Supplemental Information

Gene expression of fathead minnows (Pimephales promelas) exposed to two types of treated municipal wastewater effluents

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Tissue Sampling

At the end of the exposures, fish were anesthetized with a solution of MS-222 (0.2 g/L; Sigma-Aldrich, San Louis, MO), weighed, and measured for standard length. When anesthetized, the fish stopped moving and did not respond to gentle touch. Blood samples were taken with a heparinized syringe from the caudal vein or with hematocrit tubes; the blood was then centrifuged for plasma collection. Subsequently, the fish were humanely sacrificed using cervical dislocation. Fish handling and sacrificing procedures were conducted in accordance to institutional operating procedures, these methods have been previously published (Vidal-Dorsch et al. 2011b). The livers were removed post-mortem, weighed, and thin slices were cut and preserved in RNA later (Qiagen, Valencia, CA) for gene expression analysis. Then, the right side gonad was removed and weighed to determine the ½ GSI value. GSI data are reported as ½ GSI.

Biological Indicators

Observations were made to determine if changes in secondary sexual characteristics had occurred as a result of exposure. The appearance of the dorsal nape pad, nuptial tubercles, and the presence of ovipositors in males (an indication of male feminization and adverse reproductive system effects), were inspected following the methods of Miles-Richardson (Miles-Richardson et al. 1999). Plasma VTG was quantified using a homologous enzyme-linked immunosorbent assay (ELISA) (Eidem et al. 2006). Plasma VTG samples were analyzed in triplicate and read at a wavelength of 450 nm using a microplate reader (Multiskan Ascent 354, MTX Lab Systems, Vienna, VA). General fish condition was also evaluated using the liver somatic index (LSI was calculated as weight of liver/weight of body x 100). The gonadal

somatic index (GSI was calculated as [wet weight of one-half gonad/ wet weight body x 100]) was used to further investigate potential effluent effects on reproductive processes.

Gene Expression

Fathead minnow oligonucleotide microarrays used in this study were designed by the Denslow laboratory and purchased from Agilent (Garcia-Reyero et al. 2009). For microarray assays, the fish RNA samples were isolated from livers. Microarray hybridizations were performed according to the Agilent protocol titled "One-color microarrays-based gene expression analysis" (document no. G4140-90040 V6.5) using Cyanine 3 (Cy3) (Agilent, Palo Alto, CA). One µg of total RNA per sample (n= 4 -10 biological replicates for each fish) was used for the synthesis of cDNA, which was subsequently labeled/amplified into cRNA as per the Agilent - Quick-Amplification Amplification Kit. A one-color spike mix was prepared. Amplified cRNA containing the incorporated Cy3 label was purified using the Qiagen1RNeasy mini-spin columns (Qiagen, Valencia, CA). After purification, samples were measured for yield, and evaluated for incorporation of Cy3 dye using the NanoDrop ND-1000. Samples with a specific activity > 8.2 pmol Cy3/ml were used on the arrays. Fragmentation of the cRNA was performed in the recommended blocking agent with a volume of 2x GE Hybridization Buffer (Agilent, Gene Expression Hybridization Kit), and the reaction proceeded for 30 min at 60 ºC. A final volume of 40 µl containing fragmented cRNA was added to the 8 x 15 K microarrays, and then hybridization proceeded for 17 h at 65 ºC. Microarrays were washed the following day in GE Wash Buffer 1 at room temperature for 1 min, followed by GE Wash Buffer 2 at 37 ºC for 1 min, then with Stabilizing and Drying Solution wash at room temperature for 30 s.

Microarrays were kept in the dark until scanning on an Agilent G2505B microarray scanner. Data extraction was performed using Agilent Feature Extraction software (V9.5) and used a composite of two scans, one at 10 and the other at 100 photomultiplier tube (PMT) to extend the dynamic range (Agilent G2505 B Microarray Scanner). Raw expression data (gProcessedSignal) were imported into JMP Genomics V5 (SAS, Cary, NC). During import, data were log2-transformed, and all control and non-uniform spots were removed prior to the final analysis. Raw intensity data for each microarray were normalized by median centering before performing a one-way analysis of variance (ANOVA) to identify the genes that were significantly affected with $p < 0.05$ and fold change greater than ± 1.5.

Estrogen receptor alpha (*ESR1*), Cytochrome P450 1A, f-(*CYP1A*), Retinol binding Protein (*RBP*), and 18S ribosomal (*18S*) were quantified by qRT-PCR. The qRT-PCR primer sets for FHM VTG and *CYP1A* were previously reported (Garcia-Reyero et al. 2011); similarly, primer sets for *18S* were also previously reported (Filby and Tyler 2005). The primer for *RBP* were designed using Primer3 (Rozen and Skaletsky 2000). 500 ng of total RNA sample was DNase-treated using the TURBO DNA-free kit (Ambion), and first-strand cDNA was synthesized from 500 ng of DNAse-treated RNA using 250 ng of random primers and SuperScript™ II Reverse Transcriptase (Invitrogen) in 20µL reaction according to the manufacture's protocol. qRT-PCR analysis was carried out using 5µL of synthesized cDNA template diluted to 10 ng/L, 1 µl of each gene specific primer (10 - µM), and 12.5 µL 1x iQ SYBR Green Supermix (Bio- Rad, Hercules, CA). The two-step thermal cycling parameters were as follows: initial 1-cycle Taq activation at 95 °C for 3 min, followed by 40 cycles at 95 °C for 15 s and 58 °C for 1 min. After 40 cycles, a dissociation curve was produced starting at 55 °C (+1 °C/30 s) to 95 °C. Transcripts were assayed on an iCycler Thermal Cycler (Bio-Rad).

18S ribosomal RNA was used as the reference gene to normalize expression data. The results were analyzed using the ΔΔCt method of relative quantification (Livak and Schmittgen 2001). Standards and experimental samples were run in duplicate, along with two negative controls for each gene. A ‘‘no reverse transcriptase (-RT)’’ control was used. In this control, DNAse-treated RNA samples were pooled (n=10) and water was used in place of reverse transcriptase during the reverse transcription reaction, and a ‘‘no template control (NTC).’’ Water was used in place of template cDNA during the qPCR reaction. Melting curves for each gene indicated that a single product was formed. Significant differences ($p \leq 0.5$) between controls of exposure to POTW effluents for each gene were determined by Turkey's test with JMP software (Version 5.1.2).

Effluents and Dilution Water

Laboratory experiments were conducted with effluents from Point Loma Wastewater Treatment Plant (PL) in San Diego, CA, and the Hyperion Treatment Plant (HTP) in Playa Del Rey, CA. The effluent from PL underwent primary treatment before testing. During the treatment process the PL influent was screened and aerated, followed by chemically-assisted sedimentation for particulate removal (using ferric chloride as the primary coagulant). The HTP influent also underwent sedimentation and aeration in oxygen reactors, but was further treated with activated sludge in clarifier basins (Vidal-Dorsch et al. 2010). Effluent was collected by each treatment plant. The effluent samples represented 24 hour composites.

Treatment Controls and Effluent CEC Measurements

At the beginning and end of each experiment, aliquots of control and test solutions were taken for CEC analysis. Similarly, whole effluent samples were also collected at the beginning and after exposure days five and 10 (analytes and detection limits shown in Table SI-1). All samples were analyzed by the Southern Nevada Water Authority (Las Vegas, NV) using liquid chromatography/ tandem mass spectrometry (LC-MS/MS) and gas chromatography/ tandem mass spectrometry (GC-MS/MS). Full descriptions of the methods used for sample collection and analyses have been previously published (Trenholm et al. 2006, Vanderford and Snyder 2006, Vidal-Dorsch et al. 2011a). Quality control measures consisted of procedural blanks, duplicate analyses, and spike analyses. Laboratory spikes and blanks were analyzed with every set of 10 composites. Standard surrogates were used to determine analyte loss during processing. Isotopically-labeled standards were available for a variety of compound classes; recoveries ranged from 80 to 120%. Some blanks were contaminated with ibuprofen (0.001 µg/L), nonylphenol (0.8 µg/L) or estradiol (0.0006 µg/L). When blank contamination was found the resulting value was adjusted by subtracting the concentration found in the blanks or/and controls.

Preparation of Positive Controls and Effluent Treatments

As previously mentioned, diluted treated effluent from two POTWs, 17-β estradiol (E2) positive controls and negative water-only controls were used in the exposures. A 4 µg/L E2 positive control (nominal concentration) was used. To prepare the positive E2 control, an initial stock solution was made by dissolving 1.2 mg of E2 powder (Fisher Scientific Pittsburgh, PA, USA) in HPLC grade acetone (Fisher Scientific), in a 4 L glass beaker. Once the acetone evaporated at room temperature (after 1 hour), the originally added E2 was plated onto the

beaker surface. Then, 3 L of moderately hard water were added to the beaker to create a second stock solution which was mixed for 2 hours at room temperature. The second stock was transferred to a 20 L carboy and mixed for 5 min. with 12 L of moderately hard water to create a final E2 stock solution. A final E2 solution of 4 μg/L was manually delivered to test tanks where it was mixed with moderately hard water to achieve the target concentration. The E2 concentrations measured in the tanks using a magnetic particle ELISA kit from Abraxis Bio Science (NJ, USA) (Buehler et al. 2009) averaged 5.3 ± 3.2 μg/L (± standard deviation).

Effluents for each exposure were tested at a nominal concentration of 5%. To prepare the 5% effluent stock solution, 1.5 L of raw final effluent and 13.5 L of moderately hard water were mixed for 5 minutes in a carboy. Afterwards, 5 L of this 10% effluent solution (PL or HTP) was manually delivered to each test tank while simultaneously adding an equal volume of moderately hard water to achieve 10 L of 5% solution in each tank.

Biological Measurements: Results for the GSI and LSI Analysis

Average LSI values were not significantly different ($p > 0.05$) among fish exposed to both effluents and their respective negative (water only) controls (Table SI-3). Average LSI values were significantly higher ($p < 0.05$) in fish exposed to positive controls (E2). Average LSI values in negative controls were 0.02, compared to 0.1 in PL E2-exposed fish or 0.03 in HTP E2-exposed fish. Average ½ GSI values were not significantly different ($p >0.05$) between positive and negative controls and effluent-exposed fish during both experiments.

Statistical Pathway Analysis

We used the enrichment analysis algorithm in PathwayStudio™ which ranks specific genes that belong to pre-designated gene sets within a gene list (control or effluent exposed). Fisher's Exact Test was used to establish the statistical significance for each group of genes (Poirel et al., 2011).

The gene sets are generated by Pathway Studio using MedScan to scan the PubMed database with over 20 million abstracts regarding direct and indirect regulation and binding between entities including promoters(Yuryev et al. 2006), RNA, proteins, small molecules, cell processes and disease. In this work, FHM genes were annotated via BLAST as previously described (Weil et al. 2012)and genes with good annotation (E values < 10^{-4} against the nr database) were converted to human homologs using the RefSeq database and the GeneID database. Pathways/groups and sub-networks enriched with the differentially regulated genes (p-value <0.05; limited to the top 100 gene sets) were used. In order to reduce the analysis complexity, we focused on transcripts whose products had direct interactions with other entities. In this paper we only show the most highly enriched pathways.

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Table SI-1. Mean concentration and standard deviation (Std Dev) of contaminants of emerging concern present in the 5% effluent exposures, values are shown in µg/L. Two samples were used to calculate the means.

Chemical Name	RL	PL		HTP	
		Mean	Std Dev	Mean	Std Dev
Pharmaceuticals and Personal Care Products					
Triclosan	0.001	0.145	0.021	0.72	0.966
Sulfamethoxazole	0.0003	ND	-	0.046	0.001
Trimethoprim	0.0003	0.044	0.004	0.034	0.001
Fluoxetine	0.0005	ND	-	0.002	0
Meprobamate	0.0003	0.035	0.001	0.017	0.001
Carbamazepine	0.0005	0.041	0.031	0.016	0.003
Dilantin	0.0011	ND	-	0.004	0.005
Primidone	0.0005	ND	-	0.003	0.004
Diclofenac	0.0005	ND	-	0.006	0.007
Ibuprofen	0.001	1.149	0.071	0.001	0
Naproxen	0.0005	0.775	0.05	0.003	0.004
Atenolol	0.0011	0.195	0.035	0.067	0.002
Atorvastatin	0.0005	0.066	0.077	0.003	0.004
Musk Ketone	0.025	ND	-	ND	-
Gemfibrozil	0.0003	0.248	0.026	0.145	0.007
Caffeine	0.0053	3.8	1.131	0.028	0.031
Diazepam	0.0003	ND	-	ND	-
Iopromide	0.011	ND	-	0.026	0.03
DEET	0.0011	0.217	0.26	0.036	0.007
Current Use Pesticides					
Atrazine	0.0003	ND	-	ND	-
Hormones					
Estradiol	0.0005	ND	-	ND	-
Estrone	0.0002	0.003	0.001	ND	-
Progesterone	0.0005	0.001	0.001	ND	-
Testosterone	0.0005	0.004	0.001	ND	-
Ethinylestradiol	0.001	ND	-	ND	-
Industrial and Commercial Compounds					
Tris(2-chloroethyl) phosphate (TCEP)	0.011	ND	-	0.012	0.01
Tris(1-chloro-2-propyl) phosphate (TCPP)	0.11	ND	-	ND	-
Benzophenone	0.053	ND	-	0.325	0.248
Bisphenol A	0.005	ND	-	0.9	0.683
Nonylphenol	0.05	2.217	0.109	0.625	0.304
BHA	0.001	ND	-	0.008	0.003
Octylphenol	0.025	ND	-	ND	-

ND= Not detected.

Table SI-2. Sequences of primers used in the qRT-PCR analysis.

Gene	Forward primer 5'→3'	Reverse primer 5'→3'	Amplicon size (bp)
VTG	GCTGCTGCTCCATTTCAAAAG	GTGAGAGTGCACCTCAACGC	81
ESR1	GACAGAAACCGCAGGAAGAG	CACCCAGAAGCAACACCA	274
CYP1A	TGCAGGGAGAACTGAGAGAGAAG	TCCGTTCGGTCCGACAAG	64
RBP	TTTTGCCACCCGAAAGATAG	TTTGTCGTTGTCCCAGTTGA	199
18S	AATGTCTGCCCTATCAACTTTC	TGGATGTGGTAGCCGTTTC	117

Table SI-3. Genes differentially expressed in male fathead minnow livers after exposure to advanced primary treated or secondary treated effluent for selected biological processes.

Probe Name	NR Accession	NR E-Value	Gene Title	PL		HTP	
				p-value	Fold Change	p-value	Fold Change
Detoxification and Xenobiotic Metabolism							
UF_Ppr_AF_105382	XP_692555	0	Similar To Cytochrome P450, Family 2, Subfamily J, Polypeptide 2 /	1.7E-05	11.34	0.030	3.66
UF_Ppr_AF_113831	CAH69009	0	Cytochrome P450, Family 2, Subfamily J, Polypeptide 30 /	0.007	4.93	0.456	NS
UF_Ppr_AF_103590	XP_001331472	0	Cytochrome P450, Family 4 / Hypothetical Protein LOC791965 /	0.011	3.83	0.876	NS
UF_Ppr_AF_114713	CAO90905	3.95	Cytochrome P450, Family 26, Subfamily B, Polypeptide 1 /	0.002	2.68	0.820	NS
UF_Ppr_AF_102393	NP_001018358	0	Cytochrome P450, Family 46, Subfamily A, Polypeptide 1 /	0.007	2.15	0.882	NS
UF_Ppr_AF_111445	ABF60890	0	Cytochrome P450, Family 1, Subfamily A /	0.095	NS	0.033	2.40
UF_Ppr_AM_119570	XP_001343466	1.02E-25	Cytochrome P450, Family 2, Subfamily B, Polypeptide 6 /	0.452	NS	0.038	2.08
UF_Ppr_AF_112957	NP_001001589	0	Ubiquinol-cytochrome C Reductase Core Protein II /	0.464	NS	0.029	-1.61
UF_Ppr_AF_112901	AAR87722	0	Cytochrome P450, Family 1, Subfamily B, Polypeptide 1 /	0.890	NS	0.004	-2.25
UF_Ppr_AF_108107	NP_861447	8.64E-25	Nuclear Receptor Coactivator 7 /	0.001	1.87	6.4E-05	-2.45
UF_Ppr_AF_114632	ABV29341	1.12E-44	Nuclear Receptor Subfamily 1, Group I, Member 2 /	0.045	1.56	0.072	NS
UF_Ppr_AF_110607	AAK76396	0	Nuclear Receptor Subfamily 1, Group D, Member 2 /	0.502	NS	0.028	-1.75
UF_Ppr_AF_112153	NP_001038534	0	Similar To Nuclear Receptor Coactivator 5 /	5.E-4	-1.65	0.581	NS
UF_Ppr_AF_103223	NP_571652	0	Aryl Hydrocarbon Receptor Nuclear Translocator-like /	0.002	1.67	0.005	1.69

Table SI-3 (continued)

Probe Name	NR Accession	NR E-Value	Gene Title	PL		HTP	
				p-value	Fold Change	p-value	Fold Change
			Fatty Acid/ Lipid Metabolism/ Metabolism Processes				
UF_Ppr_AF_109792	XP_001331487	0	ELOVL Family Member 7, Elongation Of Long Chain Fatty Acids /	0.074	NS	0.017	3.95
UF_Ppr_AF_114983	AAF75683	0	Similar To Glucose Transporter 1A /	0.773	NS	0.042	1.61
UF_Ppr_AF_116320	XP_687066	0	UDP-glucose Dehydrogenase /	0.593	NS	0.009	1.60
UF_Ppr_AF_112026	AAG25711	0	Fatty Acid Desaturase 2 /	0.002	3.53	0.657	NS
UF_Ppr_AF_108360	NP_954682	2.87E-23	Serum/glucocorticoid Regulated Kinase 1 /	0.002	-2.92	0.040	2.14
UF_Ppr_AF_114197	ABC49921	0	Similar To Glucose-6-phosphatase /	0.121	NS	0.050	-2.32
UF_Ppr_AF_109012	XP_687387	0	Similar To Fatty Acid Synthase / Hypothetical Protein LOC795741 / Lethal Giant Larvae Homolog 2 / Hypothetical LOC559001 /	3.E-4	-5.30	0.002	-5.18
UF_Ppr_AF_110271	XP_699030	0	Aldehyde Oxidase 1 /	0.012	3.47	0.283	NS
UF_Ppr_AF_100038	AAK97853	0	Alcohol Dehydrogenase 8a / Protein Tyrosine Phosphatase, Receptor Type, A /	0.022	2.60	0.246	NS
UF_Ppr_AF_109357	AAH66668	0	Aldehyde Dehydrogenase 9, Subfamily A1 /	0.006	2.20	0.807	NS
UF_Ppr_AF_119011	AAH66615	0	TDP-glucose 4,6-dehydratase /	0.035	-1.57	0.683	NS
UF_Ppr_AF_112390	NP_998297	0	Fructose-1,6-bisphosphatase 1, Like / Fructose-1,6-bisphosphatase 1 /	0.007	2.48	0.638	NS
UF_Ppr_AF_113741	NP_001005927	0	Oxysterol Binding Protein-like 6 /	0.005	9.79	0.885	NS
UF_Ppr_AF_103513	XP_001341604	0	Calbindin-28K /	0.015	6.12	0.517	NS
UF_Ppr_AF_114192	AAI52079	0	Malic Enzyme 3, NADP(+)-dependent, Mitochondrial /	0.011	3.85	0.146	NS
UF_Ppr_AF_111324	XP_688483	0	Carnitine Palmitoyl transferase 1b, Muscle /	0.004	2.71	0.531	NS
UF_Ppr_AF_103926	XP_693755	0	Similar To Cytosolic Phospholipase A2 Zeta /	0.002	2.71	0.001	-3.47
UF_Ppr_AF_118045	XP_686831	0	UDP Glucuronosyl transferase 1 Family A, A /	0.003	1.92	0.335	NS
UF_Ppr_AF_118394	AAF73186	0	Glutamic Acid Decarboxylase 2 /	0.029	1.91	0.003	-2.95
UF_Ppr_AF_103350	CAM15108	0	Acyl-Coenzyme A Dehydrogenase, C-4 To C-12 Straight Chain /	0.001	1.89	0.107	NS
UF_Ppr_AF_118052	AAI54255	0	3-oxoacid CoA Transferase 1a /	0.046	1.84	0.757	NS
UF_Ppr_AF_111177	NP_997761	0	Adenylate Kinase 2 /	0.035	1.84	0.064	NS

Table SI-3 (continued)

Probe Name	NR Accession	NR E-Value	Gene Title	PL p-value	PL Fold Change	HTP p-value	HTP Fold Change
UF_Ppr_AF_100211	CAG08316	0	ATPase, Aminophospholipid Transporter, Class I, Type 8A, Member 1 /	0.048	1.80	0.022	-2.25
UF_Ppr_AF_109510	AAI54787	6.67E-10	Lipase, Gastric /	0.028	1.78	0.514	NS
UF_Ppr_AF_103889	AAI54787	0	Lipase A, Lysosomal Acid, Cholesterol Esterase /	0.020	1.78	0.587	NS
UF_Ppr_AF_108816	P46020	0	Phosphorylase Kinase, Alpha 2 /	0.004	1.77	0.228	NS
UF_Ppr_AF_118060	XP_001341627	0	Similar To Carkl Protein /	0.016	1.77	0.746	NS
UF_Ppr_AM_120293	CAG08217	7.14E-38	Mannosyl-oligosaccharide Alpha-1,2-mannosidase /	0.023	1.74	0.034	-1.83
UF_Ppr_AF_100631	BAE79606	0.596713	Phosphatidic Acid Phosphatase Type 2B /	0.004	1.72	0.023	1.64
			Endocrine Responses				
UF_Ppr_AF_116092	NP_694549	0	Retinol Binding Protein 2a, Cellular /	0.001	16.22	0.343	NS
UF_Ppr_AF_112129	CAG01984	0	Retinol Binding Protein 1b, Cellular /	0.035	4.02	0.803	NS
UF_Ppr_AF_115478	AAG30407	0	Vitellogenin 3, Phosvitinless /	0.811	NS	0.041	6.10
UF_Ppr_AF_117841	AAU87498	0	Estrogen Receptor 1 /	0.277	NS	0.044	3.43
UF_Ppr_AF_108447	NP_957175	0	Hydroxysteroid Dehydrogenase 12a /	0.058	NS	0.021	-2.38
UF_Ppr_AF_109007	CAG12657	0	Transforming Growth Factor Beta 1 Induced Transcript 1 /	0.161	NS	0.031	1.76
UF_Ppr_AM_119790	AAO17057	6.37E-31	Similar To Latent Transforming Growth Factor Binding Protein /	0.268	NS	0.039	-1.96
UF_Ppr_AF_102732	NP_997969	0	Insulin Induced Gene 2 /	0.234	NS	0.024	1.83
			Oxidative Stress				
UF_Ppr_AF_115797	NP_001007767	0	Stress-induced-phosphoprotein 1 /	0.064	NS	0.008	-1.81
UF_Ppr_AF_107611	XP_001661758	0	Oxidative-stress Responsive 1 /	0.005	-1.88	0.711	NS
UF_Ppr_AF_117939	AAO86704	0	Glutathione Peroxidase 4a /	0.003	2.41	0.263	NS
UF_Ppr_AF_100194	ABK96973	0	Glutathione S-transferase, Alpha-like / Similar To Glutathione S-transferase, Alpha-like /	0.003	10.12	0.213	NS
UF_Ppr_AF_110272	ABF55513	0	Glutathione S-transferase Pi /	0.000	2.72	0.436	NS
UF_Ppr_AF_118975	XP_689979	0	Nuclear Factor Of Kappa Light Polypeptide Gene Enhancer In B-cells Inhibitor-like 1 /	0.003	2.01	0.034	-1.76

Table SI-3 (continued)

Probe Name	NR Accession	NR E-Value	Gene Title	PL		HTP	
				p-value	Fold Change	p-value	Fold Change
Cell Stress and Apoptosis							
UF_Ppr_AF_101025	NP_571952	0	Caspase 3, Apoptosis-related Cysteine Protease A /	0.110	NS	0.043	1.78
UF_Ppr_AF_102132	NP_001076390	0	Similar To Apoptosis Associated Protein /	0.409	NS	0.042	-2.04
UF_Ppr_AF_106212	XP_001488315	0	Apoptosis Inhibitor 5 /	0.031	-1.36	0.945	NS
UF_Ppr_AF_103750	NP_851847	0	Heat Shock Protein 1 /	0.034	-1.53	0.968	NS
UF_Ppr_AF_116360	NP_571942	7.72E-33	Heat Shock Factor 2 /	0.049	-1.81	0.703	NS
UF_Ppr_AF_102760	NP_571942	0	Heat Shock Factor 2 /	0.000	-2.47	0.025	-1.52
ROS production							
UF_Ppr_AF_106533	NP_956268	0	Cytochrome C Oxidase Subunit VIIa Polypeptide 2 Like / Similar To Cytochrome C Oxidase Subunit VIIa Polypeptide 2 Like /	0.085	NS	0.029	-1.67
UF_Ppr_AF_110319	NP_001027900	8.9E-33	Cytochrome C Oxidase, Subunit VIIa 2 /	0.897	NS	0.004	-2.06
UF_Ppr_AF_100840	NP_001002068	0	Cytochrome C, Somatic /	0.027	-1.54	0.014	-1.77
UF_Ppr_AF_108024	AAI53423	0	NADH Dehydrogenase 1 Alpha Subcomplex, 9 /	0.905	NS	0.035	-1.57
UF_Ppr_AF_108887	AAI34846	0	Mitogen-activated Protein Kinase 7 Interacting Protein 3 Like /	0.024	-1.30	0.228	NS
UF_Ppr_AF_100447	NP_001038620	0	Similar To MAD Homolog 4 Interacting Transcription Coactivator 1 /	0.037	-1.41	0.076	NS
UF_Ppr_AF_106345	AAW22592	0	Tumor Necrosis Factor Superfamily, Member 10 Like 2 /	0.006	1.63	0.288	NS
UF_Ppr_AF_113670	XP_001337829	0	Similar To Proto-oncogene Tyrosine-protein Kinase /	0.103	NS	0.002	1.79
UF_Ppr_AF_114316	NP_956626	0	Tumor Necrosis Factor, Alpha-induced Protein 8 / Hypothetical LOC554951 /	0.233	NS	0.016	1.44
UF_Ppr_AF_118598	NP_001019561	0	Serine/threonine/tyrosine Interacting Protein /	0.341	NS	0.014	1.42
UF_Ppr_AF_105093	NP_001098994	0	Tubulin Tyrosine Ligase-like Family, Member 13 /	0.223	NS	0.005	-1.79
UF_Ppr_AF_113515	NP_001002749	0	Tyrosinase-related Protein 1b /	0.235	NS	0.009	-1.95
UF_Ppr_AF_119015	XP_708168	0	Tyrosyl-tRNA Synthetase /	0.324	NS	0.031	-2.24
UF_Ppr_AF_107444	XP_001332381	0	Protein Tyrosine Phosphatase, Receptor Type, U /	0.434	NS	0.024	-2.46

Table SI-3 (continued)

Probe Name	NR Accession	NR E-Value	Gene Title	PL p-value	PL Fold Change	HTP p-value	HTP Fold Change
UF_Ppr_AF_114063	NP_571918	0	Tumor Necrosis Factor Superfamily, Member 10 Like /	0.137	NS	0.001	-2.51
UF_Ppr_AF_107341	XP_699996	0	Epoxide Hydrolase 1, Microsomal /	0.183	NS	0.024	-2.72
UF_Ppr_AF_112219	CAG07763	0	Protein Tyrosine Phosphatase, Receptor Type, S /	0.843	NS	0.010	-2.79
UF_Ppr_AF_118205	NP_001098662	0	Transducin-like Enhancer Of Split 3 Homolog, Drosophila) / Groucho 1 / Transducin-like Enhancer Of Split 3, Homolog Of Drosophila E(spl) /	0.456	NS	0.038	-3.98
UF_Ppr_AF_107140	CAG09494	0	Prosaposin /	0.006	-1.41	0.545	NS
UF_Ppr_AF_117749	No Hit		Similar To Receptor Protein Tyrosine Phosphatase LAR / Si:dkey-21k10.1 /	0.037	-1.44	0.441	NS
UF_Ppr_AF_117815	NP_997770	0	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase Activation Protein, Epsilon Polypeptide /	0.017	-1.49	0.556	NS
UF_Ppr_AF_102233	NP_001002137	0	Prostaglandin E Synthase 3 /	0.046	-1.50	0.434	NS
UF_Ppr_AF_103236	AAI29204	0	Protein Tyrosine Phosphatase, Receptor Type, F Polypeptide, Interacting Protein, Alpha 1 /	0.007	-1.63	0.405	NS
UF_Ppr_AF_109029	NP_001077045	0	Protein Tyrosine Phosphatase, Receptor Type, F /	0.041	-1.66	0.267	NS
UF_Ppr_AF_107264	NP_956645	0	Tumor Necrosis Factor, Alpha-induced Protein 8-like 3 /	0.038	-1.85	0.607	NS
UF_Ppr_AF_103865	NP_001014828	7.4E-29	Prostaglandin E Synthase /	0.012	-2.30	0.181	NS
UF_Ppr_AF_116478	XP_692922	1.53E-27	Similar To Tumor Necrosis Factor, Alpha-induced Protein 3 /	0.016	-2.37	0.219	NS
UF_Ppr_AF_116701	NP_001002042	0	MYB Binding Protein 1a /	0.334	NS	0.034	1.57
			Immune System				
UF_Ppr_AF_110600	CAN88645	0	Similar To Interleukin 13 Receptor Alpha-2 /	0.034	2.78	0.474	NS
UF_Ppr_AF_104183	AAT37635	0	Interleukin-1 Receptor-associated Kinase 4 /	0.037	1.60	0.199	NS
UF_Ppr_AF_100874	NP_001018624	3.91E-35	Interleukin 17c /	0.708	NS	0.002	3.79
UF_Ppr_AF_115169	XP_690239	0	Interleukin 1 Receptor Accessory Protein-like 2 /	0.342	NS	0.004	-3.84
UF_Ppr_AF_100535	XP_686890	0	Interleukin 6 Signal Transducer / Similar To IL6ST Nirs /	0.003	-1.74	0.219	NS

Table SI-3 (continued)

				PL		HTP	
Probe Name	NR Accession	NR E-Value	Gene Title	p-value	Fold Change	p-value	Fold Change
UF_Ppr_AF_104950	XP_686371	0.036255	Similar To Interleukin-1 Receptor-associated Kinase /	0.002	-2.01	0.570	NS
UF_Ppr_AF_107550	XP_690529	9.25E-08	Similar To Novel Immune Type Receptor Protein /	0.008	4.43	0.566	NS
UF_Ppr_AF_103465	XP_692110	0	Similar To Novel Zinc Finger Protein /	0.962	NS	0.001	1.85
UF_Ppr_AF_101453	NP_001093515	0	Novel Protein Similar To UDP-GlcNAc:betaGal Beta-1,3-N-acetylglucosaminyltransferase 5 / Zgc:101733 /	0.695	NS	0.041	-1.55
UF_Ppr_AF_111643	NP_001038449	0	Zgc:63632 / Novel Protein Similar To Solute Carrier Family 24, Member 1 /	0.759	NS	0.047	-1.62
UF_Ppr_AF_113116	NP_001076285	0	Novel Protein Similar To Vertebrate Hyaluronan And Proteoglycan Link Protein 2 /	0.594	NS	0.044	-1.77
UF_Ppr_AF_106761	NP_001093492	0	Novel Protein Similar To Vertebrate Solute Carrier Family 7 /	0.225	NS	0.008	-2.51
UF_Ppr_AF_107173	XP_001333145	0	Similar To Novel Carboxylesterase Domain Containing Protein /	0.788	NS	0.038	-2.87
UF_Ppr_AM_119541	No Hit		Novel Protein Similar To Vertebrate Syntaxin Binding Protein /	0.760	NS	0.001	-3.81
UF_Ppr_AF_118337	AAI25887	0	Novel Protein Similar To Vertebrate Praja Family Protein /	0.034	-1.23	0.868	NS
UF_Ppr_AF_118430	AAI15126	0	Similar To Novel Gene Similar To Human And Rodent IER5 /	0.028	-1.46	0.512	NS
UF_Ppr_AF_107040	No Hit		Novel Protein Similar To Vertebrate Plectin 1, Intermediate Filament Binding Protein 500kDa /	0.036	-1.47	0.618	NS
UF_Ppr_AF_107259	NP_001025295	0	Novel Protein Similar To Sorting Nexin 9 / Sorting Nexin 9-like /	0.003	-1.52	0.095	NS
UF_Ppr_AF_117780	EDP37055	2.40565	CBP-B / Novel Protein Similar To Vertebrate CREB Binding Protein /	0.003	-1.61	0.193	NS
UF_Ppr_AF_102669	CAM60065	0	Coxsackie Virus And Adenovirus Receptor /	0.030	-1.63	0.506	NS
UF_Ppr_AF_101730	NP_001076338	0	Novel Protein Similar To Vertebrate Fascin Homolog 1, Actin-bundling Protein /	0.045	-1.84	0.986	NS
UF_Ppr_AF_113142	NP_001103596	0	Hypothetical Protein LOC100005755 / Novel Protein Containing A ChaC-like Protein Domain /	0.026	-2.78	0.725	NS
UF_Ppr_AF_106222	CAI11941	0	Interferon Regulatory Factor 5 /	0.000	2.92	0.006	2.11
UF_Ppr_AF_109838	NP_956381	0	Thioredoxin Interacting Protein /	0.041	1.73	0.150	NS
UF_Ppr_AF_104689	XP_699912	0	AT Rich Interactive Domain 3B /	0.144	NS	1.E-4	2.74

Table SI-3 (continued)

Probe Name	NR Accession	NR E-Value	Gene Title	PL		HTP	
				p-value	Fold Change	p-value	Fold Change
UF_Ppr_AF_105728	XP_001341794	0	Similar To Widely-interspaced Zinc Finger Motifs /	0.595	NS	0.027	2.05
UF_Ppr_AF_115515	AAI54641	0	Similar To Synuclein, Alpha Interacting Protein /	0.629	NS	0.003	2.02
UF_Ppr_AF_110147	NP_998085	0	Werner Helicase Interacting Protein 1 /	0.798	NS	0.011	1.92
UF_Ppr_AF_113078	AAH60944	0	Interphotoreceptor Retinoid-binding Protein / Similar To Interphotoreceptor Retinol-binding Protein /	0.613	NS	0.040	1.90
UF_Ppr_AF_116959	A0PJT0	0	Rab Interacting Lysosomal Protein-like 1 /	0.424	NS	0.046	1.87
UF_Ppr_AM_119543	XP_001340540	0.000284	HIRA Interacting Protein 5 /	0.568	NS	0.044	1.67
UF_Ppr_AF_113892	XP_691623	0	Hypothetical LOC573266 / Dynein, Cytoplasmic 1, Light Intermediate Chain 2 /	0.091	NS	0.036	1.64
UF_Ppr_AF_110456	AAH44361	0	RNA, U3 Small Nucleolar Interacting Protein 2 /	0.239	NS	0.032	1.63
UF_Ppr_AF_104864	XP_001335443	0	Similar To Receptor-interacting Factor 1 /	0.077	NS	0.024	-1.82
UF_Ppr_AF_104801	CAN88534	5.71E-25	Similar To Disabled Homolog 2-interacting Protein /	0.362	NS	0.009	-1.90
UF_Ppr_AF_108651	XP_695433	0	RAB3A Interacting Protein-like 1 /	0.247	NS	0.002	-2.67
UF_Ppr_AF_102888	NP_957113	0	Kv Channel Interacting Protein 3, Calsenilin / Similar To Calsenilin, Presenilin Binding Protein, EF Hand Transcription Factor /	0.147	NS	0.001	-3.04
UF_Ppr_AF_111987	XP_001341945	0	Interferon Regulatory Factor 4 /	0.052	NS	1.E-4	-3.18
UF_Ppr_AF_100553	XP_685600	0	Similar To TRAF3 Interacting Protein 2 /	0.207	NS	0.005	-5.14
UF_Ppr_AF_107040	No Hit		Novel Protein Similar To Vertebrate Plectin 1, Intermediate Filament Binding Protein 500kDa /	0.036	-1.47	0.618	NS
UF_Ppr_AF_109761	CAK10720	0	Intermedin Precursor /	0.020	-1.51	0.003	1.86
UF_Ppr_AF_113115	NP_999861	0	Synaptotagmin Binding, Cytoplasmic RNA Interacting Protein /	0.015	-1.60	0.738	NS
UF_Ppr_AF_103236	AAI29204	0	Protein Tyrosine Phosphatase, Receptor Type, F Polypeptide, Interacting Protein, Alpha 1 /	0.007	-1.63	0.431	NS
UF_Ppr_AF_105336	XP_001373115	0	Jumonji, AT Rich Interactive Domain 1C /	0.008	-1.79	0.908	NS
UF_Ppr_AF_106092	XP_683344	0	Nuclear Factor Of Activated T-cells, Cytoplasmic, Calcineurin-dependent 2 Interacting Protein /	0.001	-2.00	0.015	1.79

Table SI-3 (continued)

Probe Name	NR Accession	NR E-Value	Gene Title	PL		HTP	
				p-value	Fold Change	p-value	Fold Change
UF_Ppr_AM_119853	CAK10720	0	Intermedin Precursor /	0.032	-3.11	0.817	NS
UF_Ppr_AF_100015	CAK04140	0	Phosphotyrosine Interaction Domain Containing 1 /	0.001	-5.20	0.562	NS
UF_Ppr_AF_103066	XP_001343562	0	Similar To Interferon-inducible Protein Gig1 /	0.010	-18.72	0.152	NS
UF_Ppr_AF_108673	XP_694448	0	Endothelial Cell Growth Factor 1 /	0.040	2.20	0.521	NS

NS: Not statistical significance

Table SI-4. Pathways which were significantly enriched. Due to its length, this table is accessible as an excel file using the link below.

ftp://ftp.sccwrp.org/pub/download/DOCUMENTS/AnnualReports/2013AnnualReport/**ar13_137_151SI_TableSI_4.xlsx**

Table SI-5. Characteristics of the advanced-primary treated (PL) and the full secondary treated (HTP) wastewater influents for the year 2009. Symbols indicate significant differences between the two influents ($p < 0.05$).

Influent Constituent	PL Mean	HTP Mean
Arsenic (µg/L)	1.2	3.6*
Cadmium (µg/L)	0.2	1.1*
Chromium (µg/L)	7	8.6
Copper (µg/L)	107	513*
Lead (µg/L)	11.5*	6.7
Mercury (µg/L)	0.2	0.14
Silver (µg/L)	1.2	5*
Zinc (µg/L)	141	194*
Ammonia (mg/L)	32	41*

Table SI-6. Relative fold changes after exposure to advanced primary treated effluent or secondary treated effluent in male fathead minnow livers using qPCR. The values represent means and standard - error (SE). Symbols represent significant differences relative to controls ($p < 0.05$).

Gene ID	Mean	SE
	HTP 5% Effluent	
CYP1A	1.5	0.2
ESR1	4.9*	1
RBP	0.7	0.2
VTG	34.6*	11
	PL 5% Effluent	
CYP1A	0.7	0.1
ESR1	1.4	0.4
RBP	5*	1
VTG	4.1	2.5

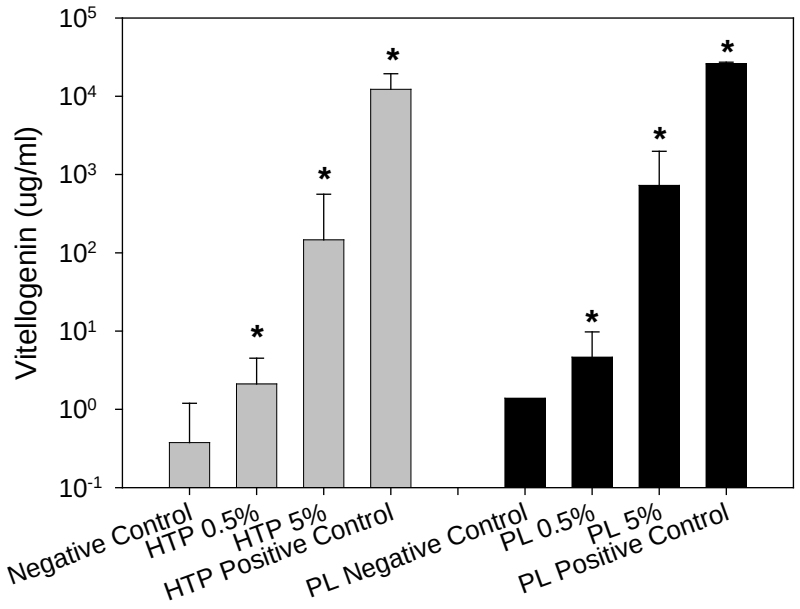
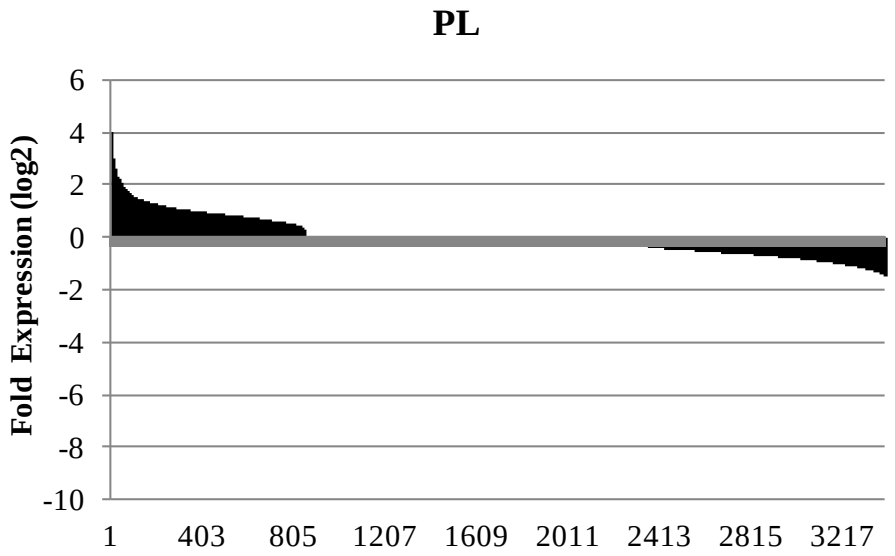


Figure SI-1. Average plasma vitellogenin concentrations for fathead minnows exposed to advanced primary treated effluent (PL) or full secondary treated effluent (HTP). The 0.5% treatment represents an environmentally realistic concentration. Symbols represent significant differences relative to their respective negative controls.

A)



B)

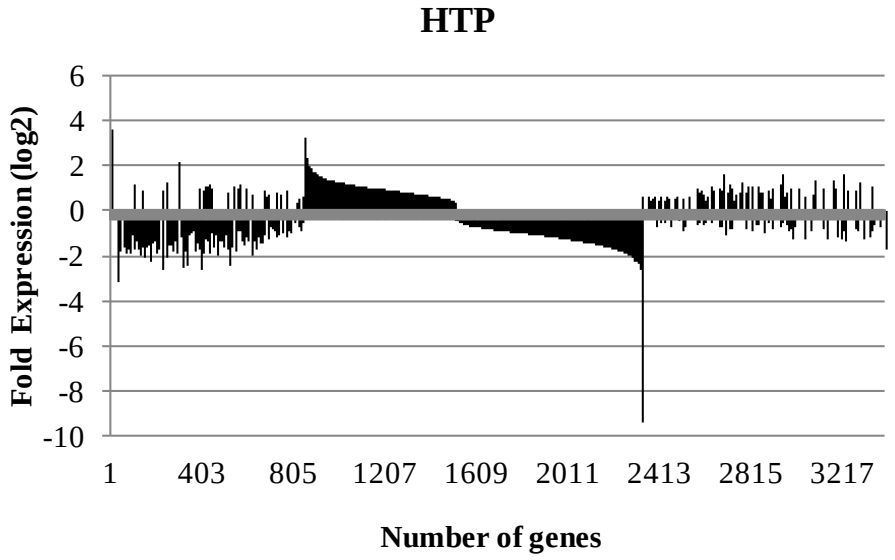


Figure SI-2. Comparison of overall differential gene expression by effluents exposure in the liver of FHM. The genes were ordered according to their expression level after exposure to PL effluent. This analysis was only conducted with genes that were differentially expressed in at least one of the exposures. Genes that were not differentially expressed in one of the exposures were set to zero. A) The fold expression (Log2) observed after exposure to PL effluent and B) the fold expression (Log2) observed after exposure to HTP effluent.

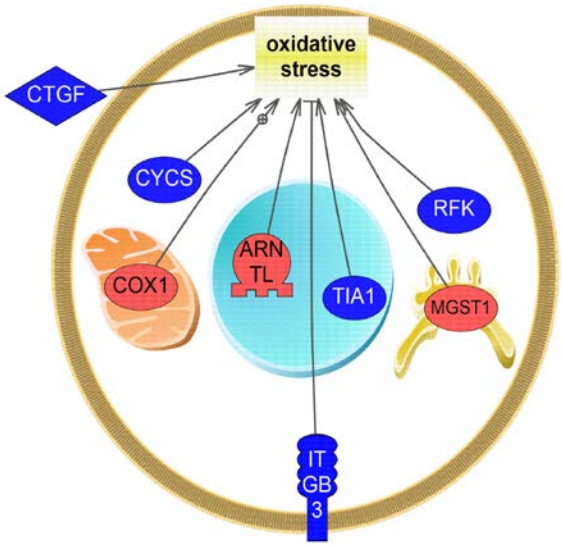
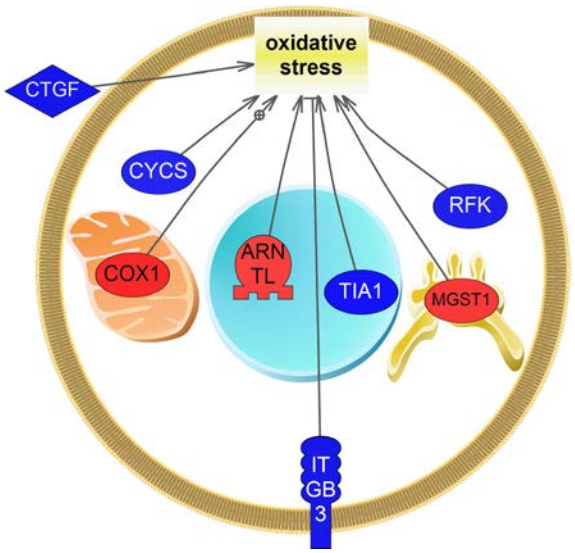
A) HTP	B) PL
	

Figure SI-3. The stress oxidative pathway was the one most potential enriched after exposure to both effluents. A) Fish exposed to HTP effluent, B) Fish exposed to PL effluent. Blue color indicates down-regulation, and red indicates up-regulation of gene expression; color intensity indicates the degree of response, more intense colors indicate a higher magnitude of response. Yellow entities indicate potential cell processes that will be most likely affected. Symbols represent protein , transcription factor , membrane receptor , and ligand . Abbreviations used: **CYCS,** cytochrome c, somatic; **ARNTL,** aryl hydrocarbon receptor nuclear translocator-like; **CTGF,** connective tissue growth factor; **COX 1,**Cyclooxygenase-1; **ITGB3,** Integrin, beta 3; **TIA1,** cytotoxic granule-associated RNA binding protein; **MGST1,** microsomal glutathione S-transferase 1; **RFK,** riboflavin kinase.

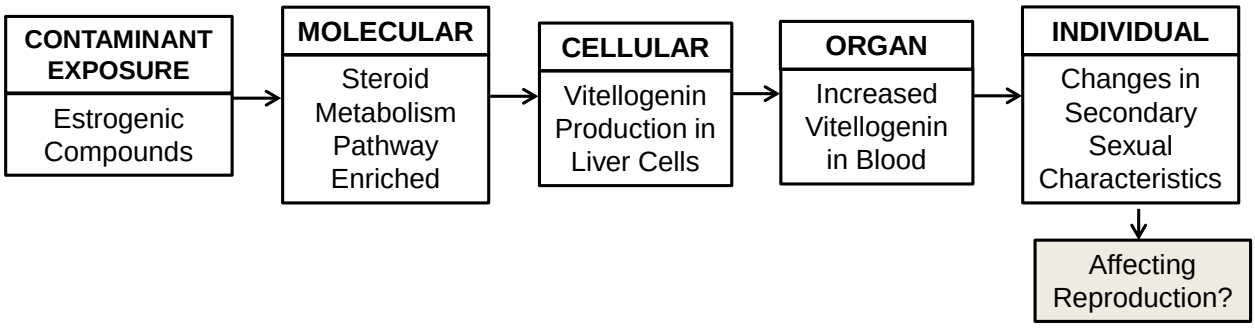
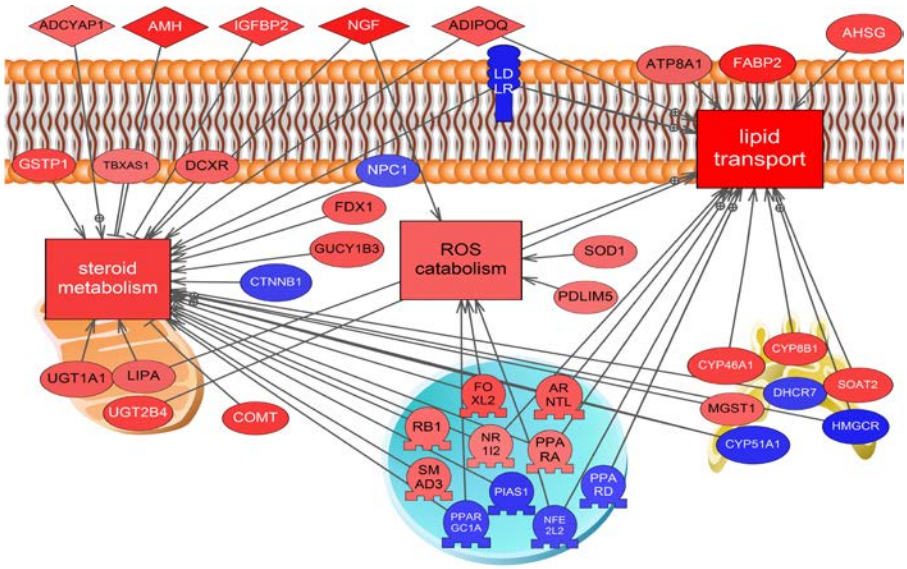


Figure SI-4. Potential adverse outcome pathway identified after effluent exposure. The VTG production in liver cells was not directly measured in this study.

ADDITIONAL INFORMATION FOR FIGURE 3 IN MAIN PAPER

A)



B)

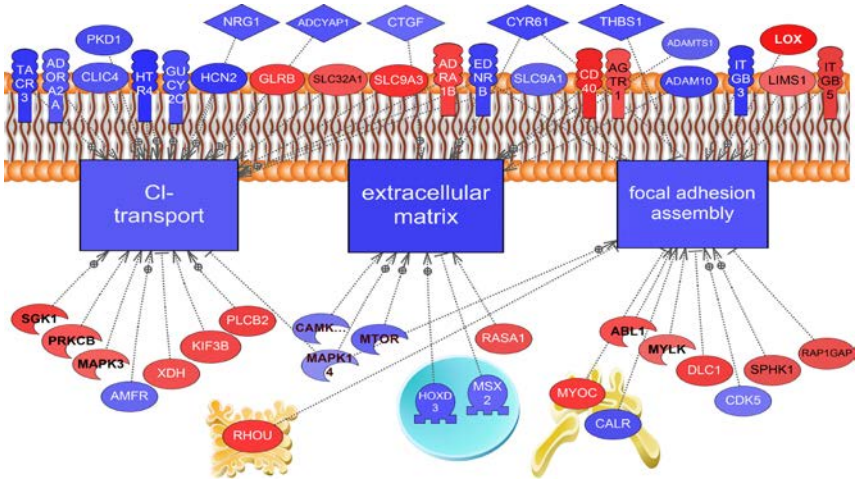


Figure SI-5. Pathway analyses of transcripts differentially regulated by the two effluents. **3A.** Pathway analysis of PL effluent. The majority of transcripts were associated with metabolic pathways like steroid metabolism, ROS catabolism, lipid transport and xenobiotic metabolism (clearance). **3B**. Most enriched pathways following exposure to HTP effluent were involved in intracellular signals associated with Cl- transport, extracellular matrix and focal adhesion assembly. Blue indicates down-regulation, and red

indicates up-regulation of gene expression. Symbols represent protein  , transcription factor  , membrane receptor , ligand  , cell process  , and kinases  . The abbreviations used are the official symbols located in http://www.ncbi.nlm.nih.gov/gene/. Abbreviations 3A: **PDLIM5,** PDZ and LIM domain 5; **FOXL2,** forkhead box L2; **RB1,** retinoblastoma 1; **SMAD3,** SMAD family member 3; **ARNTL,** aryl hydrocarbon receptor nuclear translocator-like; **NR1I2**, nuclear receptor subfamily 1, group I, member 2; **PIAS1,** protein inhibitor of activated STAT; **VDR,** vitamin D (1,25- dihydroxyvitamin D3) receptor; **TEF,** thyrotrophic embryonic factor; **PPARA,** peroxisome proliferator-activated receptor alpha-B; **PPARGC1A,** peroxisome proliferator-activated receptor gamma, coactivator 1 alpha; **VDR,** vitamin D (1,25-dihydroxyvitamin D3) receptor ; **NKRF,** NFKB repressing factor; **NPC1,** Niemann-Pick disease, type C1; **NGF,** nerve growth factor (beta polypeptide); **AMH,** anti-Mullerian hormone ; **IGFBP2,** insulin-like growth factor binding protein 2, 36kDa; **ADCYAP1,** adenylate cyclase activating polypeptide 1 (pituitary); **GUCY1B3,** guanylate cyclase 1, soluble, beta 3; **FDX1,** ferredoxin 1; **TBXAS1,** thromboxane A synthase 1 (platelet); **NPC1,** Niemann-Pick disease, type C1; **GSTP1,** glutathione S-transferase pi 1; **DCXR**, dicarbonyl/L-xylulose reductase; **COMT,** catechol-O-methyltransferase; **UGT1A1,** UDP glucuronosyltransferase 1 family, polypeptide A1; **CTNNB1,** catenin (cadherin-associated protein), beta 1, 88kDa; **SRD5A2,** steroid-5-alpha-reductase, alpha polypeptide 2 (3-oxo-5 alpha-steroid delta 4-dehydrogenase alpha 2); **GSTA1**, glutathione S-transferase alpha 1; **GSTT1,** glutathione S-transferase theta 1; **GPX4,** glutathione peroxidase 4 (phospholipid hydroperoxidase); **AOX1,** Aldehyde oxidase; **DHDH,** dihydrodiol dehydrogenase (dimeric); **ADH5,** alcohol dehydrogenase 5 (class III), chi polypeptide; **PRDX1,** peroxiredoxin 1; **ABCG2,** ATP-binding cassette, sub-family G (WHITE), member 2; **CYP2C9,** cytochrome P450, family 2, subfamily C, polypeptide 9; **OGDH,** oxoglutarate (alpha-ketoglutarate) dehydrogenase (lipoamide); **LIPA,** lipase A, lysosomal acid, cholesterol esterase; **UGT2B4,** UDP glucuronosyltransferase 2 family, polypeptide B4; **AHSG,** alpha-2-HS-glycoprotein. Alpha2-HS glycoprotein (AHSG); **SOAT2,** sterol O-acyltransferase 2. Summary; **ATP8A1,** ATPase, aminophospholipid transporter (APLT), class I, type 8A, member 1; **ADIPOQ,** adiponectin, C1Q and collagen domain containing; **CYP4B1,** cytochrome P450, family 4, subfamily B, polypeptide 1; **MGST1,** microsomal glutathione S-transferase 1; **MGST3,** microsomal glutathione S-transferase 3; **CYP8B1,** cytochrome P450, family 8, subfamily B, polypeptide 1; **CYP46A1**, cytochrome P450, family 46, subfamily

A, polypeptide 1; **DHCR7,** 7-dehydrocholesterol reductase.; **CYP51A1,** cytochrome P450, family 51, subfamily A, polypeptide 1; **HMGCR,** 3-hydroxy-3-methylglutaryl-Coenzyme A reductase; **FABP2,** fatty acid binding protein 2, intestinal; **LDLR,** low density lipoprotein receptor; **LIFR,** leukemia inhibitory factor receptor alpha; **BMP2,** bone morphogenetic protein 2; **ADIPOQ,** adiponectin, C1Q and collagen domain containing; **IGF2**, insulin-like growth factor 2 (somatomedin A); **AKT1,** similar to protein kinase N2; **ACSL4,** acyl-CoA synthetase long-chain family member 4; **LIPC,** lipase, hepatic; **AKAP1**, A kinase (PRKA) anchor protein 1; **TIMP3**, TIMP metallopeptidase inhibitor 3; **LIPA,** lipase A, lysosomal acid, cholesterol esterase; **ITGB1,** integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12); **AVPR1A,** arginine vasopressin receptor 1A; **ZFP36L1,** zinc finger protein 36, C3H type-like 1; **CYP51A1,** cytochrome P450, family 51, subfamily A, polypeptide 1. Abbreviations 3B: **TACR3**, tachykinin receptor 3; **ADORA2A,** adenosine A2a receptor; **PKD1,** polycystic kidney disease 1 (autosomal dominant); **CLIC4,** chloride intracellular channel 4; **HTR4,** 5-hydroxytryptamine (serotonin) receptor 4; **GUCY2C,** guanylate cyclase 2C (heat stable enterotoxin receptor); **NRG1,** neuregulin 1; **HCN2,** hyperpolarization activated cyclic nucleotide-gated potassium channel 2; **ADCYAP1,** adenylate cyclase activating polypeptide 1 (pituitary; **SLC32A1,** solute carrier family 32 (GABA vesicular transporter), member 1; **GLRB,** glycine receptor, beta; **SLC9A3,** solute carrier family 9 (sodium/hydrogen exchanger), member 3; **ADRA1B,** adrenergic, alpha-1B-, receptor; **EDNRB,** endothelin receptor type B; **SLC9A1,** solute carrier family 9 (sodium/hydrogen exchanger), member 1; **CD40,** CD40 molecule, TNF receptor superfamily member 5; **AGTR1,** angiotensin II receptor, type 1; **ADAM10,** ADAM metallopeptidase domain 10; **ADAMTS1,** ADAM metallopeptidase with thrombospondin type 1 motif, 1; **CYR61,** cysteine-rich, angiogenic inducer, 61; **ITGB3,** integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61); **THBS1,** thrombospondin 1; **LIMS1,** LIM and senescent cell antigen-like domains 1; **LOX,** lysyl oxidase; **ITGB5,** integrin, beta 5; **SGK1,** serum/glucocorticoid regulated kinase 1; **PRKCB,** protein kinase C, beta; **MAPK3,** mitogen-activated protein kinase 3; **AMFR,** autocrine motility factor receptor; **XDH,** xanthine dehydrogenase; **KIF3B,** kinesin family member 3B; **PLCB2**, phospholipase C, beta 2; **RHOU**, ras homolog gene family, member U; CAMK2A, calcium/calmodulin-dependent protein kinase II alpha; **MTOR,** mammalian target of rapamycin or FK506 binding protein 12-rapamycin associated protein 1; **MAPK14,** mitogen-activated protein kinase 14; **HOXD3**, homeobox D3; **MSX2,** msh homeobox 2;

RASA1, RAS p21 protein activator (GTPase activating protein); **MYOC,** myocilin, trabecular meshwork inducible glucocorticoid response; **CALR,** calreticulin; **RAP1GAP,** RAP1 GTPase activating protein; **MYLK,** similar to neuronal myosin light chain kinase 1; **ABL1,** c-abl oncogene 1, receptor tyrosine kinase; **DLC1,** deleted in liver cancer 1; **SPHK1,** sphingosine kinase 1; **CDK5,** cyclin-dependent kinase 5. **SYNE1,** spectrin repeat containing, nuclear envelope 1;