

Supplemental Material

Many Putative Endocrine Disruptors Inhibit Prostaglandin Synthesis

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Supplemental Table 1: Primers used for Real-Time PCR.

| Gene | Upstream primer | Downstream primer |
|-------------------------------|------------------------|------------------------|
| β 2-Microglobulin (B2M) | CGAGACATGTGATCAAGCATCA | TATGCTCAGCTATCTAGGATAT |
| Cyclooxygenase 1 (Cox1) | GAAGCCTTACACCTCTTTCCA | GTGCTGGGTTTCCAGTACTCT |
| Cyclooxygenase 2 (Cox2) | GAAGAAATGTGCCAATTGCTGT | CCAAAGATAGCATCTGGACGA |

Supplemental Table 2: *Cox* expression after exposure to selected endocrine disrupting compounds. Data represent fold changes (\pm s.d.) in

| Compound | Gene | Control | 0.1 μ M | 1 μ M | 10 μ M |
|-------------------------------|-------------|------------------|---------------------|--------------------|--------------------|
| Di- <i>n</i> -butyl phthalate | <i>Cox1</i> | 1 (0.87 - 1.15) | 1.17 (0.85 - 1.61) | 1.37 (1.13 - 1.67) | 1.56 (0.95-2.55) |
| | <i>Cox2</i> | 1 (0.74 - 1.34) | 0.87 (0.79 - 0.95) | 1.34 (1.1 - 1.55) | 1.34 (0.59 - 3.07) |
| <i>n</i> -Butylparaben | <i>Cox1</i> | 1 (0.59 - 1.68) | 1.16 (0.74 - 1.83) | 1.29 (0.86 - 1.93) | 1.41 (1.14 - 1.75) |
| | <i>Cox2</i> | 1 (0.60 - 1.66) | 1.42 (1.14 - 1.78) | 1.16 (0.76 - 1.76) | 1.21 (1.06 - 1.37) |
| Benzophenone 3 | <i>Cox1</i> | 1 (0.14 - 6.96) | 2.47 (1.21 - 5.07) | 5.31 (4.02 - 7.03) | 4.83(3.83 - 6.10) |
| | <i>Cox2</i> | 1 (0.08 - 13.28) | 4.85 (2.18 - 10.78) | 6.70 (5.73 - 7.83) | 7.45 (5.90 - 9.39) |
| Bisphenol A | <i>Cox1</i> | 1 (0.77 - 1.30) | 0.39 (0.07 - 2.17) | 0.87 (0.74 - 1.01) | 0.97 (0.87 - 1.08) |
| | <i>Cox2</i> | 1 (0.96 - 1.04) | 0.44 (0.12 - 1.67) | 0.84 (0.75 - 0.95) | 1 (0.86 - 1.16) |
| Diethylstilbestrol | <i>Cox1</i> | 1 (0.80 - 1.25) | 0.67 (0.49 - 0.93) | 0.97 (0.84 - 1.13) | 0.83 (0.64 - 1.07) |
| | <i>Cox2</i> | 1 (0.69 - 1.46) | 0.67 (0.48 - 0.95) | 0.91 (0.73 - 1.12) | 0.81 (0.59 - 1.13) |
| Genistein | <i>Cox1</i> | 1 (0.39 - 2.56) | 2.05 (1.27 - 3.32) | 2.53 (0.92 - 6.97) | 1.95 (0.45 - 8.41) |
| | <i>Cox2</i> | 1(0.34 - 2.97) | 1.73 (1.01 - 2.94) | 2.00 (0.74 - 5.38) | 1.36 (0.41 - 4.49) |
| Dihydrotestosterone | <i>Cox1</i> | 1(0.26 - 3.82) | 1.53 (1.14 - 2.06) | 1.51 (0.96 - 2.37) | 0.70 (0.25 - 2.01) |
| | <i>Cox2</i> | 1(0.35 - 2.89) | 1.21 (1.02 - 1.44) | 1.13 (0.73 - 1.75) | 0.68 (0.28 - 1.66) |
| Flutamide | <i>Cox1</i> | 1(0.86 - 1.16) | 0.55 (0.23 -1.31) | 1.30 (1.25 -1.34) | 0.95 (0.58 - 1.57) |
| | <i>Cox2</i> | 1 (0.83 -1.2) | 0.52 (0.25 - 1.09) | 1.34 (1.31 -1.37) | 1.24 (0.79 -1.93) |

Changes in *COX* expression were assessed with the comparative C_T method with B2M as internal control.

Supplemental Table 3a: Modeling of docking into the active site of COX2

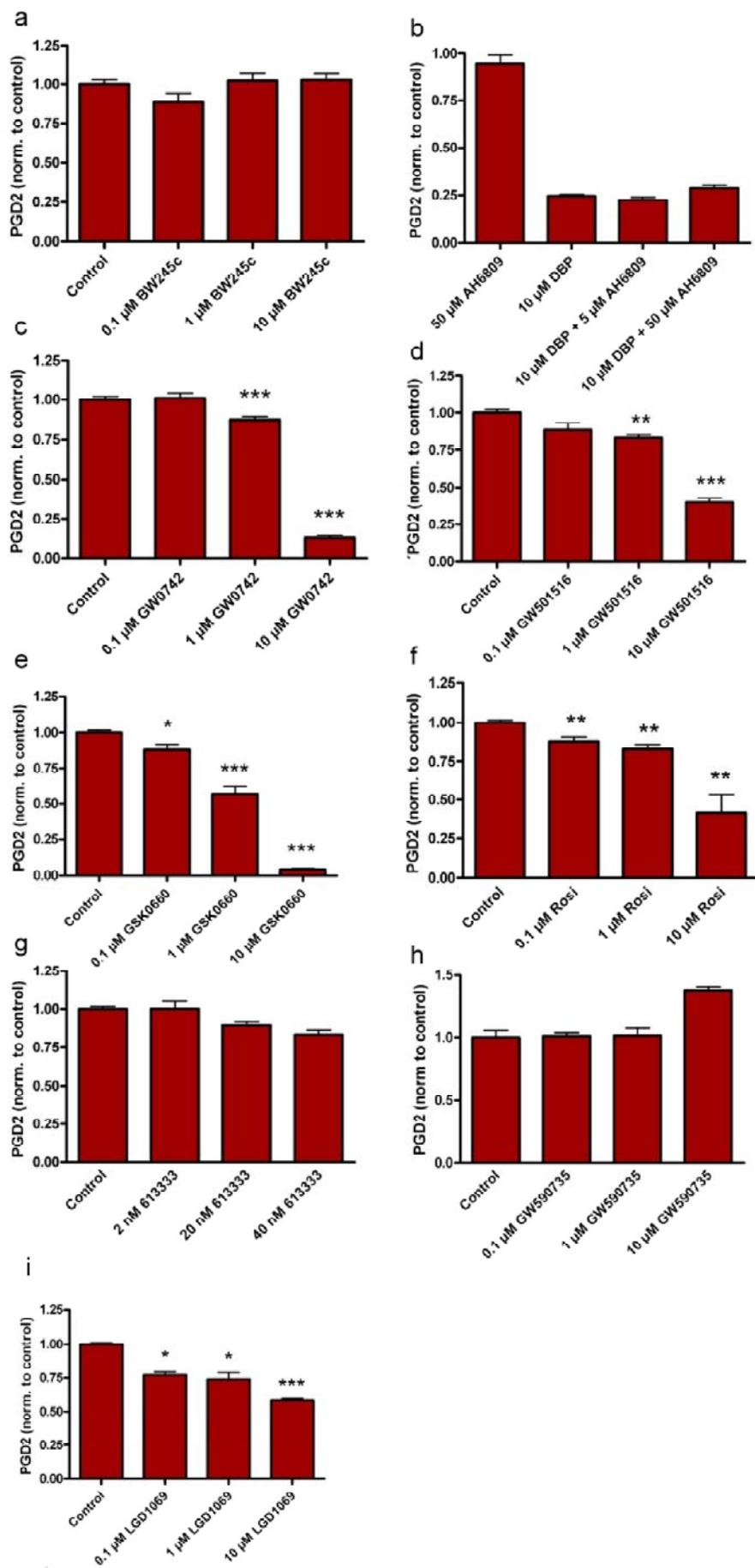
| Compound* | Andrews mean pKi** | Predicted pKi*** | Hydrogen Bonds | Hydrophobic Interaction |
|---------------------------------|-----------------------------------|-----------------------------|---------------------------|------------------------------------|
| Benzophenone 3 | -0.43 | 4.31 | 0.911 | 3.18 |
| Bisphenol A | -5.57 | 4.63 | 0.525 | 3.71 |
| Methylparaben | -5.79 | 4.35 | 0.59 | 2.92 |
| Ethylparaben | -5.71 | 4.34 | 0.52 | 3.13 |
| <i>n</i> -Propylparaben | -5.64 | 4.75 | 0.52 | 3.39 |
| <i>n</i> -Butylparaben | -5.57 | 4.63 | 0.52 | 3.71 |
| Isobutylparaben | -4.98 | 5.00 | 0.52 | 4.07 |
| Dimethyl phthalate | -7.03 | 3.40 | 0.42 | 2.44 |
| Diethyl phthalate | -6.88 | 3.97 | 0.42 | 3.3 |
| Di- <i>n</i> -propyl phthalate | -6.74 | 5.02 | 0.42 | 4.05 |
| Di- <i>n</i> -butyl phthalate | -6.59 | 6.22 | 0.42 | 4.97 |
| Di-isobutyl phthalate | -5.57 | 6.19 | 0.42 | 5.23 |
| Monomethyl phthalate | -1.61 | 4.06 | 1.11 | 2.26 |
| Monoethyl phthalate | -1.54 | 4.21 | 1.11 | 2.56 |
| <i>n</i> -Propyl phthalate | -1.46 | 4.76 | 1.11 | 2.96 |
| Mono- <i>n</i> -butyl phthalate | -1.39 | 4.79 | 1.11 | 3.41 |
| Mono-isobutyl phthalate | -0.87 | 5.02 | 1.11 | 3.22 |

* Modelling was done with compounds in similar chemical conformation in the cavity. **Andrews pKi score estimates docking to an average binding site. ***Predicted pKi score is the predicted binding of the ligand into the COX2 active site. If the predicted pKi score is higher than the Andrews pKi, the simulation suggests that the investigated ligand-compound complex is likely to occur.

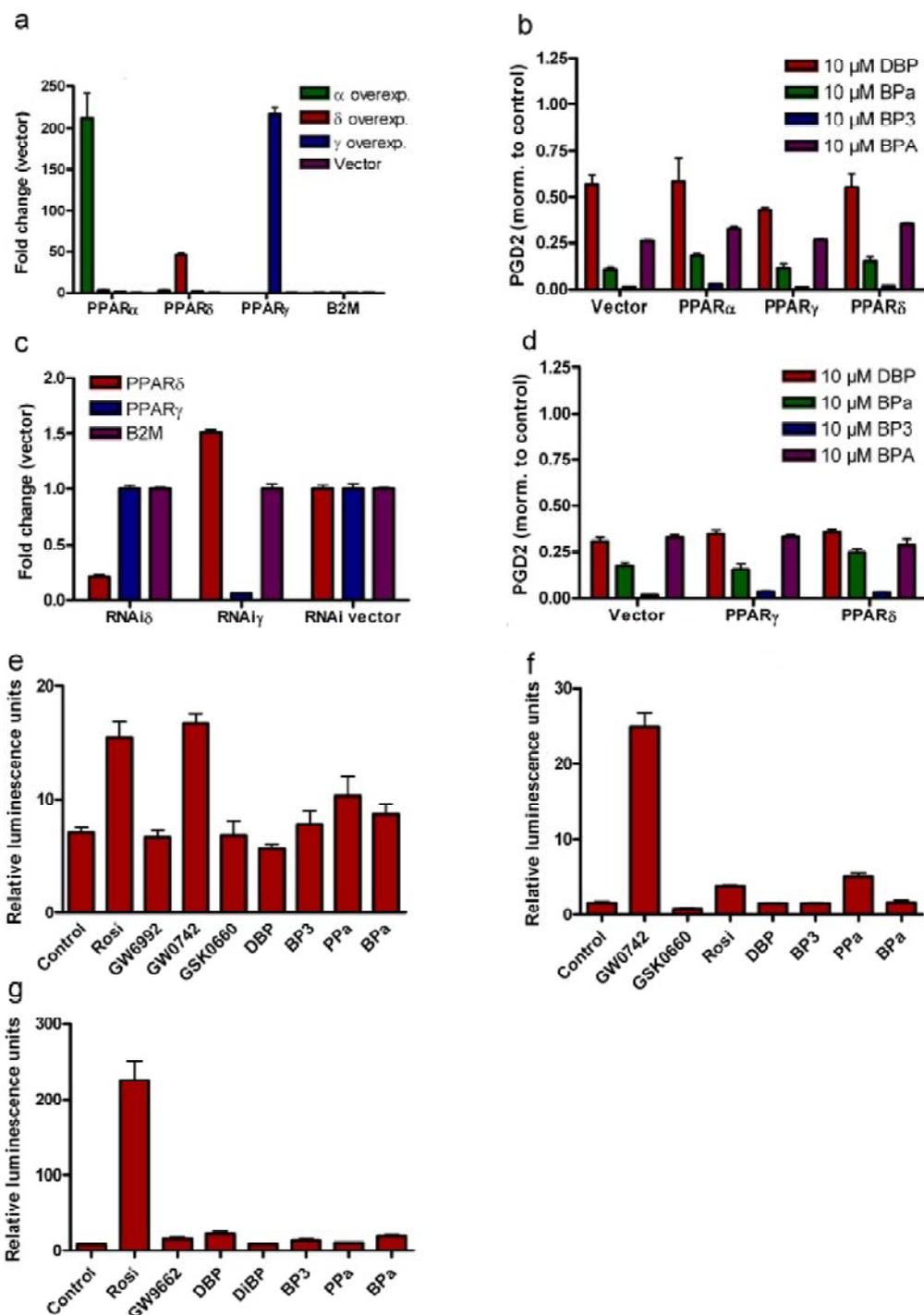
Supplemental Table 3b: Energy values obtained with docking calculations.

| Compounds | S (kcal/mol) |
|------------------|---------------------|
| DEHP | - 10.256 |
| MEHP | - 11.0041 |
| 5-oxo-MEHP | - 11.4809 |
| 5-OH-MEHP | - 12.90 |
| 5-CX-MEHP | - 13.1440 |

S is a scoring function that represents the binding energy of a compound into the binding pocket of the protein, defined in Kcal/mol. The lower the energies are, the better are the predicted affinities of the compound for the protein. Calculated by MOE software (Chemical Computing Group version 2007.09).



Supplemental Figure 1: Inhibition of prostaglandin secretion is not associated with DP and EP receptors, but mimic effect of PPAR δ ligands in SC5 cells. **a** Activation of DP receptor for 24 h with agonist BW245c had no effect of PGD2 secretion. **b**, Inhibition of both DP and EP receptors with antagonist AH6809 for 24 h had no effect and did not interfere with inhibition of PGD2 secretion by DBP after co-incubation with both compounds for 24 h. **c**, **d**, and **e**, Incubation of for 24 h with PPAR δ agonists GW0742 and GW501516, and PPAR δ antagonist GSK0660 dose-dependently inhibited PGD2 secretion. **f**, Similar results was seen with PPAR γ agonist Rosi. **e-h**, PPAR α agonists 613333 and GW590735 had not effect on PGD2 secretion after 24 h incubation. **i**, RXR agonist LGD1069 weakly inhibited PGD2 secretion from SC5 cells after 24 h. Data represent mean \pm s.e.m. for three experiments done in triplicate. *** P <0.001, ** P <0.01, * P <0.05 versus controls by two-tailed Student's t -test.



Supplemental Figure 2: The inhibition of prostaglandin synthesis by endocrine disrupting compounds is independent of PPARs. **a**, Real time PCR for *PPAR α* , *PPAR δ* , and *PPAR γ* showing overexpression of the respective *PPAR* transcripts in SC5 cells. **b**, Retroviral *PPAR* overexpression did not significantly alter the inhibitory action of DBP, BP α , BP3, and BPA after 24 h compared to vector controls. **c**, Real time PCR for *PPAR δ* and *PPAR γ* demonstrating the expected reduction of the transcripts in SC5 cells after knock-down. **d**, Lentivirus-mediated shRNA knock down

of PPAR γ and PPAR δ did not significantly alter the inhibitory action of DBP, BPa, BP3, and BPA on PGD2 secretion after 24 h incubation compared to vector controls. **e**, SC5 cells transfected with a PPAR responsive luciferase reporter plasmid did not show any consistent increase in transcriptional activity after 24 h incubation with the above mentioned compounds and PPa. Positive controls Rosi and GW0742 increased transcriptional activity. **f-g**, Transfection of SC5 cells with expression vectors containing the mouse PPAR δ (**f**) and PPAR γ (**g**) ligand-binding domain fused to Gal4 did not result in any increased transcriptional activity after incubation with compounds for 24 h, further indicating that PPARs were not activated in SC5 cells after exposure. Data are shown in mean \pm s.e.m. for three experiments done in triplicate.