In Vitro Metabolism of the Brominated Flame Retardants 2-Ethylhexyl-2,3,4,5-Tetrabromobenzoate (TBB) and Bis(2-Ethylhexyl) 2,3,4,5-Tetrabromophthalate (TBPH) in Human and Rat Tissues

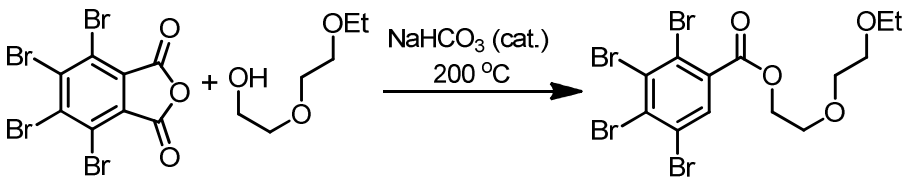
Simon C. Roberts[1], Laura J. Macaulay[1], and Heather M. Stapleton[1*]

Nicholas School of the Environment, Duke University, Durham, NC 27708

*Corresponding Author: heather.stapleton@duke.edu

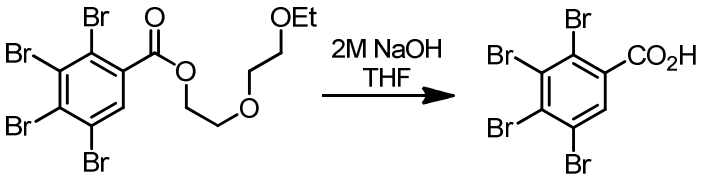
Supplemental Information.

***Synthesis of 2-(2-ethoxyethoxy)ethyl-2,3,4,5-tetrabromobenzoate*:** A mixture of 3,4,5,6-tetrabromophthalic anhydride (967 mg, 2 mmol) and diethylene glycol monoethyl ether (1.35 g, 10 mmol) and solid sodium bicarbonate (16.8 mg, 0.2 mmol) was gradually heated to 200°C (oil bath temperature) for 16 h. The reaction mixture was cooled to room temperature and partitioned between water (20 mL) and ethyl acetate (20 mL). The aqueous layer was extracted with ethyl acetate (20 mL). The combined organic extracts were washed with water (2×20 mL) and brine (20 mL) and dried over anhydrous sodium sulfate. The drying agent was removed by filtration. Silica gel (2 g) was added to the filtrate, and the mixture was concentrated to dryness under reduced pressure. Flash column chromatography (RediSepR_f SiO_2 (24 g), ethyl acetate/hexane) gave 2-(2-ethoxyethoxy)ethyl-2,3,4,5-tetrabromobenzoate as a colorless thick liquid (850 mg, 77% yield). 1H NMR (300 MHz, $CDCl_3$) **δ** 7.88 (s, 1H), 4.50 (t, J = 4.8 Hz, 2H), 3.83 (t, *J* = 4.8 Hz, 2H), 3.58-3.72 (m, 4H), 3.53 (q, *J* = 7.0 Hz, 2H), 1.21 (t, *J* = 7.0 Hz, 3H).

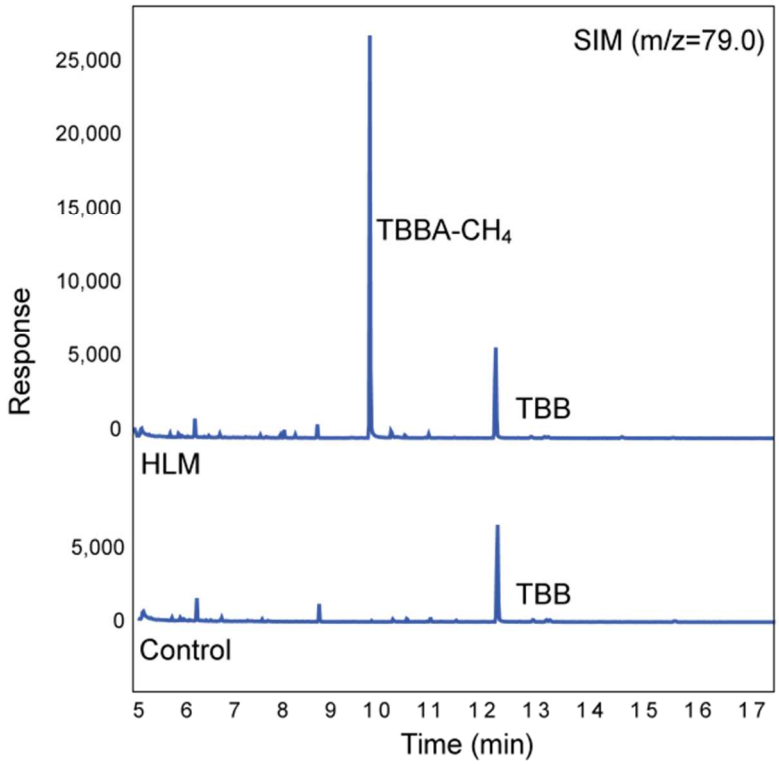


Supplemental Figure 1: Synthesis of 2-(2-ethoxyethoxy)ethyl-2,3,4,5-tetrabromobenzoate from 3,4,5,6-tetrabromophthalic anhydride and diethylene glycol monoethyl ether.

Synthesis of 2,3,4,5-tetrabromobenzoic acid: A solution of 2-(2-ethoxyethoxy)ethyl-2,3,4,5-tetrabromobenzoate (780 mg, 1.41 mmol) in THF (10 mL) was combined with 2 M NaOH (2.5 mL), and the mixture was stirred at room temperature. After 2 h, the reaction mixture was acidified with 1 M HCl to pH 3-4 (universal pH paper). The mixture was then partitioned between water (25 mL) and ethyl acetate (25 mL). The aqueous layer was extracted with ethyl acetate (20 mL). The combined organic extracts were washed with water (3×30 mL) and brine (20 mL) and dried over anhydrous sodium sulfate. The drying agent was removed by filtration. Silica gel (1 g) was added to the filtrate, and the mixture was concentrated to dryness under reduced pressure. Flash column chromatography (RediSepR_f SiO_2 (24 g), CH_2Cl_2/MeOH) gave 2,3,4,5-tetrabromobenzoic acid as a white solid (500 mg, 81% yield). 1H NMR (400 MHz, DMSO-d_6); single peak: **δ** 8.03 (s, 1H). MS (ESI): 438 $[M+H]^+$.



Supplemental Figure 2: Synthesis of 2,3,4,5-tetrabromobenzoic acid from 2-(2-ethoxyethoxy)ethyl-2,3,4,5-tetrabromobenzoate.



Supplemental Figure 3. GC/MS SIM chromatograms of methyl-derivatized extracts of incubations with HLM and without HLM (Ctrl) and TBB showing the formation of TBBA and the absence of other brominated potential metabolites.