Supporting Information

Sulfation of lower chlorinated polychlorinated biphenyls increases their affinity for the major drug-binding sites of human serum albumin

Eric A. Rodriguez[1], Xueshu Li[2], Hans-Joachim Lehmler[2], Larry W. Robertson[2], and Michael W. Duffel[1]

[1]Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, The University of Iowa, Iowa City, Iowa, USA

[2]Department of Occupational and Environmental Health, College of Public Health, The University of Iowa, Iowa City, Iowa, USA

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Experimental Section

The chemical structures and abbreviations for each PCB derivative included in this study are shown in Figures 2 to 4. With exception of the two PCB 52 metabolites, their synthesis and characterization has been described in several earlier papers. Individual PCB congeners were synthesized using the Suzuki coupling of the appropriate chlorinated benzene boronic acids with brominated chlorobenzenes[1] or purchased from AccuStandard (New Haven, CT, USA). All hydroxylated PCB metabolites were similarly synthesized by the Suzuki coupling of chlorinated benzene boronic acids with appropriate brominated (chloro-)anisoles, followed by demethylation with boron tribromide.[2-4] PCB sulfates were prepared from the hydroxylated PCBs by reaction with 2,2,2-trichloroethyl chlorosulfate in the presence of DMAP as base.[5-9] The resulting 2,2,2-trichloroethyl protected PCB sulfates were subsequently deprotected with zinc powder/ammonium formate yielded the ammonium salts of the desired PCB sulfate.[5-9] 2,2',5,5'-Tetrachlorobiphenyl-4-ol (4 OH PCB52) and the corresponding sulfate monoester (4 PCB52 sulfate) were synthesized and characterized as described in detail below (Scheme S1; see Figures S1-S5 for the corresponding spectra).

Silica gel for column chromatography (40–64 μm) for the purification of all PCB derivatives was obtained from Sorbent Technologies (Atlanta, GA, USA). Melting points were determined using a MelTemp apparatus and are uncorrected. All ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DRX-400 spectrometer in the University of Iowa Central NMR Research Facility (Iowa City, IA, USA) using tetramethylsilane (TMS) as an internal standard. Combustion analyses were performed by Atlantic Microlab Inc. (Atlanta, GA, USA). The purity of all PCB intermediates was determined either using an Agilent 6890 gas chromatograph (Agilent Technologies, CA, USA) equipped with flame ionization detector (FID) or an Agilent 6890N gas

chromatograph coupled with an Agilent 5975 Mass spectrometer (Agilent Technologies).[10] Both instruments were equipped with a HP-5 MS or equivalent column (5 % Phenyl Methyl Silox, 30 m x 250 μM x 0.25 μm). The following temperature program used for the gas chromatographic analysis: starting temperature, 80 °C; hold at 80 °C for 1 minute; 10 ºC/min to 150 ºC; 5 ºC/min to 280 ºC; hold at 280 ºC for 6 minutes; 10 ºC/min to 300 ºC; injector temperature, 280 °C; detector temperature, 280 °C. Accurate mass determinations were performed by the High Resolution Mass Spectrometry Facility of the University of Iowa (Iowa City, IA, USA).

Synthesis of 2,2',5,5'-tetrachloro-4-methoxybiphenyl

A mixture of 2,5-dichloro-4-iodoanisole[11] (2.0 g, 6.7 mmol), 2,5-dichlorobenzene boronic acid (1.26 g, 6.7 mmol), $Pd_2(dba)_3$ (122 mg, 0.134 mmol), 2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl (83 mg, 0.2 mmol) and K_3PO_4 (4.2 g, 20 mmol) in 10 mL of toluene was heated to 100 ºC for approximately 16 h as described earlier.[2] The reaction mixture was allowed to cool to room temperature and diluted with 50 mL of ethyl acetate. The organic phase was washed with water and brine, dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel with hexane-ethyl acetate (20:1, *v/v*) as eluent to give 1.8 g (85% yield) of 2,2',5,5'-tetrachloro-4-methoxybiphenyl as an off-white powder. M.P.: 91-92 ºC. ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 7.41 (d, 1H, J = 8.6 Hz), 7.32 (dd, 1H, J = 2.4 Hz, J = 8.6 Hz), 7.28 (s, 1H), 7.27 (d, J = 2.4 Hz, 1H), 7.05 (s, 1H), 3.05 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD): δ/ppm 155.4, 138.4, 132.4, 132.3, 131.8, 131.3, 130.6, 130.0, 129.5, 121.0, 113.1, 56.5. MS (EI, 70eV): *m/z* calcd. for $C_{13}H_8Cl_4O$, 319.9, found 320.0 ($M^{\bullet+}$). Anal calcd for $C_{13}H_8Cl_4O$, C 48.49; H 2.50. Found: C 49.02, H 2.41.

Synthesis of 2,2',5,5'-tetrachlorobiphenyl-4-ol (4 OH PCB52)

BBr_3 (1 M in heptane, 14 mL, 14 mmol) was added slowly to a solution of 2,2',5,5'-tetrachloro-4-methoxybiphenyl (2.2.6 g, 7 mmol) in 30 mL of dichloromethane and the solution was stirred at room temperature for 16 h.[3] The reaction mixture was quenched with water, the organic layer was washed with water and brine, dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel with hexane-ethyl acetate (10:1, *v/v*) as eluent to give 1.94 g (90% yield) of 2,2',5,5'-tetrachlorobiphenyl-4-ol as a white powder. M.P. 104-105 ºC. ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 7.41 (d, 1H, *J* = 8.6 Hz), 7.32 (dd, 1H, *J* = 2.4 Hz, *J* = 8.6 Hz), 7.27 (d, *J* = 2.4 Hz, 1H), 7.25 (s, 1H), 7.19 (s, 1H), 5.69 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ/ppm 151.8, 138.3, 133.0, 132.4, 132.3, 131.3, 130.6, 130.5, 130.4, 129.5, 118.3, 117.2. MS (EI, 70eV): *m/z* calcd. for $C_{12}H_6Cl_4O$, 305.9, found 306.0 ($M^{\bullet+}$). Anal calcd for $C_{12}H_6Cl_4O$, C 46.80; H 1.96. Found: C 46.93, H 1.86.

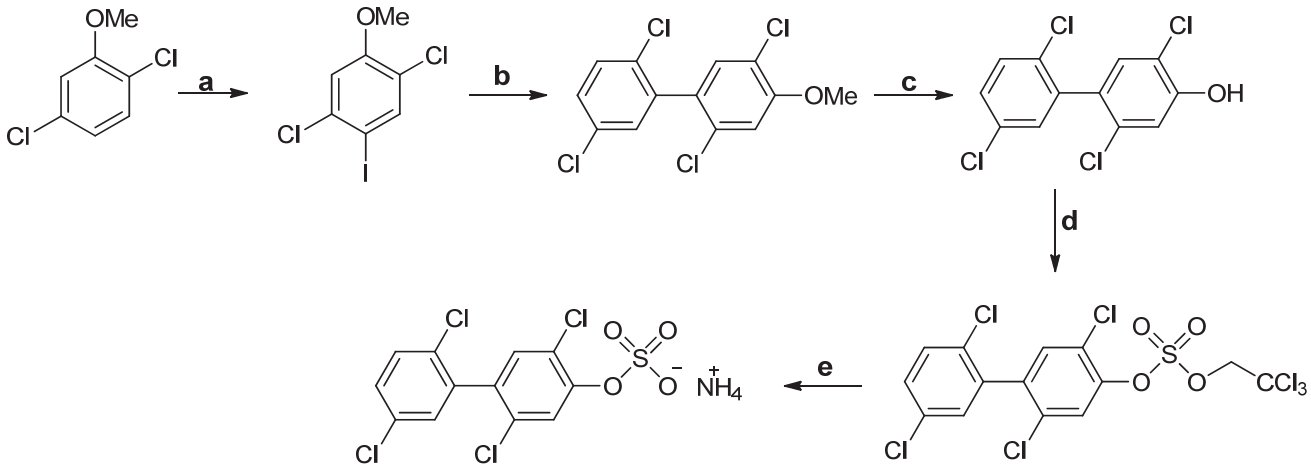
Synthesis of 2,2',5,5'-tetrachlorobiphenyl-4-yl 2,2,2-trichloroethyl ester

2,2,2-Trichloroethyl chlorosulfate[12] (0.75 g, 3 mmol) was added slowly to a mixture of 2,2',5,5'-tetrachlorobiphenyl-4-ol (0.77 g, 2.5 mmol) and DMAP (0.37 g, 3 mmol) in 10 mL of dichloromethane following a published procedure.[5,6] The reaction mixture was stirred at room temperature until the starting material was consumed (approximately 2 h). The reaction mixture was diluted with dichloromethane (50 mL), the organic layer was washed with water and brine, dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with hexane-ethyl acetate (20:1) as eluent to give 1.35 g (87% yield) of 2,2',5,5'-tetrachlorobiphenyl-4-yl 2,2,2-trichloroethyl ester as an off-white solid. M.P. 106-107 ºC. ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 7.72 (s, 1H), 7.45 (d, J = 8.4 Hz, 1H), 7.44 (s, 1H), 7.38 (dd, J = 2.4 Hz, *J* = 8.6 Hz, 1H), 7.27 (d, *J* = 2.4 Hz, 1H), 4.98 (s, 2H). ^{13}C NMR (100 MHz, CD_3OD): δ/ppm 145.6, 137.9, 137.2, 133.1, 132.78, 132.76, 131.8, 130.9, 130.8, 130.3,

125.2, 92.1, 80.9. MS (EI, 70eV): *m/z* calcd. for $C_{14}H_7Cl_7O_4S$, 515.79, found 515.7 ($M^{\bullet+}$). Anal calcd for $C_{14}H_7Cl_7O_4S$, C 32.37; H 1.36, S 6.17. Found: C 32.47, H 1.36, S 6.00.

Synthesis of sulfuric acid mono-(2,2',5,5'-tetrachlorobiphenyl-4-yl) ester, ammonium salt (4 PCB52 sulfate)

Zinc dust (250 mg, 3.85 mmol) was added to a solution of PCB52 TCE diester sulfate (1.0 g, 1.92 mmol) and ammonium formate (0.73 g, 3.85 mmol) in 20 mL of methanol and the reaction mixture was stirred at room temperature until all starting material had been consumed.[5,6] The solvent was evaporated and the crude product was further purified by column chromatography on silica gel with DCM-MeOH-NH_3/H_2O (10 : 2 : 0.1, *v/v*) as eluent to give 635 mg (82% yield) of 4 PCB52 sulfate as a white solid. M.P. 101-103 ºC (Dec.). ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 7.81 (s, 1H), 7.49 (d, J = 8.6 Hz, 1H), 7.42 (d, J = 8.6 Hz, 1H), 7.36 (s, 1H), 7.32 (d, J = 2.3 Hz, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ/ppm 150.6, 139.7, 135.2, 133.7, 133.3, 132.81, 132.83 132.20, 132.0, 131.00, 126.0, 124.1. HRMS–ESI: *m/z* $[M\text{-}NH_4]^-$, calcd. for $C_{12}H_5Cl_4O_4S$: 384.8668, found 386.8632.



Scheme S1. Synthesis of sulfuric acid mono-(2,2',5,5'-tetrachlorobiphenyl-4-yl) ester, ammonium salt (4 PCB52 sulfate): (a) I_2, $AgBF_4$, 10h, 86%; (b) 2,5-dichloroboronic acid, $Pd_2(dba)_3$,DPDB, K_3PO_4, Toluene, 10h, 85%; (c) BBr_3, DCM, 10h, 90%; (d) 2,2,2-trichloroethyl sulfonyl chloride, DMAP, DCM, 2h, 87%; (e) Zn, $HCOO_2NH_4$, MeOH, 1h, 82%.

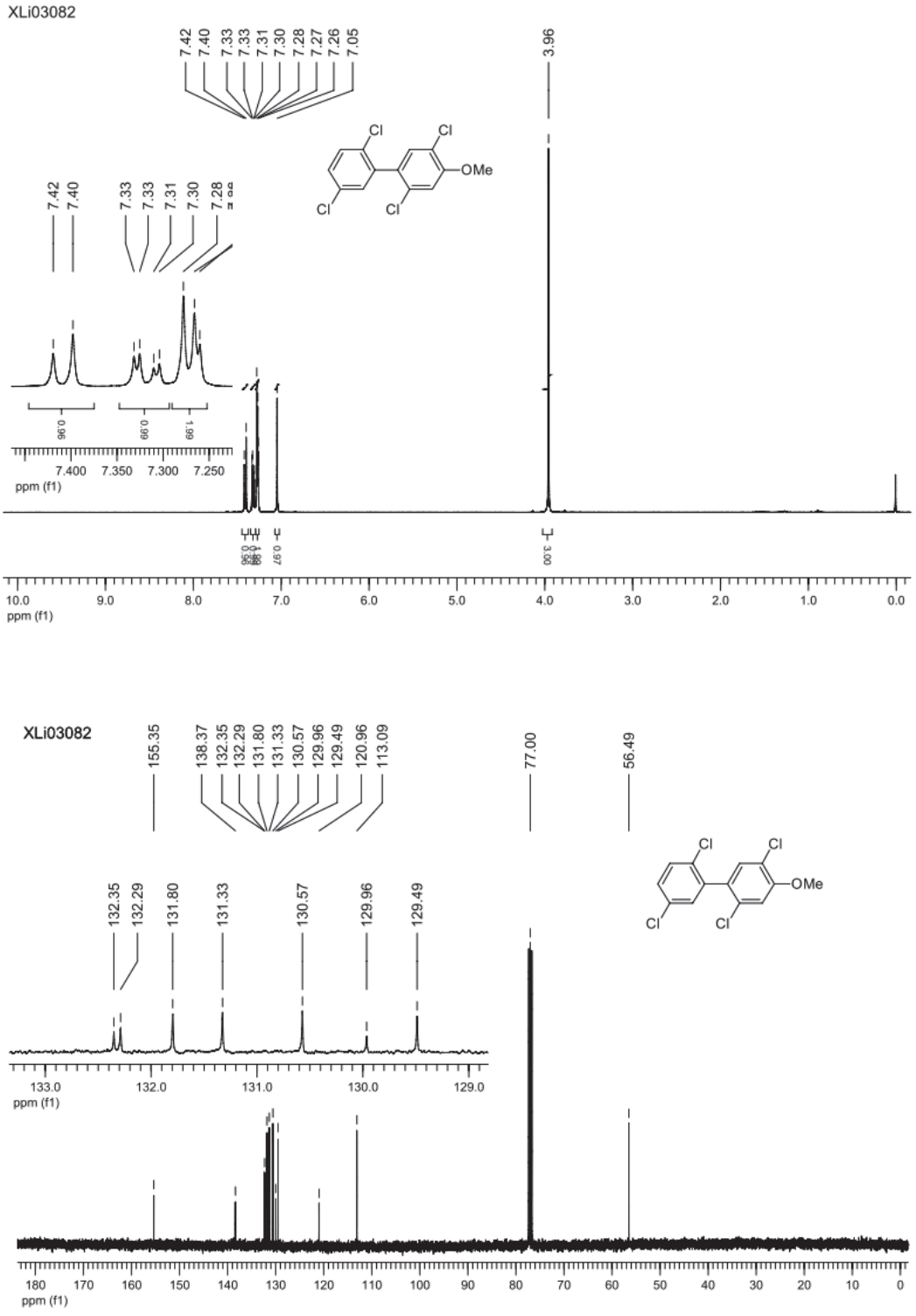


Figure S1. ^{1}H and ^{13}C NMR spectra of 2,2',5,5'-tetrachloro-4-methoxybiphenyl.

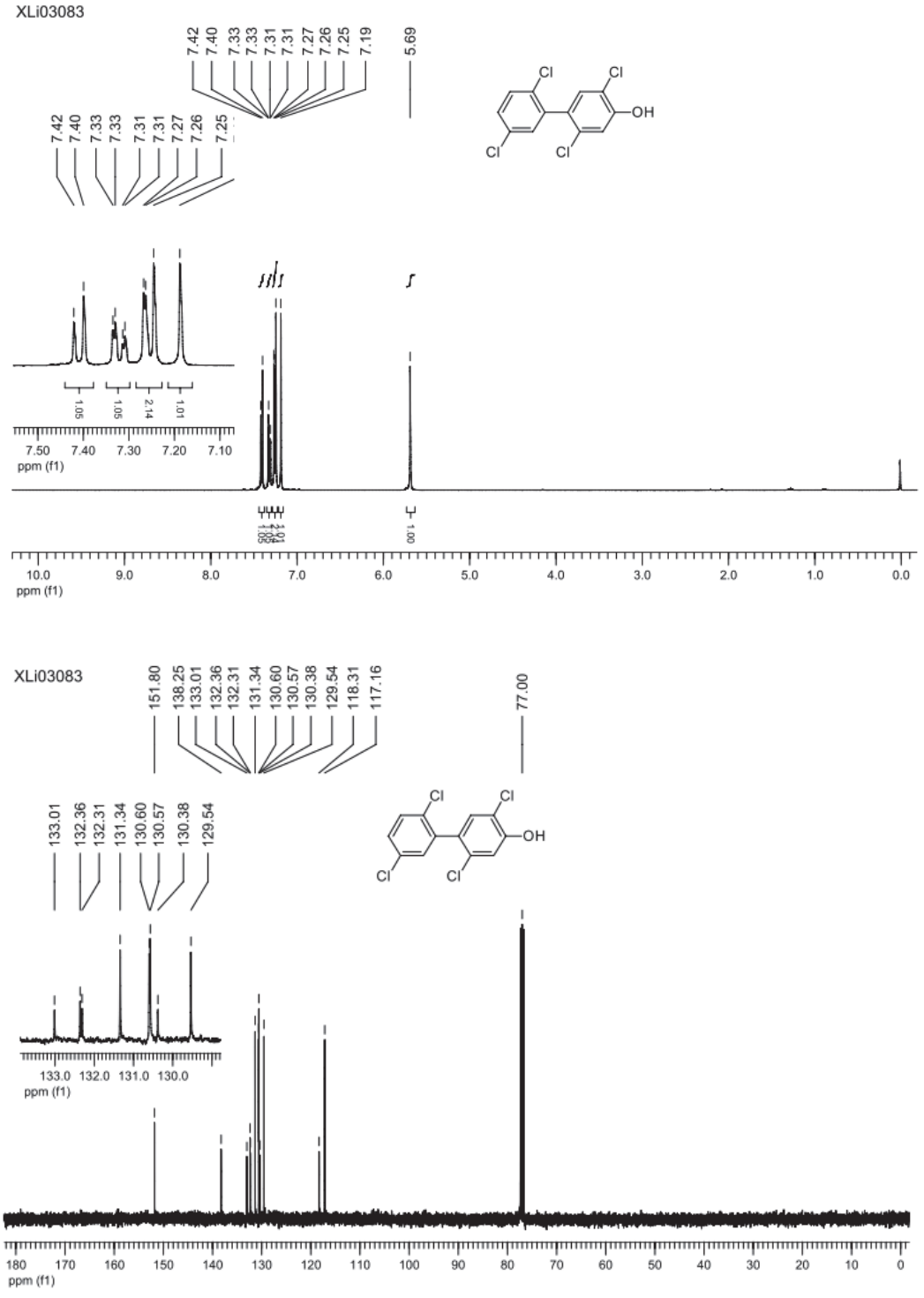


Figure S2. ^{1}H and ^{13}C NMR spectra of 2,2',5,5'-tetrachlorobiphenyl-4-ol (4 OH PCB 52).

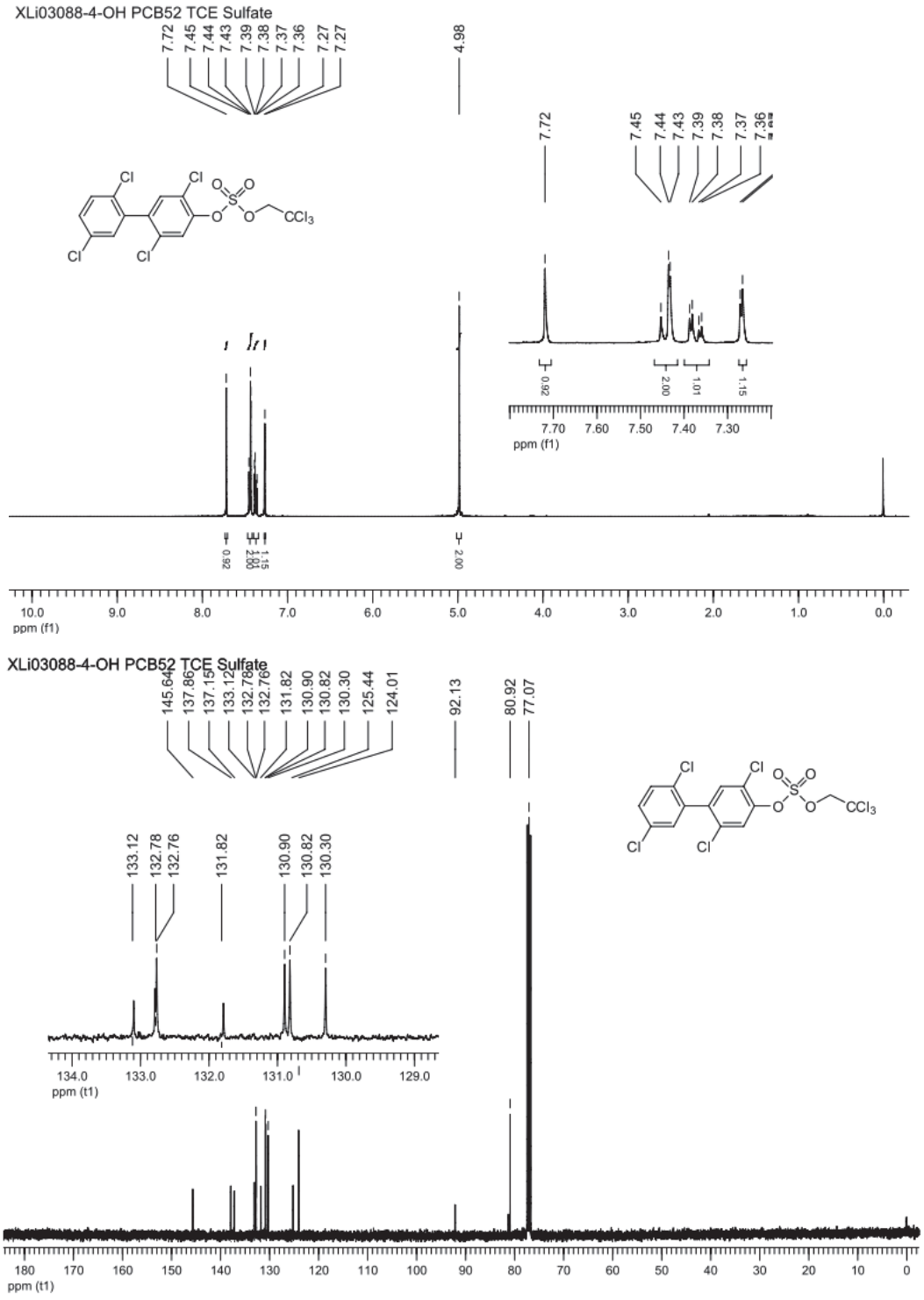


Figure S3. ^{1}H and ^{13}C NMR spectra of 2,2',5,5'-tetrachlorobiphenyl-4-yl 2,2,2-trichloroethyl ester.

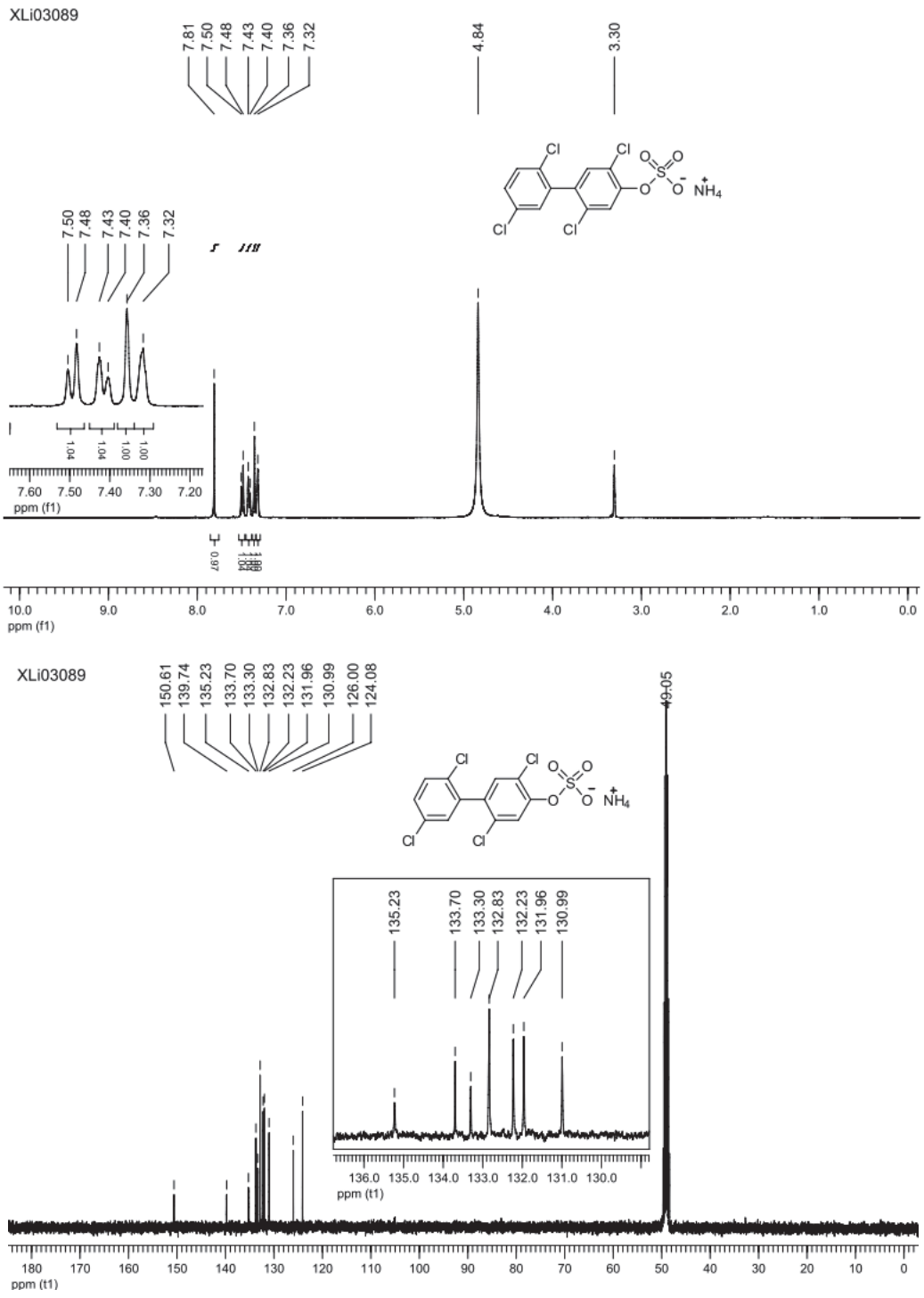
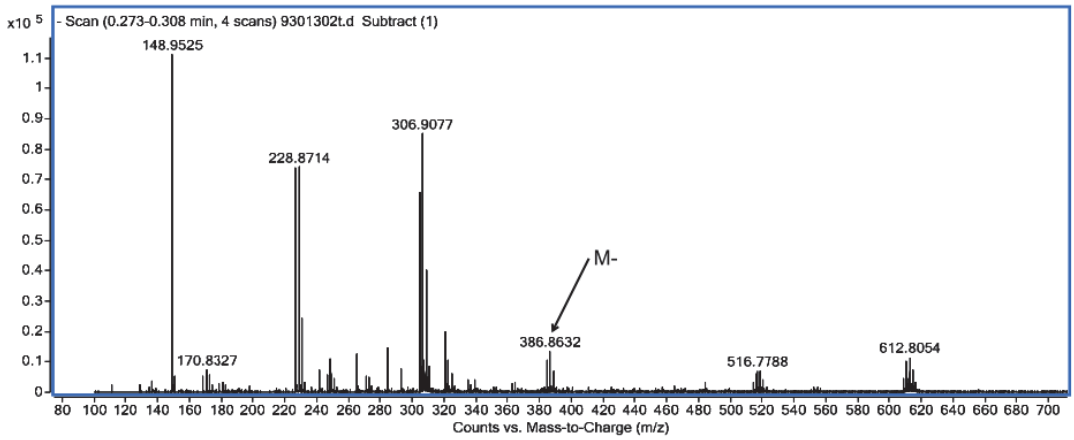
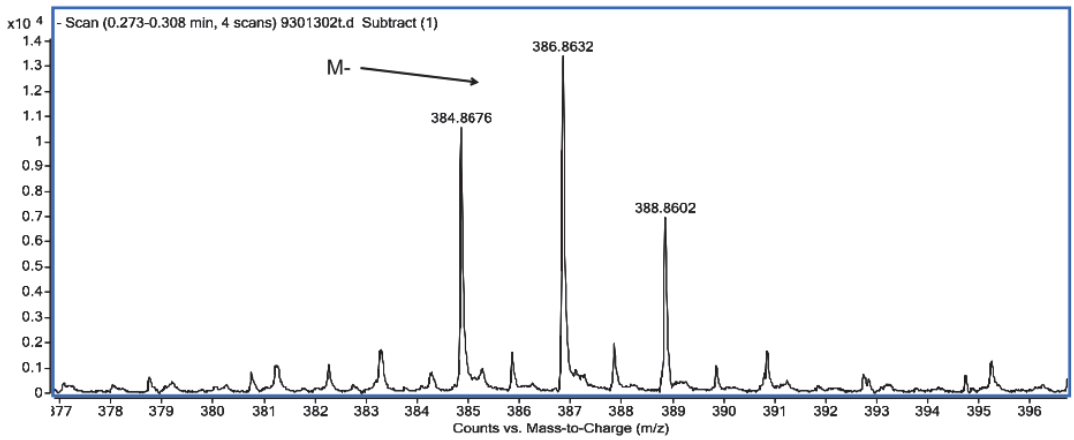


Figure S4. ^{1}H and ^{13}C NMR spectra of sulfuric acid mono-(2,2',5,5'-tetrachlorobiphenyl-4-yl) ester, ammonium salt (4 PCB52 sulfate).

XLi03089





Measured Mass	384.8676	
Element	Low Limit	High Limit
C	7	17
H	0	20
O	2	6
S	0	2
35Cl	3	5

Formula	Calculated Mass	mDaError	ppmError	RDB
C12 H5 O4 S 35Cl4	384.8668	0.8	2.0	8.5

Figure S5. Accurate mass determination of sulfuric acid mono-(2,2',5,5'-tetrachlorobiphenyl-4-yl) ester, ammonium salt (4 PCB52 sulfate).

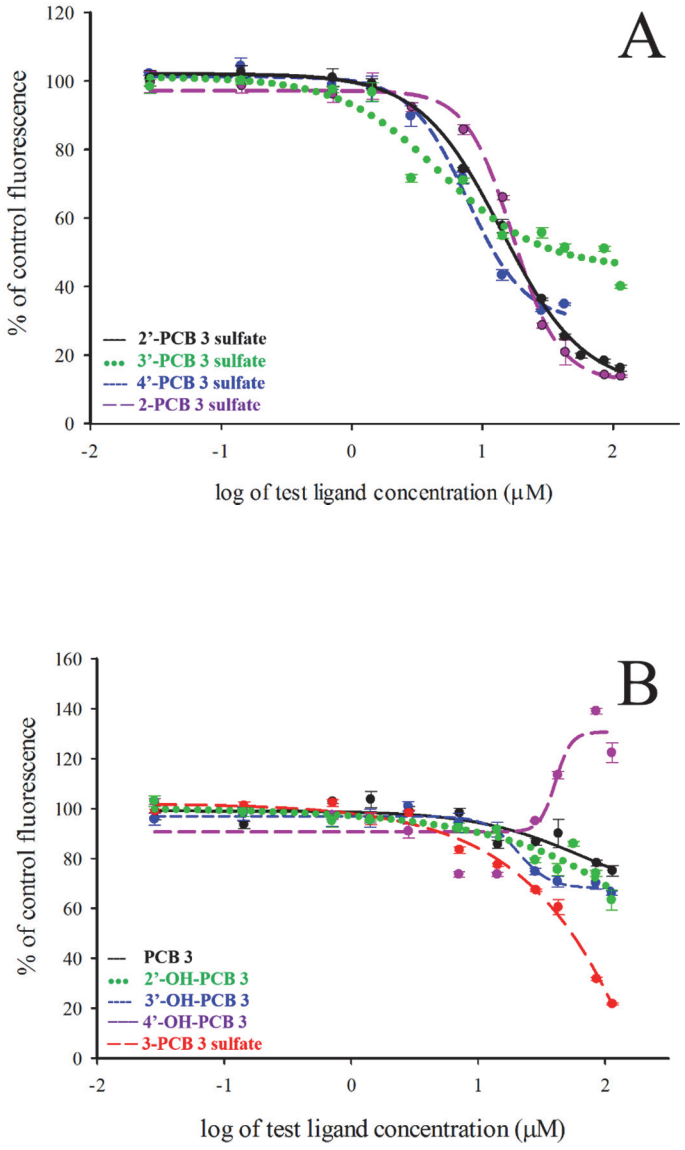


Figure S6. Site I binding curves of monochlorinated LC-PCBs, OH-LC-PCBs, and LC-PCB sulfates plotted as percent of control fluorescence vs. increasing ligand concentration. A) ligands exhibiting dose dependent displacement of the fluorescent probe. B) ligands not exhibiting dose-dependent displacement of the fluorescent probe. Each experiment consists of 10μM HSA and 20μM DNSA. Data were fit to a sigmoidal dose response ligand-binding algorithm (SigmaPlot v.11.0, Systat Software, Chicago, IL) and EC_{50} values are reported in Figure 3-7. Mean ± SE, n=3

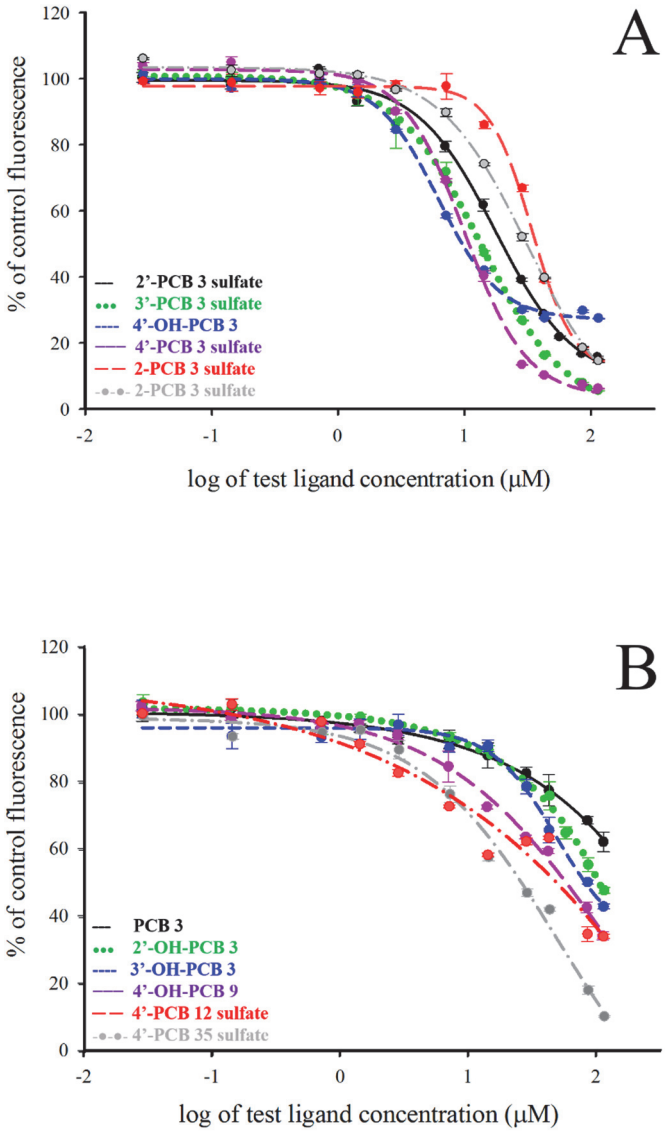


Figure S7. Site II binding curves of LC-PCBs, OH-LC-PCBs, and LC-PCB sulfates plotted as percent of control fluorescence vs. increasing ligand concentration. A) monochlorinated compounds exhibiting dose dependent displacement of the fluorescent probe. B) all ligands not exhibiting dose-dependent displacement of the fluorescent probe from Site II. Each experiment consists of 10μM HSA and 5μM DP. Data were fit to a sigmoidal dose response ligand-binding algorithm (SigmaPlot v.11.0, Systat Software, Chicago, IL) and EC_{50} values are reported in Figure 3-7. Mean ± SE, n=3

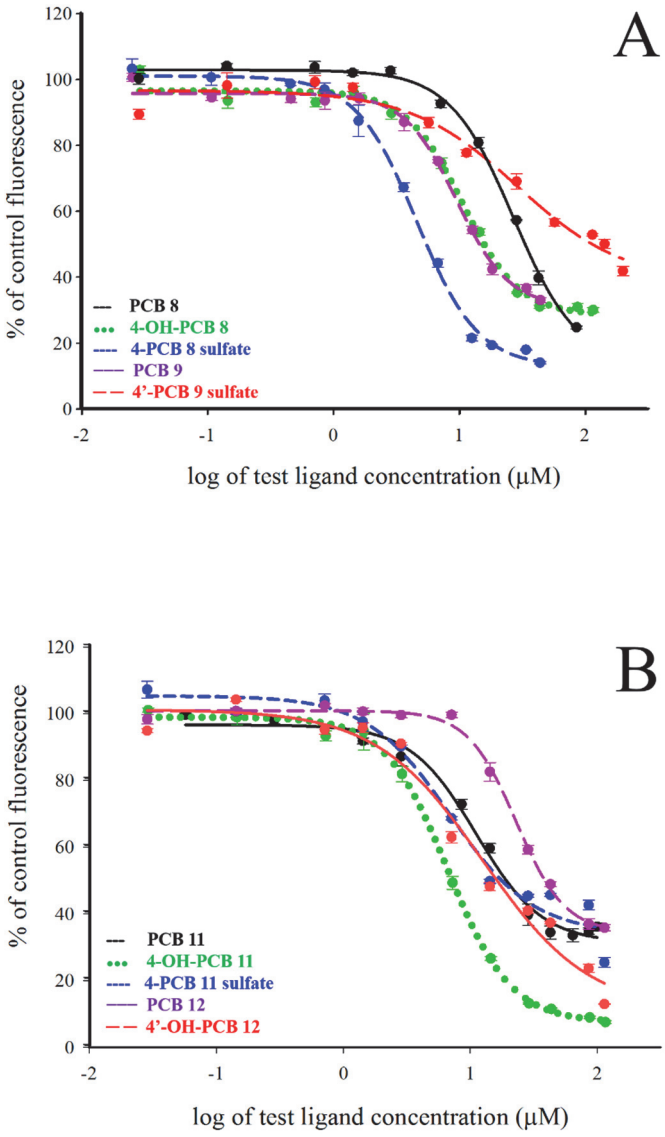


Figure S8. Site II binding curves of dichlorinated LC-PCBs, OH-LC-PCBs, and LC-PCB sulfates plotted as percent of control fluorescence vs. increasing ligand concentration. A and B) ligands exhibiting dose dependent displacement of the fluorescent probe. Each experiment consists of 10μM HSA and 5μM DP. Data were fit to a sigmoidal dose response ligand-binding algorithm (SigmaPlot v.11.0, Systat Software, Chicago, IL) and EC_{50} values are reported in Figure 3-7. Mean ± SE, n=3

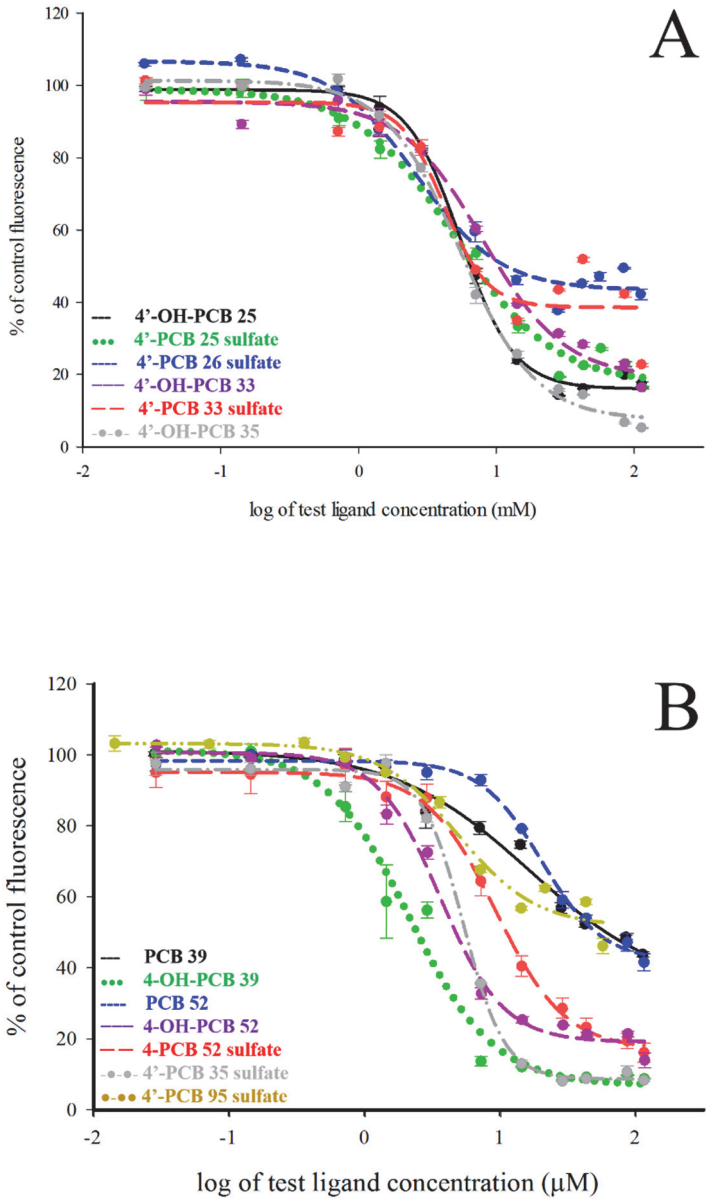


Figure S9. Site II binding curves of tri-, tetra-, and pentachlorinated LC-PCBs, OH-LC-PCBs, and LC-PCB sulfates plotted as percent of control fluorescence vs. increasing ligand concentration. A and B) ligands exhibiting dose dependent displacement of the fluorescent probe. Each experiment consists of 10µM HSA and 5µM DP. Data were fit to a sigmoidal dose response ligand-binding algorithm (SigmaPlot v.11.0, Systat Software, Chicago, IL) and EC_{50} values are reported in Figure 3-7. Mean ± SE, n=3

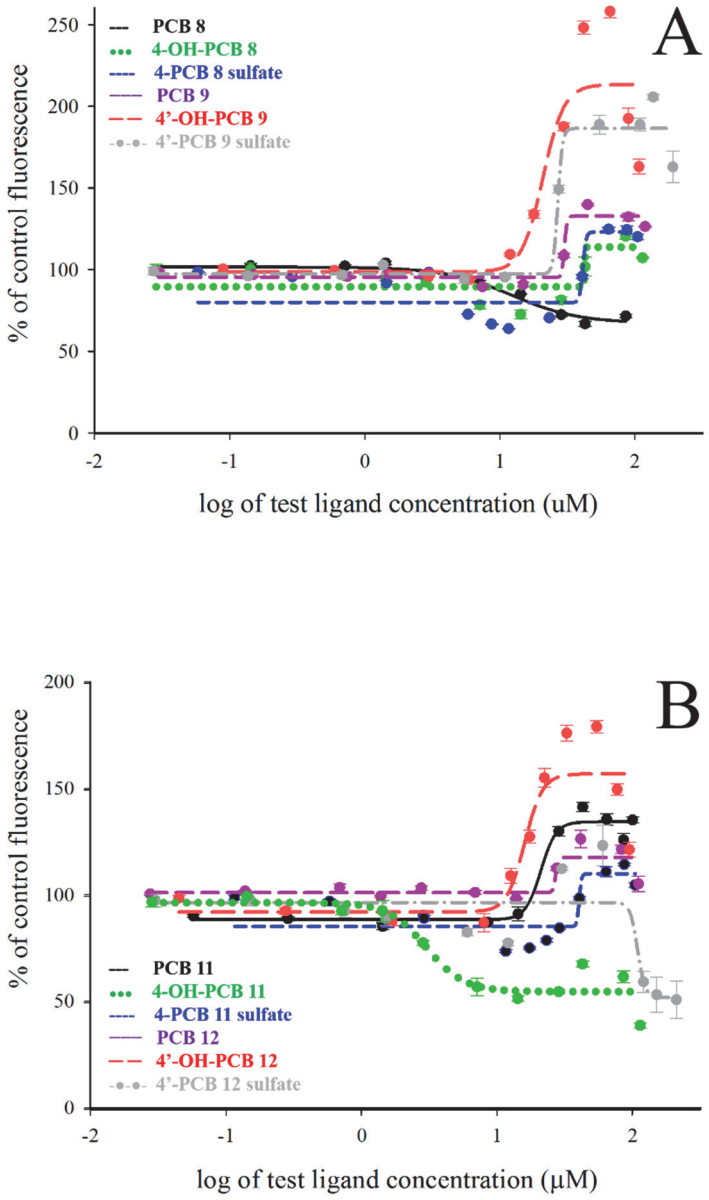


Figure S10. Site I binding curves of dichlorinated LC-PCBs, OH-LC-PCBs, and LC-PCB sulfates plotted as percent of control fluorescence vs. increasing ligand concentration. A and B) ligands not exhibiting dose-dependent displacement of the fluorescent probe from Site I. Each experiment consists of 10µM HSA and 20µM DNSA. Data were fit to a sigmoidal dose response ligand-binding algorithm (SigmaPlot v.11.0, Systat Software, Chicago, IL) and EC_{50} values are reported in Figure 3-7. Mean ± SE, n=3

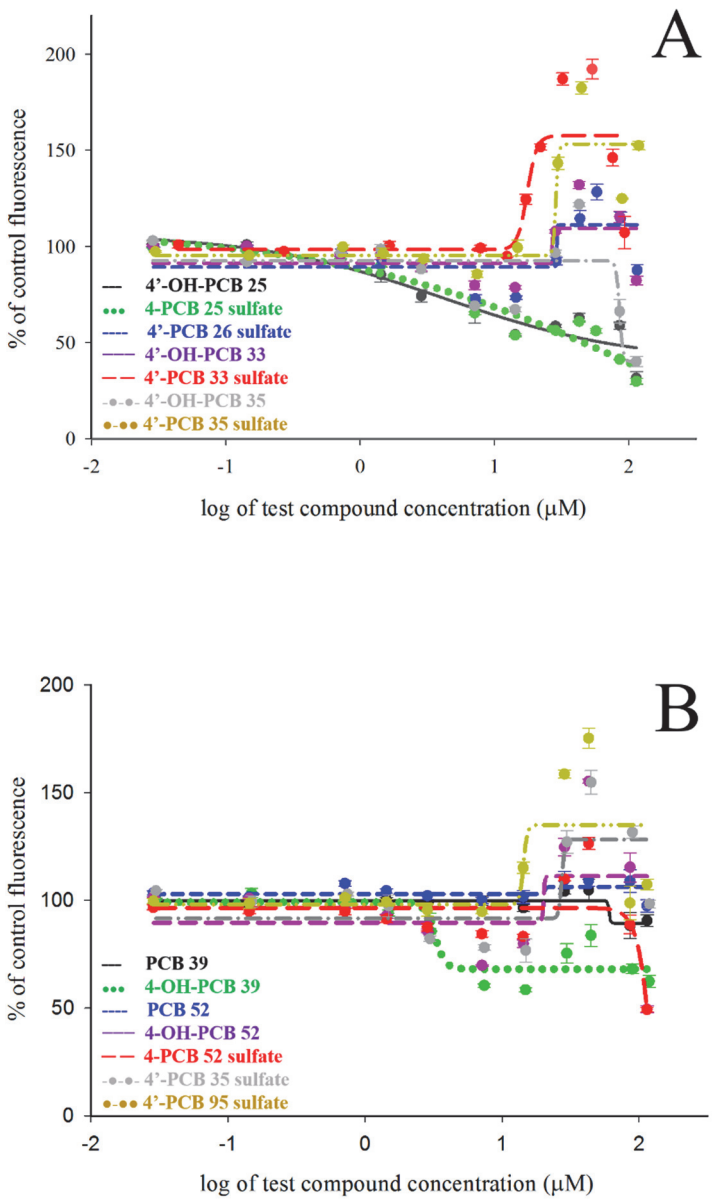


Figure S11. Site I binding curves of tri-, tetra-, and pentachlorinated LC-PCBs, OH-LC-PCBs, and LC-PCB sulfates plotted as percent of control fluorescence vs. increasing ligand concentration. A and B) ligands not exhibiting dose-dependent displacement of the fluorescent probe from Site I. Each experiment consists of 10μM HSA and 20μM DNSA. Data were fit to a sigmoidal dose response ligand-binding algorithm (SigmaPlot v.11.0, Systat Software, Chicago, IL) and EC_{50} values are reported in Figure 3-7. Mean ± SE, n=3

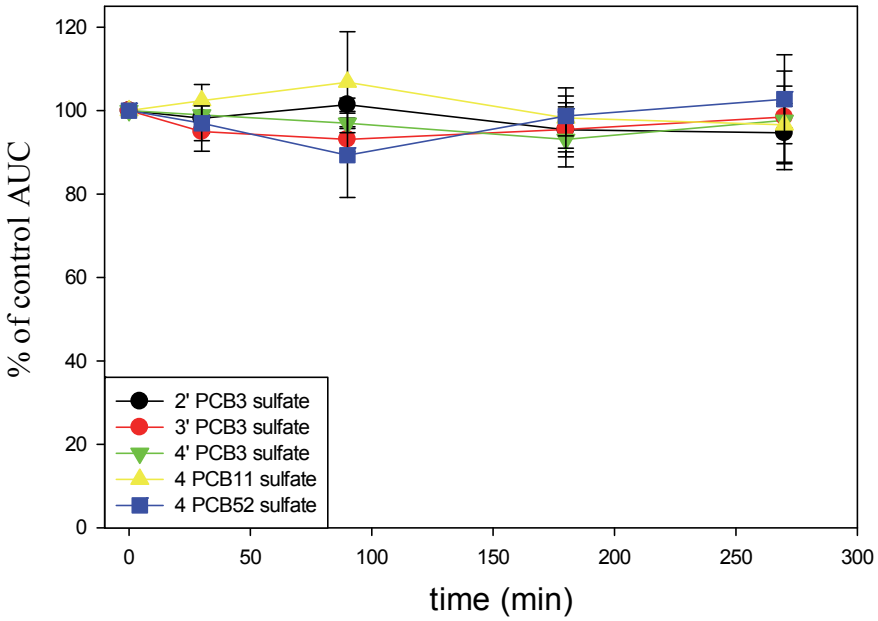


Figure S12: HPLC analysis of the recovery and reversibility of HSA-binding for representative PCB sulfates. HSA (50µM) and LC-PCB sulfate (50µM) incubated in potassium phosphate buffer (pH=7.4) and extracted into acetonitrile and analyzed by HPLC. Data are presented as Mean ± SE.

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