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Effects of Tris(1,3-dichloro-2-propyl) Phosphate on Growth, Reproduction, and Gene Transcription of *Daphnia magna* at Environmentally Relevant Concentrations

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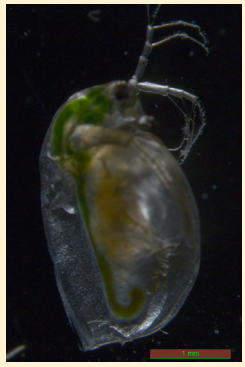
Supporting Information

ABSTRACT: The synthetic flame retardant tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) has been frequently detected in natural waters, and its maximum concentration ever reported is 377 ng/L. However, information on the adverse effects of environmentally relevant concentrations of TDCIPP on aquatic organisms are totally unknown. In this study, <12-h old water fleas, *D. magna*, were exposed to concentrations of 0, 65 ± 7.1, 550 ± 33, or 6500 ± 1400 ng/L TDCIPP, and dose- and time-dependent effects on reproduction and development were evaluated. Sequences of genes of *D. magna* were obtained from the National Center for Biotechnology Information and were used to develop PCR arrays for *D. magna*. Arrays were then used to study transcriptional responses of *D. magna* to TDCIPP. Exposure to environmentally relevant concentrations of TDCIPP significantly decreased fecundity as well as length of F_0 and F_1 generations. Transcriptional responses showed that, of the 155 genes tested, expressions of 57 genes were significantly changed, and some changes occurred following exposure to environmentally relevant concentrations (i.e., 65 ± 7.1 and 550 ± 23 ng/L). Furthermore, pathways related to protein synthesis and metabolism and endocytosis were considered to be significantly affected in a dose- and time-dependent manner and might be responsible for TDCIPP-induced reproductive and developmental toxicities.



INTRODUCTION

Tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) is an organophosphate (OP) triester flame retardant (OPFR), and has been used in various products, including plastics, foams, textiles, varnishes, electronics equipment, and furniture.[1] It has been estimated that during the period 1998−2006, annual production of TDCIPP ranged from 4500 to 22 700 tons in the United States.[1]

Environmental monitoring has demonstrated that TDCIPP is widely distributed in indoor air, indoor dust, surface waters, sediments, wildlife, and tissues of humans, and is considered to be an (re)emerging environmental pollutant considering it has been in use now for several decades.[1−13] For example, in China, it has been reported that concentrations of TDCIPP range from 2.5 to 40 ng/L in water from the Songhua River.[4,5] TDCIPP has also been detected in seawater along the coast of China near the cities of Qingdao and Xiamen, with concentrations ranging from 24 to 377 ng/L.[6] In Germany, a maximum concentration of 50 ng/L has been reported in surface water from the River Ruhr.[2] Furthermore, TDCIPP has also been detected in aquatic organisms, such as freshwater perch, with concentrations of 36−140 μg/kg lipid mass.[7] TDCIPP has been detected in human milk and urine of office workers.[7−9] Therefore, considering the wide distributions of TDCIPP, studies are needed to evaluate its environmental risks.

Despite its frequent detection in environmental media, wildlife, and humans, information about threshold and mechanisms of toxic effects of TDCIPP is limited. Published data suggest that exposure to TDCIPP can cause endocrine

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disruption,[14−17] neural toxicity,[18−22] hepatoxicology,[23−26] and developmental and reproductive toxicity in various organisms or cultured cells.[15,24,27−31] For example, two studies have reported that exposure to TDCIPP causes developmental toxicity in embryos of zebrafish by delaying remethylation of the zygotic genome and embryonic epiboly.[27,28] Treatment of embryos of chicken with TDCIPP results in significantly lesser lengths of head and bill, masses and size of gallbladder, and alterations in expressions of genes involved in immune response and lipid and steroid metabolism.[24−26] Furthermore, acute or chronic exposure of zebrafish to TDCIPP resulted in fewer numbers of eggs being produced by changing concentrations of estradiol and testosterone and expression of genes involved in hypothalamic−pituitary−gonadal axis.[15,31] Recently, to assess risk of TDCIPP on species of lower trophic levels, *Tetrahymena thermophila* was exposed in a multigeneration study. Exposure to relatively small concentrations of TDCIPP (0.01, 0.1, or 1 μM) for 5 days affected growth and reproduction by targeting the ribosome. This result suggested the need for further studies to evaluate risk of TDCIPP in other low-trophic-level species.[32]

Previous studies reported that exposure to relatively low concentrations of TDCIPP led to developmental and reproductive toxicities,[15,31,32] however, effects on development and reproduction in aquatic organisms after environmentally relevant concentrations of exposure remain unknown. The water flea, *Daphnia magna*, a freshwater branchiopod crustacean, is frequently used as a model organism in toxicological studies.[33−36] In this study, *D. magna* was used as a model to examine the effects of TDCIPP on development and reproduction after environmentally relevant concentrations of exposure. To explore possible mechanisms of toxic action, real-time polymerase chain reaction (RT-PCR) arrays were developed based on the genome of *D. magna*, and were used to examine effects of TDCIPP on the expression of 155 genes involved in 40 pathways. We hypothesized that exposure to environmentally relevant concentrations of TDCIPP would significantly alter the expressions of genes, decrease body length of F_0 and F_1 generations, and cause fewer offspring, leading to developmental and reproductive toxicities.

MATERIALS AND METHODS

Chemicals and Reagents. Tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) was purchased from Sigma (St. Louis, MO, USA), and a stock solution was prepared in dimethyl sulfoxide (DMSO). TRIzol reagent and reverse transcription and SYBR Green kits were obtained from Takara (Dalian, Liaoning, China). All the other reagents used in this study were of analytical grade.

Animals and TDCIPP Exposure Protocol. *D. magna* was obtained from School of the Environment, Nanjing University, China, and cultured for at least 10 generations before use in studies. To evaluate effects of TDCIPP on development and reproduction of *D. magna* exposed to environmentally relevant concentrations, 60 <12-h old *D. magna* were exposed to 0, 50, 500, or 5000 ng TDCIPP/L for 28 days. Exposure solutions were renewed daily with fresh water containing corresponding concentrations of TDCIPP, and each concentration included three replicated beakers. Each experimental unit (beaker) contained 5 animals and 100 mL of exposure solution. Before producing offspring, *D. magna* were fed with 0.5 mL of a mixture of *Chlorella pyrenoidosa* and *Scenedesmus obliquus* at a concentration of 2.5×10^6 cells/mL, and feeding quantity was increased to 1 mL after production of neonates commenced. Fecundity was monitored and recorded daily. Exposure solutions were sampled before and after renewal of the water solutions at the last day of exposure, and concentrations of TDCIPP were quantified. After exposure, lengths of individuals (from the apex of the helmet to the base of the tail spine) of F_0 and F_1 (from the last-three-day offspring) generations was measured. Solvent control was used, and all treatment groups received 0.01% DMSO.

To validate our findings in the dose-dependent experiment above, a time course of exposure was performed. One hundred and 20 <12-h old *D. magna* were exposed to 0 or 5000 ng TDCIPP/L, and each experimental unit (beaker) contained 20 animals and 400 mL of exposure solution. Exposure solutions were renewed daily as described above, and each concentration included three replicated beakers. During exposure, *D. magna* were fed using the same protocol as described above. Lengths of individuals of F_0 generation and expressions of genes related to protein synthesis and metabolism and endocytosis were measured after 7-, 14-, 21-, or 28-day exposure. Solvent control was used, and all treatment groups received 0.01% DMSO.

Quantification of TDCIPP in Exposure Solutions. Triplicates of beakers were conducted for each concentration, and concentrations of TDCIPP or its potential metabolite (i.e., bis(1,3-dichloro-2-propyl) phosphate (BDCIPP)) were measured. Detailed protocols for the identification and quantification of these two residues can be found elsewhere.[37,38] In brief, analyses were conducted using a Waters ACQUITY UPLC I-Class system (UHPLC) coupled to a Waters Xevo TQ-S mass spectrometer (TQ-S/MS) (Milford, MA, USA) using electrospray ionization (ESI(+)) in the multiple reaction monitoring (MRM) mode. Given the sensitivity of BDCIPP in the ESI source,[37] 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.

Development of Real-time PCR Arrays for D. magna. To explore possible toxic mechanisms of TDCIPP, real-time PCR arrays were developed for *D. magna* (Figure 1). First, all gene and EST sequences for *D. magna* were downloaded from the National Center for Biotechnology Information (NCBI) Web site (http://www.ncbi.nlm.nih.gov/) and then gene splicing and redundancy removing were performed by use of EGassembler (http://www.genome.jp/tools/egassembler/) and CD-HIT (http://weizhongli-lab.org/cd-hit/) Web sites, respectively, using all sequences available. After that, KEGG Automatic Annotation Server (KAAS) (http://www.genome.jp/tools/kaas/) was used to conduct a pathway analysis with eukaryotes

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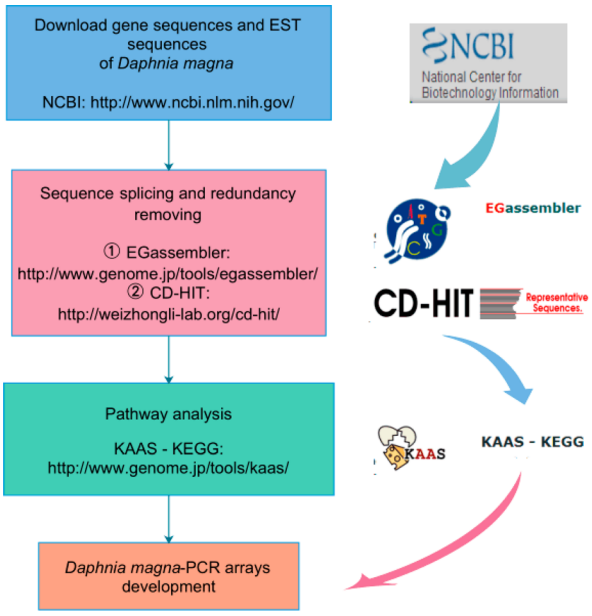

Figure 1. Flow diagram of development of *D. magna* PCR arrays. Related images were obtained from NCBI (http://www.ncbi.nlm.nih.gov/), EGassembler (http://www.genome.jp/tools/egassembler/), CD-HIT (http://weizhongli-lab.org/cd-hit/), and KAAS (http://www.genome.jp/tools/kaas/) Web sites.

as background. Finally, pathways that contained ≥5 genes were chosen for PCR arrays development.

Real-Time PCR Reactions. Real-time PCR reactions were performed according to a previously published method.[39] Briefly, isolation of total RNA was performed using TRIzol reagent (Takara, Dalian, Liaoning, China) following the manufacturer's instructions, and then purities of RNA were examined by measuring 260/280 nm ratios and concentrations were determined by absorbance at 260 nm using Epoch Microplate Spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA). Then 500 ng of total RNA for each sample was used for reverse transcription by use of the Prime ScriptTM RT reagent kit (Takara, Dalian, Liaoning, China). Quantitative real-time PCR was performed using SYBR Green Premix Ex *Taq*II kits (Takara, Dalian, Liaoning, China) according to instructions from the manufacturer, and melting curves were employed to check purities. For reference and targeted genes, two and three technical replicates were performed in each plate, respectively. Reference gene and treatment groups including solvent control were included in all plates, and intra-assay coefficients of variance were <2%. Primer sequences were developed for each gene to be amplified in the PCR arrays, by use of Primer Premier 6 software (Premier Company, Canada) (Supporting Information Table S1). On the basis of the results of the PCR, expression of gene *ndufc2* (NADH dehydrogenase (ubiquinone) 1 subunit C2) did not change after exposure to various concentrations of TDCIPP so this gene was used as an internal control or housekeeping reference gene. PCR thermal cycling was 95 °C for 3 min, followed by 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. Expression of genes was calculated by $2^{-\Delta\Delta Ct}$ method, and was presented as fold change relative to control with three biological replicates for each concentration.

Statistical Analyses. Statistical analyses were performed using Kyplot Demo 3.0 software (Tokyo, Japan). Normality and homogeneity of variances for parameters were checked by use of Kolmogorov–Smirnow and Levene's tests, respectively. If necessary, data were log-transformed to approximate normality. Significant differences of parameters tested between control and exposure groups were determined using one-way analysis of variance. The level of significance for all statistical analyses was set at $P < 0.05$.

RESULTS

Measured Concentrations of TDCIPP in Exposure Solutions. The nominal concentrations of TDCIPP in the exposure solutions were 50, 500, and 5000 ng TDCPP/L. The analytical measured and actual TDCIPP concentrations in the same three solutions were 61 ± 7.8, 550 ± 23, and 7000 ± 49 ng/L before water renewing, and 69 ± 4.6, 550 ± 47, and 6000 ± 2000 ng/L after water renewing, respectively. Mean concentrations of TDCIPP for samples taken before and after water renewing were 65 ± 7.1, 550 ± 33, and 6500 ± 1400 ng TDCIPP/L. No TDCIPP was detected in controls (Supporting Information Figure S1). Concentrations of BDCIPP in all three exposures were also monitored, but were consistently less than the MLOQ (0.015 ng BDCIPP/mL).

TDCIPP Caused Fewer Offspring to be Produced and Lesser F_0- and F_1-Generation Body Length. No mortality was observed during exposures. Exposure to 6500 ± 1400 ng/L TDCIPP for 28 days slightly but significantly decreased fecundity (cumulative production of F_1) compared with the control, while no significant effects were observed when *D. magna* were exposed to lesser concentrations (65 ± 7.1 or 550 ± 33 ng/L) or shorter times (e.g., 7, 14, or 21 days) (Figure 2). In dose exposure

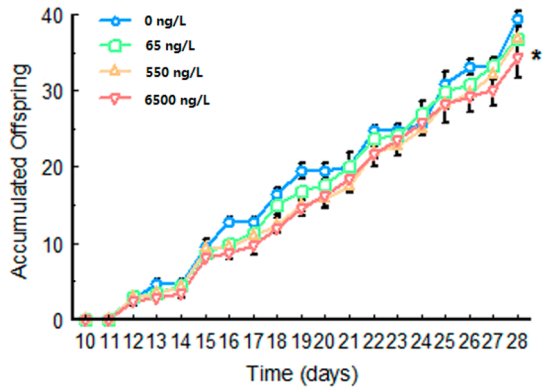

Figure 2. Effects on accumulated number of offspring in *D. magna* exposed to 0, 65 ± 7.1, 550 ± 23, or 6500 ± 1400 ng/L TDCIPP for 28 days. Values represent mean ± SD ($n = 3$). *$P < 0.05$.

experiment, lengths of bodies of F_0 individuals were unchanged after treatment with 65 ± 7.1 ng/L TDCIPP for 28 days, but significantly lesser lengths of bodies of F_0 individuals were observed after exposure to 550 ± 23 or 6500 ± 1400 ng/L (Figure 3A). For the F_1 generation, body length was significantly less by 4.25%, 4.65%, and 8.24% in 65 ± 7.1, 550 ± 23, and 6500 ± 1400 ng/L exposure groups, respective to the controls (Figure 3B). In time course study, exposure to 6500 ± 1400 ng/L TDCIPP significantly decreased body length of F_0 individuals by 6.71% and 6.92% after 21- and 28-day exposure, respectively, while no significant effect was observed after 7- and 14-day exposure (Figure 3C).

Development of D. magna PCR Arrays. PCR arrays were developed for *D. magna* to explore possible toxic mechanisms of TDCIPP. After sequence splicing and removing redundant sequences, 1549 nucleotide sequences and 15 367 EST

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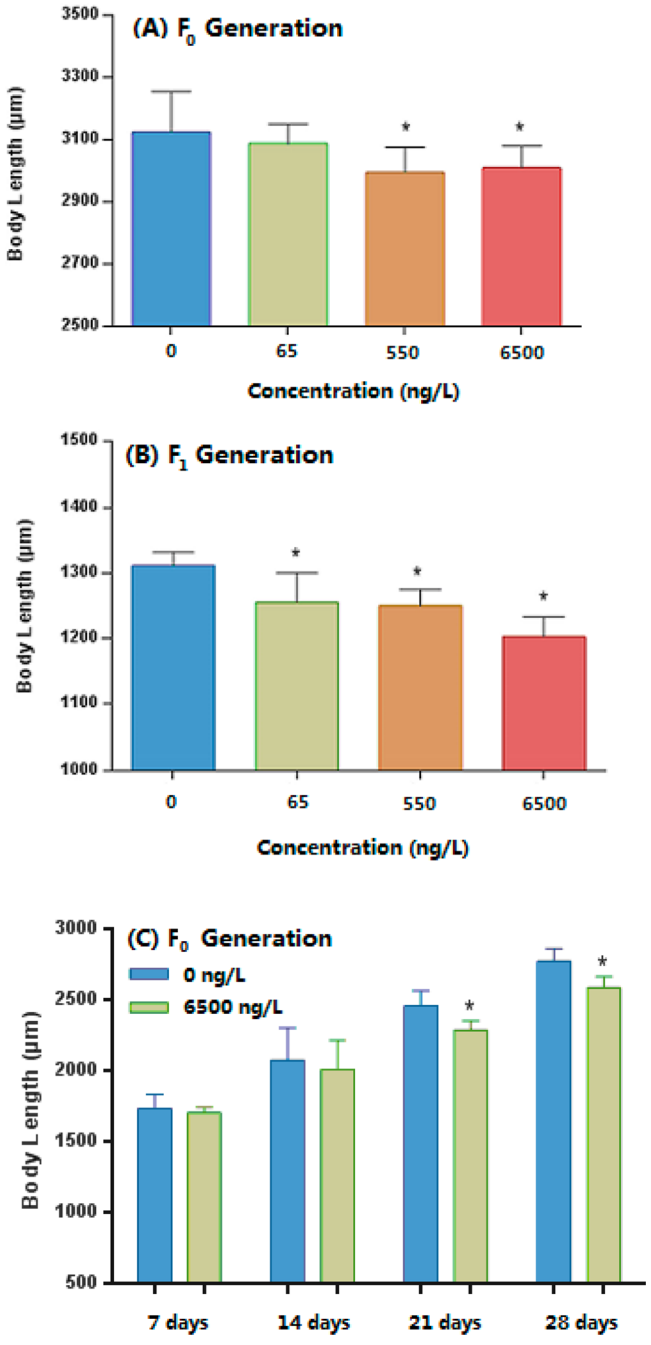

Figure 3. Dose-dependent effects on body length of F_0- (A) and F_1-generation (B) *D. magna* after exposure to 0, 65 ± 7.1, 550 ± 23, or 6500 ± 1400 ng/L TDCIPP for 28 days, and time-dependent effects on body length of F_0-generation (C) *D. magna* after exposure to 0 or 6500 ± 1400 ng/L TDCIPP for 7, 14, 21, or 28 days. Values represent mean ± SD (n = 15 for F0 generation; n = 30 for F1 generation). *P < 0.05.

sequences were acquired from the NCBI Web site and then a total of 1203 gene sequences were obtained for *D. magna.* Using these gene sequences, a pathway analysis was performed. A total of 220 pathways were obtained, and 40 pathways containing ≥5 genes were selected for development of *D. magna* PCR arrays (Figure S2).

Transcriptional Responses to TDCIPP. Expression of 155 genes involved in 40 pathways were examined in *D. magna* exposed to 0, 65 ± 7.1, 550 ± 23, or 6500 ± 1400 ng/L TDCIPP for 28 days (Table S2). In total, of the 155 genes tested, expressions of 57 genes were significantly changed, and some changes occurred following exposure to environmentally relevant concentrations (e.g., 65 ± 7.1 and 550 ± 23 ng/L) (Table S2). The 57 altered genes were involved in 30 pathways (Table S2).

To further elucidate effects of TDCIPP on pathways, expression was required to be changed by >50% or expression of three genes needed to be significantly changed before it was concluded that a particular pathway was significantly affected by exposure to TDCIPP. On the basis of these criteria, nine pathways were determined to be altered (Figures 4−6). Among the nine pathways, four (ribosome, protein processing in endoplasmic reticulum, aminoacyl-tRNA biosynthesis, and proteasome) were related to protein synthesis and metabolism (Figure 4), and three (phagosome, endocytosis, and regulation of actin cytoskeleton) were related to endocytosis (Figure 5), and the other two pathways were thyroid hormone synthesis and biosynthesis of secondary metabolites (Figure 6).

For the ribosome pathway, 20 genes were involved and expression of 4 genes was significantly down-regulated, including *rp-l26e* (large subunit ribosomal protein L26e), *rp-l30e* (large subunit ribosomal protein L30e), *rp-l38e* (large subunit ribosomal protein L38e), and *rp-s8e* (small subunit ribosomal protein S8e). There were 12 genes in the pathway related to protein processing in the endoplasmic reticulum, and expression of 4 genes (*man1* (mannosyl-oligosaccharide alpha-1,2-mannosidase), *hsp90b* (heat shock protein 90 kDa beta), *dnajc3* (DnaJ homologue subfamily C member 3) and *pdia1* (protein disulfide-isomerase A1)) were significantly changed. For the aminoacyl-tRNA biosynthesis pathway, transcription of six of seven genes including *aars* (alanyl-tRNA synthetase), *vars* (valyl-tRNA synthetase), *mars* (methionyl-tRNA synthetase), *hars* (histidyl-tRNA synthetase), *sepsecs* (*O*-phospho-L-seryl-tRNA-Sec:L-selenocysteinyl-tRNA synthase), and *eprs* (bifunctional glutamyl/prolyl-tRNA synthetase) were altered. Expression of *psmd2* (26S proteasome regulatory subunit N1), *psmc2* (26S proteasome regulatory subunit T1), and *psme4* (proteasome activator subunit 4) involved in the proteasome pathway was significantly up-regulated after exposure to TDCIPP. In total, five genes were included in this pathway. For pathways related to endocytosis expression, 11 genes were altered. These included *atpev1c* (V-type H+-transporting ATPase subunit C), *tuba* (tubulin alpha), *tubb* (tubulin beta), and *hgs* (hepatocyte growth factor-regulated tyrosine kinase substrate) in phagosome pathway, *smurf* (E3 ubiquitin ligase SMURF1/2), *fgfr3* (fibroblast growth factor receptor 3), *aip5* (atrophin-1 interacting protein 5), *ap2a* (AP-2 complex subunit alpha), and *hgs* (hepatocyte growth factor-regulated tyrosine kinase substrate) in endocytosis pathway, and *fgfr3* (fibroblast growth factor receptor 3), *cyfip* (cytoplasmic FMR1 interacting protein), and *nckap1* (NCK-associated protein 1) in regulation of actin cytoskeleton pathway.

In the time course study, exposure to 6500 ± 1400 ng/L TDCIPP caused time-dependent down-regulations of genes selected. Treatment with 6500 ± 1400 ng/L TDCIPP for 7 days did not change expressions of 29 genes selected (Tables 1 and 2). However, extended exposure to TDCIPP for 14 days significantly down-regulated the expressions of 5 genes (Tables 1 and 2). Of the 29 genes selected, expressions of 26 genes related to protein synthesis and metabolism and endocytosis were significantly down-regulated after 21-day exposure (Tables 1 and 2). Exposure to 6500 ± 1400 ng/L TDCIPP for 28 days significantly down-regulated expressions of all 29 genes selected (Tables 1 and 2).

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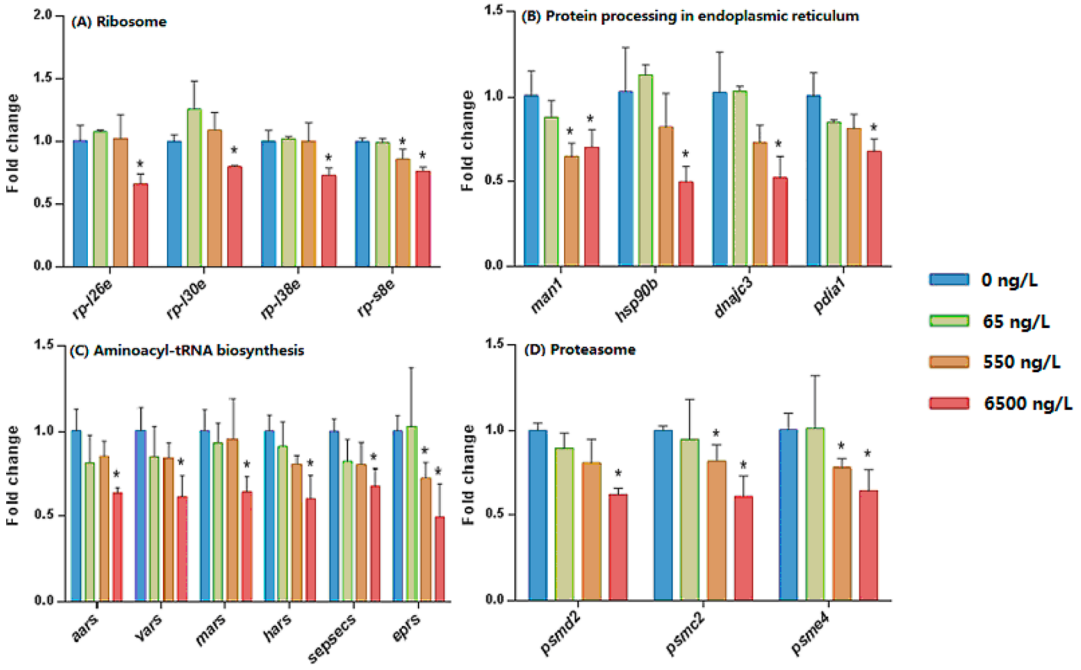

Figure 4. Effects of different concentrations of TDCIPP on the expression of genes related to protein synthesis and metabolism. These genes are included in 4 pathways: (A) ribosome, (B) protein processing in endoplasmic reticulum, (C) aminoacyl-tRNA biosynthesis, and (D) proteasome. Values represent mean ± SD (n = 3). *P < 0.05.

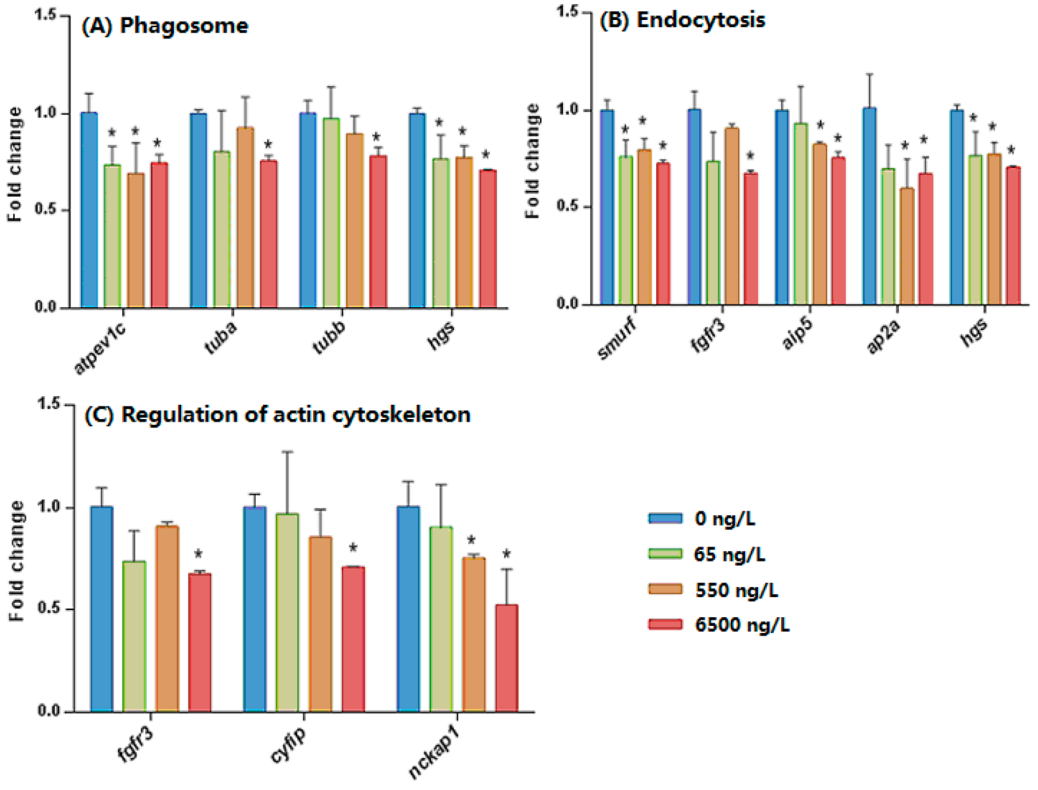

Figure 5. Effects of different concentrations of TDCIPP on the expression of genes related to endocytosis. These genes are included in 3 pathways: (A) phagosome, (B) endocytosis, and (C) regulation of actin cytoskeleton. Values represent mean ± SD (n = 3). *P < 0.05.

DISCUSSION

No significant differences were observed between TDCIPP concentrations before and after water renewing at any of three exposure concentrations (t test, p > 0.05), implying that significant depletion of TDCIPP did not occur in the present exposure system. In a previous in vitro study of effects of TDCIPP on chicken (*Gallus gallus domesticus*) embryonic hepatocytes, it was found to be completely (>98%) metabolized during a 36-h exposure period, with formation of BDCIPP metabolite.[40] However, in the present study, concentrations of

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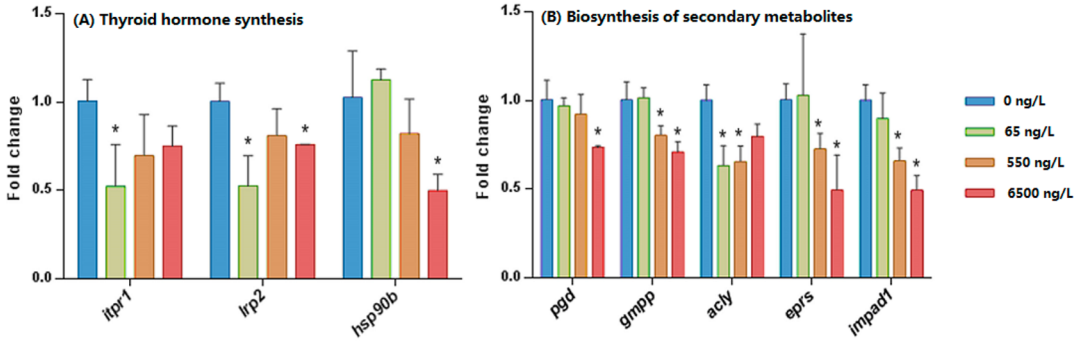

Figure 6. Effects of different concentrations of TDCIPP on the expression of genes included in (A) thyroid hormone synthesis and (B) biosynthesis of secondary metabolites. Values represent mean ± SD (n = 3). *P < 0.05.

Table 1. Time-Dependent Effects on the Expression of Genes Related to Protein Synthesis and Metabolism and Endocytosis in *D. magna* after Exposure to 0 or 6500 ± 1400 ng/L TDCIPP for 7, 14, 21, or 28 Days by Pathway[a]

	mRNA expression values							
	7 days		14 days		21 days		28 days	
gene	0 ng/L	6500 ng/L	0 ng/L	6500 ng/L	0 ng/L	6500 ng/L	0 ng/L	6500 ng/L
ribosome								
rpl26e	1.00 ± 0.08	1.12 ± 0.05	1.00 ± 0.08	1.01 ± 0.05	1.00 ± 0.03	0.87 ± 0.04[b]	1.00 ± 0.03	0.87 ± 0.03[b]
rpl30e	1.00 ± 0.04	1.09 ± 0.07	1.01 ± 0.15	0.90 ± 0.17	1.00 ± 0.08	0.84 ± 0.10	1.00 ± 0.06	0.80 ± 0.03[b]
rpl38e	1.00 ± 0.07	1.06 ± 0.04	1.01 ± 0.16	0.94 ± 0.07	1.01 ± 0.13	0.82 ± 0.02	1.00 ± 0.08	0.73 ± 0.02[b]
rps8e	1.00 ± 0.09	1.11 ± 0.04	1.00 ± 0.05	0.93 ± 0.17	1.00 ± 0.07	0.78 ± 0.03[b]	1.00 ± 0.09	0.81 ± 0.06[b]
protein processing in endoplasmic reticulum								
man1	1.00 ± 0.07	1.16 ± 0.04	1.02 ± 0.26	0.87 ± 0.18	1.00 ± 0.10	0.76 ± 0.03[b]	1.00 ± 0.03	0.82 ± 0.05[b]
hsp90b	1.00 ± 0.13	1.10 ± 0.12	1.01 ± 0.12	0.89 ± 0.20	1.00 ± 0.04	0.76 ± 0.03[b]	1.01 ± 0.15	0.66 ± 0.12[b]
dnajc3	1.00 ± 0.07	1.11 ± 0.10	1.01 ± 0.14	0.89 ± 0.11	1.00 ± 0.08	0.75 ± 0.06[b]	1.00 ± 0.11	0.77 ± 0.08[b]
pdia1	1.00 ± 0.10	1.09 ± 0.09	1.00 ± 0.10	0.97 ± 0.30	1.01 ± 0.20	0.72 ± 0.08	1.01 ± 0.17	0.65 ± 0.05[b]
aminoacyl-tRNA biosynthesis								
aars	1.00 ± 0.17	1.15 ± 0.12	1.01 ± 0.18	0.80 ± 0.19	1.00 ± 0.09	0.55 ± 0.01[b]	1.00 ± 0.10	0.60 ± 0.07[b]
vars	1.07 ± 0.46	1.12 ± 0.08	1.01 ± 0.18	0.82 ± 0.16	1.00 ± 0.10	0.65 ± 0.06[b]	1.00 ± 0.10	0.76 ± 0.05[b]
mars	1.04 ± 0.33	0.84 ± 0.04	1.01 ± 0.16	0.86 ± 0.11	1.00 ± 0.02	0.68 ± 0.04[b]	1.01 ± 0.13	0.69 ± 0.09[b]
hars	1.01 ± 0.13	1.02 ± 0.09	1.01 ± 0.19	0.85 ± 0.09	1.00 ± 0.04	0.72 ± 0.04[b]	1.02 ± 0.22	0.62 ± 0.08[b]
sepsecs	1.00 ± 0.12	0.97 ± 0.01	1.00 ± 0.09	0.82 ± 0.16	1.00 ± 0.12	0.72 ± 0.08[b]	1.01 ± 0.14	0.84 ± 0.10[b]
eprs	1.01 ± 0.20	1.13 ± 0.02	1.01 ± 0.16	0.82 ± 0.20	1.00 ± 0.06	0.70 ± 0.06[b]	1.00 ± 0.11	0.64 ± 0.14[b]
proteasome								
psmd2	1.01 ± 0.17	1.11 ± 0.07	1.00 ± 0.01	0.87 ± 0.04[b]	1.00 ± 0.12	0.64 ± 0.05[b]	1.01 ± 0.13	0.74 ± 0.10[b]
psmc2	1.01 ± 0.14	1.19 ± 0.05	1.00 ± 0.06	0.88 ± 0.08	1.00 ± 0.05	0.81 ± 0.08[b]	1.00 ± 0.01	0.72 ± 0.08[b]
psme4	1.00 ± 0.08	1.02 ± 0.04	1.00 ± 0.11	0.91 ± 0.04	1.00 ± 0.07	0.83 ± 0.07[b]	1.00 ± 0.02	0.76 ± 0.05[b]
phagosome								
atpev1c	1.00 ± 0.11	1.18 ± 0.06	1.00 ± 0.07	0.97 ± 0.12	1.00 ± 0.05	0.85 ± 0.04[b]	1.00 ± 0.06	0.71 ± 0.04[b]
tuba	1.00 ± 0.11	1.16 ± 0.02	1.00 ± 0.06	0.86 ± 0.09	1.00 ± 0.06	0.82 ± 0.03[b]	1.00 ± 0.06	0.71 ± 0.08[b]
tubb	1.01 ± 0.17	1.02 ± 0.06	1.00 ± 0.07	0.91 ± 0.16	1.00 ± 0.04	0.75 ± 0.10[b]	1.01 ± 0.17	0.63 ± 0.06[b]
hgs	1.01 ± 0.12	1.16 ± 0.08	1.01 ± 0.14	0.80 ± 0.14	1.01 ± 0.14	0.70 ± 0.05[b]	1.00 ± 0.04	0.72 ± 0.10[b]
endocytosis								
smurf	1.01 ± 0.12	1.12 ± 0.04	1.00 ± 0.08	0.83 ± 0.03[b]	1.00 ± 0.07	0.85 ± 0.02[b]	1.00 ± 0.05	0.88 ± 0.16[b]
fgfr3	1.00 ± 0.08	1.16 ± 0.12	1.01 ± 0.14	0.89 ± 0.12	1.00 ± 0.07	0.84 ± 0.02[b]	1.00 ± 0.04	0.71 ± 0.11[b]
aip5	1.01 ± 0.14	1.16 ± 0.04	1.01 ± 0.14	0.80 ± 0.05	1.00 ± 0.10	0.74 ± 0.06[b]	1.00 ± 0.04	0.74 ± 0.11[b]
ap2a	1.01 ± 0.16	1.13 ± 0.04	1.00 ± 0.08	0.81 ± 0.08[b]	1.00 ± 0.03	0.74 ± 0.04[b]	1.00 ± 0.06	0.73 ± 0.13[b]
hgs	1.01 ± 0.12	1.16 ± 0.08	1.01 ± 0.14	0.80 ± 0.14	1.01 ± 0.14	0.70 ± 0.05[b]	1.00 ± 0.04	0.72 ± 0.10[b]
regulation of actin cytoskeleton								
fgfr3	1.00 ± 0.08	1.16 ± 0.12	1.01 ± 0.14	0.89 ± 0.12	1.00 ± 0.07	0.84 ± 0.02[b]	1.00 ± 0.04	0.71 ± 0.11[b]
cyfip	1.00 ± 0.08	1.01 ± 0.02	1.01 ± 0.12	0.75 ± 0.05[b]	1.00 ± 0.02	0.86 ± 0.07[b]	1.00 ± 0.04	0.73 ± 0.05[b]
nckap1	1.00 ± 0.05	0.97 ± 0.08	1.00 ± 0.09	0.76 ± 0.06[b]	1.00 ± 0.02	0.73 ± 0.03[b]	1.00 ± 0.09	0.75 ± 0.06[b]

[a]Values represent mean ± SD (n = 3). [b]P < 0.05.

BDCIPP were consistently less than its MLOQ, which suggested that O-dealkylation of TDCIPP was not a metabolic pathway in exposed *D. magna*. Furthermore, the differences in metabolism of TDCIPP in these avian and water flea models might be partly due to (1) differences among metabolism capabilities of these two species (chicken and *D. magna*), and/or (2) that cell densities in

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Table 2. Summary of Effects on Gene Expression, Body Length, and Accumulated Offspring in *D. magna* (F_0 Generation) after Exposure to 0 or 6500 ± 1400 ng/L TDCIPP for 7, 14, 21, or 28 Days[a,b]

exposure time	gene expression[c,d]	body length	accumulated offspring
7 days	× (0.0%)	×	×
14 days	√ (17.2%)	×	×
21 days	√ (89.6%)	√	×
28 days	√ (100.0%)	√	√

[a] √: Significant differences were observed compared to DMSO control. [b] × : No significant differences were observed compared to DMSO control. [c]Expressions of total 29 genes involved in protein synthesis and metabolism and endocytosis were examined. [d]Values in the brackets are percentages of genes whose expressions were significantly changed upon TDCIPP exposure.

the present study largely differed with those in the previous study.[40]

Exposure to environmentally relevant or greater concentrations of TDCIPP significantly decreased fecundity as well as length of F_0 and F_1 *D. magna*. Results of previous studies suggest that exposure to TDCIPP causes developmental and reproductive toxicities in zebrafish and chicken embryos and adult zebrafish and *T. Thermophila*,[15,24,27−32] however exposure concentrations used in these studies are greater than environmental concentrations, and thus can not provide reliable risk assessment for TDCIPP exposure. In the present study, for the first time, it was found that exposure to environmentally relevant concentrations of TDCPP (65 ± 7.1 and 550 ± 23 ng/L) significantly decreased lengths of F_0 and F_1 in *D. magna*, and greater concentrations (6500 ± 1400 ng/L) affected fecundity, which suggested that as a result of TDCIPP exposure, development was more susceptible than reproduction. Similar effects were also observed in a previous study using zebrafish as a model, although the concentrations used in that study were higher than environmental concentrations, where the authors found that exposure to nominal concentrations of 4, 20, or 100 μg/L TDCIPP for 6 months significantly decreased body weight of female and male zebrafish, but egg production was only affected by exposure to 20 or 100 μg/L.[15]

Down-regulation of genes related to protein synthesis and metabolism might be involved in TDCIPP-induced effects on development and reproduction. Real-time PCR arrays, such as fish hypothalamic−pituitary−gonadal axis PCR array and zebrafish embryo receptor-associated PCR array have been successfully used in some studies to evaluate effects of chemicals on expression of mRNA or explore possible toxic mechanisms.[17,39,41,42] Even though they are not apical assessment end points, results of PCR arrays can be used to support some aspects of regulatory decision-making in ecotoxicology.[43] Different from previous studies where PCR arrays were developed by collecting genes included in certain pathway(s),[17,44,45] in this study, we collected all gene and EST sequences for *D. magna* from NCBI Web site and developed multipathway *D. magna* RT-PCR arrays, which allowed us to simultaneously examine pathway- and gene-level effects of TDCIPP. Exposure to TDCIPP primarily altered expression of genes involved in ribosome, protein processing in endoplasmic reticulum, aminoacyl-tRNA biosynthesis pathways, and proteasome, suggesting disruption of protein synthesis and metabolism. Syntheses of proteins are essential for organism reproduction and development and are finished by processing under the assistance of aminoacyl-tRNA and other biological substances in rough endoplasmic reticula of ribosomes.[46−48] Proteasome is present in nearly all the eukaryotic organisms and responsible for metabolism of proteins and degradation when proteins are redundant or disrupted.[49,50] Therefore, down-regulation of genes related to protein synthesis and metabolism in this study might be responsible for TDCIPP-induced developmental and reproductive toxicities. Using transcriptomic sequencing and transmission electron microscopy, it has been previously reported that exposure to TDCIPP down-regulated genes encoding for proteins in ribosomes that resulted in fewer ribosomes in endoplasmic reticulum and cytoplasm, which in turn affected growth and reproduction of *T. Thermophila*.[32]

The observed lesser transcription of genes involved in endocytosis might be also responsible for TDCIPP-induced developmental and reproductive toxicities. Endocytosis is a complex process of cellular ingestion by which the plasma membrane folds inward to bring substances into the cell.[51−53] It is responsible for homeostasis of the cell and signal transduction.[53,54] Endocytosis includes phagocytosis, pinocytosis, and receptor-mediated endocytosis, and actin plays a key role in phagocytosis.[55,56] In this study, exposure to TDCIPP resulted in lesser abundances of genes involved in endocytosis, phagosome, and regulation of actin cytoskeleton pathways. Down-regulation of these genes would decrease endocytosis process and disrupt nutrition absorption and utilization and might be also responsible for TDCIPP-induced reproductive and developmental toxicities. It was also observed that TDCIPP down-regulated expression of genes involved in thyroid hormone synthesis and biosynthesis of secondary metabolites pathways. These results suggested that complicated toxic mechanisms were included in TDCIPP-induced developmental and reproductive toxicities.

In summary, in this study, effects of TDCIPP on reproduction and development in *D. magna* were evaluated. For the first time, the results of this study suggest that exposure to environmentally relevant or greater concentrations of TDCIPP can result in significantly lesser fecundity and adversely affect development of *D. magna*. Development seems to be a more sensitive measurement end point for TDCIPP. When *D. magna* PCR arrays were developed and applied to examine transcriptional responses of 155 genes involved in 40 pathways, it was found that exposure to TDICPP resulted in down-regulation of genes related to synthesis of proteins and metabolism and endocytosis in a dose- and time-dependent manner, and our data suggested that, at least for some genes (e.g., *psmd2*, *ap2a*, *cyfip*, and *nckap1*, etc.), their down-regulations might be specific for TDCIPP exposure and involved in effects of TDICPP on fecundity and development. On the basis of the data generated from the present study, it is not possible to build direct links between individual and molecular responses, but our findings are valuable in elucidating the environmental risks of TDCIPP.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b03294.

(1) Sequences of primers for selected genes (Table S1); (2) *D. magna* PCR arrays and effects of TDCIPP on the expression of 155 genes involved in 40 pathways (Table S2) (PDF).

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Notes

The authors declare no competing financial interest.

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Supporting Information

Effects of Tris(1,3-dichloro-2-propyl) Phosphate on Growth, Reproduction and Gene Transcription of *Daphnia magna* at Environmentally Relevant Concentrations

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Number of pages: 13; Number of figures: 2; Number of tables: 2

Table S1 Sequences of primers for the genes tested.

Gene Name	Sense primer (5'-3')	Anti-Sense primer (5'-3')	Product length (bp)
idh1	ATGAGCCTTTGAAACGGTTCTG	GTTACGCCATTCATTCCTTTGATG	115
pgd	GGCATCGGTAGGCATCATAGA	TGTCAAGAGTCGTGGCGTAA	103
g6pd	CGGACCTCTTGAGCCATACTT	ACGCCTACGAACGCTTGAT	164
meth	CGTCGTATTTGCCATCCTTGAT	TCGGTGGTTGTTGTGGAACTA	231
e2.2.1.1	GTGCGAAGGCTGATGAAGTC	CAAGGCGAGTAGCAACCATT	177
gnpnat1	GACATCTTCTAACCTTGCTCTCA	TTGTGTTCGTCCACTCTACATG	253
e2.3.1.9	ACCAGCCACCATCACACTT	ACCACACCAACAACTACAATCA	108
e2.4.1.1	TCCGAACTCAACAACACTACTATG	CTTCCATCTCTTCCAACTCTTCAA	178
pk	GCCAAGGTTGAGATGCTAGAA	CGAACAGAGGCAACGGTATT	110
pgk	GCCCATTCCTTGCCATTCTC	ATGCCACCGCCAATAATCATT	103
gmpp	TGTAAGCGTGATTCCTAACTTGTC	TCTTATTCTTGTTGGAGGCTATGG	220
uap1	GGCGTTGATGGAGCGAGTA	GGAAGTATTGCGAGAGGATGAAT	110
e3.1.3.5	TAGTCCGCATCCTGTCCAAT	GTTAGCAATGAGCCGACGAT	107
e3.2.1.14	CTACCGTGTCAACAGTTACATCAA	TGGAATCAGGAGCAGCAGTC	120
e4.1.1.32	GAAGGCGGCAGCAATGTATC	GGTATGGCGGCAATTCGTTAT	151
acly	TCGTTCCGTCGCTAACCAA	CCTGTCCAAGAACAAGGCTTTA	113
e4.2.1.2b	TGTCCGTTGCTTCCACCAA	GAACCTGGCTCCTCTATTATGC	122
ears	CAGCCCTACTGGTTACATTCATT	TCTGGTCTGTGTCCTCAATCC	113
hsd17b12	CGTGGTGCTAGTCAGTAGGA	CGAGTTGCCATCTGTGAAGTC	118
aldh7a1	CAATCTCTGCTCCGCTGGTT	GCTATCGCCATCAACAATAGTGT	148
eprs	GCTCGCAATACCGTTCAATCA	CAAAGTAGTTGTCCGTTTCCCT	257
impad1	TCCTGAACATGACTTGGTGAGA	GCCGTCGCTATCTTCCTGAT	283
rp-l13e	TCGTCGTCGCAACAAGTCT	CCTCAGCAGTAGCATCAGTCT	128
rp-l19e	AGTGTTGCGTCGTCTCCTTA	GCCTTCTCTGCCTTCTTCTTG	150
rp-l26e	AGTTGATGACGGCTCCTCTC	CTTACGGTAGCACTGAATGACTT	146
rp-l30e	CATTCGTCCATTCGCCTCAG	GAACACCAGTCTTAGCCAACAT	289
rp-l31e	ACTCGTAAGTCAGCCATCAATG	TTATGCGGTGAATCTTCATCCTC	269
rp-l35ae	CGAGCCAAGTTCAAGTCCAA	TGCGAGCAATCATCCACATT	101
rp-l36e	CTCGTCGCTTCCTCTGTTCT	ATGGTTCCTCCGTTGGCTAAT	229
rp-l38e	CGCTGCTCCCGATTTCTTTAC	CACTTGAGTTCCTTGACCTGAAG	104
rp-l3e	TTTGTAGAAACGGCGACGG	GTCATTGTAGGCATTGTTGGTT	108
rp-l7	CGTAGTGTCCAGCAGCATTC	GCAACCTCGATGAGAGTCAATT	106
rp-l7e	GTCGTCGCATTCCGCTTAC	AAGGAAGTTGGAGGCGTGTT	134
rp-l8e	GTCACAATACCGATACCAAGAAGA	TCCGCCACCAGCAACAATA	107
rp-s12e	CTGATGAAGGTGGACAATAACAAG	GACAGCAACGCAAGAGCAT	108
rp-s16e	TGCGGTCATAGTTGATGAGGAT	CGACAAGCCATTGCCAAGT	100
rp-s21e	TCCGCCAGCAACAGAATCA	CGAATAGAGCCACAGATAGCATAA	128
rp-s27ae	TACACCACTCCTAAGAAGAACAAG	CTATCGCTCATTCATCGGACTC	229
rp-s27e	GTTCAACATCCCAACAGTTACTTC	TGAGCAACCAGCACATACAAC	108
rp-s5e	TGGCTGTCCGAATCCTGAAG	CACGAGGTCCAGAGTTGATAATG	105
rp-s8e	GCTCGCCGTATTCACACTG	TAATGTCCTTCATACCACTGTCTG	233
rp-s9e	GCTTCATCGTTCGGTTGGATT	GCCTACTCGTCATCTTCTTCAC	148

Table S1-*Continued a*

Gene Name	Sense primer (5'-3')	Anti-Sense primer (5'-3')	Product length (bp)
suox	GTCAACGGATTGGGACAAAGT	GTTTCACTTTCCCGCCGTC	104
serc	GAATACGGAATGGAATGGTGACA	CAGTCTGTATGTTATGGCATTGGT	185
allb	ACTCTGAACTCTGTAGCGTCTA	GAGTGCTGTTCTGCCGTAAG	141
fah	GACCATCTCGCTTACAATATCCT	GGCACACCATACTGTCACTG	212
fahd1	CAACTCCATTAACTGAGCACCAA	TGACTTCCAAGATGAGGCAAAG	149
cyc	CTGGTCAGGCTGCTGGATAT	CAATCAAGTCTGCTCGCTCAT	168
man1	GTCATCTTGTGTGGGTTCATCA	ATACTTCGTGCTCTGGAGGTTA	148
ganab	GCTTCCGCCTCCATCTCTT	ATCAACTCGTCATCTCAGTCAAC	238
sar1	GCTGAAGGATGACAGGATGG	GCCGAGTATAAGAATAGGACAATG	268
hsp90b	CCTTGGTAATCGCCTTGTAGAAT	ACTGAGGAAGAGGAAGATGCTAA	184
dnajc3	GCGTGATGGTGGTCCTTGT	TCTGGATGAGGCACATATTGAGT	150
sec63	TCAGATCCGCACACTAGAACA	ACTCATATCCACATAAGGCATTCC	133
pdia1	TGTGTAAGTTCCTCAGAAGATGG	AGTCATGCCGATGCCGATA	218
sec61a	GCATCACCATCTGTTGTTCCT	GGCTGTGTTACTACCTGTCTC	186
ost3	ACACCATAGTAGCCAACTCATTC	CGGTCTCAGGACACGGATT	260
sec24	GCCTGTCAATCCACACTTTCC	AACTTGTAGACCAAATCCTGCG	101
shp1	CATCTTCCTCCGTTAATCCTACAG	ACCATCAGCCAAGCGTATCT	116
sel1	ACCGTATCGCTTCTGACTAACT	GCTTCAGCAGCCATATCGTAA	130
cyc1	GAGCAGCGGTGATGTAGGT	TCGTCGTGGCTATGAAGTGTA	281
atpef0b	ACCTTCTGTTGCCACTTATCTTG	TTCCACCTGGTAGTCCAATCG	256
atpef1g	GCACTCATCCATCGGTCGTA	GCAACAAGAGCAGCGTCAC	180
cox2	CCAACTAACAATCTACAGCCACAA	GAACAGCATCAGCCTTAATTCCTA	157
cox3	CTAATCAGTCTCTTTGCTACCTCT	CTACTCCTACTTGAATCGTGTGAA	103
cox5a	CTTGGATTGAGCACACCTGAA	CTCTCACATAGATGACGGACTTC	110
rpb8	CCGTGGAGATTGTTGGCATC	TGGCAAGGTGTATCGTATTGAAG	123
ndufb5	CTTCAAGTCGTAAGGCAGATGA	GGATGGCTCCTAACATGATATAGA	115
ndufc2	AGTGGTATCCAACGCCATATTG	TGCTGCATGTGCAAGATAGTAA	267
itpr1	TGAGACCCGAGAGGTGAGT	GCGAATACCTGACGAATGACA	294
ap2a	TGGACATCACGCATCATTCATT	GACGAGACACGGCTAGACTAA	136
atpev1c	AATAGCCCTGTATCTTGGTGAAG	TTGTCCTACTTGCTTGCTGATT	181
atpev0a	ATGAGGACGAGAATACTGACTGT	TCTGTGGCTGATGGTGCTG	117
adam17	TCATTGCCGTCTCGCCATA	TGCTGTTGGTTGCCGACTA	136
ppih	CTTAATGGTGATGGAACTGGATGT	ACAAGCAAGCCGTCAAGAAC	212
lsm4	TGCCACTACTTCTTCTTTTACC	AACGGGGAGACCTACAATG	195
sf3b3	GCATCATCCAGGCGGTTCA	ACACCTCTTGACTGAGAAGTATGT	138
phf5a	GAGGATTTGCGATGAATGTAACT	GGATAATGACTTGATGGGAA	242
eftud2	TCGGTCGCTTATGGATCTATGA	GTGGAGAGCAACGGATATGATT	277
wbp11	GCGATGGAAGTTCAGTATGAGAA	TGATCCTGGAGTACCTGACAAC	132
crn	GACGCCGACATTGAGGTAGT	TAATCTTCCGCTTCTAGTTCTTCG	143
magoh	CAATTCCTGTCTTCCAACACGAT	GACCTGACGGCAAACTGAGA	185
thoc2	CTCGGATGCTTCAATGCCTC	ATGGACCTCACTGGACTACTTCA	117

Table S1-*Continued b*

Gene Name	Sense primer (5'-3')	Anti-Sense primer (5'-3')	Product length (bp)
ncbp1	GCTATTCACCACTATTACCTCCAA	CAGTTAGAGCCTGCACTTGAG	102
hnrnpk	CTGTCCGAGAAGCACCGAT	CATAACCGCCGTATTCCTGAG	173
ube1	CCGTCAAAGTGATGAACCCATAT	AAGTGCCAGACTCCAGTAAGG	198
mmsa	GTAATCCAGCCACCAATGAAGT	CAACAACCTGAAGTCCTCTAAGTA	258
e2.7.1.20	CCAAGAATGCCACATCCGTAG	TCACCCAAGAATGTCAGGAGATA	107
enpp1_3	CAGGATTAGTCTGCTGTGAAGA	AATGTTGGTGGTGTGGAAGAA	232
rrm2	CCTCTGCTCCGTCCTAATCC	AGTTTATCCCAGTCGTTCAAGTC	146
pde6d	CTGGCATCCGTCGCTTATCT	CTGCTACTGGCGTCCTTGT	152
prss	TGCGAATATCAGTCCTTGTGTT	TGTTGGAGCCGTTATTGTTGTA	229
p38	AAGTGTGCTCGGCAGTTGA	AGCCTTAGTTCTCTGTAAGTCCTT	112
actb_g1	CGACTTGACTGACTACTTGATGA	GGAATCGCTCGTTGCCAAT	218
kpna2	GCCGTAACCTTGAAGTTGATGA	TCCACTGTTGATGCCATTGAC	110
ns1bp	CGAGTGCTTGCGAACAGTC	GTCCATTCCATCCACCAATAACA	283
hnrnpul1	TTGTGCGAGTCGGATGGT	CTCCTCAGATGCGTCCAAGTA	175
e1.11.1.9	TGATGTCAACGGAGCGAGAG	GGAGATGAGCGATTCAATGTCTT	235
gst	CAGCATCATCAATAACAGGGACTT	AAGGAGACCAACAGGAGAATAATG	202
carp	ATCGCCATTGCTCCAACCA	GGACCTATCGAGAAGACCATCTAT	194
anpep	GTGGTCACTGGCAGATTGATG	GGCTTAATGAAGGATTCGCTACT	160
tuba	GCCACCAACAGCAACATCC	ATCAAGTTAGCGTCTCAGCCT	177
tubb	CGTGGCTCGCAACAATACC	TCTGCTCGTCAACTTCCTTCA	163
hgs	TTGACAAGCAGCTAGAGAAGG	TTGATGGCAGTGATGGCATAT	127
gng13	ACATGAAGTTCCAGGCGACTA	GGCGTGAATAAGTGGATCTGTT	107
fgfr3	AGGACACGCTGACGCATAA	CGGCAATCTTCAGGACATAATCT	147
lamb1	ATTGTCAACCATGTGGCGAAT	AGTAGAGTCCGTACTTCTGTCAA	178
ppmt5	ATGGTATGAATGGTGCGTGAC	AGTGGAATCAGTAGGCATCAAC	125
eif3d	CTGGCATTGGAAGCAACCTT	CACCTCACCTACTTCATCTTCTG	123
eif4a	GAAGGAGAAGCTGAGGACCAT	GCATAGATACCACGCAACAACT	143
cyfip	ACATAAGGTAACCAGCTCGTAGT	CCAGCCTCCTATACACCACTC	233
atp1a	CAGACAGAGGATGGCACCAA	CGCTCGGCTCAATACGGAT	265
atp2b	TGGAACTGCGAGGTCAAGAG	CTGCGACTTCAAGGATGATGAG	237
rap1b	TAAATGCCACCATGCCGATT	CTATCTGCTATCCAGTTGCTACT	120
rap11a	GCACCAGAGACGATGAATACG	CTCCACGATAGTAGGCTGATGT	246
cpa1	ATATGGAACTGACGCCGAGAT	GAGCAAGGTAGGTTGGAGGAT	219
smurf	ATAGTTGAGTCCTACAGCGAAGA	GCGTCTATCAGGTGGATGGT	149
aip5	AGGTGCTCGGACAGTTGAC	ATTCCTCGGTTCATTCTCCAATC	121
chmp1	AGTGAGTCGTTGCTATATTGTC	GGCTTCATTCTTCTTACGGATT	206
lipa	ACTGACGATGGATACATTCTTGAG	AGAGGAACGCTAGTGAGTTGT	169
aga	GGCGTTCACCAATGCTACAA	GCAACACTGATGGCACTCTT	297
ap1g1	TGGAACGCATACCGCCTAT	TTGTCGTCACCATTGCTAACTA	100
ap1b1	TACGGCACTGAGCACTACG	TGGTGGTTGCTAATGCTGTTG	260
ap1s1_2	TGGAAGTGTCTGTGAGTTGGA	AGGATGGAGTTGCGTATCGT	217

Table S1-*Continued c*

Gene Name	Sense primer (5'-3')	Anti-Sense primer (5'-3')	Product length (bp)
aars	GAGTGGTGTAGAATGCGGAATT	TCTTGACAGTGAACTCGGTTTC	125
vars	GGTCTTCAGTCTGTCAACGAAT	CAGGTGGAAGTTGTGGAATCTC	290
mars	TAAGTTCTTGGTCTAGCCAGGAT	CAAAGGGTCTCAACGGAATCAA	172
hars	GCCGATACGAAGTTGTGTTCT	TTCCAGTGTAGTAGTCCAATCCA	148
sepsecs	TTCCGTTCACTCCATCCTTCTT	CGTGGCGAACCTATGTCAGT	276
gbl	AAGGAGAACTGTTGGTAGGAGA	TGTCTTGGATTGAGGCATCTTG	108
mapkapk2	ATCCTGCGATATGTGGTCCTTA	CGTACTGTCCTGTGCGAATG	128
h4	TCCGAGATGCTGTCACCTATAC	GATAAGAACCGTTGTCAAGTCAGA	182
hdac6	GTCTCTTCTGGCTTCGATTCTG	TGCTAAGTGACGGCTCATCA	238
dlg1	TTCTTGGACGAGACTTCTTGGT	TCATCAGGTCGGTGCTATCAG	147
psmd2	CGTAATGAGGATTGGTGCTATTCT	CGCTCTTCTCCATGAGTGTCT	215
psmd14	CTACACTGACACCCGTTCCT	AGTCTGCTCCTCCAACTGATG	210
psmc2	TAGTTCCTCTGCTCCTGTGTAG	TGCTTTGGTGTATTGTCCTTGT	193
csnk2a	GTCGCCTGAAGCCTTGGAT	GTAAGAGTGTCGGAGAGGTTGA	155
myh6_7	ATGCCGTGTCCGAGATGAG	CCGCCTTGTCACTGCTAAC	138
tln	CGGCGGAGACGAATGACAT	TCTTGGCGGCAGCATCTATA	300
cpt1	TCAGTGGTGAACAAGCAATGC	AGTTGGACAGATACGGTGAGAG	175
psmb3	GCTGTCCTAATGTGCCTGAAG	GCCTGGTTGTTACTTTGTCCTT	213
psme4	CGACGCTATCCACTCCATCC	CAGAGAAGTTGCTCATCAAGGTT	122
chs1	CGAGGAGGAATACACGGAAGA	CGAGAGGACGACGACGAAT	220
acadl	CTACGGTGGAAGCAATGAAGTG	GCGGACTCGGAGAATACTCG	134
fabp3	GAGTTGAACAGGACGATGAGAC	AGCGGTGTGAGTGTATTGGAT	288
ubc	GTTGAACCATCTGACACTATTG	GACCATCCTCCAGTTGTTTACC	110
nckap1	TTCAACAGCGTCTTGCTTCAG	TGCCACATAAGTGTCTCATTCAG	281
arpc4	TTCAGCACAGACACGAGCA	CTCCATTAATTCAGTCCGCATAAG	292
lrp2	CCACCTCCATCGTTGTCAATC	TGCCGTCTCGCTTCATCAT	225
flna	ATCCAGGGTTCGCCATTCAA	ACAGTGACAGACCACCATAGC	160
ccnb	GGCAACGACAGTTCCATCAA	TCCGCTTCCTCTTCATTTCTTAC	114
plk1	ATTTCTCGGCAAGGGTGGTT	TCTGGCTTCTGGTTCAGTAACA	292
csnk1e	CCTTCAGAGCCACACCACTT	CTTGCCCTCCATTTGTTTACCA	296
fabd	GCCAACTACCTGTATCCTGAATG	GTGGAACGCTCCGCTAACT	129
e1.1.2.4	GTGTCGTGCCATTATTACAGATG	TCCAATTCCAATTCCGTGTTCT	265

Table S2 Effects of different concentrations of TDCIPP on the expression of genes involved in 40 pathways.

Pathways	Gene	mRNA expression values			
		0 ng/L	65 ng/L	550 ng/L	6500 ng/L
Biosynthesis of secondary metabolites	*idh1*	1.01±0.13	1.12±0.18	0.90±0.12	0.77±0.00
	pgd	1.00±0.11	0.97±0.05	0.92±0.11	0.74±0.01
	g6pd	1.01±0.20	0.88±0.08	0.93±0.06	0.85±0.11
	meth	1.01±0.19	0.69±0.22	0.87±0.38	0.91±0.22
	e2.2.1.1	1.02±0.23	0.77±0.21	0.77±0.22	0.80±0.13
	gnpnat1	1.05±0.36	1.60±0.29	1.08±0.03	0.91±0.12
	e2.3.1.9	1.01±0.16	1.17±0.22	0.88±0.03	0.84±0.02
	e2.4.1.1	1.01±0.15	0.72±0.08	0.70±0.22	0.85±0.10
	pk	1.02±0.26	0.76±0.18	0.72±0.19	0.92±0.10
	pgk	1.01±0.14	0.86±0.08	0.83±0.12	0.87±0.11
	gmpp	1.00±0.10	1.02±0.05	0.80±0.05 *	0.71±0.06 *
	uap1	1.02±0.23	1.01±0.07	0.95±0.08	0.80±0.21
	e3.1.3.5	1.01±0.18	0.79±0.23	0.72±0.18	0.80±0.02
	e3.2.1.14	1.01±0.20	0.83±0.09	0.96±0.15	0.90±0.03
	e4.1.1.32	1.02±0.24	1.06±0.11	1.06±0.14	0.97±0.09
	acly	1.00±0.09	0.63±0.11 *	0.66±0.09 *	0.80±0.07
	e2.4.1.2b	1.00±0.05	1.01±0.09	0.98±0.17	0.94±0.02
	ears	1.02±0.22	1.08±0.08	0.78±0.04	0.84±0.09
	hsd17b12	1.00±0.06	0.83±0.09	0.92±0.15	0.90±0.14
	aldh7a1	1.00±0.11	1.05±0.06	0.97±0.09	0.86±0.08
	eprs	1.00±0.09	1.03±0.34	0.73±0.09 *	0.49±0.20 *
	impad1	1.00±0.09	0.90±0.14	0.66±0.07 *	0.50±0.08 *
Ribosome	*rp-l13e*	1.00±0.08	1.37±0.37	1.21±0.61	0.90±0.08
	rp-l19e	1.01±0.18	0.88±0.10	0.83±0.27	0.65±0.04
	rp-l26e	1.01±0.12	1.08±0.01	1.02±0.19	0.66±0.08 *
	rp-l30e	1.00±0.05	1.26±0.22	1.09±0.14	0.80±0.01 *
	rp-l31e	1.01±0.16	1.22±0.05	0.99±0.13	0.71±0.03
	rp-l35ae	1.00±0.11	0.97±0.06	1.02±0.09	0.79±0.00
	rp-l36e	1.24±0.79	1.59±0.08	1.39±0.30	1.03±0.11
	rp-l38e	1.00±0.09	1.02±0.02	1.00±0.15	0.73±0.06 *
	rp-l3e	1.01±0.18	1.06±0.08	0.83±0.17	0.75±0.03
	rp-l7	1.01±0.13	1.37±0.59	1.76±0.68	1.10±0.28
	rp-l7e	1.02±0.22	1.19±0.08	1.05±0.08	0.77±0.01
	rp-l8e	1.03±0.30	1.14±0.17	0.91±0.16	0.66±0.04
	rp-s12e	1.01±0.17	1.00±0.16	1.05±0.09	0.84±0.12
	rp-s16e	1.02±0.24	1.07±0.14	0.93±0.10	0.71±0.07
	rp-s21e	1.02±0.25	1.25±0.44	1.05±0.13	0.70±0.14
	rp-s27ae	1.01±0.20	1.14±0.16	0.89±0.12	0.69±0.09
	rp-s27e	1.01±0.16	0.94±0.08	0.76±0.07	0.81±0.12
	rp-s5e	1.00±0.11	1.11±0.09	0.99±0.31	0.85±0.11
	rp-s8e	1.00±0.03	0.99±0.03	0.86±0.08 *	0.76±0.03 *
	rp-s9e	1.01±0.15	0.98±0.12	1.02±0.05	0.97±0.09
Microbial metabolism in diverse environments	*idh1*	1.01±0.13	1.12±0.18	0.90±0.12	0.77±0.00
	pgd	1.00±0.11	0.97±0.05	0.92±0.11	0.74±0.01 *
	g6pd	1.01±0.20	0.88±0.08	0.93±0.06	0.85±0.11
	suox	1.01±0.15	1.02±0.08	0.80±0.09	0.91±0.09
	e2.2.1.1	1.02±0.23	0.77±0.21	0.77±0.22	0.80±0.13
	e2.3.1.9	1.01±0.16	1.17±0.22	0.88±0.03	0.84±0.02
	serc	1.01±0.17	1.37±0.36	0.90±0.14	0.71±0.15
	pk	1.02±0.26	0.76±0.18	0.72±0.19	0.92±0.10
	pgk	1.01±0.14	0.86±0.08	0.83±0.12	0.87±0.11
	allb	1.01±0.17	0.99±0.05	0.92±0.06	1.04±0.16
	fah	1.00±0.08	1.05±0.15	0.90±0.11	0.78±0.07 *
	fahd1	1.03±0.32	0.91±0.19	0.83±0.07	0.69±0.06
	e4.1.1.32	1.02±0.24	1.06±0.11	1.06±0.14	0.97±0.09
	acly	1.00±0.09	0.63±0.11 *	0.66±0.09 *	0.80±0.07
	e2.4.1.2b	1.00±0.05	1.01±0.09	0.98±0.17	0.94±0.02

Table S2-*Continued a*

Pathways	Gene	mRNA expression values			
		0 ng/L	65 ng/L	550 ng/L	6500 ng/L
Microbial metabolism in diverse environments	*cyc*	1.00±0.05	0.93±0.26	0.85±0.11	0.98±0.15
	aldh7a1	1.00±0.11	1.05±0.06	0.97±0.09	0.86±0.08
Biosynthesis of antibiotics	*idh1*	1.01±0.13	1.12±0.18	0.90±0.12	0.77±0.00
	pgd	1.00±0.11	0.97±0.05	0.92±0.11	0.74±0.01 *
	g6pd	1.01±0.20	0.88±0.08	0.93±0.06	0.85±0.11
	e2.2.1.1	1.02±0.23	0.77±0.21	0.77±0.22	0.80±0.13
	e2.3.1.9	1.01±0.16	1.17±0.22	0.88±0.03	0.84±0.02
	serc	1.01±0.17	1.37±0.36	0.90±0.14	0.71±0.15
	pk	1.02±0.26	0.76±0.18	0.72±0.19	0.92±0.10
	pgk	1.01±0.14	0.86±0.08	0.83±0.12	0.87±0.11
	uap1	1.02±0.23	1.01±0.07	0.95±0.08	0.80±0.21
	e4.1.1.32	1.02±0.24	1.06±0.11	1.06±0.14	0.97±0.09
	acly	1.00±0.09	0.63±0.11 *	0.66±0.09 *	0.80±0.07
	e2.4.1.2b	1.00±0.05	1.01±0.09	0.98±0.17	0.94±0.02
	aldh7a1	1.00±0.11	1.05±0.06	0.97±0.09	0.86±0.08
Protein processing in endoplasmic reticulum	*man1*	1.00±0.14	0.88±0.10	0.65±0.08 *	0.68±0.14 *
	ganab	1.00±0.12	0.71±0.22	0.69±0.24	0.73±0.23
	sar1	1.00±0.06	1.16±0.09	1.01±0.08	0.84±0.11
	hsp90b	1.03±0.26	1.13±0.06	0.82±0.19	0.50±0.09 *
	dnajc3	1.02±0.24	1.03±0.03	0.73±0.10	0.53±0.12 *
	sec63	1.02±0.26	1.27±0.33	0.82±0.17	0.59±0.14
	pdia1	1.01±0.13	0.85±0.02	0.81±0.08	0.68±0.07 *
	sec61a	1.00±0.05	0.72±0.16	0.89±0.22	0.90±0.14
	ost3	1.01±0.17	1.09±0.04	0.91±0.12	0.80±0.19
	sec24	1.20±0.92	1.98±1.09	1.18±0.68	1.61±0.86
	shp1	1.02±0.21	1.14±0.19	0.96±0.05	0.70±0.12
	sel1	1.01±0.16	1.30±0.20	0.84±0.08	0.78±0.23
Huntington's disease	*cyc1*	1.00±0.12	1.00±0.10	0.94±0.10	0.92±0.03
	atpef0b	1.01±0.13	0.94±0.07	0.92±0.13	0.92±0.07
	atpef1g	1.02±0.24	1.03±0.27	0.91±0.17	0.87±0.02
	cox2	1.03±0.26	1.29±0.29	0.93±0.06	1.12±0.37
	cox3	1.00±0.11	0.83±0.06	0.82±0.05	0.83±0.04
	cox5a	1.00±0.09	1.14±0.07	1.09±0.15	1.05±0.10
	rpb8	1.01±0.16	0.89±0.29	0.79±0.09	0.81±0.05
	ndufb5	1.10±0.17	1.11±0.06	0.94±0.10	0.85±0.06
	itpr1	1.01±0.12	0.52±0.24	0.70±0.23	0.75±0.11
	cyc	1.00±0.05	0.93±0.26	0.85±0.11	0.98±0.15
	ap2a	1.01±0.17	0.70±0.12	0.60±0.15 *	0.68±0.08 *
Alzheimer's disease	*cyc1*	1.00±0.12	1.00±0.10	0.94±0.10	0.92±0.03
	atpef0b	1.01±0.13	0.94±0.07	0.92±0.13	0.92±0.07
	atpef1g	1.02±0.24	1.03±0.27	0.91±0.17	0.87±0.02
	cox2	1.03±0.26	1.29±0.29	0.93±0.06	1.12±0.37
	cox3	1.00±0.11	0.83±0.06	0.82±0.05	0.83±0.04
	cox5a	1.00±0.09	1.14±0.07	1.09±0.15	1.05±0.10
	ndufb5	1.10±0.17	1.11±0.06	0.94±0.10	0.85±0.06
	itpr1	1.01±0.12	0.52±0.24	0.70±0.23	0.75±0.11
	amad17	1.00±0.07	0.81±0.14	0.75±0.14	0.76±0.06 *
	cyc	1.00±0.05	0.93±0.26	0.85±0.11	0.98±0.15
Spliceosome	*ppih*	1.02±0.23	1.11±0.05	1.00±0.11	0.67±0.17
	lsm4	1.01±0.16	1.11±0.37	1.07±0.12	0.86±0.05
	sf3b3	1.01±0.14	0.73±0.25	0.77±0.30	0.80±0.12
	phf5a	1.04±0.32	1.38±0.12	1.11±0.13	0.76±0.19
	eftud2	1.01±0.19	0.79±0.32	0.69±0.22	0.65±0.01 *
	wbp11	1.01±0.14	0.88±0.20	0.74±0.16	0.81±0.13
	crn	1.02±0.23	0.98±0.09	0.71±0.10	0.67±0.14
	magoh	1.02±0.24	1.43±0.28	1.05±0.15	0.65±0.09
	thoc2	1.00±0.09	0.70±0.15 *	0.60±0.18 *	0.68±0.06 *

Table S2-*Continued b*

Pathways	Gene	mRNA expression values			
		0 ng/L	65 ng/L	550 ng/L	6500 ng/L
Spliceosome	*ns1bp*	1.00±0.02	0.68±0.14	0.78±0.19	0.74±0.05 *
	hnrnpk	1.00±0.06	0.63±0.25	0.69±0.23	0.77±0.14
Parkinson's disease	*cyc1*	1.00±0.12	1.00±0.10	0.94±0.10	0.92±0.03
	atpef0b	1.01±0.13	0.94±0.07	0.92±0.13	0.92±0.07
	atpef1g	1.02±0.24	1.03±0.27	0.91±0.17	0.87±0.02
	cox2	1.03±0.26	1.29±0.29	0.93±0.06	1.12±0.37
	cox3	1.00±0.11	0.83±0.06	0.82±0.05	0.83±0.04
	cox5a	1.00±0.09	1.14±0.07	1.09±0.15	1.05±0.10
	ube1	1.00±0.09	0.77±0.13	0.79±0.06 *	0.66±0.02 *
	ndufb5	1.10±0.17	1.11±0.06	0.94±0.10	0.85±0.06
	cyc	1.00±0.05	0.93±0.26	0.85±0.11	0.98±0.15
Carbon metabolism	*idh1*	1.01±0.13	1.12±0.18	0.90±0.12	0.77±0.00
	pgd	1.00±0.11	0.97±0.05	0.92±0.11	0.74±0.01 *
	g6pd	1.01±0.20	0.88±0.08	0.93±0.06	0.85±0.11
	mmsa	1.00±0.10	0.82±0.21	0.82±0.12	0.75±0.03 *
	e2.2.1.1	1.02±0.23	0.77±0.21	0.77±0.22	0.80±0.13
	e2.3.1.9	1.01±0.16	1.17±0.22	0.88±0.03	0.84±0.02
	serc	1.01±0.17	1.37±0.36	0.90±0.14	0.71±0.15
	pk	1.02±0.26	0.76±0.18	0.72±0.19	0.92±0.10
	pgk	1.01±0.14	0.86±0.08	0.83±0.12	0.87±0.11
	e2.4.1.2b	1.00±0.05	1.01±0.09	0.98±0.17	0.94±0.02
Oxidative phosphorylation	*cyc1*	1.00±0.12	1.00±0.10	0.94±0.10	0.92±0.03
	atpef0b	1.01±0.13	0.94±0.07	0.92±0.13	0.92±0.07
	atpef1g	1.02±0.24	1.03±0.27	0.91±0.17	0.87±0.02
	atpev1c	1.00±0.10	0.74±0.10 *	0.69±0.16 *	0.75±0.04 *
	atpev0a	1.00±0.08	0.88±0.19	0.85±0.10	0.85±0.10
	cox2	1.03±0.26	1.29±0.29	0.93±0.06	1.12±0.37
	cox3	1.00±0.11	0.83±0.06	0.82±0.05	0.83±0.04
	cox5a	1.00±0.09	1.14±0.07	1.09±0.15	1.05±0.10
	ndufb5	1.10±0.17	1.11±0.06	0.94±0.10	0.85±0.06
Influenza A	*prss*	1.01±0.16	1.23±0.20	1.00±0.10	1.23±0.28
	p38	1.00±0.09	1.06±0.21	0.86±0.07	0.90±0.29
	actb_g1	1.02±0.26	0.58±0.14	1.02±0.12	1.33±0.28
	cyc	1.00±0.05	0.93±0.26	0.85±0.11	0.98±0.15
	dnajc3	1.02±0.24	1.03±0.03	0.73±0.10	0.53±0.12 *
	kpna2	1.00±0.08	0.80±0.27	0.92±0.13	0.89±0.11
	ns1bp	1.00±0.02	0.68±0.14	0.78±0.19	0.74±0.05 *
	hnrnpul1	1.01±0.14	1.05±0.32	0.77±0.08	0.58±0.14 *
Purine metabolism	*e2.7.1.20*	1.00±0.12	0.85±0.20	0.75±0.17	0.62±0.10 *
	pk	1.02±0.26	0.76±0.18	0.72±0.19	0.92±0.10
	e3.1.3.5	1.01±0.18	0.79±0.23	0.72±0.18	0.80±0.02
	allb	1.01±0.17	0.99±0.05	0.92±0.06	1.03±0.16
	enpp1_3	1.01±0.19	0.59±0.13 *	0.64±0.13 *	1.01±0.11
	rpb8	1.01±0.16	0.89±0.29	0.79±0.09	0.81±0.05
	rrm2	1.01±0.17	1.01±0.20	0.83±0.13	0.67±0.14
	pde6d	1.01±0.18	0.61±0.16 *	0.64±0.12 *	0.48±0.04 *
Glutathione metabolism	*idh1*	1.01±0.13	1.12±0.18	0.90±0.12	0.77±0.00
	pgd	1.00±0.11	0.97±0.05	0.92±0.11	0.74±0.01 *
	g6pd	1.01±0.20	0.88±0.08	0.93±0.06	0.85±0.11
	e1.11.1.9	1.00±0.11	0.76±0.15	0.87±0.08	0.83±0.03
	gst	1.01±0.20	0.96±0.39	0.69±0.06	0.60±0.04
	carp	1.00±0.12	0.72±0.09 *	0.66±0.05 *	0.75±0.03
	rrm2	1.01±0.17	1.01±0.20	0.83±0.13	0.67±0.14
	anpep	1.01±0.13	0.47±0.03 *	0.70±0.14 *	0.75±0.11
Phagosome	*atpev1c*	1.00±0.10	0.74±0.10 *	0.69±0.16 *	0.75±0.04 *
	atpev0a	1.00±0.08	0.88±0.19	0.85±0.10	0.85±0.10
	actb_g1	1.02±0.26	0.58±0.14	1.02±0.12	1.33±0.28

Table S2-*Continued c*

Pathways	Gene	mRNA expression values			
		0 ng/L	65 ng/L	550 ng/L	6500 ng/L
Phagosome	*tuba*	1.00±0.02	0.81±0.21	0.93±0.16	0.76±0.03 *
	tubb	1.00±0.07	0.97±0.16	0.89±0.09	0.78±0.04 *
	sec61a	1.00±0.05	0.72±0.16	0.89±0.22	0.90±0.14
	hgs	1.00±0.03	0.77±0.12 *	0.78±0.06 *	0.71±0.00 *
Non-alcoholic fatty liver disease	*cyc1*	1.00±0.12	1.00±0.10	0.94±0.10	0.92±0.03
	cox2	1.03±0.26	1.29±0.29	0.93±0.06	1.12±0.37
	cox3	1.00±0.11	0.83±0.06	0.82±0.05	0.83±0.04
	cox5a	1.00±0.09	1.14±0.07	1.09±0.15	1.05±0.10
	ndufb5	1.10±0.17	1.11±0.06	0.94±0.10	0.85±0.06
	cyc	1.00±0.05	0.93±0.26	0.85±0.11	0.98±0.15
RNA transport	*prmt5*	1.01±0.20	1.03±0.09	0.87±0.21	0.89±0.22
	eif3d	1.01±0.13	0.92±0.09	0.86±0.07	0.63±0.12 *
	eif4a	1.01±0.15	0.85±0.15	0.83±0.16	0.86±0.22
	cyfip	1.00±0.06	0.97±0.30	0.86±0.13	0.71±0.00
	magoh	1.02±0.24	1.43±0.28	1.05±0.15	0.65±0.09
	thoc2	1.00±0.09	0.70±0.15 *	0.60±0.18 *	0.68±0.06 *
	ncbp1	1.01±0.14	0.76±0.07 *	0.71±0.08 *	0.73±0.07 *
Biosynthesis of amino acids	*idh1*	1.01±0.13	1.12±0.18	0.90±0.12	0.77±0.00
	meth	1.01±0.19	0.69±0.22	0.87±0.38	0.87±0.25
	e2.2.1.1	1.02±0.23	0.77±0.21	0.77±0.22	0.80±0.13
	serc	1.01±0.17	1.37±0.36	0.90±0.14	0.71±0.15
	pk	1.02±0.26	0.76±0.18	0.72±0.19	0.92±0.10
	pgk	1.01±0.14	0.86±0.08	0.83±0.12	0.87±0.11
	aldh7a1	1.00±0.11	1.05±0.06	0.97±0.09	0.86±0.08
Pancreatic secretion	*prss*	1.01±0.16	1.23±0.20	1.00±0.10	1.23±0.28
	atp1a	1.00±0.05	0.95±0.25	0.95±0.25	1.10±0.42
	itpr1	1.01±0.12	0.52±0.24	0.70±0.23	0.75±0.11
	atp2b	1.02±0.21	0.92±0.19	0.93±0.08	1.04±0.26
	rap1b	1.02±0.23	1.56±0.96	0.97±0.13	0.97±0.39
	rap11a	1.02±0.21	1.11±0.40	0.89±0.10	1.00±0.32
	cpa1	1.03±0.28	1.08±0.15	0.78±0.13	0.99±0.46
Endocytosis	*smurf*	1.00±0.05	0.76±0.08 *	0.80±0.06 *	0.73±0.02 *
	fgfr3	1.00±0.09	0.74±0.15	0.91±0.02	0.68±0.01 *
	aip5	1.00±0.05	0.93±0.19	0.83±0.01 *	0.76±0.03 *
	rab11a	1.02±0.21	1.11±0.40	0.89±0.10	1.00±0.32
	ap2a	1.01±0.17	0.70±0.12	0.60±0.15 *	0.68±0.08 *
	hgs	1.00±0.03	0.77±0.12 *	0.78±0.06 *	0.71±0.00 *
	chmp1	1.04±0.34	1.19±0.37	0.80±0.09	0.82±0.37
Aminoacyl-tRNA biosynthesis	*aars*	1.01±0.12	0.81±0.17	0.85±0.09	0.64±0.03 *
	vars	1.01±0.13	0.85±0.18	0.84±0.09	0.62±0.12 *
	mars	1.00±0.12	0.93±0.11	0.95±0.24	0.65±0.09 *
	hars	1.00±0.10	0.91±0.14	0.80±0.05 *	0.60±0.14 *
	ears	1.02±0.22	1.08±0.08	0.78±0.04	0.84±0.09
	sepsecs	1.00±0.07	0.82±0.13	0.80±0.13	0.68±0.10 *
	eprs	1.00±0.09	1.03±0.34	0.73±0.09 *	0.49±0.20 *
Pathways in cancer	*e2.4.1.2b*	1.00±0.05	1.01±0.09	0.98±0.17	0.94±0.02
	gng13	1.01±0.18	0.97±0.39	0.84±0.11	0.84±0.12
	fgfr3	1.00±0.09	0.74±0.15	0.91±0.02	0.68±0.01 *
	lamb1	1.00±0.11	0.69±0.11	1.07±0.13	1.17±0.52
	cyc	1.00±0.05	0.93±0.26	0.85±0.11	0.98±0.15
	hsp90b	1.03±0.26	1.13±0.06	0.82±0.19	0.50±0.09 *
Lysosome	*lipa*	1.14±0.64	1.28±0.42	1.43±0.14	1.19±0.61
	aga	1.01±0.17	1.04±0.24	0.70±0.04	0.84±0.23
	atpev0a	1.00±0.08	0.88±0.19	0.85±0.10	0.85±0.10
	ap1g1	1.00±0.07	0.85±0.14	0.80±0.07 *	0.67±0.09 *
	ap1b1	1.00±0.12	0.84±0.20	0.93±0.14	0.95±0.20
	ap1s1_2	1.01±0.16	1.15±0.46	0.88±0.07	0.88±0.26

Table S2-*Continued d*

Pathways	Gene	mRNA expression values			
		0 ng/L	65 ng/L	550 ng/L	6500 ng/L
PI3K-Akt signaling pathway	*e4.1.1.32*	1.02±0.24	1.06±0.11	1.06±0.14	0.97±0.09
	gng13	1.01±0.18	0.97±0.39	0.84±0.11	0.84±0.12
	fgfr3	1.00±0.09	0.74±0.15	0.91±0.02	0.68±0.01 *
	lamb1	1.00±0.11	0.69±0.11	1.07±0.13	1.17±0.52
	gbl	1.00±0.06	0.87±0.17	0.76±0.07 *	0.58±0.07 *
	hsp90b	1.03±0.26	1.13±0.06	0.82±0.19	0.50±0.09 *
Viral carcinogenesis	*pk*	1.02±0.26	0.76±0.18	0.72±0.19	0.92±0.10
	mapkapk2	1.00±0.07	0.93±0.29	0.77±0.01 *	0.63±0.03 *
	h4	1.01±0.13	1.07±0.13	1.05±0.09	1.05±0.20
	hdac6	1.00±0.09	0.73±0.11	0.80±0.09	0.73±0.01 *
	dlg1	1.00±0.07	0.94±0.26	0.82±0.06	0.96±0.26
	hnrnpk	1.00±0.06	0.63±0.25	0.69±0.23	0.78±0.14
Epstein-Barr virus infection	*rpb8*	1.01±0.16	0.89±0.29	0.79±0.09	0.81±0.05
	psmd2	1.00±0.04	0.89±0.09	0.81±0.14	0.62±0.04 *
	psmd14	1.01±0.14	0.98±0.20	0.82±0.01	0.84±0.34
	psmc2	1.00±0.03	0.95±0.23	0.82±0.10	0.61±0.12 *
	csnk2a	1.01±0.14	0.91±0.23	0.79±0.05	0.59±0.10 *
	p38	1.00±0.09	1.06±0.21	0.86±0.07	0.90±0.29
Cardiac muscle contraction	*cyc1*	1.00±0.12	1.00±0.10	0.94±0.10	0.92±0.03
	atp1a	1.00±0.05	0.95±0.25	0.95±0.25	1.10±0.42
	cox2	1.03±0.26	1.29±0.29	0.93±0.06	1.12±0.37
	cox3	1.00±0.11	0.83±0.06	0.82±0.05	0.83±0.04
	cox5a	1.00±0.09	1.14±0.07	1.09±0.15	1.05±0.10
	myh6_7	1.07±0.48	0.54±0.17	1.09±0.28	2.02±0.73
Pyruvate metabolism	*e1.1.2.4*	1.00±0.06	0.89±0.42	0.73±0.12	0.82±0.17
	e2.3.1.9	1.01±0.16	1.17±0.22	0.88±0.03	0.84±0.02
	pk	1.02±0.26	0.76±0.18	0.72±0.19	0.92±0.10
	e4.1.1.32	1.02±0.24	1.06±0.11	1.06±0.14	0.97±0.09
	e2.4.1.2b	1.00±0.05	1.01±0.09	0.98±0.17	0.94±0.02
	aldh7a1	1.00±0.11	1.05±0.06	0.97±0.09	0.86±0.08
Platelet activation	*p38*	1.00±0.09	1.06±0.21	0.86±0.07	0.90±0.29
	itpr1	1.01±0.12	0.52±0.24	0.70±0.23	0.75±0.11
	actb_g1	1.02±0.26	0.58±0.14	1.02±0.12	1.33±0.28
	tln	1.01±0.17	0.71±0.15	0.91±0.16	1.12±0.31
	rap1b	1.02±0.23	1.56±0.96	0.97±0.13	0.97±0.39
Glucagon signaling pathway	*e2.4.1.1*	1.01±0.15	0.72±0.08	0.70±0.22	0.85±0.07
	pk	1.02±0.26	0.76±0.18	0.72±0.19	0.92±0.10
	e4.1.1.32	1.02±0.24	1.06±0.11	1.06±0.14	0.97±0.09
	itpr1	1.01±0.12	0.52±0.24	0.70±0.23	0.75±0.11
	cpt1	1.06±0.41	0.61±0.10	0.81±0.14	1.11±0.22
Proteasome	*psmb3*	1.01±0.15	1.00±0.24	0.88±0.08	0.66±0.14
	psmd2	1.00±0.04	0.89±0.09	0.81±0.14	0.62±0.04 *
	psmd14	1.01±0.14	0.98±0.20	0.82±0.01	0.84±0.34
	psmc2	1.00±0.03	0.95±0.23	0.82±0.10 *	0.61±0.12 *
	psme4	1.00±0.10	1.01±0.31	0.78±0.05 *	0.65±0.13 *
Amino sugar and nucleotide sugar metabolism	*gnpnat1*	1.05±0.36	1.60±0.29	1.08±0.03	0.91±0.12
	chs1	1.01±0.19	0.93±0.12	1.27±0.09	1.36±0.33
	gmpp	1.00±0.10	1.02±0.05	0.80±0.05 *	0.71±0.06 *
	uap1	1.02±0.23	1.01±0.07	0.95±0.08	0.80±0.21
	e3.2.1.14	1.01±0.20	0.83±0.09	0.96±0.15	0.90±0.03
PPAR signaling pathway	*acadl*	1.01±0.22	1.06±0.45	0.81±0.07	1.11±0.14
	e4.1.1.32	1.02±0.24	1.06±0.11	1.06±0.14	0.97±0.09
	fabp3	1.00±0.08	0.79±0.25	0.81±0.13	0.83±0.36
	cpt1	1.06±0.41	0.61±0.10	0.81±0.14	1.11±0.22
	ubc	1.01±0.19	1.04±0.23	0.89±0.19	0.99±0.27

Table S2-*Continued e*

Pathways	Gene	mRNA expression values			
		0 ng/L	65 ng/L	550 ng/L	6500 ng/L
Regulation of actin cytoskeleton	*fgfr3*	1.00±0.09	0.74±0.15	0.91±0.02	0.68±0.01 *
	actb_g1	1.02±0.26	0.58±0.14	1.02±0.12	1.33±0.28
	cyfip	1.00±0.06	0.97±0.30	0.86±0.13	0.71±0.00 *
	nckap1	1.01±0.12	0.90±0.21	0.75±0.02 *	0.52±0.17 *
	arpc4	1.01±0.17	1.41±0.67	0.97±0.20	0.9027±0.40
Thyroid hormone synthesis	*e1.11.1.9*	1.00±0.11	0.76±0.15	0.87±0.08	0.83±0.03
	atp1a	1.00±0.05	0.95±0.25	0.95±0.25	1.10±0.42
	itpr1	1.01±0.12	0.52±0.24 *	0.70±0.23	0.75±0.11
	lrp2	1.00±0.10	0.53±0.17 *	0.81±0.15	0.76±0.00 *
	hsp90b	1.03±0.26	1.13±0.06	0.82±0.19	0.50±0.09 *
Rap1 signaling pathway	*p38*	1.00±0.09	1.06±0.21	0.87±0.07	0.90±0.29
	fgfr3	1.00±0.09	0.74±0.15	0.91±0.02	0.68±0.01 *
	actb_g1	1.02±0.26	0.58±0.14	1.02±0.12	1.33±0.28
	tln	1.01±0.17	0.71±0.15	0.91±0.16	1.12±0.31
	rap1b	1.02±0.23	1.56±0.96	0.97±0.13	0.97±0.39
MAPK signaling pathway	*flna*	1.03±0.31	0.54±0.08	0.98±0.25	1.32±0.28
	p38	1.00±0.09	1.06±0.201	0.87±0.07	0.90±0.29
	mapkapk2	1.00±0.07	0.93±0.29	0.77±0.01 *	0.63±0.03 *
	fgfr3	1.00±0.09	0.74±0.15	0.91±0.02	0.68±0.01 *
	rap1b	1.02±0.23	1.57±0.96	0.97±0.13	0.97±0.39
FoxO signaling pathway	*e4.1.1.32*	1.02±0.24	1.06±0.11	1.06±0.14	0.97±0.09
	p38	1.00±0.09	1.06±0.21	0.86±0.07	0.90±0.29
	ccnb	1.01±0.17	1.06±0.32	0.90±0.11	0.86±0.11
	plk1	1.02±0.24	0.82±0.16	0.66±0.04	0.72±0.23
	csnk1e	1.00±0.05	0.62±0.12 *	0.63±0.07 *	0.52±0.03 *
Fatty acid metabolism	*Acadl*	1.01±0.22	1.06±0.45	0.81±0.07	1.11±0.14
	e2.3.1.9	1.01±0.16	1.17±0.22	0.88±0.033	0.84±0.02
	fabd	1.03±0.33	1.16±0.49	0.83±0.06	0.82±0.41
	cpt1	1.07±0.41	0.61±0.10	0.81±0.14	1.11±0.22
	hsd17b12	1.00±0.06	0.83±0.09	0.92±0.15	0.90±0.14
Focal adhesion	*flna*	1.03±0.31	0.54±0.08	0.98±0.25	1.32±0.28
	lamb1	1.00±0.11	0.69±0.11	1.07±0.13	1.17±0.52
	actb_g1	1.02±0.26	0.58±0.14	1.02±0.12	1.33±0.28
	tln	1.01±0.17	0.71±0.15	0.91±0.16	1.12±0.31
	rap1b	1.02±0.23	1.57±0.96	0.97±0.13	0.97±0.39

Values represent mean±SD (n=3). *$P < 0.05$.

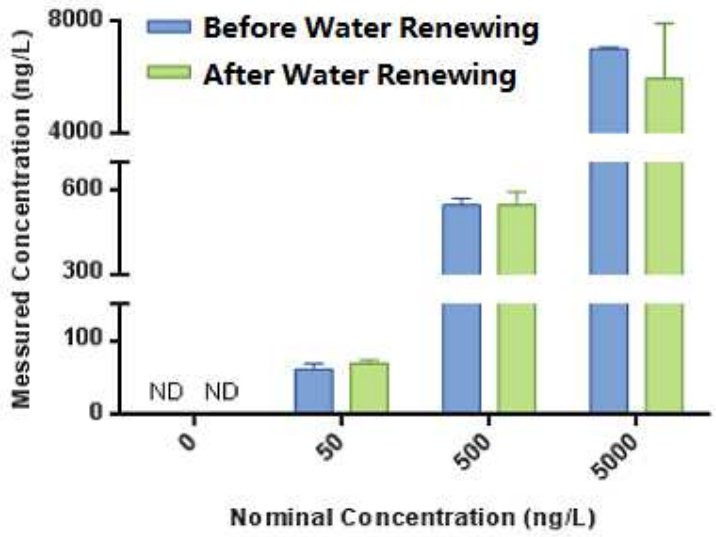

Figure S1: Measured concentrations of TDCIPP in exposure solutions at the last day of treatment. Value represent mean ± SD (n=3).

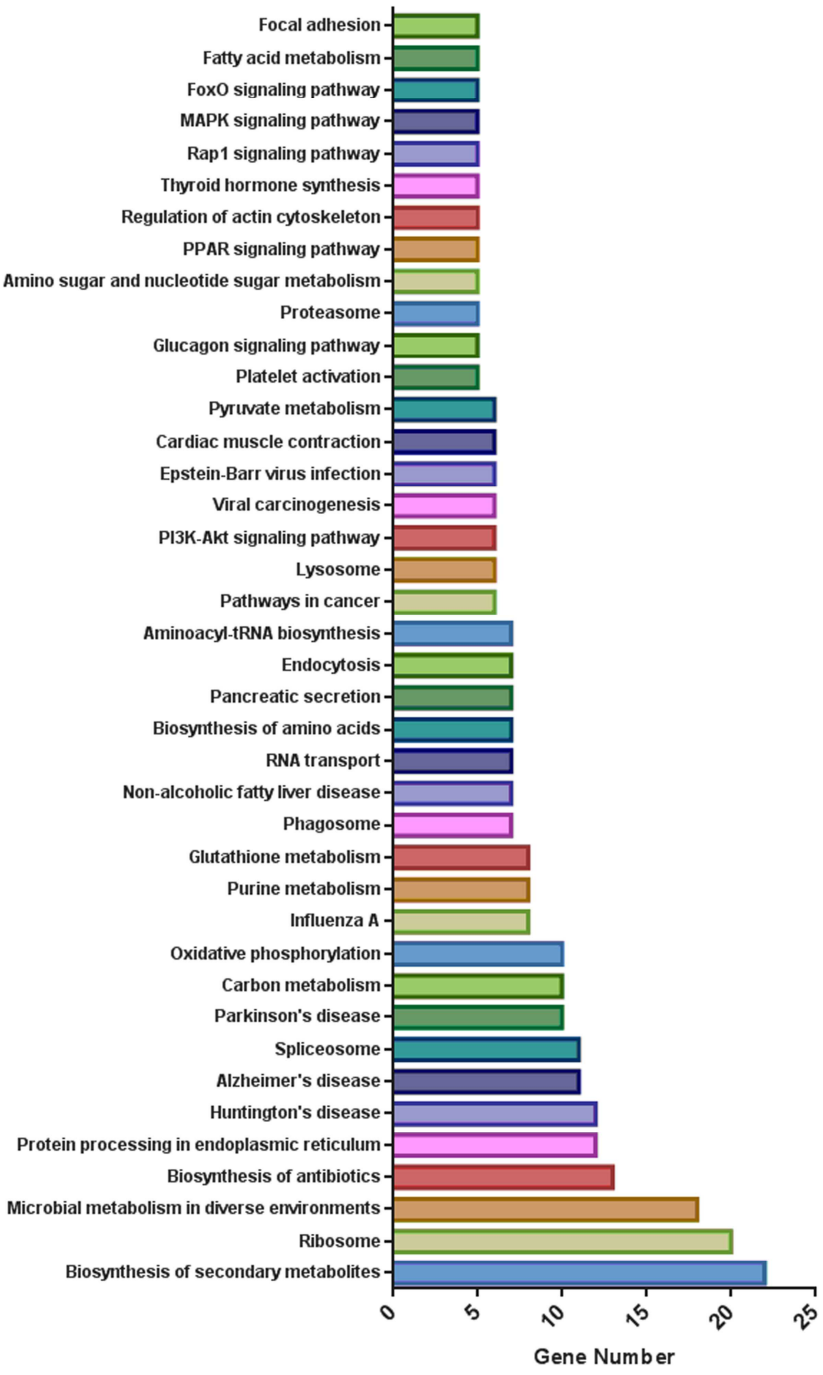

Figure S2: Gene number and the corresponding pathways in *D. magna* PCR arrays.