

Quantitative Real-Time PCR Analysis

We performed relative quantification of gene expression by fluorescent quantitative PCR (TIB8600, Triplex International Biosciences, China). Initially, qualified and quantified total RNA was reverse transcribed into cDNA using the PrimeScript™ 1st stand cDNA Synthesis Kit. The gene-specific qRT-PCR primers for *D. magna* mRNA quantification are presented in Table S1. The PCR reaction system consisted of 10 µL of 2 × SYBR real-time PCR premixture, 0.4 µL of LPCR-specific primer F (10 µM), 0.4 µL of PCR-specific primer (10 µM), 1 µL of cDNA and 8.2 µL of RNase free dH₂O per 20 µL. The reaction procedure was conducted in 3 replicates under the following conditions: first thermal denaturation at 95 °C for 5 min, after which 40 cycles were performed following the steps 95 °C for 15 s, 60 °C for 30 s. In addition, melting curve analysis was performed on all samples at the end of every run.

Table S1. Primers utilised for quantitative polymerase chain reaction (qPCR) validation of gene expression.

| Gene ID | Gene | Forward Primers | Reverse Primers |
|--------------|-------------|----------------------|----------------------|
| LOC116931413 | <i>NPC2</i> | CGTGTCTGAGCCCATTACCA | CCAGATTGCCTTTGCGTTCA |
| LOC116927431 | <i>abat</i> | ACACCTGGATGGGTGATCCT | GCTTCAAAAGGTTGTGCGCT |

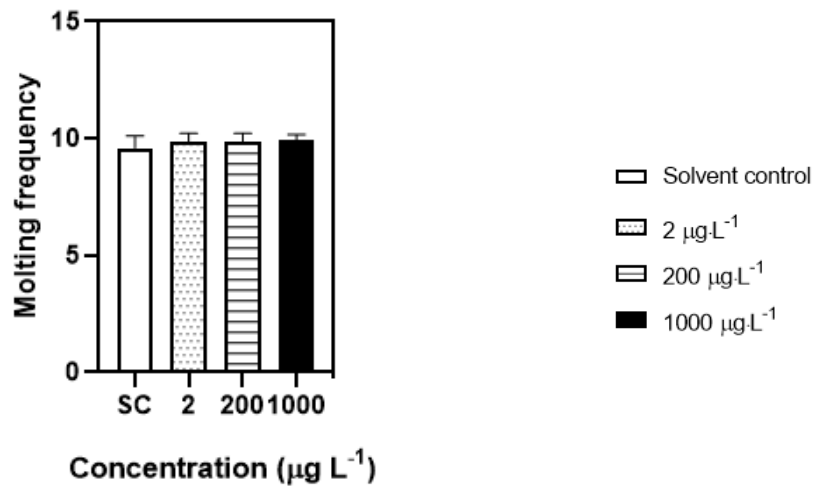


Figure S1. Effects of DES exposure to *D. magna* in molting frequency. ($p < 0.05$; $n = 20$).

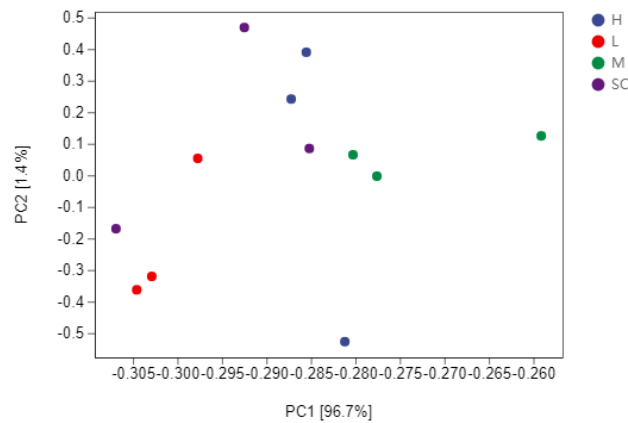


Figure S2. Principal component analysis (PCA) of DEGs of *D. magna* exposed to DES for 9d in transcriptomic analysis.

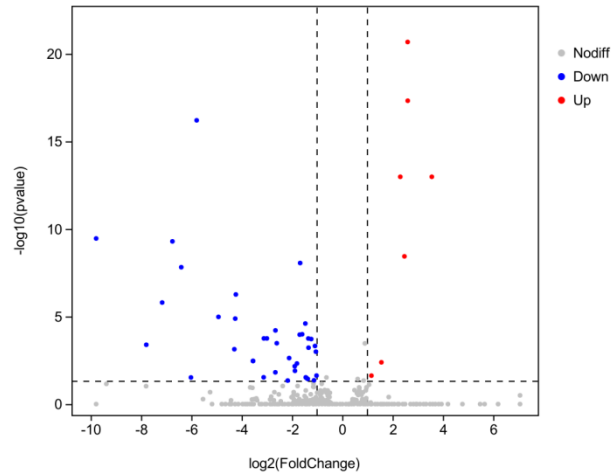


Figure S3. Gene expression profiles of *D. magna* after 9 d exposure to DES at 2 µg L⁻¹ volcano plot compared with solvent control groups.

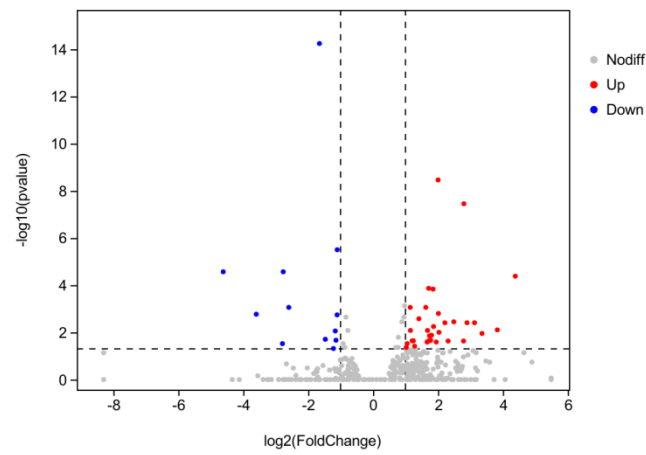


Figure S4. Gene expression profiles of *D. magna* after 9 d exposure to DES at 200 µg L⁻¹ volcano plot compared with solvent control groups.

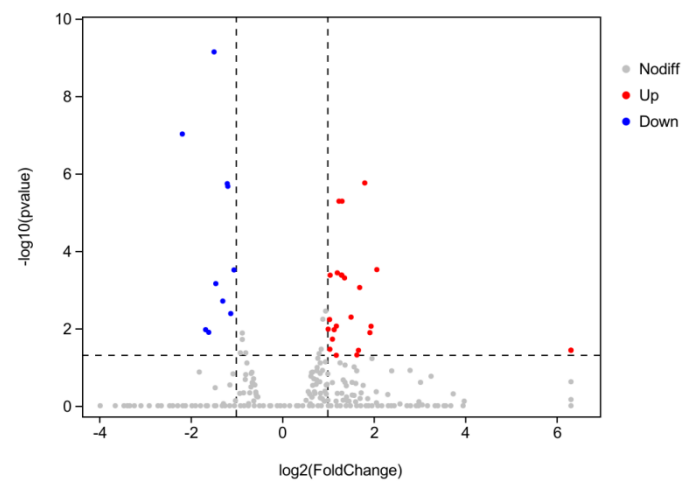


Figure S5. Gene expression profiles of *D. magna* after 9 d exposure to DES at 1000 µg L⁻¹ volcano plot compared with solvent control groups.

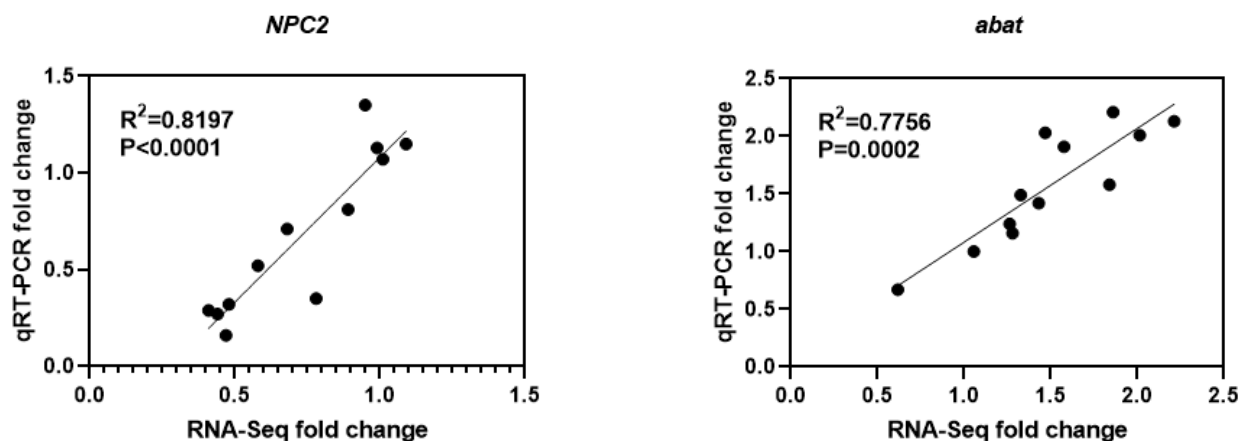


Figure S6. qRT-PCR and RNA-seq correlation analysis results diagram of selected genes. *NPC2*: Niemann-Pick C2 protein; *abat*: 4-aminobutyrate aminotransferase.

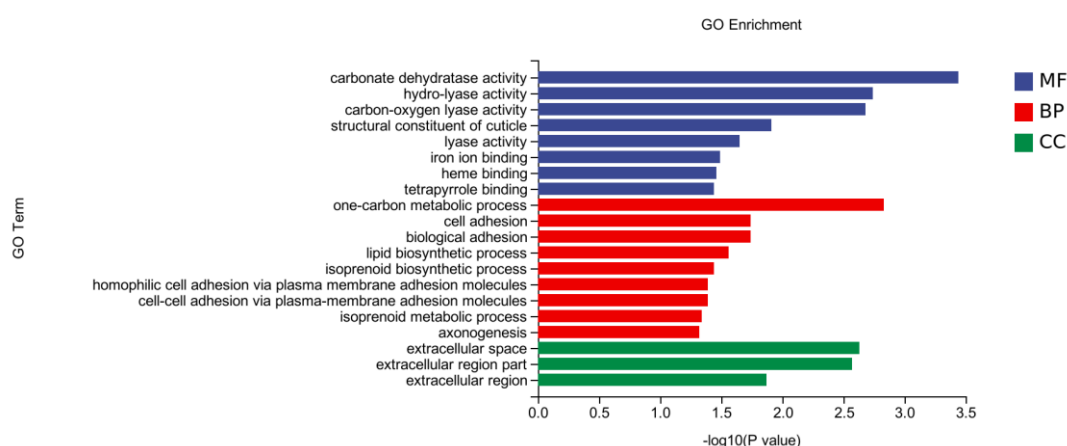


Figure S7. After a 9-d of exposure to different concentrations of DES, the top 20 enrichment terms for each category were significantly higher in the $2 \mu\text{g L}^{-1}$ treatment group than in the solvent control.

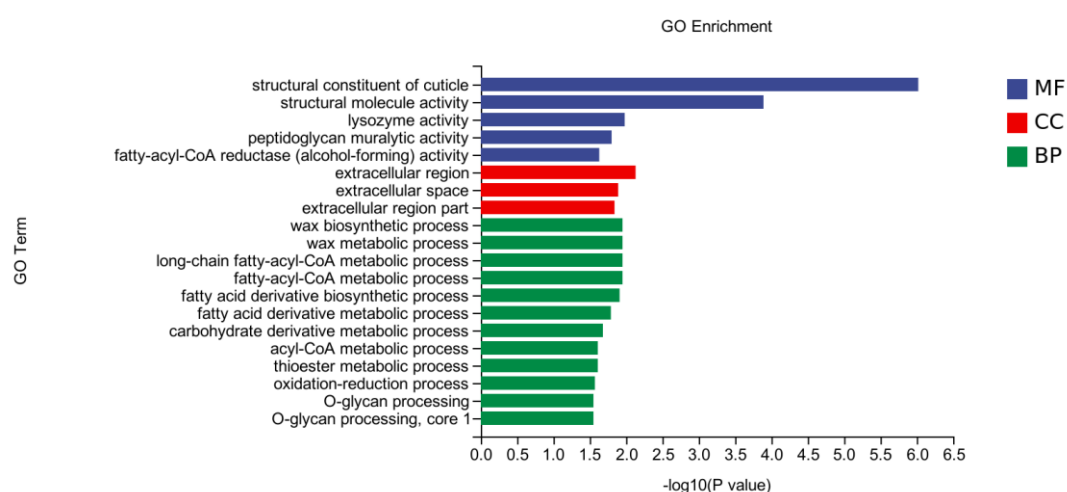


Figure S8. After a 9-d of exposure to different concentrations of DES, the top 20 enrichment terms for each category were significantly higher in the $200 \mu\text{g L}^{-1}$ treatment group than in the solvent control.

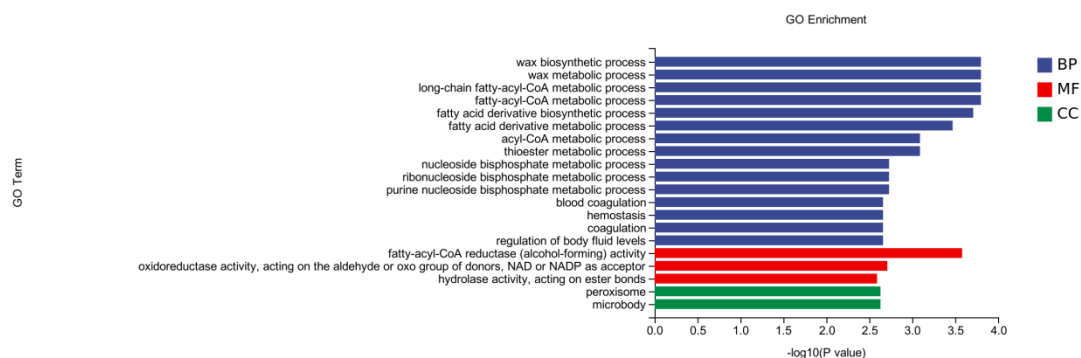


Figure S9. After a 9-d of exposure to different concentrations of DES, the top 20 enrichment terms for each category were significantly higher in the 200 $\mu\text{g L}^{-1}$ treatment group than in the solvent control.

Table S2. List of fundamental pathways affected by a 9-day exposure to DES ($p < 0.05$).

| Pathway ID | Pathway | Category | Up-Gene | Down-Gene | <i>p</i> -Value | FDR |
|------------|--------------------------------------|--|---------------|---------------------|-----------------------|-----|
| SC vs L | | | | | | |
| ko00910 | Nitrogen metabolism | Energy metabolism | - | <i>ca</i> | 0.34×10^{-3} | |
| ko00900 | Terpenoid backbone biosynthesis | Metabolism of terpenoids and polyketides | - | <i>pdss1</i> | 0.036 | |
| ko00100 | Steroid biosynthesis | Lipid metabolism | - | <i>meso1, erg25</i> | 0.041 | |
| ko04612 | Antigen processing and presentation | Immune system | - | <i>ctsb</i> | 0.042 | |
| SC vs M | | | | | | |
| ko00073 | Cutin, suberine and wax biosynthesis | Lipid metabolism | <i>far</i> | - | 0.012 | |
| ko04142 | Lysosome | Transport and catabolism | <i>hgsnat</i> | <i>NPC</i> | 0.014 | |
| ko00531 | Glycosaminoglycan degradation | Glycan biosynthesis and metabolism | <i>hgsnat</i> | - | 0.016 | |
| ko04979 | Cholesterol metabolism | Digestive system | - | <i>NPC2</i> | 0.043 | |
| SC vs H | | | | | | |
| ko04979 | Cholesterol metabolism | Digestive system | <i>LAL</i> | <i>NPC1, NPC2</i> | 3.07×10^{-5} | |
| ko00073 | Cutin, suberine and wax biosynthesis | Lipid metabolism | <i>far</i> | - | 0.13×10^{-3} | |
| ko04142 | Lysosome | Transport and catabolism | <i>lipa</i> | <i>NPC1, NPC2</i> | 0.002 | |
| ko04212 | Longevity regulating pathway - worm | Aging | <i>far</i> | - | 0.005 | |

| | | | | | |
|---------|---|--------------------------|-----------------------|---|-------|
| ko04146 | Peroxisome | Transport and catabolism | <i>far</i> | - | 0.014 |
| ko04974 | Protein digestion and absorption | Digestive system | <i>prss, collagen</i> | - | 0.018 |
| ko00250 | Alanine, aspartate and glutamate metabolism | Amino acid metabolism | <i>abat</i> | - | 0.049 |