Microplastic Contamination in the Human Placenta

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Abstract

Introduction: The widespread presence of plastics in our environment poses a growing concern as they may pose risks to human and environmental health. Microplastics (MPs) are small particles generated through fragmentation of larger plastic items. The presence of MPs particles has been reported in the human placenta, an organ essential for pregnancy and fetal development. The presence of MP contamination of the womb raises the possibility of adverse effects on the developing fetus with potential life-long consequences. This thesis seeks to investigate this issue through: 1) A review aimed to examine the current state of knowledge on the effects of exposure to MP on maternal and fetal health within the DOHaD framework; 2) A study conducted to confirm and further the reports of microplastics in human placentas through a study, in a Canadian setting, comparing MPs exposure to delivery methods.

Methods: 1) A review was conducted of the current literature on microplastic contamination in human reproductive tissues, and the resulting reproductive consequences of exposure. 2) Placentas (n=10) were collected from singleton, uncomplicated pregnancies. Placentas were collected from vaginal (n=5) and cesarean section (n=5) deliveries within a plastics-reduced clinical setting. Placental tissue biopsies were micro-dissected under plastic-reduced conditions - from basal plate, chorionic villous and chorionic plate. Samples were chemically digested and filtered through glass microfiber filters and the retained particles were identified and characterized using Raman microspectroscopy.

Results: 1) The review reports multiple lines of evidence that suggest that MP-exposure prior to or during pregnancy can contaminate various internal tissues (including those of the fetus) and may result in potential adverse effects on fertility, fetal development and long-term health of the exposed fetus. More importantly, the available evidence is limited and several significant gaps in knowledge were identified. 2) Microplastics composed of various polymer types were detected in placentas from both delivery types (vaginal or caesarian), with polyethylene being the most common. In addition, non-plastic foreign particles including graphite, lead oxide and black carbon were observed in a higher frequency than microplastics. Notably, both microplastic and non-microplastic particles were found in all placentas sampled with variations in the number of particles. Particles both plastic and non-plastic were observed in placenta regions of maternal and fetal circulation suggesting that these can pass through the placenta into fetal tissues.

Conclusion: This thesis provides evidence that the human placenta can serve as a reservoir for the accumulation of a variety of foreign particles during pregnancy. The potential human health impacts of such particles in general or on fetal development, in particular, are unknown but is a critical question for future work to understand the health consequences of plastic pollution.

Keywords: Placenta, Microplastic, Particles, Raman Micro-spectroscopy, Pregnancy

Résumé

Introduction: La présence de plastique dans notre environnement suscite une préoccupation croissante car ils peuvent présenter des risques pour la santé humane et environnementale. Les microplastiques (MPs) sont des petites particules générées par la fragmentation d'objets en plastique plus gros. La présence de particule MPs a été reportée dans le placenta humain, un

organe essentiel à la grossesse et au développement fœtal. La présence d'une contamination dans l'utérus par MPs soulève la possibilité d'effets indésirables sur le développement du fœtus, avec des conséquences potentielles à vie long terme. Cette thèse cherche à étudier cette question à travers : 1) Une revue visant à examiner l'état actuel des connaissances sur les effets de l'exposition aux MP sur la santé maternelle et fœtale dans le cadre du DOHaD ; 2) Une étude menée pour confirmer et approfondir les rapports sur les microplastiques dans le placenta humain, dans un contexte canadien, comparant l'exposition aux méthodes d'accouchement.

Méthodogie: 1) Une revue de la littérature actuelle sur la contamination par les microplastiques dans les tissus reproducteurs humains et les conséquences de l'exposition sur la reproduction a été réalisée. 2) Des placentas (n = 10) ont été collectés lors des grossesses sans complications. Les placentas ont été collectés lors d'accouchements par voie vaginale (n = 5) et par césarienne (n = 5) dans un cadre clinique à réduction de plastique. Des biopsies de tissus placentaires ont été microdisséquées dans des conditions de réduction du plastique - à partir de la plaque basale, des villeuses choriales et de la plaque choriale. Les échantillons ont été digérés chimiquement et filtrés à travers des filtres en microfibre de verre et les particules retenues ont été identifiées et caractérisées par microspectroscopie Raman.

Résultats: 1) La revue rapporte plusieurs sources de données suggérant que l'exposition aux MP avant ou pendant la grossesse peut contaminer divers tissus internes (y compris ceux du fœtus) et entraîner des effets néfastes potentiels sur la fertilité, le développement du fœtus et la santé à long terme du fœtus. Plus important encore, les preuves disponibles sont limitées et plusieurs lacunes importantes dans les connaissances ont été identifiées. 2) Des microplastiques composés

de divers types de polymères ont été détectés dans les placentas des deux types d'accouchement (vaginal ou césarienne), le polyéthylène étant le plus courant. De plus, des particules étrangères non plastiques, notamment du graphite, de l'oxyde de plomb et du noir de carbone, ont été observées à une fréquence plus élevée que les microplastiques. Notamment, des particules microplastiques et non microplastiques ont été trouvées dans tous les placentas échantillonnés avec des variations dans le nombre de particules. Des particules plastiques et non plastiques ont été observées dans les régions placentaires de la circulation maternelle et fœtale, ce qui suggère qu'elles peuvent traverser le placenta pour atteindre les tissus fœtaux.

Conclusion: Cette thèse apporte la preuve que le placenta humain peut servir de réservoir pour l'accumulation de diverses particules étrangères pendant la grossesse. Les impacts potentiels de ces particules sur la santé humaine en général ou sur le développement fœtal en particulier sont inconnus, mais constituent une question cruciale pour les travaux futurs visant à comprendre les conséquences sur la santé de la pollution plastique.

Mots clés : Placenta, Microplastique, Particules, Microspectroscopie Raman, Grossesse

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Authorship Contribution

Manuscript 1 (Chapter 2): *Microplastic exposure in pregnancy: Implications for maternal and offspring health*

Dr. Shannon Bainbridge and Dr. Mike Wade were the principal investigators for this study, responsible for the conceptualization and oversight of this manuscript. All authors took part in the review of the literature. The manuscript was prepared by Rewa Zurub and edited by Yusmaris Cariaco, Dr. Mike Wade and Dr. Shannon Bainbridge.

Manuscript 2 (Chapter 4): *Microplastic exposure and accumulation in human placenta*

Dr. Shannon Bainbridge, Dr. Sabina Halappanavar and Dr. Mike Wade and were the principal investigators for this study, responsible for the conceptualization, funding and oversight of the study. Alysha Harvey and members of the OMNI team were responsible for patient recruitment. Dr. Luna Rahman provided Raman microspectroscopy analysis training. Rewa Zurub was responsible for placenta collection, dissection and microplastic analysis. The manuscript was prepared by Rewa Zurub and revised by Dr. Shannon Bainbridge and Dr. Mike Wade.

Ethics Statement

Ethics approval for the completion of this study was granted by the Health Canada-Public Health Agency of Canada Research Ethics Board (REB 2021-033H) and the University of Ottawa Research Ethics Board (#H-03-22-7960). Copies of these approvals can be found in Appendix 1.

Table of Contents

List of Figures

List of Tables

Chapter 4.

Chapter 1 – Introduction

Plastic Pollution

Plastics are low-cost, lightweight, and long-lasting materials easily shaped into various kinds of objects for a number of applications [1] . Advancements in plastic production and use have enhanced convenience, hygiene, and safety but have also led to a dramatic increase in plastic waste and pollution [2] . A substantial proportion of plastic products are disposed after a single use, resulting in significant volume of waste that is primarily resistant to degradation[3] . Over 80% of the predicted 6.5 tones of plastic waste produced throughout Canada in 2019 ended up in landfills, with an estimated 7% treated as mismanaged waste [3] . These data show the increasing rise of plastic waste in the environment, raising concerns for population-level health, as detailed in Chapter 2.

Microplastics

Microplastics (MPs) are formed primarily as a result of the breakdown or fragmentation of larger plastic materials. Mechanical or chemical weathering, degradation by UV radiation, abrasion, synthetic fiber shedding and various manufacturing and industrial processes can expedited the breakdown of plastic products into microplastics, which range in size from 5mm and 1 µm in diameter[4,5] . Microplastics can occur in a variety of shapes including fragments, films, or fibers, made up of common polymers used in the manufacturing of common plastic products (i.e. polyethylene, polystyrene, polypropylene and polyvinyl chloride) [5]. A detailed description of microplastics can be found in Chapter 2 (Fig 1).

Raman Microspectroscopy

The study of microplastic contamination and human exposure requires the use of technology that can be used to visualize and accurately identify distinct polymer plastics from other particulates. Raman spectroscopy offers a useful method to determine the composition of small (1 – 1,300 μm) particles, determining the chemical composition and molecular structure of materials based on their scattering of laser light. For analysis of particulates in environmental or biological samples, it provides a fast, non-destructive, and high spatial resolution of structural and biochemical information via point spectras [6].

Raman spectroscopy begins with a monochromatic light source, typically a laser. The laser beam is directed onto the sample of interest. When the laser light interacts with the molecules in the sample, most of it is elastically scattered, referred as a Rayleigh Scatter, meaning it scatters at the same wavelength as the incident laser light [6,7]. However, a small fraction of the incident light (about 1 in 10^7 photons) undergoes inelastic scattering, resulting in a shift in frequency (wavelength) known as Raman scattering [7]. This shift in frequency occurs because the energy of the scattered photon is either increased or decreased compared to the incident photon, corresponding to the vibrational and rotational energy levels of the molecules in the sample. The scattered light, including both the Rayleigh and Raman scattered photons, is collected by a spectrometer. The scattered light separates into its various wavelengths, creating a Raman spectrum with unique peaks of intensity and wavelength position, with each peak corresponding to a specific molecular bond vibration. The distribution of these peaks provides a unique signature that can be used to identify the dominant material that makes up the particle being

analyzed by comparing the observed Raman peaks with reference spectra in databases, such as the KnowItAll spectral libraries [7,8].

Raman microspectroscopy is the combination of conventional light microscopy and a unique chemical identification by Raman spectroscopy[7]. Raman microspectroscopy is applied in a variety of contexts such as microbiology [9], biological tissue analysis[10] determination of biopharmaceutical or environmental pollutant constituents, and many more, as a rapid and nondestructive technique to assess the chemical composition of unknown material in near real time [9]. Due to the particle detection size range of Raman spectroscopy, it is an ideal modality to employ for the detection and characterization MP particles[11] . In the current thesis, the identification and characterization of MPs in human placenta will be carried out using this methodology.

Microplastics exposure in humans

Microplastics enter the body via one of three routes: inhalation, ingestion, and dermal contact[12]. It is not entirely clear how these routes compare with respect to their contribution to total MP bioaccumulation in human tissues, however this topic is reviewed in detail in Chapter 2. Some proportion of MPs that are ingested, inhaled, or come in contact with the skin can penetrate these barriers via some yet, poorly defined mechanism(s) and enter internal tissues and circulation. Recent evidence indicates that MP exposure can increase intestinal permeability [13,14] suggesting MPs toxicity to the gut lining may enhance their own passage across the gut barrier. Nonetheless, the routes of exposure, particle characteristics or other factors that enhance the capability to

travel across physiological barriers to contaminate internal tissues and fluids remain largely unknown.

Human Placenta

The placenta is considered the most important organ of pregnancy. During human embryogenesis, it is the first organ to develop[15]. The placenta, together with the fetal membranes and amniotic fluid, aid in the fetus's regular development and growth[16]. The placenta is responsible for all maternal-fetal exchange of nutrients, gases and waste products, and produced hormones necessary for proper maternal adaptation to pregnancy and fetal growth[16]. The placenta further acts as a selective barrier with a goal to protect the fetus from xenobiotics present in maternal circulation. However, certain pollutants have been observed to cross the placenta barrier[16].

The placenta is fetal in origin, arising from the trophectoderm of the early embryo. Functionally, the placenta can be described as having two compartments – the extravillous compartment and the villous compartment. The extra villous compartment is composed of extra villous trophoblast cells that interact with the maternal decidua to : 1) anchor the placenta to the uterine wall – forming the **basal plate** of the placenta; 2) actively remodel the uterine spiral arteries, establishing a robust utero-placental circulation required to support fetal growth[17]. The villous compartment of the placenta is composed of hundreds of **chorionic villi** – delicate tree like structures which are bathed in maternal blood. These villi encase the feto-placental vasculature and serve as the primary site of maternal-fetal exchange. The feto-placental capillary beds within these structures coalesce into larger chorionic vessels on the fetal surface of the placenta – the

chorionic plate – further fusing to become the umbilical cord blood vessels that travel to and from the fetus[16].

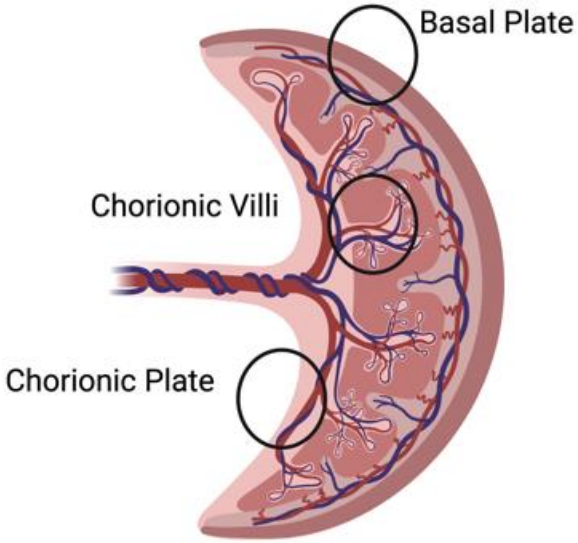

Figure 1.1. Cross-sectional schematic of the human placenta, demonstrating key functional structures and compartments, including: A) Basal plate (maternal surface); B) Chorionic villi; and C) Chorionic plate (fetal surface).

As the placenta serves as the interface between the fetus and the maternal environment, any adverse environment exposure experienced by the mother during gestation can illicit placental damage and dysfunction leading to adverse pregnancy outcomes, such as pre-term delivery and fetal growth restriction (FGR). Further, considering the well-established developmental origins of health and disease (DOHaD) framework, it is clear that adverse in utero exposures can have lasting programming effects on the developing fetus, associated with adverse health outcomes in

later life. The development and maintenance of a healthy placenta can help to optimize the in utero environment for the developing fetus, contributing to healthy short- and long-term health outcomes for the offspring.

Microplastic in the Human Placenta - Current State of Knowledge

A small number of recent studies have revealed the presence of MPs in human placentas collected at delivery. Plastic particles were first reported in a small number of placentas from healthy pregnancies that delivered vaginally in an Italian population[18]. This study received considerable attention world-wide, demonstrating the presence of 12 microplastic fragments in 4 of the 6 placentas examined, found across distinct placental biopsy sites (fetal surface, maternal surface and chorioamniotic membranes) and sizes ranging from 5 to 10 um. The particles were identified as plastic in origin, based on the presence of pigments except for some polypropylene particles. A second study[19] , detected MPs in placentas (n=2) following caesarean deliveries from uncomplicated term pregnancies. In addition, these authors also identified the presence of MPs within matched fetal meconium samples – indicative of transplacental transfer of MPs to the fetal compartment. In both placenta and meconium samples, the authors observed the presence of 10 common types of microplastics (> 50um). Since these landmark studies in 2021, a small handful of additional studies (n=3) have been published confirming the presence of MPs in placental tissue from obstetrical populations around the globe, however to date no evaluation of gestational MP exposure in a Canadian population has been undertaken[20–22]

While the majority of studies to date examining MP accumulation in the human placenta have focused on healthy pregnancies, data is beginning to arrive suggesting a potential association between placental MP accumulation and adverse pregnancy outcomes. In cases of fetal growth restriction (FGR), an inverse correlation between the accumulation of MPs in the placenta and birthweight (correlation coefficient, $r = -0.82$, $p < 0.001$) has been described, with similar associations observed for neonatal length at birth, head circumference, and 1-minute APGAR scores[22]. All 13 cases of FGR examined exhibited the presence of MPs, with each sample containing a range of 2.9 to 34.5 um-sized MPs, primarily composed of polyethylene (PE) and polystyrene (PS) polymers [22]. Coupled to a large body of work carried out in rodent models (reviewed in detail in Chapter 2) which demonstrates a causal relationship between gestational MP exposure and poor placental development, compromised fetal growth profiles and adverse long term health outcomes, these findings certainly are a cause for concern given the ubiquitous nature of plastic pollution and MP exposure in our environments. It is important to note that the literature on this topic in human populations remains small and exposure estimation is qualitative at best. Also, there remains limitations in experimental designs used to date, including: small sample sizes (n= 2 – 43), limited exploration on the distribution of MPs across the various functional compartments of the placenta, a lack of comparison of MP contamination according to mode of delivery and serious considerations of post-delivery contamination. The current thesis project will specifically address these limitations and gaps in knowledge, aiming to identify and characterize placental MP accumulation, according to polymer character and placental distribution, from pregnancies delivered by C-section and vaginal delivery in a Canadian obstetrical population.

References

1. Hopewell J, Dvorak R, Kosior E. Plastics recycling: challenges and opportunities. *Philos Trans R Soc B Biol Sci*. 2009;364(1526):2115-2126. doi:10.1098/rstb.2008.0311

2. Kibria MG, Masuk NI, Safayet R, Nguyen HQ, Mourshed M. Plastic Waste: Challenges and Opportunities to Mitigate Pollution and Effective Management. *Int J Environ Res*. 2023;17(1):20. doi:10.1007/s41742-023-00507-z

3. Plastic pollution is growing relentlessly as waste management and recycling fall short, says OECD. Accessed October 11, 2023. https://www.oecd.org/environment/plastic-pollution-is-growing-relentlessly-as-waste-management-and-recycling-fall-short.htm

4. Hartmann NB, Hüffer T, Thompson RC, et al. Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris. *Environ Sci Technol*. 2019;53(3):1039-1047. doi:10.1021/acs.est.8b05297

5. Hirt N, Body-Malapel M. Immunotoxicity and intestinal effects of nano- and microplastics: a review of the literature. *Part Fibre Toxicol*. 2020;17(1):57. doi:10.1186/s12989-020-00387-7

6. Butler HJ, Ashton L, Bird B, et al. Using Raman spectroscopy to characterize biological materials. *Nat Protoc*. 2016;11(4):664-687. doi:10.1038/nprot.2016.036

7. Ivleva NP, Kubryk P, Niessner R. Raman microspectroscopy, surface-enhanced Raman scattering microspectroscopy, and stable-isotope Raman microspectroscopy for biofilm characterization. *Anal Bioanal Chem*. 2017;409(18):4353-4375. doi:10.1007/s00216-017-0303-0

8. D'Souza M, Whitley G, Clines N, Kunitsky K, Bethancourt-Hughes G, Huma Z. KnowItAll Microplastic Classification.

9. Lee KS, Landry Z, Pereira FC, et al. Raman microspectroscopy for microbiology. *Nat Rev Methods Primer*. 2021;1(1):1-25. doi:10.1038/s43586-021-00075-6

10. Förster M, Bolzinger MA, Montagnac G, Briançon S. Confocal Raman microspectroscopy of the skin. *Eur J Dermatol EJD*. 2011;21(6):851-863. doi:10.1684/ejd.2011.1494

11. Käppler A, Fischer D, Oberbeckmann S, et al. Analysis of environmental microplastics by vibrational microspectroscopy: FTIR, Raman or both? *Anal Bioanal Chem*. 2016;408(29):8377-8391. doi:10.1007/s00216-016-9956-3

12. Landrigan PJ, Raps H, Cropper M, et al. The Minderoo-Monaco Commission on Plastics and Human Health. *Ann Glob Health*. 2023;89(1):23. doi:10.5334/aogh.4056

13. Toto B, Refosco A, O'Keeffe M, et al. Intestinal permeability and gene expression after polyethylene and polyamide microplastic ingestion in Wistar rats. *Toxicol Lett*. 2022;370:35-41. doi:10.1016/j.toxlet.2022.09.002

14. Okamura T, Hamaguchi M, Hasegawa Y, et al. Oral Exposure to Polystyrene Microplastics of Mice on a Normal or High-Fat Diet and Intestinal and Metabolic Outcomes. *Environ Health Perspect*. 2023;131(2):27006. doi:10.1289/EHP11072

15. Dusza HM, van Boxel J, van Duursen MBM, Forsberg MM, Legler J, Vähäkangas KH. Experimental human placental models for studying uptake, transport and toxicity of micro- and nanoplastics. *Sci Total Environ*. 2023;860:160403. doi:10.1016/j.scitotenv.2022.160403

16. Gude NM, Roberts CT, Kalionis B, King RG. Growth and function of the normal human placenta. *Thromb Res*. 2004;114(5-6):397-407. doi:10.1016/j.thromres.2004.06.038

17. Burton GJ, Fowden AL. The placenta: a multifaceted, transient organ. *Philos Trans R Soc Lond B Biol Sci*. 2015;370(1663):20140066. doi:10.1098/rstb.2014.0066

18. Ragusa A, Svelato A, Santacroce C, et al. Plasticenta: First evidence of microplastics in human placenta. *Environ Int*. 2021;146:106274. doi:10.1016/j.envint.2020.106274

19. Braun T, Ehrlich L, Henrich W, et al. Detection of Microplastic in Human Placenta and Meconium in a Clinical Setting. *Pharmaceutics*. 2021;13(7):921. doi:10.3390/pharmaceutics13070921

20. Liu S, Lin G, Liu X, et al. Detection of various microplastics in placentas, meconium, infant feces, breastmilk and infant formula: A pilot prospective study. *Sci Total Environ*. 2022;854:158699. doi:10.1016/j.scitotenv.2022.158699

21. Liu S, Liu X, Guo J, et al. The association between microplastics and microbiota in placentas and meconium: The first evidence in humans. *Environ Sci Technol*. Published online 2022.

22. Amereh F, Amjadi N, Mohseni-Bandpei A, et al. Placental plastics in young women from general population correlate with reduced foetal growth in IUGR pregnancies. *Environ Pollut 1987*. 2022;314:120174. doi:10.1016/j.envpol.2022.120174

Chapter 2 - Microplastic Exposure in Pregnancy: Implications for Maternal and Offspring Health

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The manuscript was prepared by R.Z. All authors have read and agreed to the published version of the manuscript.

Abstract

Plastics found in our everyday environment are becoming an increasing concern for individual and population-level health, the extent of exposure and potential toxic effects of these contaminants on numerous human organ systems are becoming clear. Microplastics (MPs), tiny plastic particles, appear to have many of the same biological effects as their plastic precursors and have the compounded effect of potential accumulation in different organs. Recently, microplastic accumulation was observed in the human placenta, raising important questions related to the biological effects of these contaminants on the health of mothers and their offspring. These concerns are particularly heightened considering the developmental origins of health and disease (DOHaD) framework, which postulates that *in utero* exposure can programme the lifelong health of the offspring. The current review examines the state of knowledge on this topic and highlights important avenues for future investigation.

Keywords: Plastics pollution, Microplastic, Pregnancy, Placenta, DOHaD,

Plastic Pollution

Plastics are synthetic or semi-synthetic polymers, developed after the 19th-century Industrial Revolution. Due to their many useful characteristics, including being light weight, infinitely mouldable, having low production cost, broad chemical resistance, and ease to manufacture and transport [1], they are widely used in food packaging (i.e., containers, plastic bags), building products (i.e., pipes, vinyl cladding), electronics, and transportation materials [1]. The development of plastics has also revolutionised medicine with life-saving devices and the availability of sterile, single use instruments and personal protective equipment. However, the excessive use of plastics has led to a throw away culture that reveals the materials dark side.

Plastic pollution is an accumulation of synthetic plastic products in the environment, disrupting the habitats and health of wildlife and humans alike. The rapidly rising output of disposable plastic goods is currently exceeding our capacity to handle its disposal, leading to the emergence of plastic pollution as one of the most urgent environmental issues [2]. As previously reviewed by Hirt et al. [3], plastic waste reached 359 million metric tons in 2018 [3], with estimates that between 4.8 and 12.7 million metric tonnes are reaching the ocean each year, contributing to 80% of the plastic pollution in the world's oceans and seas [3]. Plastic trash is also carried to sea by major rivers, which can act as a conveyor belt, picking up more and more garbage as it moves downstream. When plastic trash gets caught up in an ocean's current, it can be transported around the world. Many single use plastic products have a functional lifespan of minutes to hours, yet they may persist in the environment for hundreds of years. Plastic degradation is a very slow process, with fragmentation and degradation of plastic polymers occurring by physical forces, ultraviolet (UV)

rays, temperature changes and biodegradation in the environment. The resulting breakdown products are smaller plastic fragments, known as micro and Nanoplastics [4]

Microplastics and Nanoplastics

Microplastics (MPs) are generated by the breakdown of larger plastic products, and are defined as being less than 5 mm in size. Microplastics are omnipresent in our environment, being found in large quantities in oceans, rivers, ground water, sediments and soil environments, sewage, and even the air we breathe [5]. Most plastics in use have a strong resistance to biodegradation [6]. However, they are susceptible to mechanical and photochemical processes that can break them down into micro and nanoscale particles [6]. Nanoplastics (NPs) are plastic particles ranging in size from 1nm – 1um [7]. MPs and NPs demonstrate similar characteristics and biological effects; however, NPs demonstrate a higher biological mobility and bioavailability because of their small size, which enables them to pass through biological membranes with ease [8]. For the purposes of the current review, the abbreviation "MNP" will be used to describe all plastic fragments < 5 μm – and, as such, will include both MPs and NPs.

MNPs can be further characterized by their polymer composition and shape – characteristics that are intimately linked to the plastic product source from which they were derived (Figure 1). Plastics are made up of various polymers, including polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET), polycarbonate (PC), polymethacrylate (PMMA), and polyurethane (PU) [3]. However, polyethylene (PE), polypropylene (PP) and polystyrene (PS) are the three most common occurring polymers [5], being found in a

countless number of household and personal care products [9,10], cosmetic products [11], toothpaste [10,12,13] and plastic food containers [14]. The shape of MNPs is also varied, and includes fibres, microbeads, fragments, nurdles and Styrofoam [3]. The types and sources of plastic pollution have been reviewed in detail elsewhere. [15–21]

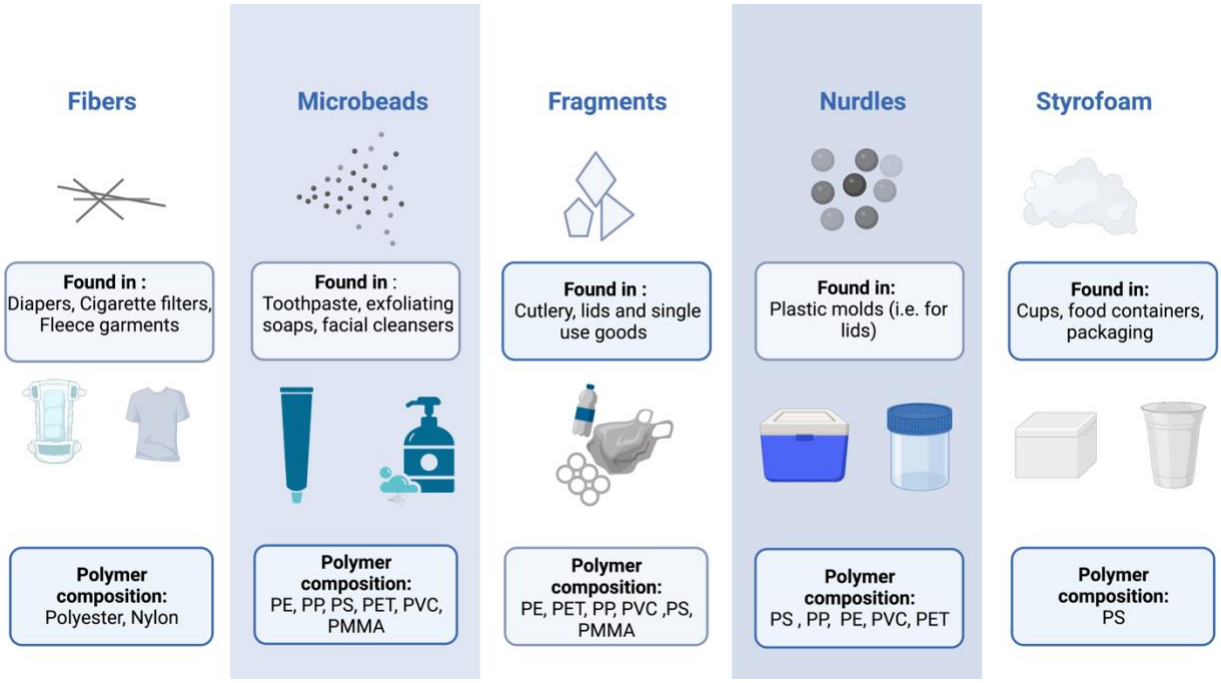

Figure 2.1. The shapes, compositions, and potential sources of common micro- and nano-plastics (MNPs). PE = polyethylene; PP = polypropylene; PS = polystyrene; PET = polyethylene terephthalate; PVC= polyvinyl chloride; PMMA = polymethacrylate.

It is important to note that MNPs are not just pure plastic polymers, rather they are associated with a diverse mixture of organic molecules and/or metals. Commercial plastics contain many additives that can leech out of plastic into the surrounding environment or tissue(s), as they are not covalently linked to the polymer matrix. A recent review estimated that over 10,000 unique chemicals are used at various stages in plastics manufacturing, of which roughly 2,400 have been identified as chemicals of regulatory concern . Further, the hydrophobic surface of MNPs can absorb environmental contaminants, particularly polyaromatic hydrocarbons [22]. There is concern

that chemicals contained within MNPs, or those absorbed to their surface, can be carried into the human body and released into various tissue beds [23]. In this way, MNPs act as a vehicle for toxic exposure to a number of xenobiotics, which may bypass typical physiological defences such as drug metabolizing enzymes in the gut and liver and induce direct effects to the cells/tissues surrounding the internalized MNPs [24]. Several chemicals known to leach from plastics are well known to induce a variety of adverse health conditions in humans, including to developing fetuses exposed in utero [25,26]. However, investigations specifically exploring the developmental toxicity MNPs, and the many associated chemicals, are limited.

Routes of Exposure and Adverse Human Health Outcomes

There are three routes through which the human body is exposed to MNPs – inhalation, ingestion and dermal contact [27] (Figure 2). It is estimated that an individual will be exposed to approximately 74,000-121,000 MPs per year, with ingestion and inhalation considered the primary routes of exposure [28]. As this estimate does not consider NPs, it is likely that total MNP particle exposure is in fact considerably higher. Importantly, most MNPs can cross the physiological barriers of the lungs, gut, and skin. The mechanisms underlying this translocation are poorly understood and are beyond the scope of the current work, however they have been reviewed in detail elsewhere [27].

In humans, MNPs have been found in a diverse range of biological samples, including blood [29,30], urine [31], sputum [32], feces [33]; [34], and breast milk [3536]. Further, MNP accumulation has been identified in numerous organ systems including lung [37–42], colon and spleen [43]. Microplastics have more recently been identified in human placenta tissue [44–49] and meconium [34,36] demonstrating

direct exposure to the fetus and raising concerns for developmental toxicity and long-term health consequences for the offspring. While the scope of MNP contamination and human exposure has become widely apparent within the last decade, relatively few studies have focused explicitly on the reproductive consequences of MNP exposure, particularly in humans. The limited body of work in this area, specifically that focused on the effects of MNP exposure on mammalian reproduction, is summarized below.

Effects of MNP Exposure on Mammalian Reproduction

Reproductive Effects in Adult Males

Several adverse reproductive effects are observed in male mammals following oral exposure to MNPs of various size and with varying duration. For example, in male rodents oral exposure to PS-MNP leads to accumulation within the testis [50–54], coupled to disruption of the seminiferous epithelium [50,51,53,55–59], evidence of localized oxidative stress and mitochondrial dysfunction [46], and over-expression of pro-inflammatory cytokines in the testis [51,52]. This same exposure is associated with disruption of the blood-testis barrier [51,57,58,60], with *in vitro* studies demonstrating oxidative stress, endoplasmic reticulum stress and misfolding/degradation of tight junctional proteins in Sertoli cells [61,62]. There are clear functional consequences of these exposures, as MNP exposure in rodent models leads to reduced sperm quantity and quality [50–53,56,58,60,63,64] in addition to reduced testicular production [56] and circulating levels of testosterone [50,53,55,56,60] and luteinizing hormone (LH) [50,53,55,56], suggesting that MNP exposure may have important implications in the pituitary-gonadotropin endocrine signalling pathways, testis function and sperm quality in male mammals [see [65] for a detailed review on this topic]. It is noteworthy that an exponential rise in

global plastic production [4] coincides with a well-documented population-wide decline in human sperm production which appears to be accelerating since 2000 [66].

Male fertility, fetal health and the long-term health of offspring are dependent on the epigenetic programming events that occur during spermatogenesis, events that can be adversely disrupted by exposures to various testicular toxicants [67,68]. Epigenetic modifications play a crucial role in regulating gene expression and developmental processes, including germ cell differentiation and sperm production. While there are currently no studies to date examining the toxicant effects of MNPs on the sperm epigenome in mammals, there is strong evidence that common additives found within MNPs (i.e. phthalates and BPA) can in fact disrupt this critical developmental process. In rodent models, exposure to phthalates and BPA can induce alterations in DNA methylation patterns [69–72], histone modifications [72,73], and non-coding RNA expression within the germline. These changes can disrupt normal epigenetic programming during critical windows of spermatogenesis, leading to impaired sperm development, reduced sperm quality, and compromised fertility [69,72–74]. Similar associations have been observed in human populations, with several studies demonstrating a correlation between urine phthalate and/or BPA metabolite concentrations and differential methylation patterns in the sperm, often in promoter regions of genes related to cellular growth and development, coupled to poor sperm quality and fertility outcomes [73,75–78]. Furthermore, the intergenerational and transgenerational effects of phthalates and BPA on germ cell epigenetic marks have been observed, indicating the potential for long-lasting impacts on future generations [69,70].

Reproductive Effects in Adult Females

Like most studies investigating the reproductive impact of MNPs in males, PS-MNPs are among the most widely studied plastic particles in relation to female reproductive toxicity in mammals [79.] In both a rat and mouse model, oral exposure to PS-MNPs results in the accumulation of these particles within uterine tissue [80] and in various ovarian compartments, including within growing follicles [53,79–84]. Ovaries of these exposed rodents have reduced weight, decreased expression of cytoskeletal proteins, and demonstrate altered follicle dynamics, with a reduction in the number of growing and mature follicles and increased atretic and cystic follicles [79]. In parallel, distinct changes in reproductive hormone signalling are observed, with reductions in the circulating concentrations of estradiol (E_2) and anti-mullerian hormone (AMH), and increased concentrations of LH, follicle stimulating hormone (FSH) and testosterone [53,79,83]. Exposed rodents demonstrate functional/fecundity consequences of this MNP exposure, with measurable changes in estrous cycle duration, decreased ovarian reserve, lower embryo implantation rates and smaller litter sizes [53,83]. The mechanistic underpinning of this reproductive dysfunction is thought to be in large part driven by MNP-induced oxidative stress. Ovarian tissues of exposed rodents demonstrate markers of oxidative stress, such as malondialdehyde (MDA) [79,80,83,85], with noted disturbances in total antioxidant capacity and increased evidence of apoptosis [83]. Similarly, human granulosa cells (COV434) exposed to PS-MNPs *in vitro* likewise demonstrate increased evidence of lipid peroxidation, with decreased protein expression of super oxide dismutase (SOD2) and glutathione (GSH) antioxidant systems, and decreased cell viability [83]. The accumulation of MNPs in female reproductive organs and the resulting oxidative stress are thought to promote excess fibroblasts proliferation and fibrosis [86–90]. Furthermore, evidence of pro-inflammatory signalling is observed in these exposed tissues [80]. It should be noted that very little work to date has

examined the presence or toxicity of MNP exposure in human female reproductive tissues, and this should be a prioritized focus of research endeavours moving forward. However, MNPs were recently detected in follicular fluid of 7 patients undergoing fertility treatment [91]. The mean concentration of the MNPs in these samples was ~ 120 MNP/ml, with the most predominant polymers present being PVC, PE, PS, PP and PU, found in conjunction with several common plastic-related particles, including common pigments, solubilizers and fillers. Importantly, the authors demonstrated compromised bovine oocyte maturation when cultured in the presence of these same MNPs in vitro, at similar concentrations to those measured. Further, these exposed oocytes demonstrated significant proteomic alteration, with differential expression of proteins involved in oocyte function, oxidative stress, and DNA damage [91]. To date, there have been no investigations examining the impact of MNPs on the oocyte epigenome in any mammalian species, however the epigenome of female mammalian gametes is likewise known to be adversely impacted by many additive compounds found in MNPs [92,93]. As such, there is very likely additional adverse effects of these plastic particles on oocyte health, and the health of subsequent generations, driven in part by altered oocyte epigenetic imprinting. Collectively, this patchwork of findings collected across mammalian species provide compelling evidence of the toxic effects of MNPs on female reproductive health and fecundity.

MNP Exposure in Pregnancy

MNPs in the Placenta

Mounting evidence suggest that MNPs accumulate within and affect the proper functioning of the placenta – the vital organ of pregnancy responsible for all maternal-fetal exchange [94]. The

presence of MNP accumulation in placental tissue of rats treated with PS-MNPs was first described in 2020 [95] and has since been reproduced in many studies in mice [96–98], with observed structural and functional consequences [95–100]. Exposed females (MNPs between 100 nm-10 µm) have smaller placentas [95,97], reduced numbers of glycogen-containing cells within the placental endocrine-functioning junctional zone [99], and poorly developed feto-placental vasculature [99]. The remodelling of the uterine spiral arteries is also compromised, likely the result of uterine and placental immune cell imbalances (i.e., decreased uterine natural killer cells, altered macrophage 1/2 ratio) [96]. In addition, transcriptomic and metabolic analyses of MNP-exposed placentas demonstrate disturbed amino acid, glucose and cholesterol metabolism and complement/coagulation cascades pathways [99,100].

More recently, MNP accumulation has been observed in human placenta tissue of otherwise healthy pregnancies delivered both vaginally and by C-section [36,44–49]. The number of MNPs measured varied across patients, ranging from 0.3-9.6 MNPs/g tissue [49], with the most common polymers identified as PE, PS, PA, PU, and PVC [36,45,46,49]. Grossly, MNPs were found in both the fetal and maternal compartments of the placenta, along with the chorioamniotic membranes [47]. More detailed investigations identified microplastic-like particles within the syncytiotrophoblast cellular layer of the placenta, both free within the cytoplasm and encapsulated within structures located below the plasma membrane (i.e. vacuoles, lipid droplets, vesicular bodies, lysosomes, peroxisomes), as well as within the pericytes and fetal vascular endothelial cells located within the chorionic villous structures [48].

While *in vivo* functional investigations pose an ethical and logistical challenge in human populations, *in vitro* studies carried out using different human placenta cell lines demonstrate a clear potential for human placental MNP uptake and functional alterations. Using the immortalized HTR-8/SVneo extravillous cytotrophoblast cell line, PS-MNP exposure resulted in MNP accumulation within the cytoplasm, followed by increased production of reactive oxygen species (ROS), enhanced production of pro-inflammatory cytokines (e.x., TNF-α and IFN-γ), cell cycle arrest and ultimately reduced cellular viability [101]. These cells also demonstrate altered gene expression profiles, with up-regulation of genes required for regulation of leukocyte differentiation, cell cycle, apoptotic process, and cellular adhesion. Functionally, these MNP-exposed cells demonstrated impaired cell motility and invasion capacities, indicating that MNP exposure may negatively impact the invasive placentation process, required for the establishment of a robust utero-placental circulation needed to support adequate fetal development [101,102] Studies have also been carried out using BeWo and JEG-3 cells [103,104] – both choriocarcinoma cell lines representative of the chorionic villous cytotrophoblast and syncytiotrophoblast cell lineages, that directly facilitate maternal-fetal exchange. These studies likewise demonstrate placental cell uptake of MNPs in a size and concentration-dependant fashion, with MNP-induced ROS production, inflammation, DNA damage, cell cycle arrest, and apoptosis [103,104].

A combination of animal, human observational and experimental data has distinctly demonstrated the potential for placental transfer of MNPs into the fetal compartment. Observations in both maternal mice and rats exposed to PS-NPs ranging in size from 20 to 500nm during gestation demonstrated the presence of these particles in fetal liver [95,105,106], heart [95,106], brain [98,105,106], lung [105,106], and kidney [106]. Interestingly, a similar exposure using PE-MPs (10-45

um) observed accumulation exclusively in fetal kidneys [107]. In humans, MNPs have been measured in fetal meconium [36,45,46], and using an *ex vivo* human placenta perfusion model, a size dependent transfer of MNPs from the maternal to fetal circulation has been described [108,109]. These exposed human placenta tissues also demonstrated dysregulated expression of genes and proteins related to inflammation and iron homeostasis [110]. Collectively, these data demonstrate placental accumulation and translocation of MNPs into the fetal compartment, leading to concern that maternal MNP exposure during pregnancy may initiate adverse fetal programming events, resulting in short- and long-term health concerns for the offspring.

MNP Exposure during Pregnancy and Early Life Health Effects on Progeny

Given the vital importance of placental health and function for fetal development, it is unsurprising that MNP exposure in pregnancy is associated with altered fetal growth profiles. Mouse models of gestational exposure to PS-MNPs, ranging in size from 90 nm – 5 um, demonstrate pronounced fetal growth restriction in the last half of pregnancy (E15.5-E17), with fetal weights on average 12-15% smaller than non-exposed fetuses [97,99,111]. The feto-placental weight ratio is also reduced [111], a finding consistent with fetal growth restriction (FGR) suggesting inadequate nutrient transfer capacity to support fetal weight gain [112]. These authors also observed decreased umbilical cord length in the MNP-exposed fetuses [111], a finding described in murine models of hypoxia-mediated FGR [113,114] and found in human cases of FGR and fetal distress [115]. Most studies have reported no impact of maternal MNP exposure on total litter size, however some reports of embryonic lethality and resorption have been reported in both murine and chick embryo model systems [97,116], the latter attributed to significant embryonic malformations and developmental delays.

MNP-induced FGR is further extended to observations of reduced birth and neonatal body weight. In both rat and mouse models, exposure to PS-MNPs (70-100 nm) during pregnancy reduced neonatal pup weight by 7-15%, in some cases in a sex-dependant fashion [95,117–120]. Specifically, two studies report reduced neonatal weights only for female offspring [118,119]. Interestingly, a paralleled decrease in placental expression of 11β-hydroxysteroid dehydrogenase (11β-HSD) was uniquely observed in exposed female fetuses **[118]**. As placental 11β-HSD is a critical regulator of fetal cortisol exposure in utero, the authors speculate that dysregulated glucocorticoid signalling may, in part, be responsible for the sex-specific differences in neonatal weight observed. Curiously, there is one report of altered neonatal sex ratio at birth, skewed in favor of male offspring, coupled to a reduced live birth rate in a mouse model of gestational PE-MNP exposure**.** However, in this model, the body weight of male and female pups were equally reduced at 6 hrs after birth **[120].** In human populations very little is known about the impact of maternal MNP exposure on fetal growth and offspring birthweight. However, recently, an inverse correlation between placental MNP accumulation and birthweight in FGR pregnancies was reported ($r = -0.82$, $p < 0.001$) [44]. Similar relationships were observed for neonatal length at birth, head circumference and 1 minute APGAR scores. MNPs were detected in all 13 cases of FGR examined, with up to 38 distinct MNPs measured per sample. PE and PS were the most abundant polymers identified, and the MNPs ranged in size from 2.9 to 34.5 um. While this study has a small sample size, and some methodology questions are outstanding (i.e. how much placenta tissue was examined/case?), it certainly provides concern regarding the impact of in utero MNP exposure on fetal growth and development that warrants further investigation.

An increasing body of evidence suggests that in utero exposure to MNPs not only adversely affects fetal and neonatal body weight but also compromises fetal organ development. For example, skeletal muscle tissue collected from term murine fetuses exposed to PS-MNPs in utero, demonstrate significant dysplasia, with dysregulated expression of genes involved in muscle tissue development, lipid metabolism, and skin formation [99]. In the post-natal period (day 14), mice exposed to MNPs in utero and during lactation demonstrate a substantial reduction in the number of proliferative cells within the hippocampus, with reduced numbers of neural stem cells – indicative of abnormal brain development [121]. These offspring demonstrate neurophysiological and cognitive deficits in a gender-specific manner affecting females. Evidence collected using a chick embryo model likewise points to detrimental effects of in utero MNP exposure on nervous system development, including the observation of neural tube defects [97]. Post-natal observations in both murine and chick models additionally indicate adverse effects of gestational MNP exposure on the size and histological organization of the developing liver, spleen and heart [116,117,120], with evidence of oxidative stress and dysregulated immune cell infiltration. Importantly, findings of altered fetal/neonate body and organ weight are shown to persist into adulthood in some cases [117,120], emphasizing the potential for adverse short- *and* long-term health outcomes for offspring exposed to MNPs during pregnancy.

It should be noted that not all results collected to date have likewise demonstrated reduced fetal, birth or neonatal organ weights following MNP exposure in utero. Rather, other groups have found no differences, or even increased rodent pup weight up to 1 week after delivery [106,107,121]. These observed discrepancies can likely be attributed to the wide range of MNP exposure protocols and experimental methods used across studies. Most studies summarized were carried

out using PS-MNPs, often in the nano-particle size range (25-900 nm) [97,99,106,111,116,116–119,121], however a few used larger PS- or PE-MNPs (1 um-45 um) [107,111,116,120] which may not demonstrate similar bioavailability and/or trans-placental transfer profiles. Lengths of maternal MNP exposure also varied, some beginning exposure up to 80 days prior to mating [120] and others continuing exposure until the time of weaning [117,121]. In fact, Jeong et al reported no changes in fetal weight profiles at gestational day 14, but increased pup weight at 7 days post-delivery – findings they attributed to postnatal MNP exposure via breastmilk [121]. Further, differences in route of administration (oral vs. inhalation vs injection), coupled to differences in dosing reporting practices, makes direct comparison of pregnancy outcome data from various MNP exposure models a challenge to interpret.

MNP Exposure during Pregnancy and Later Life Health Effects on Progeny

It is proposed that in utero MNP exposure may have lasting effects on the life-long health profiles of offspring, attributed to MNP-mediated adverse in utero programming events. Within the developmental origins of health and disease (DOHaD) framework, it is well established that FGR, reduced birthweights and subsequent rapid growth in early life (ie. “catch up” growth) are associated with development of cardiovascular disease, hypertension, type 2 diabetes, metabolic syndrome and insulin resistance in later life [122]. In this regard, the findings detailed in section 5.2 would certainly suggest an increased susceptibility to adult chronic disease in MNP-induced growth restricted offspring. While there is not yet a large body of work directly testing this hypothesis, the findings in support of it are beginning to trickle in.

In rodent models, lifelong metabolic dysfunction has been observed in offspring with in utero exposure to PS-NPs. Mice offspring born to mothers exposure to PS-MPs (0.5 um – 5 um) across pregnancy demonstrate increased liver weights, and alterations in lipid, metabolic, and gene expression profiles associated with fatty acid metabolism disorder at post-natal day 42, in a MNP size- and offspring sex- dependant fashion, with larger MNPs effecting female offspring more profoundly [123,124]. Even more concerning, while milder in nature, similar evidence of metabolic dysfunction was observed in the F2 generation of offspring in this model of gestational MNP exposure[124]. It is important to note, that the MNPs used in the studies were manufactured and likely did not contain any of the myriad of chemical additives present in commercial plastic products. This is an important consideration, as many of these additive chemicals are well established as adverse fetal programming chemical exposures, associated with the transgenerational propagation of chronic adult-onset metabolic disorders [125,126].

In utero MNP exposure has also been linked to adverse neurodevelopmental programming events in rodent models, with subsequent later life neurocognitive deficits. Offspring exposed to PE-MNPs (10um-20um) in utero demonstrate autistic-like traits, including reduced sociability, decreased working memory, repetitive, compulsive and anxiety-like behavior at 8 weeks of age [127]. Coupled to evidence of MNP accumulation within the fetal thalamus with in utero exposure [98], these findings certainly raise concern over the impact of gestational MNP exposure on neurodevelopment and neurocognitive health outcomes for offspring.

While not extensive in volume, the limited studies complete to date focused on the long-term health implications of in utero MNP exposure warrant concern and certainly identify a gap in knowledge that we urgently need to address.

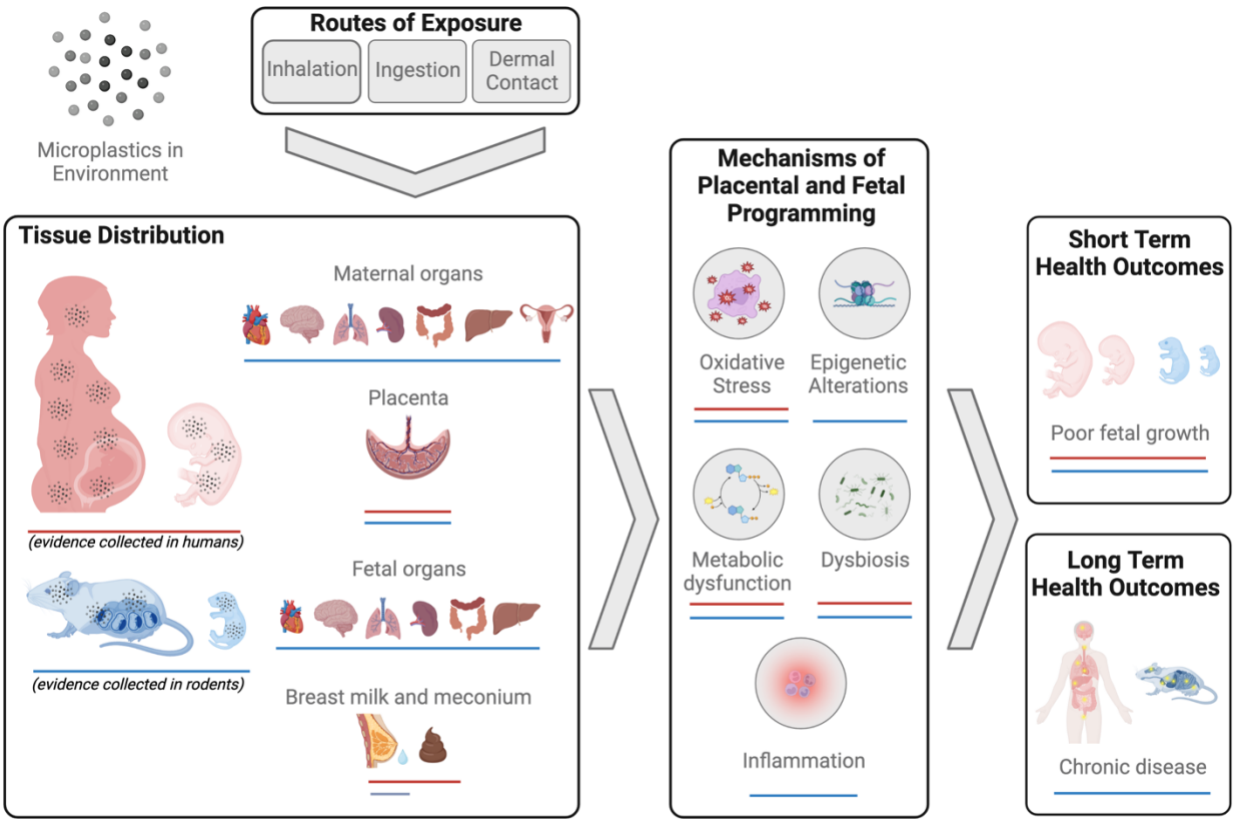

Figure 2.2. Summary of the current evidence available on MNP exposure in pregnancy, potential mechanisms of MNP-induced in utero programming, and offspring health outcomes associated with in utero MNP exposure in humans and rodent models.

Remaining Gaps in Knowledge and High Priority Research Areas

The relatively limited evidence available on reproductive & developmental effects of MNP exposures suggests that significant impacts are possible. However, there remain considerable gaps in understanding that prevent a thorough assessment of whether current MNP exposures

contribute to significant human infertility, compromised early life development and life-long disease risk. It is clear that humans are ubiquitously exposed to diverse microplastics that probably infiltrate fetal tissues. However, limitations in current methods of measuring MNP in various matrices (food, dust, tissue, etc) render accurate assessment of exposures inaccurate especially, for MNPs < 1um – likely those with the highest degree of associated biological harm.

In addition, there are very few sources of well characterized, homogeneous preparations of microplastics available in sufficient quantity to study the potential hazards to reproduction and developmental programming. As such, the vast majority of published toxicity studies have examined the effects of standardized polystyrene micro- or nano-sized plastics (PS-MNP). This contrasts with the great diversity of microplastic shapes, sizes, polymer matrices and their associated chemical content found among the microplastic pollution in the environment. It is possible that some MNP phenotypes may be more harmful than others, and the combined effect of particle size/shape and chemical composition may cause more damage than the pure polymer particle alone. The extent to which these variables may influence toxicity remains unknown and research is needed to evaluate the potential health effects on different types of polymers and additives.

The cumulative results of human observational studies and animal experimental studies have certainly revealed concern for the short-term health concerns associated with gestational MNP exposure. Unfortunately, only a handful of studies to date have explicitly focused on the potential long term consequences of MNP-induced early life programming events. As such, a focus of

future work should be placed on the investigation of long-term health outcomes of offspring exposed to environmentally relevant MNPs in utero. In parallel, improved methods to characterize MNP exposure in human populations are needed, such that clinical and epidemiological studies can begin to assess real world impacts on human populations. This will expand our ability to identify sources of MNPs exposure and develop mitigating strategies to limit exposures. Human exposure to plastic pollution will continue to grow rapidly as the rate at which plastics are used, consumed and disposed of continues to increase. As such, a deeper consideration and understanding of the threat of environmental MNPs to the health of future generations is warranted.

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Conflicts of Interest

No conflicts of interest to declare.

References

1. Andrady AL, Neal MA. Applications and societal benefits of plastics. *Philos Trans R Soc Lond B Biol Sci*. 2009;364(1526):1977-1984. doi:10.1098/rstb.2008.0304

2. Thompson RC, Moore CJ, Saal FS vom, Swan SH. Plastics, the environment and human health: current consensus and future trends. *Philos Trans R Soc LondonSeries B Biol Sci*. 2009;364(1526):2153-2166. doi:10.1098/rstb.2009.0053

3. Hirt N, Body-Malapel M. Immunotoxicity and intestinal effects of nano- and microplastics: a review of the literature. *Part Fibre Toxicol*. 2020;17(1):57. doi:10.1186/s12989-020-00387-7

4. Geyer R, Jambeck JR, Law KL. Production, use, and fate of all plastics ever made. *Sci Adv*. 2017;3(7):e1700782. doi:10.1126/sciadv.1700782

5. Sadri SS, Thompson RC. On the quantity and composition of floating plastic debris entering and leaving the Tamar Estuary, Southwest England. *Mar Pollut Bull*. 2014;81(1):55-60. doi:10.1016/j.marpolbul.2014.02.020

6. Bahl S, Dolma J, Jyot Singh J, Sehgal S. Biodegradation of plastics: A state of the art review. *Mater Today Proc*. 2021;39:31-34. doi:10.1016/j.matpr.2020.06.096

7. Wahl A, Le Juge C, Davranche M, et al. Nanoplastic occurrence in a soil amended with plastic debris. *Chemosphere*. 2021;262:127784. doi:10.1016/j.chemosphere.2020.127784

8. Yu CW, Luk TC, Liao VHC. Long-term nanoplastics exposure results in multi and trans-generational reproduction decline associated with germline toxicity and epigenetic regulation in Caenorhabditis elegans. *J Hazard Mater*. 2021;412:125173. doi:10.1016/j.jhazmat.2021.125173

9. Fendall LS, Sewell MA. Contributing to marine pollution by washing your face: microplastics in facial cleansers. *Mar Pollut Bull*. 2009;58(8):1225-1228. doi:10.1016/j.marpolbul.2009.04.025

10. Praveena SM, Shaifuddin SNM, Akizuki S. Exploration of microplastics from personal care and cosmetic products and its estimated emissions to marine environment: An evidence from Malaysia. *Mar Pollut Bull*. 2018;136:135-140. doi:10.1016/j.marpolbul.2018.09.012

11. Lei K, Qiao F, Liu Q, et al. Microplastics releasing from personal care and cosmetic products in China. *Mar Pollut Bull*. 2017;123(1-2):122-126. doi:10.1016/j.marpolbul.2017.09.016

12. Ustabasi GS, Baysal A. Occurrence and risk assessment of microplastics from various toothpastes. *Environ Monit Assess*. 2019;191(7):438. doi:10.1007/s10661-019-7574-1

13. Madhumitha CT, Karmegam N, Biruntha M, et al. Extraction, identification, and environmental risk assessment of microplastics in commercial toothpaste. *Chemosphere*. 2022;296:133976. doi:10.1016/j.chemosphere.2022.133976

14. Fadare OO, Wan B, Guo LH, Zhao L. Microplastics from consumer plastic food containers: Are we consuming it? *Chemosphere*. 2020;253:126787. doi:10.1016/j.chemosphere.2020.126787

15. Burns EE, Boxall ABA. Microplastics in the aquatic environment: Evidence for or against adverse impacts and major knowledge gaps. *Environ Toxicol Chem*. 2018;37(11):2776-2796. doi:10.1002/etc.4268

16. Hidalgo-Ruz V, Gutow L, Thompson RC, Thiel M. Microplastics in the marine environment: a review of the methods used for identification and quantification. *Environ Sci Technol*. 2012;46(6):3060-3075. doi:10.1021/es2031505

17. Andrady AL. The plastic in microplastics: A review. *Mar Pollut Bull*. 2017;119(1):12-22. doi:10.1016/j.marpolbul.2017.01.082

18. Napper IE, Bakir A, Rowland SJ, Thompson RC. Characterisation, quantity and sorptive properties of microplastics extracted from cosmetics. *Mar Pollut Bull*. 2015;99(1-2):178-185. doi:10.1016/j.marpolbul.2015.07.029

19. Carr SA, Liu J, Tesoro AG. Transport and fate of microplastic particles in wastewater treatment plants. *Water Res*. 2016;91:174-182. doi:10.1016/j.watres.2016.01.002

20. Sillanpää M, Sainio P. Release of polyester and cotton fibers from textiles in machine washings. *Environ Sci Pollut Res Int*. 2017;24(23):19313-19321. doi:10.1007/s11356-017-9621-1

21. Park HJ, Oh MJ, Kim PG, et al. National Reconnaissance Survey of Microplastics in Municipal Wastewater Treatment Plants in Korea. *Environ Sci Technol*. 2020;54(3):1503-1512. doi:10.1021/acs.est.9b04929

22. Hu X, Yu Q, Waigi MG, et al. Microplastics-sorbed phenanthrene and its derivatives are highly bioaccessible and may induce human cancer risks. *Environ Int*. 2022;168:107459. doi:10.1016/j.envint.2022.107459

23. Hu L, Zhao Y, Xu H. Trojan horse in the intestine: A review on the biotoxicity of microplastics combined environmental contaminants. *J Hazard Mater*. 2022;439:129652. doi:10.1016/j.jhazmat.2022.129652

24. Sun N, Shi H, Li X, Gao C, Liu R. Combined toxicity of micro/nanoplastics loaded with environmental pollutants to organisms and cells: Role, effects, and mechanism. *Environ Int*. 2023;171:107711. doi:10.1016/j.envint.2022.107711

25. Darbre PD. Chemical components of plastics as endocrine disruptors: Overview and commentary. *Birth Defects Res*. 2020;112(17):1300-1307. doi:10.1002/bdr2.1778

26. Maradonna F, Vandenberg LN, Meccariello R. Editorial: Endocrine-Disrupting Compounds in Plastics and Their Effects on Reproduction, Fertility, and Development. *Front Toxicol*. 2022;4:886628. doi:10.3389/ftox.2022.886628

27. Prata JC, Costa JP da, Lopes I, Duarte AC, Rocha-Santos T. Environmental exposure to microplastics: An overview on possible human health effects. *Sci Total Environ*. 2020;702:134455. doi:10.1016/j.scitotenv.2019.134455

28. Cox KD, Covernton GA, Davies HL, Dower JF, Juanes F, Dudas SE. Human Consumption of Microplastics. *Environ Sci Technol*. 2019;53(12):7068-7074. doi:10.1021/acs.est.9b01517

29. Leslie HA, Velzen MJM van, Brandsma SH, Vethaak AD, Garcia-Vallejo JJ, Lamoree MH. Discovery and quantification of plastic particle pollution in human blood. *Environ Int*. 2022;163:107199. doi:10.1016/j.envint.2022.107199

30. Salvia R, Rico LG, Bradford JA, et al. Fast-screening flow cytometry method for detecting nanoplastics in human peripheral blood. *MethodsX*. 2023;10:102057. doi:10.1016/j.mex.2023.102057

31. Pironti C, Notarstefano V, Ricciardi M, Motta O, Giorgini E, Montano L. First Evidence of Microplastics in Human Urine, a Preliminary Study of Intake in the Human Body. *Toxics*. 2022;11(1):40. doi:10.3390/toxics11010040

32. Huang S, Huang X, Bi R, et al. Detection and Analysis of Microplastics in Human Sputum. *Environ Sci Technol*. 2022;56(4):2476-2486. doi:10.1021/acs.est.1c03859

33. Schwabl P, Köppel S, Königshofer P, et al. Detection of Various Microplastics in Human Stool: A Prospective Case Series. *Ann Intern Med*. 2019;171(7):453-457. doi:10.7326/M19-0618

34. Zhang J, Wang L, Trasande L, Kannan K. Occurrence of Polyethylene Terephthalate and Polycarbonate Microplastics in Infant and Adult Feces. *Environ Sci Technol Lett*. 2021;8(11):989-994. doi:10.1021/acs.estlett.1c00559

35. Ragusa A, Notarstefano V, Svelato A, et al. Raman Microspectroscopy Detection and Characterisation of Microplastics in Human Breastmilk. *Polymers*. 2022;14(13):2700. doi:10.3390/polym14132700

36. Liu S, Lin G, Liu X, et al. Detection of various microplastics in placentas, meconium, infant feces, breastmilk and infant formula: A pilot prospective study. *Sci Total Environ*. 2022;854:158699. doi:10.1016/j.scitotenv.2022.158699

37. Amato-Lourenço LF, Carvalho-Oliveira R, Júnior GR, Dos Santos Galvão L, Ando RA, Mauad T. Presence of airborne microplastics in human lung tissue. *J Hazard Mater*. 2021;416:126124. doi:10.1016/j.jhazmat.2021.126124

38. Baeza-Martínez C, Olmos S, González-Pleiter M, et al. First evidence of microplastics isolated in European citizens' lower airway. *J Hazard Mater*. 2022;438:129439. doi:10.1016/j.jhazmat.2022.129439

39. Pauly JL, Stegmeier SJ, Allaart HA, et al. Inhaled cellulosic and plastic fibers found in human lung tissue. *Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol*. 1998;7(5):419-428.

40. Qiu L, Lu W, Tu C, et al. Evidence of Microplastics in Bronchoalveolar Lavage Fluid among Never-Smokers: A Prospective Case Series. *Environ Sci Technol*. 2023;57(6):2435-2444. doi:10.1021/acs.est.2c06880

41. Jenner LC, Rotchell JM, Bennett RT, Cowen M, Tentzeris V, Sadofsky LR. Detection of microplastics in human lung tissue using μFTIR spectroscopy. *Sci Total Environ*. 2022;831:154907. doi:10.1016/j.scitotenv.2022.154907

42. Horvatits T, Tamminga M, Liu B, et al. Microplastics detected in cirrhotic liver tissue. *EBioMedicine*. 2022;82:104147. doi:10.1016/j.ebiom.2022.104147

43. Kutralam-Muniasamy G, Shruti VC, Pérez-Guevara F, Roy PD. Microplastic diagnostics in humans: "The 3Ps" Progress, problems, and prospects. *Sci Total Environ*. 2023;856(Pt 2):159164. doi:10.1016/j.scitotenv.2022.159164

44. Amereh F, Amjadi N, Mohseni-Bandpei A, et al. Placental plastics in young women from general population correlate with reduced foetal growth in IUGR pregnancies. *Environ Pollut 1987*. 2022;314:120174. doi:10.1016/j.envpol.2022.120174

45. Braun T, Ehrlich L, Henrich W, et al. Detection of Microplastic in Human Placenta and Meconium in a Clinical Setting. *Pharmaceutics*. 2021;13(7):921. doi:10.3390/pharmaceutics13070921

46. Liu S, Liu X, Guo J, et al. The association between microplastics and microbiota in placentas and meconium: The first evidence in humans. *Environ Sci Technol*. Published online 2022.

47. Ragusa A, Svelato A, Santacroce C, et al. Plasticenta: First evidence of microplastics in human placenta. *Environ Int*. 2021;146:106274. doi:10.1016/j.envint.2020.106274

48. Ragusa A, Matta M, Cristiano L, et al. Deeply in Plasticenta: Presence of Microplastics in the Intracellular Compartment of Human Placentas. *Int J Environ Res Public Health*. 2022;19(18):11593. doi:10.3390/ijerph191811593

49. Zhu L, Zhu J, Zuo R, Xu Q, Qian Y, An L. Identification of microplastics in human placenta using laser direct infrared spectroscopy. *Sci Total Environ*. 2023;856(Pt 1):159060. doi:10.1016/j.scitotenv.2022.159060

50. Amereh F, Babaei M, Eslami A, Fazelipour S, Rafiee M. The emerging risk of exposure to nano(micro)plastics on endocrine disturbance and reproductive toxicity: From a hypothetical scenario to a global public health challenge. *Environ Pollut 1987*. 2020;261:114158. doi:10.1016/j.envpol.2020.114158

51. Jin H, Ma T, Sha X, et al. Polystyrene microplastics induced male reproductive toxicity in mice. *J Hazard Mater*. 2021;401:123430. doi:10.1016/j.jhazmat.2020.123430

52. Hou B, Wang F, Liu T, Wang Z. Reproductive toxicity of polystyrene microplastics: In vivo experimental study on testicular toxicity in mice. *J Hazard Mater*. 2021;405:124028. doi:10.1016/j.jhazmat.2020.124028

53. Wei Z, Wang Y, Wang S, Xie J, Han Q, Chen M. Comparing the effects of polystyrene microplastics exposure on reproduction and fertility in male and female mice. *Toxicol Amst*. 2022;465:153059. doi:10.1016/j.tox.2021.153059

54. Yang ZS, Bai YL, Jin CH, et al. Evidence on Invasion of Blood, Adipose Tissues, Nervous System and Reproductive System of Mice After a Single Oral Exposure: Nanoplastics versus Microplastics. *Biomed Environ Sci*. 2022;35(11):1025-1037. doi:10.3967/bes2022.131

55. Ijaz MU, Shahzadi S, Samad A, et al. Dose-Dependent Effect of Polystyrene Microplastics on the Testicular Tissues of the Male Sprague Dawley Rats. *Dose-Response Publ Int Hormesis Soc*. 2021;19(2):15593258211019882. doi:10.1177/15593258211019882

56. Jin H, Yan M, Pan C, et al. Chronic exposure to polystyrene microplastics induced male reproductive toxicity and decreased testosterone levels via the LH-mediated LHR/cAMP/PKA/StAR pathway. *Part Fibre Toxicol*. 2022;19(1):13. doi:10.1186/s12989-022-00453-2

57. Li S, Wang Q, Yu H, et al. Polystyrene microplastics induce blood–testis barrier disruption regulated by the MAPK-Nrf2 signaling pathway in rats. *Environ Sci Pollut Res Int*. 2021;28(35):47921-47931. doi:10.1007/s11356-021-13911-9

58. Wei Y, Zhou Y, Long C, et al. Polystyrene microplastics disrupt the blood-testis barrier integrity through ROS-Mediated imbalance of mTORC1 and mTORC2. *Environ Pollut 1987*. 2021;289:117904. doi:10.1016/j.envpol.2021.117904

59. Wen S, Chen Y, Tang Y, et al. Male reproductive toxicity of polystyrene microplastics: Study on the endoplasmic reticulum stress signaling pathway. *Food Chem Toxicol Int J Publ Br Ind Biol Res Assoc*. 2023;172:113577. doi:10.1016/j.fct.2022.113577

60. Xie X, Deng T, Duan J, Xie J, Yuan J, Chen M. Exposure to polystyrene microplastics causes reproductive toxicity through oxidative stress and activation of the p38 MAPK signaling pathway. *Ecotoxicol Environ Saf*. 2020;190:110133. doi:10.1016/j.ecoenv.2019.110133

61. Li S, Ma Y, Ye S, Su Y, Hu D, Xiao F. Endogenous hydrogen sulfide counteracts polystyrene nanoplastics-induced mitochondrial apoptosis and excessive autophagy via regulating Nrf2 and

PGC-1α signaling pathway in mouse spermatocyte-derived GC-2spd(ts) cells. *Food Chem Toxicol*. 2022;164:113071. doi:10.1016/j.fct.2022.113071

62. Hu R, Yao C, Li Y, et al. Polystyrene nanoplastics promote CHIP-mediated degradation of tight junction proteins by activating IRE1α/XBP1s pathway in mouse Sertoli cells. *Ecotoxicol Environ Saf*. 2022;248:114332. doi:10.1016/j.ecoenv.2022.114332

63. Liu T, Hou B, Zhang Y, Wang Z. Determination of Biological and Molecular Attributes Related to Polystyrene Microplastic-Induced Reproductive Toxicity and Its Reversibility in Male Mice. *Int J Environ Res Public Health*. 2022;19(21):14093. doi:10.3390/ijerph192114093

64. Zhou L, Yu Z, Xia Y, et al. Repression of autophagy leads to acrosome biogenesis disruption caused by a sub-chronic oral administration of polystyrene nanoparticles. *Environ Int*. 2022;163:107220. doi:10.1016/j.envint.2022.107220

65. D'Angelo S, Meccariello R. Microplastics: a threat for male fertility. *Int J Environ Res Public Health*. 2021;18(5):2392.

66. Levine H, Jørgensen N, Martino-Andrade A, et al. Temporal trends in sperm count: a systematic review and meta-regression analysis of samples collected globally in the 20th and 21st centuries. *Hum Reprod Update*. 2023;29(2):157-176. doi:10.1093/humupd/dmac035

67. Stuppia L, Franzago M, Ballerini P, Gatta V, Antonucci I. Epigenetics and male reproduction: the consequences of paternal lifestyle on fertility, embryo development, and children lifetime health. *Clin Epigenetics*. 2015;7:120. doi:10.1186/s13148-015-0155-4

68. Marcho C, Oluwayiose OA, Pilsner JR. The preconception environment and sperm epigenetics. *Andrology*. 2020;8(4):924-942. doi:10.1111/andr.12753

69. Prados J, Stenz L, Somm E, Stouder C, Dayer A, Paoloni-Giacobino A. Prenatal Exposure to DEHP Affects Spermatogenesis and Sperm DNA Methylation in a Strain-Dependent Manner. *PloS One*. 2015;10(7):e0132136. doi:10.1371/journal.pone.0132136

70. Oluwayiose OA, Marcho C, Wu H, et al. Paternal preconception phthalate exposure alters sperm methylome and embryonic programming. *Environ Int*. 2021;155:106693. doi:10.1016/j.envint.2021.106693

71. Yin L, Dai Y, Jiang X, et al. Role of DNA methylation in bisphenol A exposed mouse spermatocyte. *Environ Toxicol Pharmacol*. 2016;48:265-271. doi:10.1016/j.etap.2016.11.003

72. Ryu DY, Pang WK, Adegoke EO, Rahman MS, Park YJ, Pang MG. Abnormal histone replacement following BPA exposure affects spermatogenesis and fertility sequentially. *Environ Int*. 2022;170:107617. doi:10.1016/j.envint.2022.107617

73. Zhang T, Zhou Y, Li L, et al. Melatonin protects prepuberal testis from deleterious effects of bisphenol A or diethylhexyl phthalate by preserving H3K9 methylation. *J Pineal Res*. 2018;65(2):e12497. doi:10.1111/jpi.12497

74. Zhao TX, Wang JK, Shen LJ, et al. Increased m6A RNA modification is related to the inhibition of the Nrf2-mediated antioxidant response in di-(2-ethylhexyl) phthalate-induced prepubertal testicular injury. *Environ Pollut Barking Essex 1987*. 2020;259:113911. doi:10.1016/j.envpol.2020.113911

75. Miao M, Zhou X, Li Y, et al. LINE-1 hypomethylation in spermatozoa is associated with Bisphenol A exposure. *Andrology*. 2014;2(1):138-144. doi:10.1111/j.2047-2927.2013.00166.x

76. Wu H, Estill MS, Shershebnev A, et al. Preconception urinary phthalate concentrations and sperm DNA methylation profiles among men undergoing IVF treatment: a cross-sectional study. *Hum Reprod Oxf Engl*. 2017;32(11):2159-2169. doi:10.1093/humrep/dex283

77. Oluwayiose OA, Houle E, Wu H, et al. Urinary phthalate metabolites and their mixtures are associated with advanced sperm epigenetic aging in a general population. *Environ Res*. 2022;214(Pt 4):114115. doi:10.1016/j.envres.2022.114115

78. Tian M, Liu L, Zhang J, Huang Q, Shen H. Positive association of low-level environmental phthalate exposure with sperm motility was mediated by DNA methylation: A pilot study. *Chemosphere*. 2019;220:459-467. doi:10.1016/j.chemosphere.2018.12.155

79. Haddadi A, Kessabi K, Boughammoura S, et al. Exposure to microplastics leads to a defective ovarian function and change in cytoskeleton protein expression in rat. *Environ Sci Pollut Res Int*. 2022;29(23):34594-34606. doi:10.1007/s11356-021-18218-3

80. Liu Z, Zhuan Q, Zhang L, Meng L, Fu X, Hou Y. Polystyrene microplastics induced female reproductive toxicity in mice. *J Hazard Mater*. 2022;424(Pt C):127629. doi:10.1016/j.jhazmat.2021.127629

81. Zeng L, Zhou C, Xu W, et al. The ovarian-related effects of polystyrene nanoplastics on human ovarian granulosa cells and female mice. *Ecotoxicol Environ Saf*. 2023;257:114941. doi:10.1016/j.ecoenv.2023.114941

82. Wu H, Liu Q, Yang N, Xu S. Polystyrene-microplastics and DEHP co-exposure induced DNA damage, cell cycle arrest and necroptosis of ovarian granulosa cells in mice by promoting ROS production. *Sci Total Environ*. 2023;871:161962. doi:10.1016/j.scitotenv.2023.161962

83. Huang J, Zou L, Bao M, Feng Q, Xia W, Zhu C. Toxicity of polystyrene nanoparticles for mouse ovary and cultured human granulosa cells. *Ecotoxicol Environ Saf*. 2023;249:114371. doi:10.1016/j.ecoenv.2022.114371

84. Hou J, Lei Z, Cui L, et al. Polystyrene microplastics lead to pyroptosis and apoptosis of ovarian granulosa cells via NLRP3/Caspase-1 signaling pathway in rats. *Ecotoxicol Environ Saf*. 2021;212:112012. doi:10.1016/j.ecoenv.2021.112012

85. Zhang Y, Wang X, Zhao Y, et al. Reproductive toxicity of microplastics in female mice and their offspring from induction of oxidative stress. *Environ Pollut Barking Essex 1987*. 2023;327:121482. doi:10.1016/j.envpol.2023.121482

86. Wu H, Xu T, Chen T, Liu J, Xu S. Oxidative stress mediated by the TLR4/NOX2 signalling axis is involved in polystyrene microplastic-induced uterine fibrosis in mice. *Sci Total Environ*. 2022;838(Pt 2):155825. doi:10.1016/j.scitotenv.2022.155825

87. An R, Wang X, Yang L, et al. Polystyrene microplastics cause granulosa cells apoptosis and fibrosis in ovary through oxidative stress in rats. *Toxicology*. 2021;449:152665. doi:10.1016/j.tox.2020.152665

88. Meirow D, Dor J, Kaufman B, et al. Cortical fibrosis and blood-vessels damage in human ovaries exposed to chemotherapy. Potential mechanisms of ovarian injury. *Hum Reprod Oxf Engl*. 2007;22(6):1626-1633. doi:10.1093/humrep/dem027

89. Meirow D, Biederman H, Anderson RA, Wallace WHB. Toxicity of chemotherapy and radiation on female reproduction. *Clin Obstet Gynecol*. 2010;53(4):727-739. doi:10.1097/GRF.0b013e3181f96b54

90. Zhou F, Shi LB, Zhang SY. Ovarian Fibrosis: A Phenomenon of Concern. *Chin Med J (Engl)*. 2017;130(3):365-371. doi:10.4103/0366-6999.198931

91. Grechi N, Franko R, Rajaraman R, et al. *Microplastics Are Present in Women's and Cows' Follicular Fluid and Polystyrene Microplastics Compromise Bovine Oocyte Function in Vitro*. elife; 2023. doi:10.7554/eLife.86791.1

92. Manikkam M, Guerrero-Bosagna C, Tracey R, Haque MM, Skinner MK. Transgenerational actions of environmental compounds on reproductive disease and identification of epigenetic biomarkers of ancestral exposures. *PloS One*. 2012;7(2):e31901. doi:10.1371/journal.pone.0031901

93. Berger A, Ziv-Gal A, Cudiamat J, Wang W, Zhou C, Flaws JA. The effects of in utero bisphenol A exposure on the ovaries in multiple generations of mice. *Reprod Toxicol Elmsford N*. 2016;60:39-52. doi:10.1016/j.reprotox.2015.12.004

94. Dusza HM, van Boxel J, van Duursen MBM, Forsberg MM, Legler J, Vähäkangas KH. Experimental human placental models for studying uptake, transport and toxicity of micro- and nanoplastics. *Sci Total Environ*. 2023;860:160403. doi:10.1016/j.scitotenv.2022.160403

95. Fournier SB, D'Errico JN, Adler DS, et al. Nanopolystyrene translocation and fetal deposition after acute lung exposure during late-stage pregnancy. *Part Fibre Toxicol*. 2020;17(1):55. doi:10.1186/s12989-020-00385-9

96. Hu J, Qin X, Zhang J, et al. Polystyrene microplastics disturb maternal-fetal immune balance and cause reproductive toxicity in pregnant mice. *Reprod Toxicol*. 2021;106:42-50. doi:10.1016/j.reprotox.2021.10.002

97. Nie JH, Shen Y, Roshdy M, Cheng X, Wang G, Yang X. Polystyrene nanoplastics exposure caused defective neural tube morphogenesis through caveolae-mediated endocytosis and faulty apoptosis. *Nanotoxicology*. 2021;15(7):885-904. doi:10.1080/17435390.2021.1930228

98. Yang D, Zhu J, Zhou X, et al. Polystyrene micro- and nano-particle coexposure injures fetal thalamus by inducing ROS-mediated cell apoptosis. *Environ Int*. 2022;166:107362. doi:10.1016/j.envint.2022.107362

99. Chen G, Xiong S, Jing Q, et al. Maternal exposure to polystyrene nanoparticles retarded fetal growth and triggered metabolic disorders of placenta and fetus in mice. *Sci Total Environ*. 2022;854:158666. doi:10.1016/j.scitotenv.2022.158666

100. Aghaei Z, Mercer GV, Schneider CM, et al. Maternal exposure to polystyrene microplastics alters placental metabolism in mice. *Metabolomics Off J Metabolomic Soc*. 2022;19(1):1. doi:10.1007/s11306-022-01967-8

101. Hu J, Zhu Y, Zhang J, et al. The potential toxicity of polystyrene nanoplastics to human trophoblasts in vitro. *Environ Pollut Barking Essex 1987*. 2022;311:119924. doi:10.1016/j.envpol.2022.119924

102. Lee HS, Amarakoon D, Wei CI, Choi KY, Smolensky D, Lee SH. Adverse effect of polystyrene microplastics (PS-MPs) on tube formation and viability of human umbilical vein endothelial cells. *Food Chem Toxicol Int J Publ Br Ind Biol Res Assoc*. 2021;154:112356. doi:10.1016/j.fct.2021.112356

103. Dusza HM, Katrukha EA, Nijmeijer SM, et al. Uptake, Transport, and Toxicity of Pristine and Weathered Micro- and Nanoplastics in Human Placenta Cells. *Environ Health Perspect*. 2022;130(9):097006. doi:10.1289/EHP10873

104. Shen F, Li D, Guo J, Chen J. Mechanistic toxicity assessment of differently sized and charged polystyrene nanoparticles based on human placental cells. *Water Res*. 2022;223:118960. doi:10.1016/j.watres.2022.118960

105. Huang JP, Hsieh PCH, Chen CY, et al. Nanoparticles can cross mouse placenta and induce trophoblast apoptosis. *Placenta*. 2015;36(12):1433-1441. doi:10.1016/j.placenta.2015.10.007

106. Cary CM, DeLoid GM, Yang Z, et al. Ingested Polystyrene Nanospheres Translocate to Placenta and Fetal Tissues in Pregnant Rats: Potential Health Implications. *Nanomater Basel Switz*. 2023;13(4):720. doi:10.3390/nano13040720

107. Han Y, Song Y, Kim GW, et al. No prominent toxicity of polyethylene microplastics observed in neonatal mice following intratracheal instillation to dams during gestational and neonatal period. *Toxicol Res Seoul*. 2021;37(4):443-450. doi:10.1007/s43188-020-00086-7

108. Wick P, Malek A, Manser P, et al. Barrier capacity of human placenta for nanosized materials. *Environ Health Perspect*. 2010;118(3):432-436. doi:10.1289/ehp.0901200

109. Gruber MM, Hirschmugl B, Berger N, et al. Plasma proteins facilitates placental transfer of polystyrene particles. *J Nanobiotechnology*. 2020;18(1):128. doi:10.1186/s12951-020-00676-5

110. Chortarea S, Gupta G, Saarimäki LA, et al. Transcriptomic profiling reveals differential cellular response to copper oxide nanoparticles and polystyrene nanoplastics in perfused human placenta. *Environ Int*. 2023;177:108015. doi:10.1016/j.envint.2023.108015

111. Aghaei Z, Sled JG, Kingdom JC, et al. Maternal Exposure to Polystyrene Micro- and Nanoplastics Causes Fetal Growth Restriction in Mice. *Environ Sci Technol Lett*. 2022;9(5):426-430. doi:10.1021/acs.estlett.2c00186

112. Perry IJ, Beevers DG, Whincup PH, Bareford D. Predictors of ratio of placental weight to fetal weight in multiethnic community. *BMJ*. 1995;310(6977):436-439. doi:10.1136/bmj.310.6977.436

113. Cahill LS, Rennie MY, Hoggarth J, et al. Feto- and utero-placental vascular adaptations to chronic maternal hypoxia in the mouse. *J Physiol*. 2018;596(15):3285-3297. doi:10.1113/JP274845

114. Cahill LS, Zhou YQ, Hoggarth J, et al. Placental vascular abnormalities in the mouse alter umbilical artery wave reflections. *Am J Physiol Heart Circ Physiol*. 2019;316(3):H664-H672. doi:10.1152/ajpheart.00733.2018

115. Krakowiak P, Smith EN, de Bruyn G, Lydon-Rochelle MT. Risk factors and outcomes associated with a short umbilical cord. *Obstet Gynecol*. 2004;103(1):119-127. doi:10.1097/01.AOG.0000102706.84063.C7

116. Wang M, Rücklin M, Poelmann RE, et al. Nanoplastics causes extensive congenital malformations during embryonic development by passively targeting neural crest cells. *Environ Int*. 2023;173:107865. doi:10.1016/j.envint.2023.107865

117. Huang T, Zhang W, Lin T, et al. Maternal exposure to polystyrene nanoplastics during gestation and lactation induces hepatic and testicular toxicity in male mouse offspring. *Food Chem Toxicol*. 2022;160:112803. doi:10.1016/j.fct.2021.112803

118. Tang J, Bu W, Hu W, et al. Ferroptosis Is Involved in Sex-Specific Small Intestinal Toxicity in the Offspring of Adult Mice Exposed to Polystyrene Nanoplastics during Pregnancy. *ACS Nano*. 2023;17(3):2440-2449. doi:10.1021/acsnano.2c09729

119. Wang X, Zhao Z, Wang X, et al. Effects of polystyrene nanoplastic gestational exposure on mice. *Chemosphere Oxf*. 2023;324:138255. doi:10.1016/j.chemosphere.2023.138255

120. Park EJ, Han JS, Park EJ, et al. Repeated-oral dose toxicity of polyethylene microplastics and the possible implications on reproduction and development of the next generation. *Toxicol Lett*. 2020;324:75-85. doi:10.1016/j.toxlet.2020.01.008

121. Jeong B, Baek JY, Koo J, et al. Maternal exposure to polystyrene nanoplastics causes brain abnormalities in progeny. *J Hazard Mater*. 2022;426:127815. doi:10.1016/j.jhazmat.2021.127815

122. Darendeliler F. IUGR: Genetic influences, metabolic problems, environmental associations/triggers, current and future management. *Best Pract Res Clin Endocrinol Metab*. 2019;33(3):101260. doi:10.1016/j.beem.2019.01.001

123. Luo T, Zhang Y, Wang C, et al. Maternal exposure to different sizes of polystyrene microplastics during gestation causes metabolic disorders in their offspring. *Environ Pollut Barking Essex 1987*. 2019;255(Pt 1):113122. doi:10.1016/j.envpol.2019.113122

124. Luo T, Wang C, Pan Z, Jin C, Fu Z, Jin Y. Maternal Polystyrene Microplastic Exposure during Gestation and Lactation Altered Metabolic Homeostasis in the Dams and Their F1 and F2 Offspring. *Environ Sci Technol*. 2019;53(18):10978-10992. doi:10.1021/acs.est.9b03191

125. Martínez-Ibarra A, Martínez-Razo LD, MacDonald-Ramos K, et al. Multisystemic alterations in humans induced by bisphenol A and phthalates: Experimental, epidemiological and clinical studies reveal the need to change health policies. *Environ Pollut Barking Essex 1987*. 2021;271:116380. doi:10.1016/j.envpol.2020.116380

126. Alonso-Magdalena P, Quesada I, Nadal Á. Prenatal Exposure to BPA and Offspring Outcomes: The Diabesogenic Behavior of BPA. *Dose-Response Publ Int Hormesis Soc*. 2015;13(2):1559325815590395. doi:10.1177/1559325815590395

127. Zaheer J, Kim H, Ko IO, et al. Pre/post-natal exposure to microplastic as a potential risk factor for autism spectrum disorder. *Environ Int*. 2022;161:107121. doi:10.1016/j.envint.2022.107121

Chapter 3 - Hypothesis and Research Aims

Accumulation of environmental microplastics (MPs) across several organ systems has been documented in human populations. Experimental models have also demonstrated the ability of MPs to likewise accumulate in the placenta across pregnancy and transfer them to fetal tissues [1,2]. More recently, these same observations have been made in a few small observational studies of human placenta tissue [3–5]. What is not as clear is which placental structures are most susceptible to MP exposure and accumulation – information needed to decipher the potential impact of MPs on placental function. Further, the magnitude and geographical context of this potential health concern remains uncertain, particularly for the Canadian pregnant population. In the studies carried out to date, there remains some concern about the source of the identified MPs, and whether they may in part be the result of post-delivery contamination and potentially influenced by the mode of delivery (vaginal vs. C-section). However, MPs have been measured in human fetal meconium, findings that are highly suggestive of placental accumulation and maternal-fetal transfer of these environmental contaminants. As such, the current study aims to confirm the presence and localization of MP contamination in human placentas within a Canadian pregnant population. In the design of this study, considerable attention was given to the role of post-delivery environmental contamination, and the potential role of mode of delivery, with a comprehensive environmental sampling for contaminating MPs and the comparison of MP accumulation in placentas collected from both vaginal and C-section deliveries.

Hypothesis

The overarching hypothesis of the current study is that ***environmental microplastics can accumulate in human placenta across pregnancy and may serve as an adverse developmental***

programming exposure to the developing offspring. More specifically, it is hypothesized that MPs will be found throughout the placenta, including within the chorionic villi structures of the placenta – the site of active maternal-fetal exchange. Further, it is proposed that placentas collected from vaginal deliveries, compared to C-section deliveries, will demonstrate a higher level of MP contamination due to passage through the vaginal canal, where additional MP contamination may be present.

Specific Research Aims

In the current MSc thesis these hypotheses will be tested through the completion of the following specific research aims:

Aim 1. Complete an environmental assessment of MP contamination within a Canadian labor and delivery unit and develop a plastic reduced protocol for human placental sampling following both C-section and vaginal deliveries.

Aim 2. Measure and compare total MP contamination in human placentas following C-section and vaginal deliveries using Raman micro-spectrometry.

Aim 3. Determine the types, and potential sources, of MP contamination of the human placenta in a Canadian context.

In the completion of this thesis a comprehensive review of the literature focused on the potential impacts of MP exposure on maternal and fetal health, considered within the developmental origins of health and disease (DOHaD) framework, was carried out and will be submitted for peer review (Chapter 2). The main body of this thesis comprises an additional

manuscript (Chapter 4) that presents the results obtained in the completion of the stated research objectives 1-3, which also will be submitted for peer review.

References

1. Han Y, Song Y, Kim GW, et al. No prominent toxicity of polyethylene microplastics observed in neonatal mice following intratracheal instillation to dams during gestational and neonatal period. *Toxicol Res Seoul*. 2021;37(4):443-450. doi:10.1007/s43188-020-00086-7

2. Yang D, Zhu J, Zhou X, et al. Polystyrene micro- and nano-particle coexposure injures fetal thalamus by inducing ROS-mediated cell apoptosis. *Environ Int*. 2022;166:107362. doi:10.1016/j.envint.2022.107362

3. Amereh F, Amjadi N, Mohseni-Bandpei A, et al. Placental plastics in young women from general population correlate with reduced foetal growth in IUGR pregnancies. *Environ Pollut 1987*. 2022;314:120174. doi:10.1016/j.envpol.2022.120174

4. Ragusa A, Svelato A, Santacroce C, et al. Plasticenta: First evidence of microplastics in human placenta. *Environ Int*. 2021;146:106274. doi:10.1016/j.envint.2020.106274

5. Braun T, Ehrlich L, Henrich W, et al. Detection of Microplastic in Human Placenta and Meconium in a Clinical Setting. *Pharmaceutics*. 2021;13(7):921. doi:10.3390/pharmaceutics13070921

Chapter 4 - Microplastic Exposure and Accumulation in Human Placenta

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R.Z contributed to data collection and analysis, writing and editing. All authors have read and agreed to the published version of the manuscript.

Abstract

Microplastics (MPs) are tiny plastic particles (≤5 mm) generated by the breakdown of larger plastic products in the environment. Microplastic accumulation in human placenta has recently been described. In an extension of this landmark work, the current study describes the accumulation, distribution, and characterization of MPs within the term human placenta. Placenta tissues were collected from healthy pregnancies following vaginal (n=5) and caesarean section (n=5) deliveries at a tertiary care centre located in an urban Canadian city (Ottawa, ON), with MPs detected and characterized by Raman microspectroscopy. MPs were identified in all placentas examined, with an average of 1 ± 1.2 MP/g of tissue. Similar tissue concentrations of MPs were identified in all regions of the placenta (basal plate, chorionic villous, chorionic plate), and did not differ according to mode of delivery. MPs ranged in size (2 - 60 μm), with the most abundant MPs being polyethylene (PE), polypropylene (PP), polystyrene (PS) and Polyvinyl chloride (PVC). Several non-plastic foreign particles were also identified in all placenta samples (4 ± 2.9 non-MP particles/g tissue), most abundantly identified as carbon, graphite, and lead oxide. Collectively, these results demonstrate the accumulation of foreign particles, including MPs, throughout the human placenta. Given the vital functions of the placenta in supporting fetal growth and development, and the known biological toxicity of MPs, further investigations into the potential harmful effects of these environmental toxicants on maternal and fetal health is warranted.

Introduction

Intense plastic manufacturing and use, coupled to poor waste management, has led to an environment overwhelmed with billions of tons of plastic waste [1,2]. Plastic material abandoned in the environment leads to fragmentation and degradation [3]. This process yields smaller plastic fragments referred to as microplastics (MPs). MPs range in size, from 1 µm to 5mm [4], and are found ubiquitously in our environment, including waterways [5,6] soil [7] tap water [8], drinking water [8], household dust [9] and food products [8,10,11].

Numerous investigations, encompassing both human and animal studies, have established microplastic particles have the capacity to enter the human body and amass within various tissues and organ systems [12–18] . Microplastics have been detected in vital human biological matrices, including but not limited to, the circulatory system, exemplified by their presence in human blood [19]. Additionally, they have been found in urine [20] and fecal matter [21]. Furthermore, these investigations have illuminated the distribution of MPs within various human organ systems encompassing the lungs [12–16], spleen [22], liver [17], kidney [22], and colon [18]. Recently studies identified the presence of MPs within the human placenta [23–29] and fetal meconium [24–26]. These discoveries are causing concern regarding the potential ramifications of environmental contaminations, specifically MPs, on the health and development of the human fetus [30] .

The placenta is a transitional but vital organ of pregnancy, responsible for all maternal-fetal exchange required to support fetal growth and development. In rodent models of gestational MP exposure, placental uptake has been described, with observed consequences for placental

function and compromised fetal growth trajectories [29]. The landmark findings of MP accumulation within the human placenta have caught global attention, with ongoing queries regarding the scope and geographical context of this potential environmental health concern for mothers and infants. Given the heavy reliance on plastic polymer products within obstetrical health care delivery [30], there are additional questions surrounding the contributions of post-delivery contamination within the small observational studies reporting these findings, particularly across various delivery environments (i.e. surgical suite for C-section deliveries) [24] .

The present study aimed to evaluate the accumulation of MPs in human placentas collected from healthy pregnant Canadian women, with the use of Raman microspectroscopy to specifically identify polymer composition. Detailed investigation of MP localization across the distinct compartments of the placenta was undertaken, as well as a comparison of placental MP measurement in placentas delivered by vaginal or caesarean section, to specifically address concerns related to delivery environment and post-delivery contamination. We further present a modified protocol for MP isolation from human tissues which permits the identification of additional particles within placenta tissue samples beyond plastic polymers.

Methods

Patient Recruitment

This study received approval from Health Canada-Public Health Agency of Canada Research Ethics Board (REB 2021-033H) and the University of Ottawa Research Ethics Board (#H-03-22-7960). All recruited women had singleton, uncomplicated term pregnancies at The Ottawa

Hospital General Campus Birthing Unit. Exclusion criteria for study participation included any obstetrical complication during pregnancy or delivery, C-section delivery for any reason other than breach presentation or repeat C-section delivery, multiple (twins or more) pregnancy, or participants who did not understand either English or French. A total of 10 patients were recruited: 5 undergoing a standard vaginal delivery and 5 undergoing an elective C-section delivery.

Characterization of potential post-delivery and environmental MP contamination

A plastic-reduced protocol was developed for both vaginal and C-section deliveries. All plastic-containing materials used during a standard vaginal or C-section delivery at The Ottawa Hospital General Campus were identified, and where available a non-plastic alterative material was identified for use during delivery. For those plastic-containing materials that could not be substituted due to institutional standard of care operational procedure (i.e., plastic surgical drape, gloves), items were sampled to determine potential post-delivery contamination signal. Briefly, in a sterile environment, the plastic-containing materials were rinsed three times with 30 ml de-ionized filtered water , with the rinsate collected into sterile glass bottles. The samples were filtered through a silicon membrane (1 µm retention limit, Microplastic Sample Preparation Kit, Thermo Fisher Nepean, ON) using glass vacuum filtration funnel. Silicon filter membranes were used to collect any microplastics in these wash samples as their low optical interference and fine retention diameter (1 µm) ensured a high likelihood of identifying microplastics compared with glass fibre membranes required for the more difficult to filter

tissue samples (see below). Raman microspectroscopy was used to identify the presence and character of any background plastic contamination, as described below (Section 2.4).

Similarly, plastics reduced conditions were developed for all tissue dissection and sample processing. All plastic materials (with the exception of nitrile gloves) were kept out of the biocontainment hood in which samples were handled and sample filtering was completed. Individuals working in this hood wore only clothing (including lab coats) made from natural fibres. Prior to use all containers, sample vials, and glassware used in transporting, collecting, processing or storing placenta or blank samples were vigorously rinsed with 50% of container volume with deionized water (MilliQ,) that was filtered through glass fibre membranes (Grade F, 0.7 µm retention limit, Sterlitech, Aubern, WA).

Placenta collection

After delivery, all placentas were collected under sterile conditions, placed into a sealed metal container, and transported on ice to the laboratory for processing within 1 hour of delivery. Placentas were weighed and placed into a sterile glass Pyrex tray within a biosafety hood. A metal bowl with 10ml deionized filtered water was placed in the hood to assess the presence of contaminating MPs within the hood air (Hood Blank - HB). Collected placentas were rinsed with 100ml deionized filtered water and transferred to a second sterile glass Pyrex dish. Microdissections of placental tissue were carried out midway between the placental margin and umbilical cord insertion site. At each microdissection site, samples were collected from the basal plate (maternal surface), chorionic villous tissue (maternal-fetal exchange region), and chorionic plate (fetal surface). All samples and HBs and were placed in labelled low-particle

glass vials and sealed. The water rinsate (100ml) used in the dissection process was collected into a 125 ml glass Wheaton bottle with phenol cap with Polytetrafluoroethylene (PFTE) liner. Prior to sample collection, all vials and containers were rinsed 2 times with deionized water (50% total container volume) which was pre-filtered through fibre filter membranes (Whatman Grade GF/F, 0.7 µm pore size). All collected samples were stored at -20°C until digestion.

Digestion of placenta samples

The digestion of placenta samples was performed at the Environmental Health Science and Research Bureau at Health Canada, using a previously published method [16] with minor modifications as described. All glassware used to process tissue for MP isolation was washed, rinsed 2X with deionized water then rinsed with filtered deionized water and sealed with caps (vials) or rinsed tinfoil (flasks) prior to use. Vials containing placenta tissue were thawed at room temperature, rinsed with filtered water, and placed within a biocontainment hood. Cleaned and rinsed foil-sealed glass Erlenmeyer flasks (500 ml) were weighed to the nearest 10 mg and placed into the biocontainment hood. For each microdissected sample from each placenta, 1 g of placenta tissue was placed into a flask, resealed, and reweighed. The flask was then returned to the hood and 100 ml of filtered (GF/F) 30% H_2O_2 (>95%, Thermo Scientific) was added. The resealed flasks were then placed in a shaking incubator at 55°C for 11 days at 100 rpm. After the first 5 days of incubation an additional 50ml of filtered (GF/F) 30% H_2O_2 was added to the digest sample, which was then placed back in the incubator for an additional 6 days. For each batch of samples digested, a Procedural Blank (PB) was also processed. This

was generated by adding the filtered (GF/F) 30% H_2O_2 solution to a flask in the absence of any biological tissues and treated in the same manner as the digest samples.

At the end of the incubation period, digests and PBs were filtered through a glass fiber membrane (Whatman Grade GF/D, 2.7 µm pore size), using a glass vacuum filtration apparatus. Glass filtration assemblies and clamp were pre-warmed to 55 °C. Digests were poured through the membrane, followed by 10 ml of filtered (GF/F) deionized water to remove any remaining H_2O_2 residue. The glass fiber membranes were then placed in clean glass petri dishes with unique identification and left in the plastic-free biosafety hood to dry.

Analysis of microplastics by Raman Microspectroscopy

Dried glass fiber membranes were examined using an integrated imaging system that couples an Enhanced Dark Field (EDF) optical microscope (CytoViva, Inc. Auburn, AL, USA) with a confocal Raman imaging system (Horiba Scientific XploRa Plus, Japan). The LabSpec 6 software suite (Horiba Ltd, Piscataway, NJ, USA) was used to acquire the Raman spectral information and KnowItAll spectral database (Bio-Rad Laboratories Inc, Hercules, CA, USA) was used to identify the character of the MPs and other particulate matter. Membranes were visually scanned under 10X power in an organized systemic-grid fashion to locate all particles adherent to the glass fiber membranes. Upon observing a particle, a brightfield image of the particle was captured (under 50X power), with a Raman spectrum and KnowItAll prediction collected from mid particle focus (**Figure 4.1**).

Figure 4.1

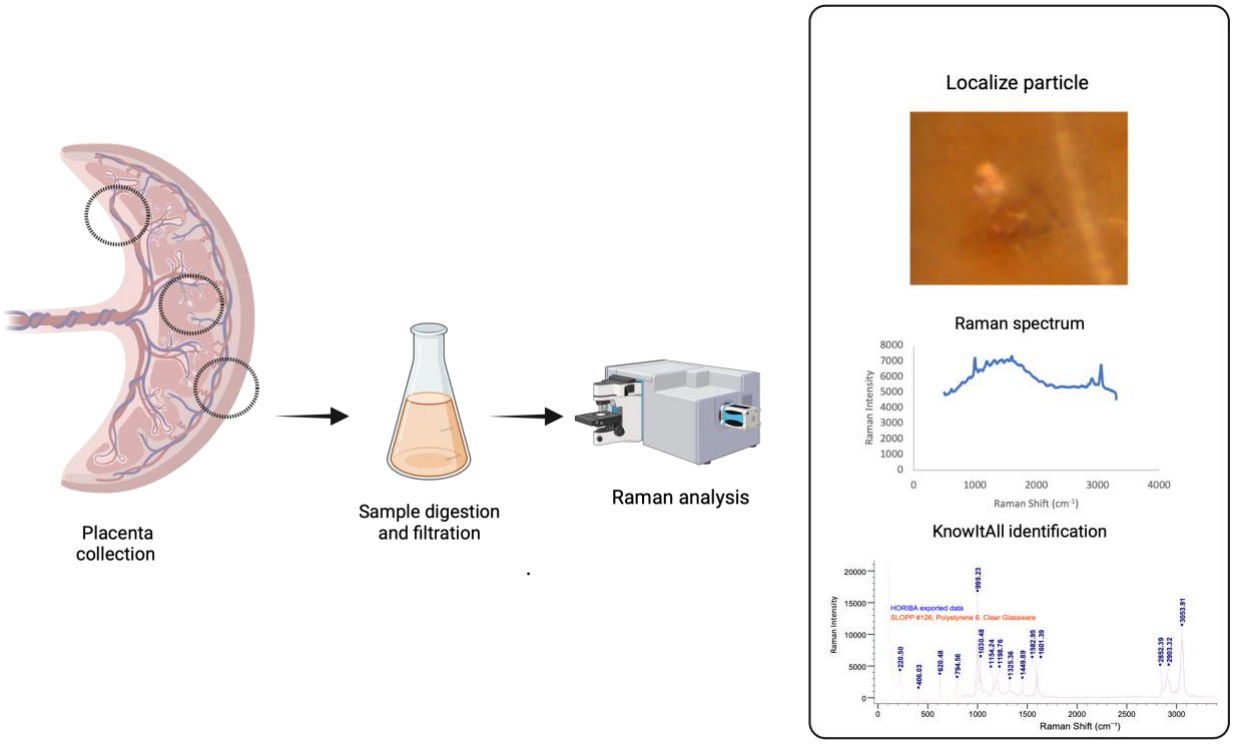

Figure 4.1. *Methodology workflow of placenta tissue sampling and processing for microplastic detection. One gram of placental tissue was collected from each anatomical site (chorionic plate, chorionic villus, and basal plate), which underwent chemical digestion and filtering through a GF/D membrane. Microplastics were identified using Raman microspectroscopy, with the generated spectra compared to the KnowItAll database to determine the particulate identity.*

Statistical Analysis

The comparison of data on MPs or non-MP numbers between regions and delivery types was done iteratively. Initially, a 2-way ANOVA was attempted but the data did not meet the essential assumptions of normality (Shapiro Wilk test) and equal variance (Brown-Forsythe test) required for a parametric test. Therefore, the difference in particle numbers (MP and non-MP) was analyzed separately in each placental region or summed across all regions by comparing the mode of delivery. Data was tested for normality using the Shapiro Wilk test. If the data was normally distributed, a 2-sided T-test was applied, and if the data was not normally distributed, the Mann-Witney rank sum test was applied. The Kruskal Wallis test and Friedman test was used to analyze the difference while comparing all anatomical regions as the data was non-normally distributed. The significance for all statistical tests was set at $p<0.05$. As no tests indicated significant differences, no post-hoc comparisons were used. All statistical analyses were carried out using SigmaPlot (v13.0, Systat Inc, San Jose, CA).

Results

Patient characteristics

Patient demographics, stratified according to mode of delivery, are presented in **Table 4.1**. Patients who delivered by C-section had slightly lower gestational ages compared to those delivered vaginally but this failed to reach statistical significance ($p = 0.07$) (38.2 $\pm$ 1.30 vs 39.8 $\pm$ 1.09) patients who delivered by C-section were carrying male fetuses (5/5), compared to 60% of

patients in the vaginal delivery group (3/5). No differences were noted for maternal age, birthweight and placental weight, and no smokers were included in either group of patients.

***Table 4.1**. Patient Demographics*

	Vaginal Delivery (n=5)	C-Section Delivery (n=5)	T-Test P-Value
Maternal Age; yrs	34 ± 3.71	33 ± 2.55	0.507
Smoker; n (%)	0	0	-
Gestational age at delivery; weeks	39.8 ± 1.09	38.2 ± 1.30	0.07
Fetal Sex; n males (% males)	3 (60)	5 (100)	-
Birthweight percentile	46 ± 34.5	72.6 ± 29.7	0.23
Placental weight; g	663 ± 133	685 ± 121	0.79

Post-delivery and environmental MP contamination

Four plastic materials were identified in the delivery and surgical suites of the birthing unit that could not be substituted during the delivery and sampling protocol and underwent MP evaluation. Only one item tested, the surgical drape used during C-section deliveries, demonstrated evidence of MP shedding, with 3 MP particles identified in 30 ml of rinsate, identified as polypropylene (PP) polymers (**Supplemental Table 4.1**).

In both HBs and PBs some contaminating fibres were identified, with cotton or human hair source origin (**Supplemental Table 4.2**). Of note, these contaminating fiber types would be completely degraded in the sample digestion protocol employed. Nevertheless, fibres were excluded from all enumeration of observed particles in our placenta samples, regardless of their composition. Other types of contaminations shown in **Supplemental Table 4.2** were observed in the HBs from C-section sample 1 (C1), C4, Vaginal sample 1 (V1), V2 and V3 and in PBs of C3, C4, V2 and V3.

However, these contaminants were infrequently observed and did not appear to be commonly found in any biological samples tested.

Identification of MPs in Placenta Samples

For each patient, 1 gram of tissue was collected from each of the three distinct anatomical regions of the placenta and processed for Raman Microspectroscopy (**Figure 4.1**). Identified MPs were noted in all placentas sampled, with 31 MP particles total detected across all ten placentas. The number of MPs per placenta varied (ranging from 1-11, **Figure 4.2**), with an average of 1 ± 1.2 MP/g of tissue. The identified MPs ranged in size from approximately 2 - 60 μm. No significant differences were observed in the number of identified MPs across the three anatomical sites sampled – the basal plate, the chorionic villous and chorionic plate ($p>0.05$; **Figure 4.2**). There was likewise no difference in the total or localized number of MPs identified according to mode of delivery ($p>0.05$; **Figure 4.2**)

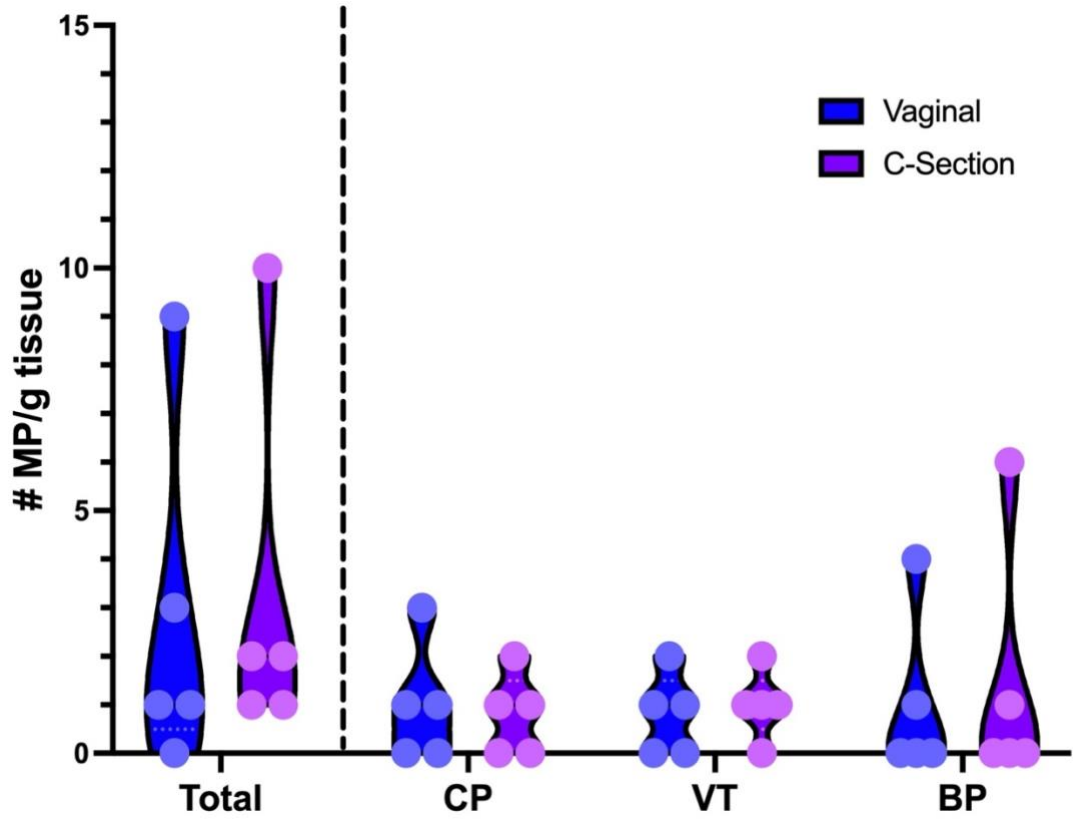

Figure 4.2. *Total and region-specific number of microplastics (MP) identified in placenta tissue collected from vaginal (blue) and C-section (purple) deliveries. One gram of tissue was collected and processed from each anatomical site for each placenta. BP = basal plate; VT = chorionic villous tissue; CP = chorionic plate.*

Characterization of MPs identified within placenta samples

Raman spectra were analyzed for each identified particle and exported into the KnowItAll database software for comparison to a library of spectra from known materials. In the collected placenta samples, the most abundant MP detected was unidentified polymers containing Copper-phthalocyanine, found in 6/10 placentas (0.85 ± 1.2 MP/g tissue), however no significant differences were observed according to anatomical location within the placenta or mode of

delivery (P=0.54; **Tables 4.2, 4.3**). Polyethylene Polystyrene (PS) and polyvinyl chloride (PVC) were each identified in 2/10 placentas (both with 0.3 particles/g tissue). Other common polymers identified in at least one placenta, included polypropylene (PP) and polymethyl methacrylate (PMMA) (both with 0.3 particles/g tissue). While polyethylene (PE) was only observed in 1/10 placentas sampled, a total of 7 particles of PE were found across all three placental compartments in that placenta (2.3 MP/g tissue). As sample numbers were too small, statistical analysis of differences in MP polymer type according to placental distribution or mode of delivery was not possible for PP, PMMA, and PE. Representative bright field images for each of these MPs are depicted in **Figure 4.3**, with detailed spectra analysis for each presented in **Supplemental Table 3**. Additional polymers were observed, including 1 styrene isoprene and 1 phthalocyanine (Mortoperm blue - polymer dye), however the polymer matrix containing the dye was not identifiable.

Table 4.2. *Total number, distribution, and polymer character of detected microplastic particles per gram tissue in placentas collected following C-section and vaginal deliveries.*

MP Polymer	C-section (n= 5)				Vaginal (n=5)			
	BP (MP/g tissue)	VT (MP/g tissue)	CP (MP/g tissue)	Size (um)	BP (MP/g)	VT (MP/g tissue)	CP (MP/g tissue)	Size (um)
PE	-	-	-	-	2	2	3	5 – 20
PP	1	-	-	60	-	-	-	-
PS	-	1	-	7	1	-	-	40
PVC	-	2	-	9 - 15	-	-	-	-
PMMA	-	-	1	3	-	-	-	-
Copper phthalocyanine#	6	3	2	2 – 40	2	1	2	12 - 44
Styrene-isopropene	-	-	1	10	-	-	-	-
Mortoperm blue#	-	-	-	-	1	-	-	7
All polymers	7	6	4	2 - 60	6	3	5	5 - 44

[#]Dye used in plastic.

MP = microplastic; g = gram tissue; BP = Basal Plate; VT = Chorionic Villous Tissue; CP = Chorionic Plate; PE = Polyethylene; PS = Polystyrene; PP = Polypropylene; PVC = Polyvinyl Chloride; PMMA = Polymethyl methacrylate.

***Table 4.3**. The number of microplastic particles identified in placenta tissue according to polymer type and mode of delivery.*

MP Polymer	**C-Section (n= 5) # MPs identified***					**Vaginal delivery (n = 5) # MPs identified***					**Total # MPs identified by polymer type**
	C1	C2	C3	C4	C5	V1	V2	V3	V4	V5	
PE	-	-	-	-	-	-	-	7	-	-	**7**
PP	-	-	-	1	-	-	-	-	-	-	**1**
PS	1	-	-	-	-	-	1	-	-	-	**2**
PVC	-	-	1	1	-	-	-	-	-	-	**2**
PMMA	-	-	1	-	-	-	-	-	-	-	**1**
Copper phthalocyanine[#]	-	1	-	-	10	1	1	2	-	1	**16**
Styrene-isopropene	-	-	-	-	1	-	-	-	-	-	**1**
Mortoperm blue[#]	-	-	-	-	-	-	-	-	1	-	**1**
Total # MPs identified/ Placenta*	**1**	**1**	**2**	**2**	**11**	**1**	**2**	**9**	**1**	**1**	**31**

*Total of 3 grams collected per placenta.

[#]Dye used in some plastic.

Polyethylene (PE); Polystyrene (PS); Polypropylene (PP); Polyvinyl Chloride (PVC); Polymethyl methacrylate (PMMA).

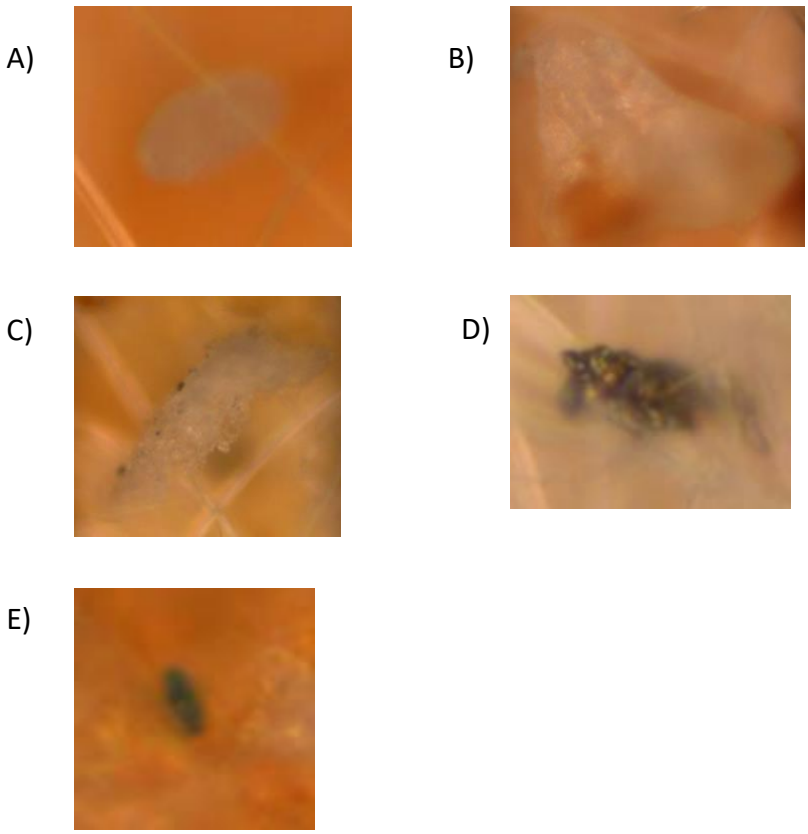

Figure 4.3. Representative brightfield images of common types of microplastic polymers found in human placenta samples. A) Polyethylene (PE), B) Polypropylene (PP), C) Polystyrene (PS), D) Polyvinyl Chloride (PVC), E) Copper phthalocyanine.

Identification of non-MP particles in placenta samples

Particles observed in digested placenta samples were not limited to microplastics, other non-microplastic particles were also detected. All placenta samples contained non-MP particles, with a total of 121 non-MP particles detected across all 10 placentas. The number of non-MP particles per placenta varied (ranging from 2-34, **Figure 4.4**), with an average of 4 $\pm$ 2.9 non-MP particles/g of tissue. The identified non-MP particles ranged in size from approximately 2 - 100 µm (**Table**

4.4). A non-significant trend of increasing particle accumulation within the chorionic villous compartment of the placenta was noted, compared to the basal and chorionic plate compartments (p = 0.151; p= 0.100; p= 0.421 **Figure 4.4**, **Table 4.4**). There was no significant difference in the total number distribution of non-MP particles identified according to mode of delivery (p= 0.155) **Figure 4.4**, **Table 4.4**).

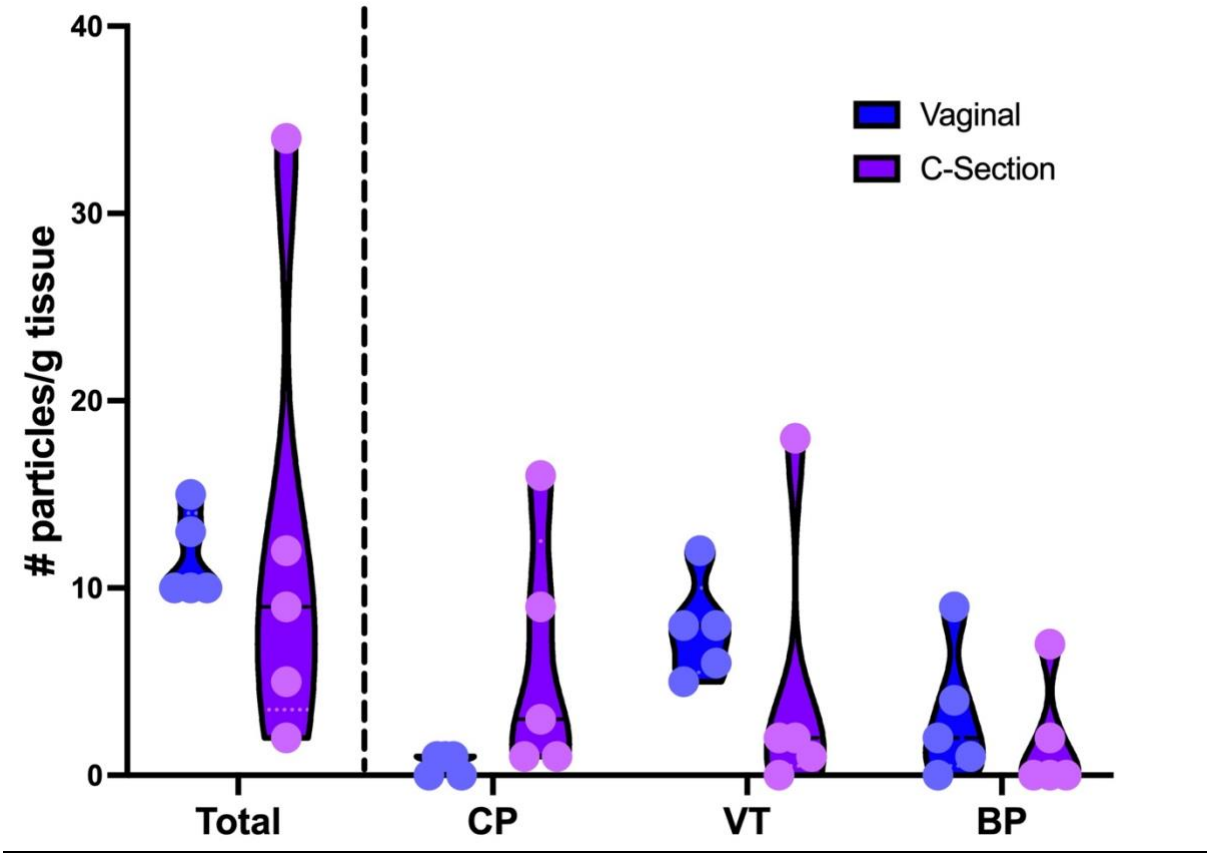

Fig 4.4. Total and region-specific number of non-microplastic particles identified in placenta tissue collected from vaginal (blue) and C-section (purple) deliveries. One gram of tissue was collected and processed from each anatomical site for each placenta. BP = basal plate; VT = chorionic villous tissue; CP = chorionic plate.

Table 4.4. Total number, distribution, and particle character of detected non-MP particles per gram tissue in placentas collected following C-section and vaginal deliveries.

Non-MP Particle	C-section (n= 5)				Vaginal (n=5)			
	BP (particles/g tissue)	VT (particles/g tissue)	CP (particles/g tissue)	Size (um)	BP (particles/g tissue)	VT (particles/g tissue)	CP (particles/g tissue)	Size (um)
Carbon[#]	5	-	-	10 - 25	-	3	2	3 -15
Graphite	5	4	-	2 - 25	-	8	1	5 -16
Lead Oxide	4	5	1	12 - 40	-	-	-	-
Bayerite	3	2	-	6 - 60	-	5	-	12 - 34
Other[^]	11	10	3	3 - 80	3	23	11	4 - 100
Unknown[+]	2	6	1	3 - 40	-	1	2	8 - 80
All non-MP particles	30	27	5	2 - 80	3	40	16	4 - 100

[#] Includes black carbon, diamond like carbon

[^]Includes less common findings, details included in **Supplemental Table 4**

[+]Spectrum could not be identified through Raman spectra and KnowItAll database.

Basal Plate (BP); Chorionic Villous Tissue (VT); Chorionic Plate (CP)

Characterization of non-MPs particles in placenta samples

Non-MP particle composition was also identified in placenta samples using Raman microspectroscopy, with confirmation of particle identity though KnowItAll database. A total of 29 unique particle identities were captured **(Supplemental Table 4.3**), with the most abundant particles identified as carbon, graphite, lead oxide and bayerite composition (**Tables 4.4** and **4.5**). Each of these particles were observed in 2-3 different placentas, ranging from 1-22 total particles per placenta (1.5 $\pm$ 1.1 particles/g of tissue). No significant differences were observed in the

specific non-MP particle type according to anatomical location within the placenta or mode of delivery (P> 0.05; **Tables 4.4** and **4.5**). Representative bright field images for each of these MPs are depicted in **Figure 4.5**, with detailed spectra analysis for each presented in **Supplemental Table 4.5**. It should be noted that there were 12 particles for which Raman spectral analysis was inconclusive and, therefore, could not be identified (**Table 4.5**).

Table 4.5. The number of non-microplastic particles identified in placenta tissue according to particle type and mode of delivery.

Particle type	**C-Section (n= 5) # particles identified***					**Vaginal delivery (n = 5) # particles identified***					**Total # of particles identified by type**
	C1	C2	C3	C4	C5	V1	V2	V3	V4	V5	
Carbon[#]	5	-	-	-	-	2	-	-	3	-	**10**
Graphite	-	-	9	-	-	-	-	8	-	1	**18**
Lead Oxide	-	-	9	1	-	-	-	-	-	-	**10**
Bayerite	-	-	4	-	1	-	-	-	5	-	**10**
Other[^]	4	-	6	11	3	11	13	2	2	9	**61**
Unknown[+]	-	2	6	-	1	2	1	-	-	-	**12**
Total # particles identified/ Placenta*	**9**	**2**	**34**	**12**	**5**	**15**	**14**	**10**	**10**	**10**	**121**

*Total of 3 grams collected per placenta.

[#] Includes black carbon, diamond like carbon

[^]Includes fewer common findings, details included in Supplemental Table 4

[+]Spectrum could not be identified through Raman spectra and KnowItAll database.

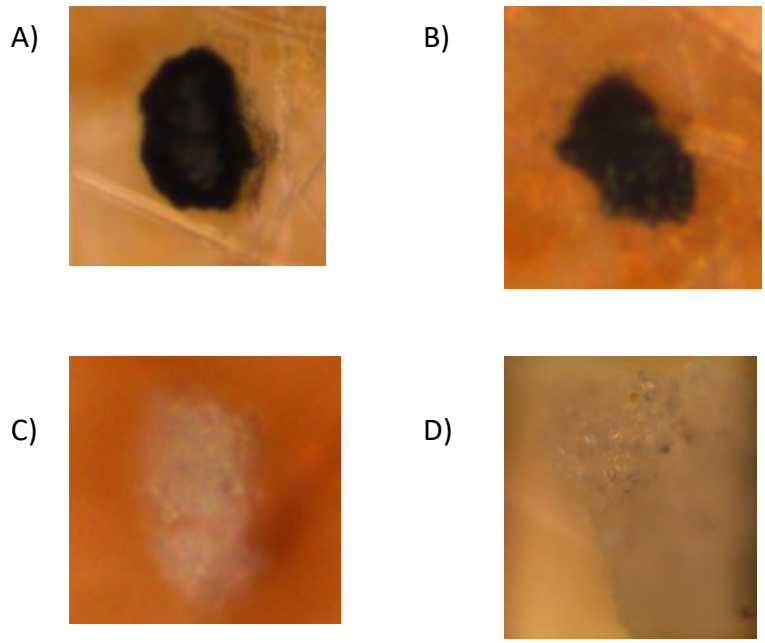

Figure 4.5. *Representative brightfield images of common types of non-microplastic particles found in human placenta samples.* ***A)*** *Carbon,* ***B)*** *Graphite,* ***C)*** *Lead Oxide,* ***D)*** *Bayerite.*

Discussion

The major findings of this study indicate the presence of both MP and non-MP particles in all placentas sampled, distributed across all placental compartments and unrelated to the mode of delivery. This small sample was selected from women+ whose singleton pregnancies were uncomplicated and who delivered within a tertiary care hospital setting within in a large Canadian city (Ottawa, Ontario). While these exposure levels may not represent the diversity of exposures across the Canadian population, it certainly provides insight regarding routine particulate exposures of adult women+ within a major urban region in Canada. To the best of our knowledge this study is the first to report presence of microplastic in human placentas within a Canadian pregnant population.

Our findings are mostly consistent with those reported in a handful of recent publications [23,24,29]. Importantly, however, the current study found MP contamination in all placentas samples (100%, n=10), whereas smaller observational studies carried out in Italian and German urban city obstetrical populations [23,24] have reported MPs in less than 70% of placentas analysed – possibly suggesting increased gestational exposure to MPs experienced by Canadian women+. The current study further identified the presence of non-MP particles in all placenta samples, many composed of synthetic materials. Few studies have reported the presence of foreign non-MP particles in human placenta tissues to date, although Ragusa et al., did describe the presence of unidentified particles bound to polymer dyes [23,28] .Carbon was also a common non-MP placental contaminant identified in the current study, findings that have been previously described by others [31] and found to correlate with levels of carbon black found in dust particulate within a women+'s home during pregnancy.

An extremely important consideration when interpreting the scope of the presented MP (1 ± 1.2 MPs/g tissue) and non-MP particle (4 ± 2.9 non MPs/g tissue) accumulation within the placenta, is the total size of this vital organ of pregnancy at the time of delivery – typically ranging from 550-650 grams. As such, in our sampled Canadian population, it can be assumed that an average placenta may contain upwards of 650 MPs and 2,600 non-MP particles at the end of gestation. A second critical consideration for data interpretation, is the current technical limitations of MP and non-MP identification by Raman Microspectroscopy at the lower end of the particle size spectrum (<1 μm). The smallest particles detected in the current study were ~2 μm – aligned to the lower threshold of detection of this current technology [32]. Due to the higher bioavailability of smaller particles throughout the human body and placenta [33], there are certainly smaller particles present

in the placenta that were not accounted for in the presented measurements, and as such reported concentrations are likely significant underestimates of total particle accumulation in the placenta. It is important to note that variation by individual was observed, with some placentas demonstrating very few total particles (0.3 particles/g tissue) and others demonstrating a high degree of particle accumulation within the placenta (11.3 particles/g tissue). No smokers were included within the sampled population, however these results would indicate vastly different particle exposure levels during pregnancy, possibly related to differences in diet/food practices, socioeconomic status, workplace and home life exposures. Future work focused on better understanding the associations between gestational MP/non-MP particle exposures and geo-demographical variables is warranted.

The chemical character of MP and non-MP particles were identified for most MP and non-MP particles detected in the current study – with the most common MPs identified as PE, PP, PS and PVC, and the most common non-MP particles identified as carbon, graphite and lead oxide. While these characterizations are important for understanding relevant gestational exposures, particularly in light of future design of toxicology and mechanistic in vitro studies, concrete determination of original sources of the exposures is not possible. Homes, workplaces, and other environments contain a wide variety of products and materials that could be sources of the detected MP particles. Polyethylene (PE) is widely used in plastic bags, plastic wraps, packaging, bottles, etc [34]. Polypropylene (PP) is widely used in plastic bottles, indoor-outdoor carpets, microwavable containers and disposable face masks [34]. Polystyrene (PS) is mainly found in styrofoam and single use cutlery [34], while polyvinyl chloride (PVC) can be found in plastic pipes, synthetic leather, clear food wrap, flooring materials and soft toys, among others [34]. Likewise, the

most abundant non-MP particles identified are pervasive in the environment. Carbon/black carbon particle is a primary constituent of air pollution, the result of incomplete combustion of fossil fuels [35] .Graphite is mainly found in lead pencils[36] , whereas lead oxide particles could arise from the use of lead in old batteries, from paint pigments (especially white), or gas sensors[37] . The wide use of these various products and materials represent a wide range of potential exposures to humans.

MPs and non-MP particles were detected in equal quantities in all three regions of the placenta sampled. Had there been a notable concentration uniquely located on the maternal surface (basal plate), one could have inferred a selective barrier function of the placenta regarding MP and non-MP transfer to the fetal compartment. However, this was not the case, as MP accumulation was also observed within the chorionic villous exchange region and the fetal surface of the placenta (chorionic plate). The presence of MPs throughout the placenta certainly warrants concern regarding the functional impact of these particles on placental integrity and function (i.e., maternal-fetal exchange, hormone production etc.) and certainly infer MP translocation into the fetal compartment. Indeed, ex vivo placenta perfusion studies have confirmed the transfer of polystyrene nanoplastics from maternal to fetal compartments [33,38]. Furthermore, the detection of MPs in the meconium of newborn infants [24–26] provides clear evidence of in utero exposure to MPs.

The widespread distribution of MPs across all placenta samples underscores the significance of investigating their potential consequences on placental function and fetal development, an area of investigation that has been relatively unexplored, particularly in human populations. Evidence

collected using rodent models of gestational MP exposure have demonstrated smaller placentas [39–41], impaired feto-placental and utero-placental vasculature development [40,42], disturbances in uterine immune cell balance (96) and placental metabolic disruption [42,43] . In vitro exposure of human placental tissue to MPs has also been shown to illicit dysregulated expression of genes and proteins related to inflammation and iron homeostasis [44] . Some limited evidence in human studies has suggested a potential adverse impact of gestational microplastic (MP) exposure on pregnancy outcomes. Studies have shown that in cases of fetal growth restriction (FGR), there is a negative relationship between the accumulation of MPs in the placenta and birthweight. This correlation is statistically significant (correlation coefficient, $r = -0.82$, $p < 0.001$). Similar associations have also been observed for neonatal length at birth, head circumference, and 1-minute APGAR scores.[29]. All 13 cases of FGR examined exhibited the presence of MNPs, with each sample containing a range of 2.9 to 34.5 um-sized MNPs, primarily composed of polyethylene (PE) and polystyrene (PS) polymers [29]. Notably non-MP, black carbon particles have also been identified in higher concentrations within placentas of pregnancies complicated by pre-term delivery and/or FGR, compared to health controls [29,31].

A comparative analysis was also conducted to assess the impact of the mode of delivery, specifically caesarean and vaginal deliveries, on the presence of both MPs and non-MP particles within placental tissues. The objective was to discern whether any disparities in the quantity or types of MPs detected in placentas from these delivery modes could shed light on whether contamination occurred post-delivery or resulted from in utero accumulation during placental development. The absence of significant variations in MPs and non-MPs levels between the two delivery modes lends credence to the hypothesis that MPs contaminate the placental tissue

during gestation rather than after birth. This outcome also reflects the effectiveness of the measures taken to minimize plastic contamination throughout the delivery process, placenta dissection, sample collection, storage, digestion, and all other phases of sample handling and analysis. This confidence in the prevention of contamination is further supported by the near absence of particle contamination of PBs, which served to monitor potential sources of contamination during sample dissection and sample processing. In instances where particles were noted in the PBs, they did not bear resemblance to the particles found within the placenta samples. Furthermore, the presence of particles within the chorionic villous region of the placenta, located deep within the organ and shielded from external contact during delivery, provides compelling evidence that these particles were introduced during pregnancy rather than post-delivery. These collective observations strengthen the conclusion that MP particles identified in placental samples are indicative of in utero exposure, dispelling concerns of post-delivery contamination.

In conclusion, this study sheds a light on the presence of MPs, as well as non-MP foreign particles, in human placentas. Remarkably, particles were found in all placentas tested, and throughout the various compartments of the placenta – demonstrating the ability of these particles to translocate into the fetal compartment. As the placenta is the vital organ of pregnancy supporting fetal development, any adverse effects on these foreign particles on the health and function of the placenta can have serious effects on the health and wellbeing of the offspring. Further research is needed to understand the repercussion of this exposure in pregnancy but more importantly the developing fetus.

Supplement

Supplemental Table 4.1. MP shedding analysis of plastic materials used in birthing suites at The Ottawa Hospital that could not be replaced in the plastic-reduced protocol.

Item	Common MPs		
	PE	PS	PP
Surgical drape	-	-	3
Placenta collection bowl	-	-	
Placenta collection bag	-	-	-
Gloves	-	-	-

Supplemental Table 4.2. Microplastic and non-microplastic particle contamination identified in hood blanks (HB) and procedural blanks (PB) for each placenta sampling.

Mode of delivery	Blanks	MPs identified (MPs/10 ml)		Non-MP particles identified. (particles/10ml)						
		HDPE	PE	Graphite	Glass	Fibers	Cotton	Unknown	Human hair	Dye
C-section samples (C1-C5)	HB-C1	-	-	-	-	-	-	2	-	-
	PB-C1	-	-	-	-	-	-	-	-	-
	HB-C2	-	-	-	-	-	-	-	-	-
	PB-C2	-	-	-	-	-	-	-	-	-
	HB-C3	-	-	-	-	2	-	-	-	-
	PB-C3	-	-	-	1	-	1	-	-	-
	HB-C4	-	1	-	-	1	-	-	-	-
	PB-C4	-	-	-	-	-	-	1	-	-
	HB-C5	-	-	-	-	-	-	-	-	-
	PB-C5	-	-	-	-	-	-	-	-	-
Vaginal samples (V1-V5)	HB-V1	-	-	1	-	8	-	-	1	-
	PB-V1	-	-	-	-	-	-	-	-	-
	HB-V2	-	-	-	-	2	-	1	-	-
	PB-V2	-	-	1	-	-	-	-	-	-
	HB-V3	1	-	-	-	2	-	1	-	-
	PB-V3	-	-	-	-	-	-	-	-	1
	HB-V4	-	-	-	-	-	-	-	-	-
	PB-V4	-	-	-	-	-	-	-	-	-
	HB-V5	-	-	-	-	1	-	-	-	-
	PB-V5	-	-	-	-	-	-	-	-	-

Supplemental Table 4.3. Raman characteristics of detected microplastic polymers

Polymer	Detected Raman Spectral Bands (cm^{-1})	Spectral Characteristics
PE	~ 1063	C-C asymmetric stretching
	~ 1296	CH_2 twisting
	~ 2846	CH_2 symmetric stretching
	~ 2881	CH_2 symmetric stretching
PP	~ 808	CH_2 rocking mode
	~ 841	CH_2 rocking mode
	~ 1153	C-C stretching and CH bending
	~ 1330	CH bending and CH_2 twisting
	~ 2884	CH_2 symmetric stretching
	~ 2954	CH_2 asymmetric stretching
PS	~ 1002	Styrene aromatic ring
	~ 1604	CH_2 skeletal stretch
	~ 3056	C-H bonds stretching on benzene ring
PVC	~ 635	C-Cl bonds stretching
	~ 688	
PMMA	~ 991	O-CH_3 rock
	~ 1456	C-H bending of α-CH_3
	~ 1736	C=O carbonyl stretching
	~ 2849	Combination band O-CH_3
	~ 2957	C-H symmetric starching of O-CH_3 with C-H stretching of α-CH_3 and anti-symmetric stretching of CH_2
CuPc	~ 590	Twisting motions of the five membered ring
	~ 679	Two sets of Nitrogen in phase
	~ 1143	Type G antisymmetric and symmetric motion
	~ 1341	Type C symmetric and antisymmetric vibration
	~ 1452	Deformation of isoindole ring
	~ 1527	Type B stretching
Styrene - Isoprene	~ 1032	CH_3 rocking
	~ 1451	CH_3 in plane deformation

	~ 2912	CH_2 in phase stretch
	~ 3052	(=CH)(C(CH_3)=CH_2)) asymmetric stretch
Mortoperm blue	~ 590	Twisting motions of the five membered ring
	~ 679	Two sets of Nitrogen in phase
	~ 1143	Type G antisymmetric and symmetric motion
	~ 1341	Type C symmetric and antisymmetric vibration
	~ 1452	Deformation of isoindole ring
	~ 1527	Type B stretching

Supplemental Table 4.4. Non-MP particles identified in placenta samples that were classified within the "other" category, according to mode of delivery.

Non-MP Particle	**C-Section (n= 5) # particles identified***					**Vaginal (n= 5) # particles identified***					**Total # of particles identified by type***
	C1	C2	C3	C4	C5	V1	V2	V3	V4	V5	
Carbon	5	-	-	-	-	2	-	-	3	-	**10**
Gallium (III) Fluoride trihydrate	1	-	-	-	-	-	-	-	-	-	**1**
Biotite	1	-	-	-	-	-	-	-	-	-	**1**
Dentin	1	-	-	-	-	-	-	-	-	1	**2**
Ammonium Hexafluorozirconate	1	-	-	-	-	-	-	-	-	-	**1**
Unknown	-	2	6	-	1	2	1	-	-	-	**12**
Unknown Dye	-	-	3	-	-	-	-	-	-	-	**3**
Graphite	-	-	9	-	-	-	-	8	-	1	**18**
Silicon	-	-	1	-	-	-	-	-	-	-	**1**
Lead Oxide	-	-	9	1	-	-	-	-	-	-	**10**
Bayerite	-	-	4	-	1	-	-	-	5	-	**10**
Raw Umber	-	-	1	-	-	-	-	-	-	-	**1**
Barium hydroxide, hydrate	-	-	1	-	-	-	-	-	-	-	**1**
Burnt Umber	-	-	-	3	-	4	-	-	-	2	**9**
Silicon Carbine 3C	-	-	-	3	-	-	-	-	-	-	**3**
Anthophyllite	-	-	-	1	-	-	-	-	-	-	**1**
Calcium oxalate	-	-	-	1	-	-	9	-	-	1	**11**

Burnt Sienna	-	-	-	1	1	2	-	-	-	-	**4**
Magnetite	-	-	-	1	-	1	-	-	-	-	**2**
Silotrans Red	-	-	-	1	-	-	-	-	-	-	**1**
Ivory	-	-	-	-	-	-	-	-	-	2	**2**
Ivory black	-	-	-	-	1	-	1	-	-	1	**3**
Rutile	-	-	-	-	1	-	-	1	-	-	**2**
Iron Oxide	-	-	-	-	-	1	-	-	-	-	**1**
Poly(ethylene) Glycole Distearate	-	-	-	-	-	2	1	1	-	-	**4**
Cadmium yellow	-	-	-	-	-	1	-	-	-	-	**1**
Red Ochre	-	-	-	-	-	-	2	-	-	-	**2**
Silicon Polycrystalline	-	-	-	-	-	-	-	-	1	1	**2**
Magnesite	-	-	-	-	-	-	-	-	1	1	**2**
Total #	**9**	**2**	**34**	**12**	**5**	**15**	**14**	**10**	**10**	**10**	**121**

*Total of 3 grams collected per placenta.

Supplemental Table 4.5. Raman characteristics of detected non-plastic particles.

Polymer	**Detected Raman Spectral Bands (cm^{-1})**	**Spectral Characteristics**
Carbon	~ 1350 ~ 1585	D band G band
Graphite	~ 1580 ~ 2700	G peak G' peak
Lead Oxide	~ 550	Reference peak
Bayerite	~ 388 ~ 545	Reference peak

Supplemental Table 4.6. Non-plastic particles and potential sources.

Particles	**Potential sources**
Carbon	Product of air pollution due to incomplete combustion of fossil and other fuels, photo copiers, printers, coloring agent.

Gallium (III) Fluoride trihydrate	water gallium source for use in oxygen-sensitive applications such as metal production
Biotite	Silicate mineral in the common mica group. Found in granite (counter tops)
Dentin	A thick dentin layer forms the bulk of dental mineralized dental tissue
Ammonium Hexafluorozirconate	chemical compound used in corrosion-resistant adherent coatings on aluminum surfaces and aqueous acid solutions for chemical conversion coatings
Graphite	Crystalline form of the element carbon ; Found in pencils, lubricants, lamps, batteries, polishes
Silicon	Used in cookware, bakeware, toys, adhesive, lubricants, gaskets, medical application
Lead Oxide	Widely used in batteries, gas sensors, pigments and paints, ceramics, glass
Bayerite	Production of aluminum metals and used in flame retardants
Raw Umber	Umber is a natural brown earth pigment color that contains iron oxide and mangases oxide. In its natural form it is called raw umber
Barium hydroxide, hydrate	Highly water insoluble crystalline barium source for uses compatible with higher basic pH environments
Burnt Umber	Umber is a natural brown earth pigment, made by heating raw umber, which dehydrates the iron oxide
Silicon Carbine 3C	Silicon Carbine 3C is found in ceramic plates, bulletproof vest, car clutches. Silicon carbine is a hard chemical compound containing silicon and carbon
Anthophyllite	Type of asbestos
Calcium oxalate	Oxalate is found in certain foods such as spinach, almonds, soy etc.. Oxalate cannot be metabolized and must be excreted through urine. High levels may lead to kidney stones
Burnt Sienna	Earth pigment from iron oxide
Magnetite	A mineral whose primary component is iron oxide; Greated use is iron ore for steel manufacture
Silotrans Red	Dye - not sure for what specifically
Ivory	Piano keys, jewelry, chess sets, also used in medicines
Ivory black	Black paint by carbonising ivory

Rutile	Oxide mineral composed titanium dioxide
Iron Oxide	Chemical composed of iron and oxygen
Poly(ethylene) Glycole Distearate	cosmetic products as surfactants, emulsifiers, cleaning agents and skin conditioners
Cadmium yellow	Yellow pigment/paint
Red Ochre	A variant of ochre containing a large amount of hematite, or dehydrated iron oxide, has a reddish tint known as red ochre
Silicon Polycrystalline	unsure
Magnesite	a refractory material, a catalyst and filler in the production of synthetic rubber, and a material in the preparation of magnesium chemicals and fertilizers.

Reference

1. Jambeck JR, Geyer R, Wilcox C, et al. Marine pollution. Plastic waste inputs from land into the ocean. *Science*. 2015;347(6223):768-771. doi:10.1126/science.1260352

2. Geyer R, Jambeck JR, Law KL. Production, use, and fate of all plastics ever made. *Sci Adv*. 2017;3(7):e1700782. doi:10.1126/sciadv.1700782

3. Andrady AL. The plastic in microplastics: A review. *Mar Pollut Bull*. 2017;119(1):12-22. doi:10.1016/j.marpolbul.2017.01.082

4. Hartmann NB, Hüffer T, Thompson RC, et al. Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris. *Environ Sci Technol*. 2019;53(3):1039-1047. doi:10.1021/acs.est.8b05297

5. Eriksen M, Mason S, Wilson S, et al. Microplastic pollution in the surface waters of the Laurentian Great Lakes. *Mar Pollut Bull*. 2013;77(1-2):177-182. doi:10.1016/j.marpolbul.2013.10.007

6. Gaylarde C, Baptista-Neto JA, Da Fonseca EM. Plastic microfibre pollution: how important is clothes' laundering? *Heliyon*. 2021;7(5):e07105. doi:10.1016/j.heliyon.2021.e07105

7. Ramos L, Berenstein G, Hughes EA, Zalts A, Montserrat JM. Polyethylene film incorporation into the horticultural soil of small periurban production units in Argentina. *Sci Total Environ*. 2015;523:74-81. doi:10.1016/j.scitotenv.2015.03.142

8. Kosuth M, Mason SA, Wattenberg EV. Anthropogenic contamination of tap water, beer, and sea salt. *PloS One*. 2018;13(4):e0194970. doi:10.1371/journal.pone.0194970

9. Zhang J, Wang L, Kannan K. Microplastics in house dust from 12 countries and associated human exposure. *Environ Int*. 2020;134:105314. doi:10.1016/j.envint.2019.105314

10. Afrin S, Rahman MM, Akbor MA, Siddique MAB, Uddin MK, Malafaia G. Is there tea complemented with the appealing flavor of microplastics? A pioneering study on plastic

pollution in commercially available tea bags in Bangladesh. *Sci Total Environ*. 2022;837:155833. doi:10.1016/j.scitotenv.2022.155833

11. Diaz-Basantes MF, Conesa JA, Fullana A. Microplastics in Honey, Beer, Milk and Refreshments in Ecuador as Emerging Contaminants. *Sustainability*. 2020;12(14):5514. doi:10.3390/su12145514

12. Amato-Lourenço LF, Carvalho-Oliveira R, Júnior GR, Dos Santos Galvão L, Ando RA, Mauad T. Presence of airborne microplastics in human lung tissue. *J Hazard Mater*. 2021;416:126124. doi:10.1016/j.jhazmat.2021.126124

13. Baeza-Martínez C, Olmos S, González-Pleiter M, et al. First evidence of microplastics isolated in European citizens' lower airway. *J Hazard Mater*. 2022;438:129439. doi:10.1016/j.jhazmat.2022.129439

14. Pauly JL, Stegmeier SJ, Allaart HA, et al. Inhaled cellulosic and plastic fibers found in human lung tissue. *Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol*. 1998;7(5):419-428.

15. Ozawa Y, Mizushima Y, Koyama I, et al. Intestinal absorption enhancement of coenzyme Q10 with a lipid microsphere. *Arzneimittelforschung*. 1986;36(4):689-690.

16. Jenner LC, Rotchell JM, Bennett RT, Cowen M, Tentzeris V, Sadofsky LR. Detection of microplastics in human lung tissue using µFTIR spectroscopy. *Sci Total Environ*. 2022;831:154907. doi:10.1016/j.scitotenv.2022.154907

17. Horvatits T, Tamminga M, Liu B, et al. Microplastics detected in cirrhotic liver tissue. *EBioMedicine*. 2022;82:104147. doi:10.1016/j.ebiom.2022.104147

18. Ibrahim YS, Tuan Anuar S, Azmi AA, et al. Detection of microplastics in human colectomy specimens. *JGH Open Open Access J Gastroenterol Hepatol*. 2021;5(1):116-121. doi:10.1002/jgh3.12457

19. Leslie HA, Velzen MJM van, Brandsma SH, Vethaak AD, Garcia-Vallejo JJ, Lamoree MH. Discovery and quantification of plastic particle pollution in human blood. *Environ Int*. 2022;163:107199. doi:10.1016/j.envint.2022.107199

20. Pironti C, Notarstefano V, Ricciardi M, Motta O, Giorgini E, Montano L. First Evidence of Microplastics in Human Urine, a Preliminary Study of Intake in the Human Body. *Toxics*. 2022;11(1):40. doi:10.3390/toxics11010040

21. Schwabl P, Köppel S, Königshofer P, et al. Detection of Various Microplastics in Human Stool: A Prospective Case Series. *Ann Intern Med*. 2019;171(7):453-457. doi:10.7326/M19-0618

22. Kutralam-Muniasamy G, Shruti VC, Pérez-Guevara F, Roy PD. Microplastic diagnostics in humans: "The 3Ps" Progress, problems, and prospects. *Sci Total Environ*. 2023;856(Pt 2):159164. doi:10.1016/j.scitotenv.2022.159164

23. Ragusa A, Svelato A, Santacroce C, et al. Plasticenta: First evidence of microplastics in human placenta. *Environ Int*. 2021;146:106274. doi:10.1016/j.envint.2020.106274

24. Braun T, Ehrlich L, Henrich W, et al. Detection of Microplastic in Human Placenta and Meconium in a Clinical Setting. *Pharmaceutics*. 2021;13(7):921. doi:10.3390/pharmaceutics13070921

25. Liu S, Lin G, Liu X, et al. Detection of various microplastics in placentas, meconium, infant feces, breastmilk and infant formula: A pilot prospective study. *Sci Total Environ*. 2022;854:158699. doi:10.1016/j.scitotenv.2022.158699

26. Liu S, Liu X, Guo J, et al. The association between microplastics and microbiota in placentas and meconium: The first evidence in humans. *Environ Sci Technol*. Published online 2022.

27. Zhu L, Zhu J, Zuo R, Xu Q, Qian Y, An L. Identification of microplastics in human placenta using laser direct infrared spectroscopy. *Sci Total Environ*. 2023;856(Pt 1):159060. doi:10.1016/j.scitotenv.2022.159060

28. Ragusa A, Matta M, Cristiano L, et al. Deeply in Plasticenta: Presence of Microplastics in the Intracellular Compartment of Human Placentas. *Int J Environ Res Public Health*. 2022;19(18):11593. doi:10.3390/ijerph191811593

29. Amereh F, Amjadi N, Mohseni-Bandpei A, et al. Placental plastics in young women from general population correlate with reduced foetal growth in IUGR pregnancies. *Environ Pollut 1987*. 2022;314:120174. doi:10.1016/j.envpol.2022.120174

30. Jummaat F, Yahya EB, Khalil H P S A, et al. The Role of Biopolymer-Based Materials in Obstetrics and Gynecology Applications: A Review. *Polymers*. 2021;13(4):633. doi:10.3390/polym13040633

31. Bové H, Bongaerts E, Slenders E, et al. Ambient black carbon particles reach the fetal side of human placenta. *Nat Commun*. 2019;10(1):3866.

32. Araujo CF, Nolasco MM, Ribeiro AMP, Ribeiro-Claro PJA. Identification of microplastics using Raman spectroscopy: Latest developments and future prospects. *Water Res*. 2018;142:426-440. doi:10.1016/j.watres.2018.05.060

33. Wick P, Malek A, Manser P, et al. Barrier capacity of human placenta for nanosized materials. *Environ Health Perspect*. 2010;118(3):432-436. doi:10.1289/ehp.0901200

34. Science of Plastics. Science History Institute. Accessed October 16, 2023. https://sciencehistory.org/education/classroom-activities/role-playing-games/case-of-plastics/science-of-plastics/

35. What is Black Carbon? Center for Climate and Energy Solutions. Accessed October 16, 2023. https://www.c2es.org/document/what-is-black-carbon/

36. Uses of Graphite - Most Important and Popular Uses of Graphite. BYJUS. Accessed October 16, 2023. https://byjus.com/chemistry/uses-of-graphite/

37. Bratovcic A. Synthesis, Characterization, Applications, and Toxicity of Lead Oxide Nanoparticles. In: *Lead Chemistry*. IntechOpen; 2020. doi:10.5772/intechopen.91362

38. Grafmueller S, Manser P, Diener L, et al. Bidirectional Transfer Study of Polystyrene Nanoparticles across the Placental Barrier in an ex Vivo Human Placental Perfusion Model. *Environ Health Perspect*. 2015;123(12):1280-1286. doi:10.1289/ehp.1409271

39. Fournier SB, D'Errico JN, Adler DS, et al. Nanopolystyrene translocation and fetal deposition after acute lung exposure during late-stage pregnancy. *Part Fibre Toxicol*. 2020;17(1):55. doi:10.1186/s12989-020-00385-9

40. Hu J, Qin X, Zhang J, et al. Polystyrene microplastics disturb maternal-fetal immune balance and cause reproductive toxicity in pregnant mice. *Reprod Toxicol*. 2021;106:42-50. doi:10.1016/j.reprotox.2021.10.002

41. Nie JH, Shen Y, Roshdy M, Cheng X, Wang G, Yang X. Polystyrene nanoplastics exposure caused defective neural tube morphogenesis through caveolae-mediated endocytosis and faulty apoptosis. *Nanotoxicology*. 2021;15(7):885-904. doi:10.1080/17435390.2021.1930228

42. Chen G, Xiong S, Jing Q, et al. Maternal exposure to polystyrene nanoparticles retarded fetal growth and triggered metabolic disorders of placenta and fetus in mice. *Sci Total Environ*. 2022;854:158666. doi:10.1016/j.scitotenv.2022.158666

43. Aghaei Z, Mercer GV, Schneider CM, et al. Maternal exposure to polystyrene microplastics alters placental metabolism in mice. *Metabolomics Off J Metabolomic Soc*. 2022;19(1):1. doi:10.1007/s11306-022-01967-8

44. Chortarea S, Gupta G, Saarimäki LA, et al. Transcriptomic profiling reveals differential cellular response to copper oxide nanoparticles and polystyrene nanoplastics in perfused human placenta. *Environ Int*. 2023;177:108015. doi:10.1016/j.envint.2023.108015

Chapter 5 - Integrated Discussion

General Discussion

The current thesis addresses the overarching concern that microplastics from the environment may accumulate in the human placenta and induce adverse developmental programming in the fetus. First, a detailed review of the primary literature relevant to this question was conducted. Specifically, a comprehensive overview of the current state of knowledge on microplastic contamination of human reproductive tissues, including placental and fetal tissues, and the reproductive consequences of these exposures was completed (Chapter 2). Next, a prospective observational study was undertaken to specifically measure and characterize microplastic accumulation within human placentas within a Canadian context (Chapter 4). The completion of this observational study included the development of plastic-reduced sampling and processing procedures (Appendix 2), along with the optimization of methods for MP identification within biological tissues using Raman microspectroscopy analysis (Appendix 3). Importantly, the results presented within this thesis demonstrate the presence of microplastic particles, along with other non-plastic particles, in ***all*** placentas examined and within both the maternal and fetal compartments of the placentas – indicative of in utero exposure to these environmental pollutants.

In addition to confirming the abundant presence of MP particles in placentas of pregnant Canadians – addressing a geographical gap in knowledge - this study also sought to establish whether the delivery method influences the overall quantity and types of microplastics measured. The rationale for this specific comparison was to determine if there might be any environmental contamination unique to the delivery and/or post-delivery environment. For example, placentas collected from vaginal deliveries may be exposed to contaminating MP

particles found in the vaginal canal or on the vulva – derived from sanitary items used by the individual at the end of pregnancy. Conversely, placentas collected from a C-section delivery may become contaminated with MPs derived from surgical procedure tools or items. A comprehensive environmental survey and sampling of all tools and items used in both a vaginal delivery suite and the surgical C-section suites was carried out in the completion of this thesis – addressing an overall lack of consideration for environmental contamination limiting the interpretation of the literature to date. Interestingly, mode of delivery was not found to be associated with any differences in MP measurements, and aside from 1 particle of polypropylene (PP) derived from the surgical drape, minimal delivery and post-delivery contamination was identified. These results strengthen the notion that reports of MP accumulation within placenta tissue are in fact valid, and certainly warrant concern over the potential impacts of these exposures in pregnancy.

The current thesis contributes to the growing evidence of the potential adverse human health impacts of plastic pollution, specifically identifying a critical window of human development (fetal life) when these environmental exposures may have substantial, long-lasting health consequences. As detailed in Chapter 2, using the DOHaD framework, we can speculate that in utero MP exposure may serve to compromise placenta health and function, compromising maternal-fetal exchange and ultimately fetal development. As demonstrated in the current thesis and by others [1–3] , the distribution of MPs extends across all compartments of the placenta, and when combined with evidence of MP measurements in fetal tissues [4,5] and fetal/ Infant meconium [3,6,7], there is strong evidence for the presence of MP transfer across the placenta into the fetal compartment, with the potential for direct MP-induced programming of developing fetal organ systems. To date, direct evidence for adverse in utero fetal programming and subsequent future

health compromise is limited to animal model investigations and should certainly be an important area for future investigation. While not the primary focus of the current thesis, it is also important to consider that other critical windows of MP exposures may be of high relevance to human reproduction, including considerations of the effects of environmental MP exposures to gamete health and fertility (pre-conception period), as well as early childhood development (postnatal period).

In addition to the confirmation of MP accumulation within the human placenta, an important finding of this thesis was the additional measurements of accumulated non-plastic particles within the placenta. To the best of our knowledge, this is one of the few studies to identify environmental pollutants in human placentas. One study investigated the effects of black carbon, an air pollutant, during pregnancy in humans. Another study investigated exposure to lead, an environmental pollutant [8,9]. Exposure to both particulates was reported to cause preterm birth, cause low birth weight, and compromise fetal mental development [8,9]. To date evidence of adverse health effects in humans induced by environmental pollutants exposure is limited and important for future work. However, investigations of particulates during pregnancy should not be limited to microplastics as other types of environmental pollutants can accumulate in the placenta compromising growth and fetal programming.

The sophisticated methods used in the current thesis allowed for the determination of the chemical composition of both the MP and non-MP particles found within the placenta, identifying Polyethylene (PE), Polystyrene (PS), Polyvinyl Chloride (PVC) and Polypropylene (PP) to be the most prevalent MPs identified and carbon, graphite, and lead oxide to be the most prevalent non-MPs identified. However, this characterization cannot inform on the original sources or routes of

exposure by which these particles entered the bodies of study participants. The types of particles observed provide hints of potential sources tempting speculation that factors of lifestyle, workplace and diet contribute to these exposures. Until the factors that drive exposure are better understood, developing strategies to limit exposure will be a challenge. While such exposures are not desirable, the lack of understanding of the risks that such exposures present to fetal and maternal health prevents an assessment of how aggressively such exposures should be limited.

Limitations

Although this thesis presents novel findings, there are several limitations that must be considered in their interpretation. To date, there is a lack of published evidence examining the relationship between MP exposures and *human* infertility, effects on early life development, and the risks of chronic illness. Further, the overwhelming majority of animal studies of MP toxicity examined the effects of highly engineered polystyrene microspheres. It is not clear if the effects of these can be extrapolated to predict the effects of environmentally relevant microplastics that make up the vast majority of microplastics to which the human population is exposed. Environmentally-relevant microplastics – as has been described in detail in Chapter 2 – arise from diverse sources, have diverse compositions, include chemical additives that may leach out of the polymers, and can exhibit altered surface chemistry due to environmental weathering, etc. Although polystyrene MPs were observed in placenta samples, as reported in Chapter 4, these are unlike the PS microspheres whose toxicity is most widely studied. Until the toxicity of a broader range of microplastics are better understood and/or studies of human health outcomes associated with

real world microplastic exposure are available, the potential impacts of MPs to health will remain poorly understood.

Within our observational study, while considerable attention was paid to the development of plastic-free protocols and contaminant measurements, the potential for sample contamination between the time of delivery and Raman analyses of filtered samples cannot be ignored and was discussed in detail in Chapter 4. It is also important to consider, that using the methods employed, particles smaller than 2 micrometers in diameter could not be detected. The need to harvest particles from dissolved tissue samples using a glass fibre filter means that the size of the apertures in the filter membrane sets a lower boundary (2.7 μm) to the size of particles that can be detected. There is no reason to believe that particles smaller than this limit are not present in tissue given that the nanoplastics are more abundant in the environment than microplastics [10]. Several lines of evidence suggest that smaller, nanosized particles are more effective than microsized particles to be absorbed through the gut and lungs [11–13] and pass through perfused human placenta [14,15]. Finally, the current study reports particles from a relatively small sample of women, examining the tissues from only 1 quadrant of the large and highly heterogenous placental organ. It should be noted that more extensive tissue sampling was conducted from each of these participants, with plans to continue the measurements and characterization of MPs on these samples in the future.

Future directions

The current MSc thesis addresses the current knowledge gap regarding microplastic exposure in human placentas within a Canadian context, with detailed attention given to tissue distribution

and potential differences in MP profiles according to mode of delivery. As previously highlighted, future work is needed to evaluate potential health impacts for the developing fetus, as some evidence in rodent studies suggests MP exposure during pregnancy can have adverse impacts on fetal development with long-lasting health effects across the lifespan. Further work should attempt to identify maternal behaviours, lifestyles, diet, or other factors that could be driving microplastic exposure. Currently, the mechanism underlying the translocation of microplastics into the human body through certain routes of exposure is poorly understood and requires further investigation. In addition, toxicity studies – either in vitro or in animal models, should be conducted to investigate the developmental impacts of exposure to a wide array of environmentally relevant microplastics. The use of manufactured pristine MPs is only scratching the surface of the potential health effects.

Interdisciplinarity of Thesis Work

The completion of this thesis was carried out under the collaborative mentorship of an academic researcher (S. Bainbridge) with expertise in placental biology and DOHaD and a federal government researcher (M. Wade, Environmental Health Sciences and Research Bureau, Health Canada) with expertise in reproductive toxicology and its alignment to regulatory policy. Further, collaborations with chemists who specialize in MP measurement and characterization and obstetrical care teams who can provide important insight into pregnancy, delivery and post-delivery environmental contaminants were critical for the success of this project. Collectively, this interdisciplinary team was able to bring together the required knowledge and expertise to carry out important research on a pressing health issue of high relevance to the Canadian population

– bridging clinical care, biological, environmental and risk policy perspectives. Additionally, the two-pronged strategy of this thesis – including a comprehensive review and an observational study permitted the collection of data needed to address current gaps in knowledge. It is hoped that the insights made in this thesis will pave the way for future beneficial collaborations in this domain.

Conclusion

In conclusion, MP and non-plastic foreign particles were identified in all compartments of the human placentas collected from a Canadian pregnant sample. It is strongly suspected that these particles entered the placenta via maternal circulation before birth. The investigations in the lab did not look into whether these particles caused any dangers to the growing fetus or mother. However, the presented detailed review of our current state of knowledge certainly indicates there is cause of concern, as MPs have been shown to exert adverse direct and indirect effects on the feto-placenta unit, in some cases with evidence of long-lasting health effects for the offspring.

Appendices

Appendix 1A. Ethics Approval – Health Canada

Health Canada and Public Health Agency of Canada | Santé Canada et l'Agence de la santé publique du Canada

Research Ethics Board | Comité d'éthique de la recherche

CERTIFICATE OF ETHICS REVIEW

Type of Review: Initial Review of Research

Principal Investigator:
Mike Wade
Research Scientist
Healthy Environments and Consumer Safety Branch
Health Canada
Frederick G Banting Building
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Project Title: Do human placentas contain micro plastics?

Project File Number: REB 2021-033H

Contact Department/Agency: Health Canada

Document Name:

Date:
November 16, 2022

List of all documents submitted to the REB:
REB Application
Research Protocol and Budget
Consent Form
CV's

Answers to the REB questions/observations and additional material provided on January 11 and January 20, 2023
Email responses from PI
Revised Consent Forms

ETHICS REVIEW:
Your application submitted to the Health Canada and Public Health Agency of Canada Research Ethics Board (REB) regarding the above-referenced research project was reviewed on November 29, 2022 and your responses to the REB members' questions were reviewed on January 17 and January 20, 2022. The most recent versions of the documents listed above were found to meet ethical requirements for research involving humans.

_____

Barbara McGillivray, MD, FRCPC, FCCMG
Chair, Research Ethics Board

Date: January 21, 2022

Certificate Expiry Date:
January 21, 2023

Appendix 1B. Ethics Approval – University of Ottawa

06/04/2022

Université d'Ottawa
Bureau d'éthique et d'intégrité de la recherche

University of Ottawa
Office of Research Ethics and Integrity

Lettre d'approbation administrative | Letter of administrative approval

Numéro de dossier / Ethics File Number	H-03-22-7960
Titre du projet / Project Title	Placenta Exposure to Microplastics
Type de projet / Project Type	Recherche de clinicien / Clinician's research project
CÉR primaire / Primary REB	Réseau de science de la santé d'Ottawa (RSSO) / Ottawa Health Science Network (OHSN)
Statut du projet / Project Status	Approuvé / Approved
Date d'approbation (jj/mm/aaaa) / Approval Date (dd/mm/yyyy)	06/04/2022
Date d'expiration (jj/mm/aaaa) / Expiry Date (dd/mm/yyyy)	01/03/2023

Équipe de recherche / Research Team

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Stephanie BOYD	Ottawa Hospital Research Institute	Coordonnateur de recherche / Research Coordinator
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Conditions spéciales ou commentaires / Special conditions or comments:

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Appendix 2. Reduced Plastic Protocol from Vaginal and Caesarean delivery.

REDUCED PLASTIC PROTOCOL: VAGINAL DELIVERY

Preparation

1. Plastic background
    a. Procedural blanks and samples of any potential environmental contaminant from the time of delivery will be run in parallel to estimate the presence/identity of potential micro plastic contamination.
    b. Background examination on plastic material, device and antiseptic that will encounter the placenta will be done by washing materials ten times with pretreated/filtered water.
    c. Pretreated/filtered water will then be placed on plastic material and poured into glass bottles.
    d. Raman Microscope will then be used to observe the presences of microplastic contamination in material.

Delivery

1. Delivery of the fetus
    a. If forceps delivery is required, metal forceps will be used as instrumentation.
    b. If episiotomy is needed, metal scissors will be used.
    c. If amniotomy is required, a metal amnio hook will be used.

2. Delivery of the placenta
    a. Metal clamps will be used for cord clamping and metal scissors will be used for cutting the umbilical cord.
    b. Placenta will be collected and transported in a metal container for further examination. Plastic collection bags will not be used.

Post-Delivery

1. Sample collection
    a. Placenta will be collected within 1 hour of delivery. Under sterile conditions to minimize plastic particle contamination placenta will be dissected into basal plate, chorionic villi, chorionic plate and chorio-amniotic membrane samples.
    b. Separate samples (~30 g) will be collected into glass bottles with glass or metal lids.

2. Sample digestion & filtration
    a. Placenta samples will be digested using 30% Hydrogen peroxide.
    a. Digested samples will be filtered through glass microfiber filters (Whatman GF/D – 2.7 µm pore size). Filters will then be rinsed with ~ 10ml prefiltered deionized water.

3. Sample examination
    a. Filters will be dried under plastic and fibre-free conditions and examined using an imaging system that couples an Enhanced Dark Field (EDF) optical microscope and hyperspectral imaging (HSI) with a confocal Raman imaging system.
    b. The EDF-HSI images will be analysed using Environment for Visualization software. LabSpec 6 software suite will be used for the acquisition of Raman spectral information and KnowItAll spectral database to identify the specific Microplastics type

REDUCED PLASTIC PROTOCOL: CAESAREAN SECTION

Preparation

1. Plastic background
    a. Procedural blanks and samples of any potential environmental contaminant from the time of delivery will be run in parallel to estimate the presence/identity of potential micro plastic contamination
    b. Plastic material that will encounter the placenta such as plastic drape, gloves, collection bowl and plastic bag will be collected prior.
    c. Background examination of these materials will be done by washing materials ten times with pretreated/filtered water.
    d. Pretreated/filtered water will then be placed on plastic material and poured into glass bottles.
    e. Raman Microscope will then be used to observe the presences of microplastic contamination.

2. Pre-operative preparation
    a. The lower abdomen will be scrubbed with prepping sponges and cleaning solution.
    b. A sterile drape with transparent plastic film will be applied to the operative area.
    c. Adequate exposure of the uterus and abdomen will be obtained using sterile metal instruments, and/or metal retractors.

Operative Procedure

1. Delivery of the placenta
    a. A metal cautery tip and/or scalpel will be used to make the incision and metal retractors will be used to adequate visualization.
    b. Metal clamps will be used for cord clamping and metal clippers will be used for cutting the umbilical cord.
    c. The placenta will be collected in a blue plastic bowl and taken for further examination; no plastic collection bags will be used.

Post-Delivery

2. Sample collection

a. Placenta will be collected within 1 hour of delivery. Under sterile conditions to minimize plastic particle contamination placenta will be dissected into basal plate, chorionic villi, chorionic plate and chorio-amniotic membrane samples.
b. Separate samples (~30 g) will be collected into glass bottles with glass or metal lids.

3. Sample digestion & filtration
    a. Placenta samples will be digested using 30 % hydrogen peroxide
    b. Digested samples will be filtered through glass microfiber filters (Whatman GF/D – 2.7 µm pore size). Filters will then be rinsed with ~ 10ml prefiltered deionized water.

4. Sample examination
    a. Filters will be dried under plastic and fibre-free conditions and examined using an imaging system that couples an Enhanced Dark Field (EDF) optical microscope and hyperspectral imaging (HSI) with a confocal Raman imaging system.
    b. The EDF-HSI images will be analysed using Environment for Visualization software. LabSpec 6 software suite will be used for the acquisition of Raman spectral information and KnowItAll spectral database to identify the specific Microplastics type

Appendix 3. Summary of the different extraction protocols tested and results achieved.

1. Filter Membranes

1.1. Silicon membrane

One advantage of using silicon membranes is that they do not interfere with the Raman signature. However, when 5-10 ml of 10% KOH was filtered through the silicon membranes, bubbles appeared during filtration and the membrane became fractioned afterwards. Additionally, the silicon membrane fused with the gaskets. As a result, it was determined that silicon membranes were not suitable for this particular application.

1.2. Gold-coated polycarbonate membrane

Polycarbonate membranes coated with gold have the benefit of not interfering with the Raman signature. To test their effectiveness, 5-10ml of 10% KOH was filtered successfully through these membranes. However, when attempting to filter digest, the total volume was unable to pass through due to clogging of the membrane. As a result, it was determined that gold coated polycarbonate membranes were not a suitable option.

1.3. Gold plated polyester

Gold-plated polyester membranes were ineffective in filtering 5-10ml of 10% KOH, rendering them unsuitable for use with tissue digest. Gold-plated polyester membranes did not affect Raman signature but were unsuitable for filtering 5-10ml of 10% KOH. Tissue digestion was not tested due to the inability of fresh 10% KOH to pass through.

1.4. GF/F – Pore size of 0.7um with 4.7cm in diameter

The grade F glass fiber (GF/F) membranes pose a problem as the glass fibers interfere with the Raman signal. These membranes have a pore size of 0.7um and a diameter of 4.7cm, which is similar to the membranes used in the Ragusa paper. During testing, around 100g of liver digest was filtered through the membrane, and it was discovered that the volume of the digest could pass through successfully. However, due to the large size of the filter, the entire membrane could not be analyzed under Raman. As a result, this particular membrane was deemed unsuitable.

1.5. GF/F – Pore size of 0.7um with 2.5cm in diameter

As the digest could pass through GF/F (4.7 cm), the same volume was tested with the same grade membrane GF/F with a smaller diameter (2.5cm) for better analyzation under Raman. GF/F (2.5cm) membranes have the same disadvantage as previous membrane. These membranes were tested adopting the same protocol as the previous GF/F (4.7cm) membranes, however the digest could not pass all the way through. Therefore, this specific membrane was not suitable.

1.6. GF/A – Pore size of 1.6um with 2.5 cm in diameter

Glass fiber membrane grade A contain a pore size of 1.6um. These membranes have the same disadvantage as previous membrane as the fibers interfere with the Raman signature. In particular these filters were utilized by *Ragusa et al* paper and is appropriate size in diameter for Raman analyzation. These membranes were tested using method as the previous GF/F (2.5cm) membranes, however the digest could not pass all the way through due to pore size. Therefore, these membranes were not utilized.

1.7. GF/D – Pore size 2.7um with 2.5cm in diameter

Glass fiber membrane grade D contain a pore size of 2.7um. These membranes have the same disadvantage as previous glass fiber membranes, the fibers interfere with the Raman signature. The advantage grade D glass fibers are the bigger pore size. These membranes were tested the same way as the previous GF/F and GF/A membranes. Total volume of digest pass through sufficiently with minimal damage to the membrane and no clogging, therefore GF/D was chosen for the study.

2. Tissue Digest with 10% KOH

2.1. Liver

In developing a method, cow liver tissue was chosen as it shares similarities with the placenta in terms of containing high levels of blood. Additionally, cow liver was easily obtainable. At the initial stage of the method development, the tissue dissolution procedure performed was used by Ragusa et al – the first study to report microplastic in human placenta [1]. A significant amount of liver (~20g) was used along with 100 ml of 10% KOH. However, this method proved to be unsuitable as the tissue was not entirely digested and ended up clogging the pore filter (0.7um) of the GF/F – 4.7 cm membrane.

After reducing the tissue amount to approximately 5g using 20 times 10% KOH (100 ml) with 60°C incubation and shaking, there was some improvement in the digestion process. However, even though the majority of the digest passed through the (0.7um pore size GF/F – 2.5 cm) membrane, there was still a significant amount of tissue residue that obstructed the Raman signal.

An amount of tissue was reduced to 1g using 20 times 10% KOH (~20ml) with 60°C incubation and shaking. This tissue amount was adopted for further development of the method. The digestion process was improved as there was no clogging when it passed through the (0.7um pore size GF/F – 2.5 cm) membrane. However, there was no change observed in the residue amount, which obstructed the Raman signal for microplastics.

3. Minimizing Tissue interference with Raman

3.1. Washes H_2O_2

In order to reduce tissue coloration and improve the Raman signal, a 10% H2O2 solution was applied after filtering a liver digest with 10% KOH. Although the glass membrane

appeared less colored after filtration and rinsing, microspectroscopy revealed that the tissue was still present and obstructed the signal, although the coloration was reduced.

3.2. Fenton's reagent

After digesting the glass membrane with a 10% KOH solution, Fenton's reagent was used as a rinse. Fenton's reagent consists of 10ml of 30% and 20ml of 0.05 M Fe (II) and is commonly used to eliminate natural organic debris. It's an effective pre-treatment for micro-spectroscopy imaging. Unfortunately, it didn't work as expected in this instance, as a considerable amount of tissue residue remained on the filter.

3.3. Alcohol

After digesting with 10% KOH on a glass membrane, a mixture of hexane and 5ml of three alcohols (4.50ml ethanol, 0.25ml methanol, 0.25ml isopropanol) was used as an alcohol rinse. This method was originally adapted during staining with Nile Red to remove excess dye and background staining [16]. However, it was later discovered that this method was not effective as the Nile Red was staining beyond MPs.

4. Identifying the Presence of MPs with Nile Red

Analyzation through a confocal microscope was used for the detection of Microplastics through Nile red staining.

4.1. Nile Red staining on the membrane

To apply the first Nile Red staining method, the liver digest containing various pristine MPs was filtered through a GF/D membrane with 10% KOH. Afterwards, an additional 10% KOH was used to wash the membrane and minimize residue. The membrane was then stained directly with about 1ml of Nile red. However, this method proved ineffective in reducing tissue residue following the KOH wash and staining.

To stain the membrane with Nile red, a second method was used which involved pre-treatment with 10% H2O2. Post-filtration treatment was then conducted using different solvents as mentioned in section 3. These treatments did not decrease tissue residue and allowed Nile red to stain beyond MPs.

The experiment tested acetone as a post-filtration treatment and Nile Red staining pre-treatment. Different volumes, ranging from 10ml to 40ml, were experimented with. However, it was observed that the solvent had an adverse impact on microplastic particles as it dissolved the polymers, particularly polyethylene[17]. This method has been previously documented in the literature as a pre-treatment for Nile Red staining [18].

4.2. Nile Red in digests

An experiment was conducted to find microplastics in a tissue digest that contained a mixture of newly produced MPs and 10% KOH. Nile red was added as a staining agent to identify the particles present in the solution and filter them through a GF/D membrane. However, it was later discovered that the Nile red was staining the organic material in the liver digest instead of the microplastics. This led to the method being deemed unsuitable for the study.

5. Tissue Digest with 30% H_2O_2

5.1. Liver and Placenta

In order to remove any remaining tissue residue on a glass membrane, the effectiveness of 30% hydrogen peroxide was tested on 1g of cow liver with approximately 200 ml of H2O2. This method was acquired through LC Jenner. The liver sample was placed in a glass flask along with 100ml of 30% H2O2 and incubated at 55°C for 11 days at 100 rpm. An additional 100 ml of 30% H2O2 was added to the mixture after 5 days to aid in digestion.

At filtration, little tissue residue was observed on the membrane did not interfere with the Raman signal, however, damage to the membrane was observed due to the high amount of H_2O_2. The overall method was altered to a total of 150ml. The altered method was adapted for placenta digest. This method has the capacity to identify and characterize MPs as well as non-plastic particles that were not reported by Ragusa et al KOH method.

References

1. Ragusa A, Svelato A, Santacroce C, et al. Plasticenta: First evidence of microplastics in human placenta. *Environ Int*. 2021;146:106274. doi:10.1016/j.envint.2020.106274
2. Amereh F, Amjadi N, Mohseni-Bandpei A, et al. Placental plastics in young women from general population correlate with reduced foetal growth in IUGR pregnancies. *Environ Pollut 1987*. 2022;314:120174. doi:10.1016/j.envpol.2022.120174
3. Braun T, Ehrlich L, Henrich W, et al. Detection of Microplastic in Human Placenta and Meconium in a Clinical Setting. *Pharmaceutics*. 2021;13(7):921. doi:10.3390/pharmaceutics13070921
4. Han Y, Song Y, Kim GW, et al. No prominent toxicity of polyethylene microplastics observed in neonatal mice following intratracheal instillation to dams during gestational and neonatal period. *Toxicol Res Seoul*. 2021;37(4):443-450. doi:10.1007/s43188-020-00086-7
5. Park EJ, Han JS, Park EJ, et al. Repeated-oral dose toxicity of polyethylene microplastics and the possible implications on reproduction and development of the next generation. *Toxicol Lett*. 2020;324:75-85. doi:10.1016/j.toxlet.2020.01.008
6. Schwabl P, Köppel S, Königshofer P, et al. Detection of Various Microplastics in Human Stool: A Prospective Case Series. *Ann Intern Med*. 2019;171(7):453-457. doi:10.7326/M19-0618
7. Liu S, Liu X, Guo J, et al. The association between microplastics and microbiota in placentas and meconium: The first evidence in humans. *Environ Sci Technol*. Published online 2022.
8. Bové H, Bongaerts E, Slenders E, et al. Ambient black carbon particles reach the fetal side of human placenta. *Nat Commun*. 2019;10(1):3866.
9. Ruano CSM, Miralles F, Méhats C, Vaiman D. The Impact of Oxidative Stress of Environmental Origin on the Onset of Placental Diseases. *Antioxid Basel Switz*. 2022;11(1):106. doi:10.3390/antiox11010106
10. Landrigan PJ, Stegeman JJ, Fleming LE, et al. Human Health and Ocean Pollution. *Ann Glob Health*. 2020;86(1):151. doi:10.5334/aogh.2831

11. Jenner LC, Rotchell JM, Bennett RT, Cowen M, Tentzeris V, Sadofsky LR. Detection of microplastics in human lung tissue using μFTIR spectroscopy. *Sci Total Environ*. 2022;831:154907. doi:10.1016/j.scitotenv.2022.154907

12. Wang S, Lu W, Cao Q, et al. Microplastics in the Lung Tissues Associated with Blood Test Index. *Toxics*. 2023;11(9):759. doi:10.3390/toxics11090759

13. Yan J, Pan Y, He J, et al. Toxic vascular effects of polystyrene microplastic exposure. *Sci Total Environ*. 2023;905:167215. doi:10.1016/j.scitotenv.2023.167215

14. Wick P, Malek A, Manser P, et al. Barrier capacity of human placenta for nanosized materials. *Environ Health Perspect*. 2010;118(3):432-436. doi:10.1289/ehp.0901200

15. Gruber MM, Hirschmugl B, Berger N, et al. Plasma proteins facilitates placental transfer of polystyrene particles. *J Nanobiotechnology*. 2020;18(1):128. doi:10.1186/s12951-020-00676-5

16. Mistri M, Sfriso AA, Casoni E, Nicoli M, Vaccaro C, Munari C. Microplastic accumulation in commercial fish from the Adriatic Sea. *Mar Pollut Bull*. 2022;174:113279. doi:10.1016/j.marpolbul.2021.113279

17. Prata JC, Reis V, Matos JTV, Da Costa JP, Duarte AC, Rocha-Santos T. A new approach for routine quantification of microplastics using Nile Red and automated software (MP-VAT). *Sci Total Environ*. 2019;690:1277-1283. doi:10.1016/j.scitotenv.2019.07.060

18. Prata JC, Sequeira IF, Monteiro SS, et al. Preparation of biological samples for microplastic identification by Nile Red. *Sci Total Environ*. 2021;783:147065. doi:10.1016/j.scitotenv.2021.147065