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Literature Review assignment on Brominated flame retardants and legacy organochlorines in archived human placenta samples: Sex differences, temporal analysis and associations with infant birth weight.

CHEM 3140

March 11, 2024

Ruis, M.; Hoffman, K.; Stapleton, H. M. Brominated Flame Retardants and Legacy Organochlorines in Archived Human Placenta Samples: Sex Differences, Temporal Analysis and Associations with Infant Birth Weight. *Chemosphere* **2023**, *322*, 138170

<https://doi-org.ezproxy.tru.ca/10.1016/j.chemosphere.2023.138170>

Introduction and Analytical Question

The goal of this research paper was quantitatively determine placental Persistent Organic pollutants (POP) and Brominated flame retardants (BFR) concentrations and how these concentrations differ with fetal sex differences or temporal changes in POP levels.

The analytical question:

Can POP and BFR concentrations be quantitatively determined in placental tissues collected over the period of 2009-2015 and how do the concentrations vary over time.

Several classes of POPs were examined in these placental tissues including polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and several organochlorine pesticides. I decided to focus on the brominated flame-retardant class of organic pollutants. BFRs are not only located in firefighting applications, but they can also be found on various consumer products including furniture and electronics to prevent fires from starting. The current commercial flame retardant used is called Firemaster 550 (FM550) which includes POPs such as 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB) and Bis(2-ethylhexyl)tetrabromophthalate (BEH-TBPH). The FM550 replaced an old commercial mixture called PentaBDE which was phased out beginning in 2005 because of concern of its long-term impacts. The EH-TBB and BEH-TBPH are the two BFRs which were quantified in this study. BFRs have recently been identified as POPs due to their capability of long-range transport and bioaccumulate within ecosystems. They have also been associated with adverse health effects including endocrine disruption and neurodevelopment delays.

Analytical Method

Sampling

- Placental tissues were collected during routine births at the Duke University Medical center in Durham, NC between 2009-2015. They were collected through a placenta biobanking program, so the authors did not actually sample the placenta themselves.
- Placental tissues were sub-sampled at delivery to isolate a full-thickness core of 1-2 cm in diameter
 - The placenta was sampled using a coring device to obtain a representative sample of the placenta ensuring all parts are sampled
- Samples were frozen at -80°C
 - Samples were frozen to eliminate any reactions from occurring and to preserve the integrity of the sample
- Samples were stored in screw-top cryovials until analysis
 - Sealed vials to prevent any contamination during storage

Sample Preparation

- The placental tissues (1-2g) were thawed, and their masses were recorded to three decimal places
 - Samples were thawed to be able to work/cut the tissues
 - The masses were recorded to ensure all samples were roughly close in mass and potentially to determine % moisture
- The tissue samples were cut into pieces less than 1cm in size using pre-cleaned, solvent rinsed forceps and dissecting scissors
 - Tissue samples were cut up to ensure homogeneity and making them easier to work with in further steps
 - The forceps and scissors were pre-cleaned, and solvent rinsed to ensure they would not contaminate the sample by potentially introducing analytes
- The cut tissue samples were then crushed using a mortar
 - This was to further homogenize the samples and increase surface area for extraction procedures
- Then ~10g of sodium sulphate was added to the mortar
 - Sodium sulphate is a drying agent so this would be to remove any water content from the samples
- The sample was added to a centrifuge tube and spiked with 10 ng of internal standard mixture
 - Internal standards were used to minimize effects of random and systematic errors introduced during analysis, also helps improve precision of results

- A mixture of Hexane: dichloromethane (1:1 v/v) was added to the centrifuge tube and left overnight
 - This was the extraction step (batch extraction) to extract the analytes from the placental samples. The Hexane: dichloromethane mixture was the organic solvent used for the extraction.
- The solvent-tissue solution was then centrifuged, and the supernatant was collected
 - This was done to separate the tissue from the solvent after the extraction
- The extraction was repeated two more times, and the collected solvent extracts were combined
 - This was done to increase the extraction efficiency and ensure no analytes were left in the tissues after extracting
- The three combined solvent extractions were then concentrated under a stream of nitrogen
 - This was done to reduce the volume of the solution while maintaining the concentration of the analytes
- The concentrated sample was then put through a SupleClean ENVI-Florisil solid phase extraction (SPE) cartridge using hexane: dichloromethane (1:1 v/v) as a solvent
 - This was to clean-up the sample to remove any unwanted interferences present
- Eluent of SPE was concentrated under gentle stream of nitrogen
 - This was again to decrease the volume of the sample while maintaining its concentration
- Solvent was exchanged for hexane
 - Exchange solvent to prepare for gas chromatography
- Sample was spiked with recovery standard ¹³C-CDE-141
 - Samples were spiked with recovery standard to determine the column chromatography cleanup efficiency. Standard was similar to the analytes but contained isotopically labelled carbon

Analysis

- Prepared samples were analyzed using a high-resolution Hybrid Quadropole-Orbitrap (GC-MS/MS) system which was operated in both electron ionization and negative chemical ionization mode
 - Since samples and the solvent are volatile, they could be analyzed by gas chromatography

Quality Control/quality assurance

- Laboratory blanks were used and processed alongside samples
 - These were used to determine any analyte contamination present through the analytical method
 - They were also used to calculate the method detection limits

Critique of the Method

The procedure for this paper was successfully able to quantitatively determine placental Persistent Organic pollutants (POP) and Brominated flame retardants (BFR) concentrations and how these concentrations overlap with fetal sex differences or temporal changes in POP levels. The extraction procedure was very simple and could be performed in most laboratories. The procedure was also done in a way to minimize any potential contaminants as the laboratory blanks only ranged from 0.0004 ng/g to 0.0493 ng/g. There was no mention of specific green chemistry principles within the report, but some could be deduced. For example, the extraction procedure did not use any heat/reflux and was just left to interact overnight which saves energy. Each sample only contained a small mass of placenta, which reduces the need for large extraction volumes of solvent which minimizes waste for this step. All steps within the method contained a useful purpose and was quite simple allowing for the results to be reproduced elsewhere. The one critique I would include for the method involves the use or mention of more blanks within the procedure. The only blanks mentioned were method blanks. Since POPs can be present almost anywhere, I would also include equipment blanks, field blank, reagent blank, trip blank and an instrument blank.