Distribution and speciation of bromine in mammalian tissue and fluids by X-ray fluorescence imaging and X-ray absorption spectroscopy – Supplementary data.

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Supplementary Figure S1: XRF spectrum of a bovine ovarian section (green) showing fit (red) and background (violet). The K lines of Br are marked in orange.

Supplementary Experimental

TBBPA analysis of ovarian tissue by LC-MS/MS

The extraction method was modified from that previously reported by Johnson-Restrepo *et al.* $(2008)^1$. Approximately 2 g of wet mass ovarian tissue was ground with anhydrous sodium sulphate and extracted with dichloromethane and hexane (3:1; 400 mL) for 16 h in a Soxhlet apparatus.

Liquid chromatography separations occurred using a Surveyor MS plus pump consisting of four solvent pumps, a Surveyor autosampler plus and a Rheodyne injector with a 25 μ L loop. Separation was carried out at 30°C on a RESTEK ultra IBD column (3 μ m, 100 x 2.1 mm, Restek Bellefonye, PA USA) with a C18 guard column (4 mm × 4 mm i.d., Restek). 10 μ L of reconstituted sample (0.5 mL acetonitrile) was injected onto the column. The mobile phase used was (A) acetonitrile and (B) a mixture of 0.1% formic acid and 10 mM ammonium formate, at a flow rate of 0.25 mL/min. The gradient started with 5% of solvent A, increased to 95% in 2 min, kept isocratic for 6 min, and then returned to initial conditions within 0.1 min, the column was then equilibrated for 15 min before injecting the next sample.

Identification and quantification of TBBPA was carried out with a Thermo-FINNIGAN TSQ triple quadrupole mass spectrometer (Thermo-FINNIGAN, USA), fitted with ESI interface operating in negative mode. The capillary temperature was 350°C and spray voltage was 4000 V. Nitrogen was used as both drying and nebulising gas and argon were used as the collision gas. The collision voltage, as well as other MS parameters, were optimised by direct infusion of TBBPA and HBCD standards in acetonitrile at a flow rate of 5 μ L/min. Multiple Reaction Monitoring (MRM) mode was used for MS detection. The parent ions [M-H]- and their respective product ions were monitored at the following transitions: 540.78 > 419.9 and 540.78 > 445.8 for TBBPA and 640.30 > 78.90 and 640.30 > 81 for HBCD.

1. B. Johnson-Restrepo, D. H. Adams and K. Kannan, *Chemosphere*, 2008, 70, 1935-1944.

The following figures depict the Compton scatter maps corresponding to Figures 1 and 2 in the main text, respectively. Presenting this data is designed to give the reader an appreciation for the density of the imaged ovarian tissues and how cell density can correspond to perceived elevation of analysed elements, in particular Br.



Supplementary Figure S2: XRF generated Compton map for Figure 1. Scale bar: 300 µm.



Supplementary Figure S3: XRF generated Compton map for Figure 2. Scale bar: 200 μ m.

The following chromatograms depict three TBBPA calibration standards (1, 10 and 100 μ g/L) (Figs. S4 to S6), a sample blank (Fig. S7), a spiked (100 μ g/L TBBPA)-sample (Fig. S8) and two bovine ovarian samples (R1 and R2) (Figs. 9 and 10). Neither TBBPA nor hexabromocyclododecane (HBCD) were detected in the ovarian tissues.



Supplementary Figure S4: Plot of relative abundance versus time of the 1 μ g/L TBBPA calibration standard



Supplementary Figure S5: Plot of relative abundance versus time of the 10 $\mu\text{g/L}$ TBBPA calibration standard .



Supplementary Figure S6: Plot of relative abundance versus time of the 100 $\mu g/L$ TBBPA calibration standard .



Supplementary Figure S7: Plot of relative abundance versus time for the sample blank.



Supplementary Figure S8: Plot of relative abundance versus time for the spiked (100 µg/L TBBPA) sample.



Supplementary Figure S9: Plot of relative abundance versus time for the bovine sample R1 indicating no TBBPA was detected.



Supplementary Figure S10: Plot of relative abundance versus time for the bovine sample R2 indicating that no TBBPA was detected