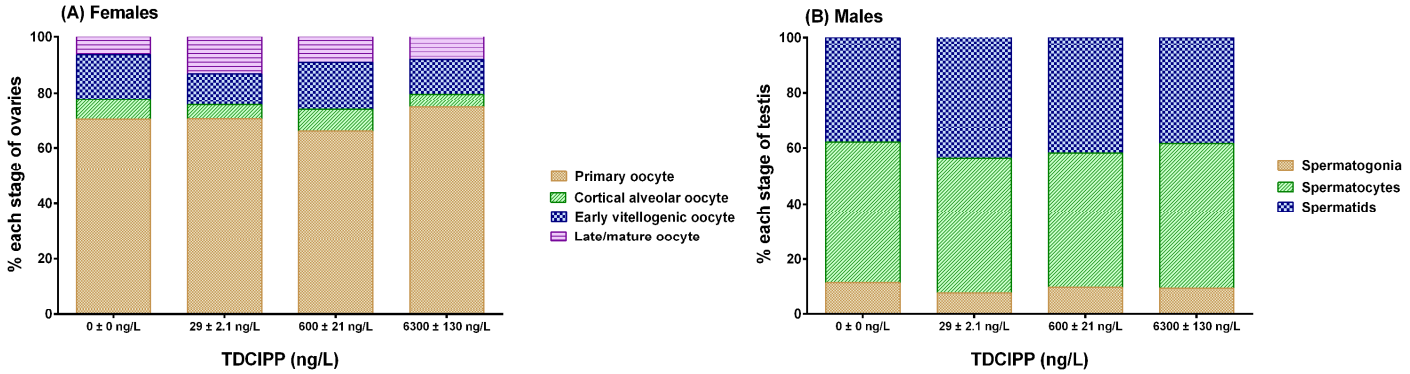

Figure S1: Histological examination of gonad development in female (A) and male (B) zebrafish after exposure to various concentrations of TDCIPP for 120 days. Values represent the mean ± SE of six individual fish from 3 replicate tanks.

Figure S2

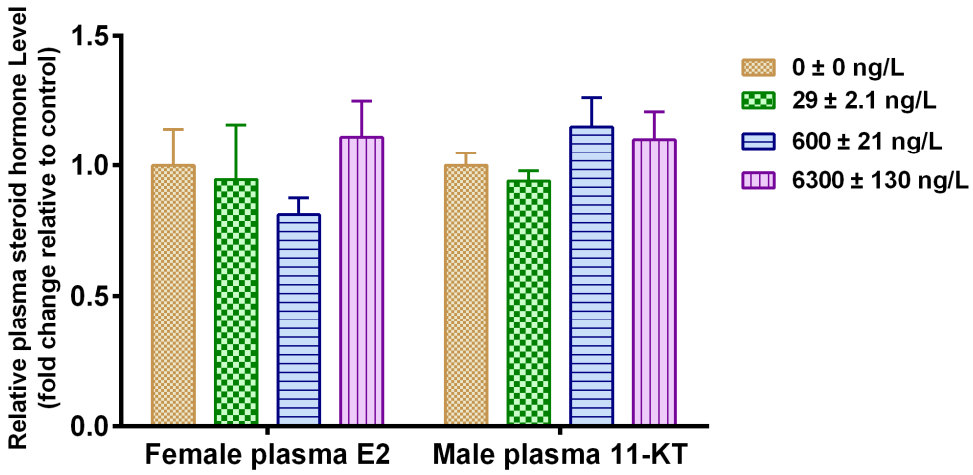

Figure S2: Effects on plasma estradiol (E2) in females and 11-ketotestosterone (11-KT) in males after exposure to various concentrations of TDCIPP for 120 days. Value represent mean ± SE (n=3).