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## **Supplemental Material**

## Effects of Crude Oil/Dispersant Mixture and Dispersant Components on PPARγ Activity in Vitro and in Vivo: Identification of Dioctyl Sodium Sulfosuccinate (DOSS; CAS #577-11-7) as a Probable Obesogen

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Figure S1. PPARγ ligand binding activity of DWAF in a GAL4-UAS system. Dilutions of mixtures were prepared, HEK293T/17 cells were transfected, exposed in triplicate for 18 hours, and luciferase activities were measured as detailed in *Materials and Methods*. Dose dependent ligand binding activities were not detected in any of the DWAF dilutions tested. Data are expressed as Mean  $\pm$  SD; n = 3 per group (no statistically significant differences).

**Figure S2. Detection of prevalent COREXIT components Tween 80 and DOSS in the 50:50 ethanol:water fraction.** (Top) Total ion chromatogram in full scan positive mode of the CWAF ethanol/water SPE extractable fraction. Inset A, corresponding to peak at 5.33 min, displays a positive mode precursor ion scan of m/z 309.3 that produces a mass spectral pattern (sorbitan monooleates with 16-27 polyoxy-ethylene units) previously found in Tween 80 (Zhang et al. 2012). (Bottom) Total ion chromatogram in full scan negative mode of the CWAF ethanol/water SPE extractable fraction. Inset B, corresponding to peak at 2.45 min, displays a product ion scan

for m/z 421.0. Examining the fragmentation profile, noting the fragment ion m/z 81.0, indicates the presence of DOSS, as previously reported (Mathew et al. 2012; Ramirez et al. 2013).

**Figure S3. Molecular modeling of PPARγ with COREXIT components Span 80, Tween 80 and dioctyl sodium sulfosuccinate (DOSS).** A) Average E Score for COREXIT components analyzed for PPARγ ligand binding using MOE as in *Materials and Methods*. B) DOSS modeled in the PPARγ ligand-binding pocket.

**Figure S4. PPAR**γ activity of COREXIT components other than DOSS. Dilutions of mixtures were prepared, HEK293T/17 cells were transfected, exposed in triplicate for 18 hours, and luciferase activities were measured as detailed in *Materials and Methods*. Dose-dependent ligand binding activities were not detected in any of the dilutions tested for A) Span 80 or C) ICP:Propylene Glycol (PG). Modest ligand binding activity was detected only at the highest dose of B) Tween 80 (50ppm). Robust dose-dependent ligand binding activities were detected in D) ICP:PG:DOSS (4 ppm and 8 ppm). Collectively, these results indicate that DOSS is the principle obesogen in COREXIT. Data are expressed as Mean  $\pm$  SD; n = 3 per group (\* p < 0.05 versus untreated controls).

Figure S5. DOSS has Human PPAR $\gamma$ , PPAR $\alpha$ , PPAR $\beta$ /δ, and RXR $\alpha$  transactivation activity. Dilutions of mixtures and positive controls were prepared, HEK293T/17 cells were transfected, exposed in triplicate for 18 h, and luciferase activities were measured. A) PPAR $\gamma$ , B) PPAR $\alpha$ , C) PPAR $\beta$ /δ and D) RXR $\alpha$ . Dose-dependent transactivation activities by DOSS were detected for PPAR $\gamma$  and PPAR $\beta$ /δ. Data are expressed as Mean ± SD; n = 3 per group (\* p < 0.05 versus not treatment control).

Figure S6. Dlk1 and Fabp4 mRNA expression in 3T3-L1 cells at day three of adipogenesis. 3T3-L1 cells were treated for 72 h as described in *Materials and Methods* and qPCR was performed as described in *Supplemental Materials and Methods*. Data are represented as a fold change in expression over the housekeeping gene HPRT for A) preadipocyte marker Pref-1/Dlk1 and B) adipocyte marker Fabp4 (\* p < 0.05 versus MIM control, n = 6 per group).

**Table S1.** Primers used for qPCR analysis of 3T3-L1 cells.

Species	Gene	Forward Primer	Reverse Primer
Mouse	Hprt	AGGCCAGACTTTGTTGGATTTG	TTCAACTTGCGCTCATCTTAGG
Mouse	Pref-1/Dlk1	CCTGGCTGTGTCAATGGAGT	CAAGTTCCATTGTTGGCGCA
Mouse	Fabp4	ATGTGTGATGCCTTTGTGGGA	GATGATCATGTTGGGCTTGGC

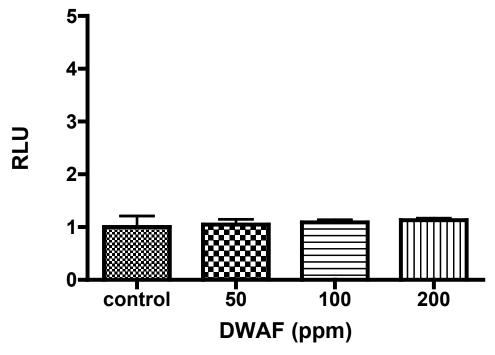
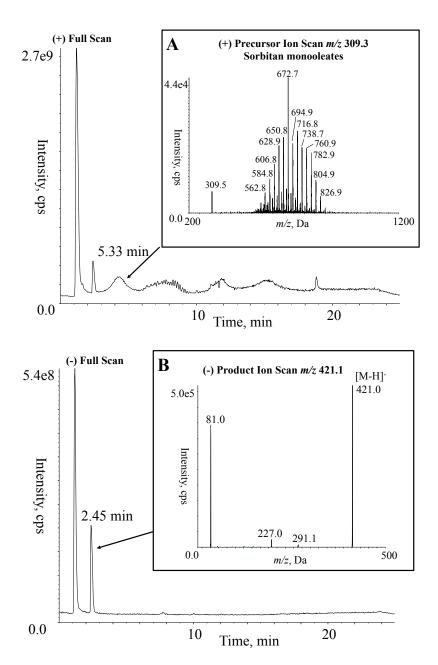
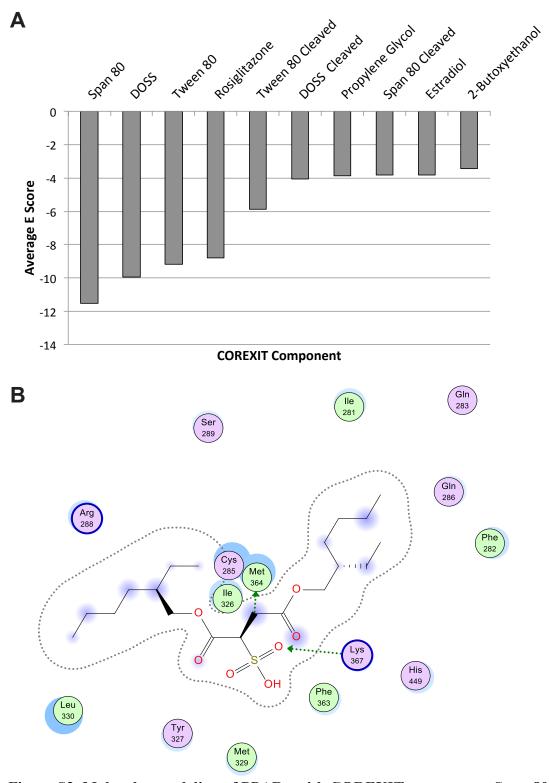


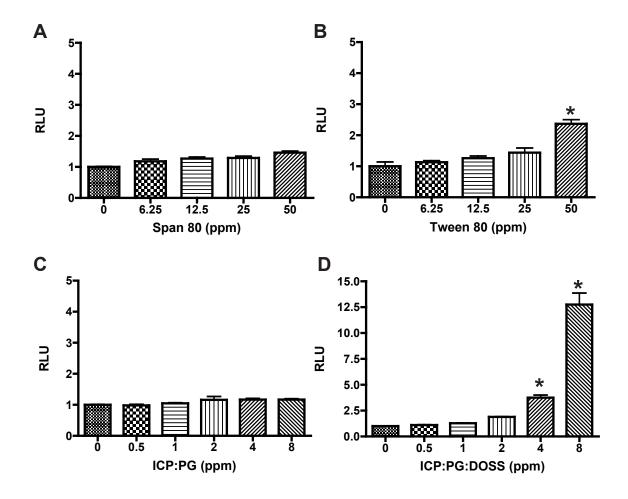
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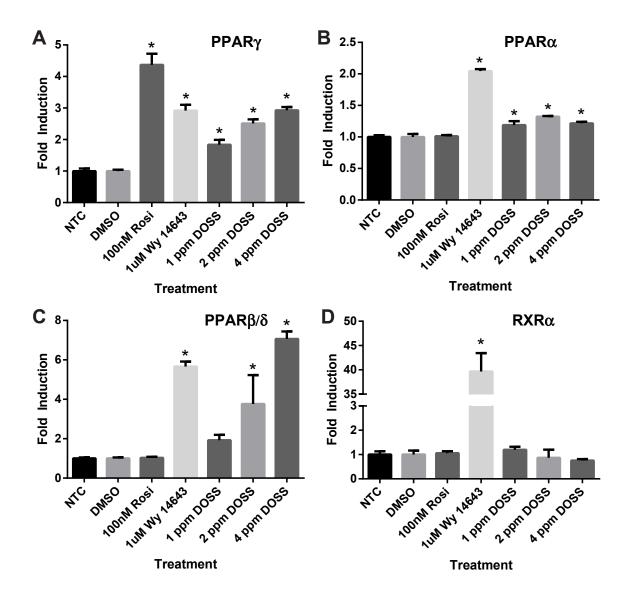


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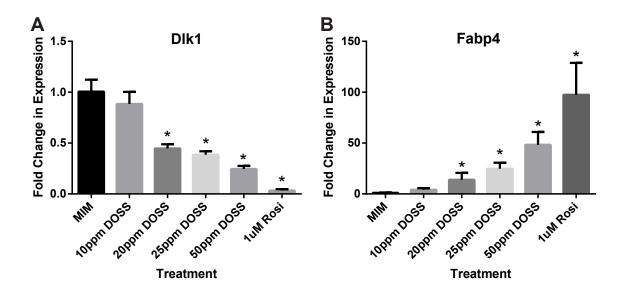


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