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Supplemental Material

Integrated Studies on Male Reproductive Toxicity of Decabromodiphenyl Ethane in Zebrafish Spermatozoa *Ex Vivo*, Male Zebrafish *in Vivo*, and GC-1 Cells *in Vitro*

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Table of Contents

Figure S3. Effects of DBDPE in vivo exposure on different parameters of reproductive behavior in male zebrafish. (A) Cumulative mating duration. (B) Mating rate. (C) Mating frequency. (D) Cumulative body contact duration. (E) Body contact rate. (F) Body contact frequency. n = 19-21. Data represented as the means ± standard error of the mean (SEM). No statistical significance was observed in all the DBDPE-exposed group compared to the control, by one-way analysis of variance (ANOVA) followed by the post hoc least significant difference (LSD) test. Data are reported in Excel Table S12.

Figure S4. Area ratio of interstitial space/whole testicular section in male zebrafish testes after 2-month *in vivo* exposure to DBDPE. Data represented as the means ± standard error of the mean (SEM), n = 7-9. No statistical significance was observed in all the DBDPE-exposed group compared to the control, by one-way analysis of variance (ANOVA) followed by the post hoc least significant difference (LSD) test. P = 0.843, 0.149, 0.056, 0.116 in 0.1, 1, 10,100 nM DBDPE exposure group, respectively. See Excel Table S13 for summary data.

Figure S5. Whole proteome and phosphoproteome male in male zebrafish testes after exposure to 100 nM DBDPE. (A) Distribution of the phosphorylated serine, threonine, and tyrosine residues among the identified phosphorylation sites in the phosphoproteome. (B) Numbers and percentages of up-regulated and down-regulated proteins, and those that did not significantly differ in 100 nM DBDPE treated zebrafish testes relative to the control. (C) Numbers and percentages of up-regulated and down-regulated phospho-protiens and those that did not significantly differ in 100 nM DBDPE treated zebrafish testes relative to the control. (D) GO enrichment analysis of DEPs. (E) GO enrichment analysis of proteins harboring differentially phosphorylated sites. See Excel Table S14 for summary data.

Figure S6. The relative mRNA expression levels of target genes in testes of zebrafish exposed to lower concentrations of DBDPE (0, 0.1, 1, 10 nM) for two months (n =3 in each group). Data were represented as the means ± standard error of the mean (SEM). *P < 0.05, **P < 0.01 and ***P < 0.01indicate significant differences between the exposure groups and the control group by one-way analysis of variance (ANOVA) followed by the post hoc least significant difference (LSD) test. Red and blue colors indicate upregulation and downregulation of target genes in exposed zebrafish testes relative to control, repectively. Color intensity is proportional to $\log_2$(fold change). The values in each box indicate $\log_2$(fold change) of mRNA expression levels of target genes relative to control. See Excel Table S15 for summary data.

Figure S7. Primary investigation on the effects of DBDPE on MMP and ATP contents in GC-1 cells by setting a series of concentrations *in vitro*. (A) Mitochondrial membrane potential (MMP) of GC-1 cells following exposure to 0.01-10 μM DBDPE for 48 h (n = 4-5). (B) ATP contents of GC-1 cells following exposure to 0.01-10 μM DBDPE for 48 h (n = 3). Data were represented as the means ± standard error of the mean (SEM). *P < 0.05 indicates significant differences between the exposure groups and the control group. Data are reported in Excel Table S10.

Figure S8. Correlation analysis of the investigated parameters in GC-1 cells between the 1 uM DBDPE exposure group and the oxamate exposure group. For most of the investigated parameters were significantly changed in 1 μM DBDPE exposure group and oxamate exposure group, with similar change trends observed. A pearson correlation analysis were conducted to further figure out their changing correlationship. *"R"* and *"P"* indicate the correlation coefficient and the significant difference, respectively; their values are shown in the figure. Red and blue colour represent positive and negative correlation, respectively.

Table S1. The measured sperm motility parameters and accompanying definitions in this study.

Table S2. CASA input parameters for zebrafish.

Table S3. Detailed informations of primers for qRT-PCR used in the present study.

Table S4. DBDPE contents detected in zebrafish testes.

Table S5. Basic developmental parameters of exposed male fish and F1 offspring larvae.

Additional File- Excel Document