Nanopolystyrene Translocation and Fetal Deposition After Acute Lung Exposure During Late-Stage Pregnancy

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Research

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Abstract

Background: Plastic is everywhere. It is used in food packaging, storage containers, electronics, furniture, clothing, and common single-use disposable items. Microplastic and nanoplastic particulates are formed from bulk fragmentation and disintegration of plastic pollution. Plastic particulates have recently been detected in indoor air and remote atmospheric fallout. Due to their small size, microplastic and nanoplastic particulate in the atmosphere can be inhaled and may pose a risk for human health, specifically in susceptible populations. When inhaled, nanosized particles have been shown to translocate across pulmonary cell barriers to secondary organs, including the placenta. However, the potential for maternal-to-fetal translocation of nanosized-plastic particles and the impact of nanoplastic deposition and accumulation on fetal health remain unknown. In this study we investigated whether nanopolystyrene particles can cross the placental barrier and deposit in fetal tissues after maternal pulmonary exposure.

Results: Pregnant Sprague Dawley rats were exposed to 20 nm rhodamine-labeled nanopolystyrene beads (2.64×10^{14} particles) via intratracheal instillation on gestational day (GD) 19. Twenty-four hours later on GD 20, maternal and fetal tissues were evaluated using fluorescent optical imaging. Fetal tissues were fixed for particle visualization with hyperspectral microscopy. Using isolated placental perfusion, a known concentration of nanopolystyrene was injected into the uterine artery. Maternal and fetal effluents were collected for 180 minutes and assessed for polystyrene particle concentration. Twenty-four hours after maternal exposure, fetal and placental weights were significantly lower (7% and 8%, respectively) compared with controls. Nanopolystyrene particles were detected in the maternal lung, heart, and spleen. Polystyrene nanoparticles were also observed in the placenta, fetal liver, lungs, heart, kidney, and brain suggesting maternal lung-to-fetal tissue nanoparticle translocation in late stage pregnancy.

Conclusion: These studies confirm that maternal pulmonary exposure to nanopolystyrene results in the translocation of plastic particles to placental and fetal tissues and renders the fetoplacental unit vulnerable to adverse effects. These data are vital to the understanding of plastic particulate toxicology and the developmental origins of health and disease.

Background

Plastics are ubiquitous in modern society, used worldwide in a variety of applications ranging from manufacturing, packaging materials, personal products, and medical devices. Growing production and post-consumer plastic waste disposal contribute to the accumulation of plastic in landfills, waterways, and oceans [1]. In the natural environment, material fragmentation of bulk plastic waste by a combination of physical, chemical, and biological processes produces smaller particles referred to as microplastics (< 5 mm in a single dimension [2]) and nanoplastics (< 100 nm in a single dimension). Recent literature identified microplastics in remote atmospheric fallout [3, 4] and as a significant component of indoor air pollution [3]. These have raised concerns for potential adverse health effects of human nanoplastic particle inhalation [5]. In an occupational setting the potential for unintentional

exposure to aerosolized micro- and nanoplastics is a critical issue. According to the National Institute for Occupational Safety and Health (NIOSH) there are presently no occupational exposure limits for micro- or nano-sized plastic particles. Current data on exposure to micro- and nanoplastics in a consumer or occupational context are very limited as the quantification of emissions in a background of ambient air particles cannot be accurately measured with existing technology [6] but may be estimated based on fragmentation of microplastics in the environment. A recent meta-analysis demonstrated that adult women are exposed to an average of 258 microplastic particles per day, of which inhalation accounts for 132 microplastic particles [7].

Compared with like particles of a larger size, nanoparticles have the propensity to access deeper regions of the lung and cross biological barriers [8]. Gold was detected in the blood and urine of healthy volunteers following acute inhalation of engineered gold nanoparticles [9]. Titanium was identified in the spleen and liver of young adult (12–13 weeks) and aged (19 months) rats exposed to a TiO_2 nanostructured aerosol [10]. Similarly, we have reported particle translocation of multi-walled carbon nanotubes (MWCNT) to the heart, kidneys, and other systemic tissues after inhalation of MWCNT aerosols in young adult rats [11]. While investigations of the impact of micro- and nanoplastic pollution in terrestrial ecosystems are limited, numerous studies have documented the effects of micro- and nanoplastics on the aquatic environment [5, 12, 13]. Mattsson et al. reported trophic transfer from prey to predator within a laboratory-simulated food chain where 53 nm polystyrene particles transferred from algae to the zooplankter *Daphnia magna*, and then to a freshwater fish [14]. Polystyrene nanoparticles were identified in the yolk sac, gastrointestinal tract, liver, and pancreas of F1 embryos and larvae of adult female zebrafish fed polystyrene nanoparticles, providing evidence of maternal-offspring transfer [15]. Microplastic polystyrene particles (20 um) accumulated (size-dependent) in the liver, kidney, and gut of mice exposed via drinking water. Further, investigators identified alterations in biomarkers of oxidative stress, lipid metabolism, and neurotoxicity, suggesting widespread health risks of polystyrene nanoparticle exposure in mice [16]. Cellular uptake of polystyrene nanoparticles (25 nm and 70 nm) has been reported in human alveolar epithelial A549 cells. Nanoplastic exposure reduced cell viability, induced cell cycle S phase arrest, and up-regulation of pro-inflammatory cytokines and pro-apoptotic proteins. Importantly, exposure duration, particle diameter, and concentration were key determinants of the toxicological effects of polystyrene nanoparticle exposure on alveolar epithelial cells [17].

While information about the risk of airborne micro- and nanoplastic particles to human health is limited, recent studies in mammalian models have identified maternal-to-offspring translocation of nanoplastic particles raising concerns for the risk of adverse health effects in dams and embryos/fetuses during pregnancy [18]. Using an *ex vivo* human placental perfusion model, Wick et al. confirmed size-dependent maternal-to-fetal placental translocation of fluorescent polystyrene particles (50 nm, 80 nm, and 240 nm) [19]. Furthermore, Grafmueller et al. examined the bidirectional transfer of polystyrene particles using the *ex vivo* human placental perfusion model and observed placental translocation and particle accumulation in the syncytiotrophoblast of the placental tissue [20]. Investigators reported that nanoparticle translocation across the human placenta was dependent on particle physio-chemical

characteristics and functionalization and was likely to involve an active, energy-dependent transport pathway [20]. While it is understood that nanoplastic particles are likely to reach the fetal compartment after maternal inhalation, the impact of maternal lung exposure to nanoplastic particles on fetal pup growth and particle deposition within the fetus remains unclear. In this study, we examine the translocation and accumulation/deposition of nanopolystyrene particles in maternal and fetal tissues after a maternal pulmonary exposure in rats during late gestation. Further, we assess the impact of nanopolystyrene particles on fluid flow in real-time across the live placenta using an isolated *ex vivo* utero-placental perfusion system.

Materials And Methods

Polystyrene Nanobeads: Stock solutions of commercially available 20 nm rhodamine-labeled polystyrene beads (8.8×10^{14} particles/mL; PS20-RB-2; NanoCS, New York, NY) were suspended in distilled water and 0.01% surfactant and sonicated for 15 minutes prior to measurement. The size of the nanoparticles was measured with Non-Invasive Backscatter optics (NIBS) using a 4 mW, 633 nm laser. The ENM ζ-potential was also measured via Zetasizer Nano ZS. Independent verification of particle size revealed an average particle agglomerate size of 21.86 nm ± 0.026 and a zeta potential of -0.0874 ± 0.195.

Animals: Time-pregnant Sprague Dawley rats were ordered from Charles River Laboratories (Kingston, NY). Animals were delivered on gestational day (GD) 15 and allowed to acclimate within an AAALAC accredited vivarium at Rutgers University for at least 72 hours. Animals had access to food and water *ad libitum.* All procedures were approved by the Institutional Animal Care and Use Committee of Rutgers University.

Exposure: Rhodamine-labeled nanopolystyrene particles were prepared by vortexing 300 µl of manufacturer's 1.0% aqueous suspension for 2 minutes, followed by ultra-sonication on ice, for 5 minutes as previously described [21, 22]. Rats were anesthetized with isoflurane gas (5.0% induction). Animals were placed on an angled board by suspending the upper incisor teeth on an incisor loop at a 45° angle. The tongue was retracted using forceps and a cotton-tipped applicator. Using a veterinary operating otoscope fitted with a speculum, the epiglottis was visualized, and a 20 gauge, 4-inch stainless steel ball-tipped oral gavage needle was inserted via the mouth to the trachea. The rats received intratracheal instillation of 300 µL (2.64×10^{14} particles) of nanopolystyrene suspension or vehicle (0.9% NaCl). Rats were monitored after instillation until they regained consciousness and normal physiological activity (e.g., walking, eating, drinking, grooming, and resting).

To extrapolate nanopolystyrene particle dosage, we considered an average 1 mm^3 atmospheric microparticle and mathematically converted this to nanosized particles representative of our spherical 20 nm polystyrene beads. Therefore, a single microparticle represents 2.39×10^{14} nanopolystyrene particles (Fig. 1a). Cox et al. reported that the average women inhales 132 microplastic particles per day [7]. Given that maternal minute ventilation, or the volume of gas inhaled during pregnancy, increases by up to 48% while the respiration rate remains unchanged [23], it is likely that daily total exposure is closer

to the upper bound of 279 microplastics identified in the study [7]. These data suggest that the average pregnant woman could be exposed to 6.67×10^{16} nanoplastic particles per day (Fig. 1b). When the surface area of the lung between human (62.7 m^2) and our laboratory rat (0.409 m^2) model [24] is considered, the appropriate experimental exposure amounts to 4.34×10^{14} nanoplastic particles (Fig. 1c). This value is much greater than the exposure dose of 2.64×10^{14} nanoplastic particles and therefore, the exposure dose used in this study is within real-world considerations.

Fluorescent Optical Imaging: Twenty-four hours after exposure, dams were fully anesthetized with 3–5% isoflurane in oxygen and Nair was applied to the abdominal region to remove hair prior to imaging. The animal was transferred into the Bruker In-Vivo Multispectral (MS) FX PRO Imager (Bruker, Billerica, MA, USA) imaging chamber with nose cone attached to the manifold and placed in the prone position. The MSFX Pro Bruker detects bioluminescence, fluorescence, radio isotope, and X-ray.

A brightfield was taken to confirm positioning and provide a snapshot/photo of the scan. The primary scans consisted of an excitation of 480 nm with an emission of 535 nm for a 1-minute exposure. Later scans consisted of an excitation of 550 nm with an emission of 600 nm. For these scans, the detectable light refracting off the contrast was recorded. The final scan in this series was an X-ray of the sample that assisted with co-registration of the signal with organ tissues. Following live imaging, animals were sacrificed by removal of the heart according to the Rutgers IACUC approval. Maternal tissues, fetal pups, and fetal tissues were harvested and placed on a polycarbonate tray. After tissue scans were complete, the regions of interest were measured using Bruker MSFX PRO Image software.

Hyperspectral-enhanced darkfield microscopy: Formalin fixed fetal tissues were processed, embedded in paraffin, and sectioned to 4 microns. Slides were visualized via transmitted darkfield hyperspectral images and data captured using CytoViva optics at 60x magnification with oil objective. Dual Mode Fluorescence (DMF) and full fluorescence images were captured with Texas Red excitation filter and triple pass emission filter for further particle confirmation. Data was processed using ENVI 4.8 (CytoViva, Inc., Auburn, AL).

Placental Isolation and Perfusion: A separate cohort of naïve gravid rats were anesthetized with isoflurane (5% induction and 3% maintenance) on GD 20. The right uterine horn was isolated, removed, and placed into a dish of cold (4 °C) physiological salt solution (PSS). Briefly, the uterine horn was dissected, placental unit was identified, amniotic sac opened, fetal pup removed, and umbilical vessels were ligated and unraveled as previously described [25, 26]. The placental unit was removed and placed into a modified isolated vessel chamber (Living Systems Instrumentation, Burlington, VT) filled with warmed (37 °C), oxygenated (21% O_2 – 5% CO_2 – 74% N_2), circulating PSS. The placental vasculature (uterine artery and umbilical artery and vein) were secured to glass pipettes or 26 gauge, 4-inch stainless steel blunt needles, respectively. The uterine artery was perfused with a peristaltic pump at 80 mmHg and the umbilical artery was perfused at 50 mmHg. After a 30-minute equilibration and 10-minute baseline, a bolus of 900 µL (7.92×10^{14} particles/mL) of nanopolystyrene particles were slowly injected into the uterine artery. Effluents were collected and weighed from the distal uterine artery and umbilical vein

cannula at 10-minute intervals for a total of 180 minutes. The remaining fluid within the stainless-steel needle cannulating the umbilical vein was collected.

Quantification of Nanopolystyrene particles: 25 µL of effluent from each sampling time point was pipetted in duplicate on a 96-well clear bottom plate. Positive control was identified as 25 µL of stock solution and negative control as PSS only. All samples were diluted by adding 100 µL of PSS into each well. Fluorescence was measured by a spectrophotometer at 546/575 nm (excitation/emission) using a SpectraMax M3 fluorescent microplate reader (Molecular Devices, Sunnyvale, CA). Data were collected using SoftMax Pro 6.3 software.

To confirm that the rhodamine tag remained attached to the polystyrene beads throughout the perfusion, maternal and fetal effluents were pooled together for 4 representative experiments. The samples were centrifuged at 100,000 x g for 1 hour in an ultracentrifuge (Beckman Coulter Max-XP tabletop Ultracentrifuge) to pellet polystyrene ENM. 25 µL of supernatant was removed from each sample and placed in a 96-well clear bottom plate and read at 546/575 nm (excitation/emission) using a SpectraMax M3 fluorescent microplate reader (Molecular Devices, Sunnyvale, CA). Data were collected using SoftMax Pro 6.3 software.

Histology: Representative placentas from the perfusion experiments were fixed in 10% neutral buffered formalin, processed and sectioned to 4 µm. Hematoxylin and eosin (H&E) stained slides were assessed by an ACVP board-certified veterinary pathologist.

Statistics: Outliers were identified as above or below 2 standard deviations from the mean and removed. All data were analyzed by Student's T-test. Statistical significance was set to $p < 0.05$ and is indicated with an asterisk (*). Trends were identified as $p < 0.10$ and are indicated with a (T).

Results

Litter Characteristics

Maternal and fetal parameters including maternal weight, litter size, fetal pup weight, placental weight, placental efficiency, and sites of resorption are reported in Table 1. In treated dams, fetal pup and placenta weights were significantly lower in the exposed group compared with control. Resorption sites in the exposed group were also significantly greater compared with control. There were fewer fetal pups in dams treated with nanopolystyrene particles, however, this difference did not reach significance in our cohort.

Table 1
Effect of maternal nanopolystyrene pulmonary exposure on litter characteristics. Values are shown as mean ± SEM. n number of dams. Statistics were analyzed with a one-way analysis of variance (*$p \leq 0.05$; $T \leq 0.10$).

Treatment	n	Maternal Weight (g)	Number of Fetal Pups	Fetal Pup Weight (g)	Placental Weight (g)	Placental Efficiency	Number of Resorption Sites
Saline	12	353 ± 11	13.3 ± 0.3	2.71 ± 0.05	0.47 ± 0.01	5.79 ± 0.15	0.42 ± 0.14
PS	11	362 ± 17	12.4 ± 0.6	*2.52 ± 0.06	*0.43 ± 0.01	6.08 ± 0.26	*1.13 ± 0.30

Fluorescent Optical Imaging

Primary whole animal scan yielded null results as the skin was too dense to visualize any fluorescence (data not shown). Representative images obtained from secondary scans of maternal and fetal tissues and recorded at the same intensity are given in Fig. 2a and Fig. 2b, respectively. Graphical representations of optical intensities are represented for maternal tissues in Fig. 2c and fetal tissues in Fig. 2d. These data indicate significate nanopolystyrene deposition in the maternal lung, heart, spleen, and a trend toward significance in the gravid uterus. Deposition of polystyrene was elevated in all fetal tissues evaluated. These were significantly higher in the isolated GD 20 fetal pup, fetal abdomen, and isolated liver. There was an elevated trend in the fetal pup and placenta in its entirety within the amniotic sac, isolated placenta, and isolated fetal hearts. These studies indicate nanopolystyrene particle translocation from the maternal lungs to systemic tissues, including the fetus and fetal organs.

Hyperspectral Darkfield Microscopy

Enhanced darkfield imaging of fetal tissue sections readily demonstrated polystyrene nanoparticle deposition, illustrated in Fig. 3. Polystyrene nanoparticles were visualized in the fetal liver, lung, kidney, heart, and brain. In representative images, these particles appear as white dots/spots. These studies further demonstrate nanoplastic particle deposition within fetal tissues.

Placental Perfusion

Polystyrene nanoparticle transfer through the maternal vasculature from the proximal to the distal uterine artery was confirmed within 10 minutes of bolus infusion (Fig. 4a). Nanoparticle transfer across the maternal uterine artery peaked at 20 minutes, remained significantly above baseline for 50 minutes, and was elevated at 90 minutes. Effluent fluorescence returned to normal levels after 100 minutes. Further, elevated concentrations of polystyrene nanoparticles were detected in umbilical effluent within 90 minutes of uterine artery bolus infusion (Fig. 4b). Concentration of the nanoparticles in the umbilical effluent were significantly high after 150 minutes through 180 minutes after infusion and significant concentrations remained in the umbilical cannula after 180 minutes of perfusion. These results confirm the capacity of 20 nm nanopolystyrene plastic particles to pass from the maternal to the fetal

compartment within 90-minutes of uterine artery infusion. Interestingly, while fluid flow from the maternal to fetal compartment decreased after both saline control and nanopolystyrene injection, there were no significant differences in fluid flow between groups during the 180-minute perfusion (Fig. 4c). This suggests no reduction in blood flow through the placenta within 180-minutes of particles reaching the uterine artery. Placenta were evaluated after perfusion and no histopathological alterations were identified.

Discussion

In this study we observed reduced fetal pup weight and reduced placental weight 24 hours after maternal nanopolystyrene particle pulmonary exposure. We also identified nanopolystyrene translocation from the maternal lungs, across the placenta, to the fetal compartment. Deposition of nanoplastic particles was observed in the fetal liver, lung, heart, and brain in late-stage pregnancy. Further, we observed the transfer of nanopolystyrene particles from the maternal uterine circulation, across the placenta to the fetal circulation within 90 minutes without compromising fluid dynamics, using an *ex vivo* placental perfusion system.

The results of this study corroborate data from previous investigations from our group that have shown reduced fetal weight after chronic maternal inhalation of nano-sized particles [27]. We postulate that reductions in fetal development, as reported here and in previous studies, are associated with indirect vascular deficiencies leading to ischemia and reduced nutrient/waste exchange in late-stages of pregnancy [27, 28, 29]. Nanoplastic particle deposition within the placental vasculature could result in a reduction in blood flow through the placenta due to a physical blockage; however, we did not observe a reduction in fluid flow to the fetal compartment after direct infusion of nanopolystyrene particles into the uterine artery. We have observed decreased fluid flow during *ex vivo* placental perfusion previously after gold nanoparticle infusion [25]. Future studies to assess the impact of a chronic exposure to nanoplastic particles on fetal growth and development are required for a comprehensive understanding of the health hazards associated with airborne nanoplastics.

Similarly, Grafmueller et al. demonstrated the placental transfer of fluorescently-labeled nanopolystyrene particles from the maternal to the fetal compartment [19, 20]. Upon further study utilizing the *ex vivo* human placental perfusion model, the authors identified a bidirectional, size-dependent transfer of nanopolystyrene beads without cytotoxicity [20]. While it is recognized that the concentration of particles that translocate from the primary site of exposure to the fetal compartment and tissues is low [30] and that this methodology is not without limitation [31]; it is likely that nanoplastic particle transfer across the placenta involves energy-dependent uptake, material transfer, and particle efflux to the fetal side [20]. As it pertains to nanoplastic particle deposition within the tissues, it remains unclear if the nanopolystyrene particles have been taken up by the fetal cells, if they remain in the vasculature, migrate to the interstitial space, or if they are returned to the maternal circulation in bulk. The uptake and passage of nanosized materials is highly dependent on the physio-chemical properties of the particles including size, functionalization, chemical construct, and surface charge [5].

Furthermore, cellular uptake and subsequent toxicity of nanoplastic particles is dependent on the unique protein and chemical corona that forms on the surface during contact with biological fluids (e.g. pulmonary surfactant, interstitial fluid, plasma) and environmental chemicals; in the case of plastics these chemicals may be adsorbed and serve and a vehicle for chemical transport. Chemical additives adsorbed to the surface or added to plastics during the polymerization process can leach or be transferred from polystyrene products with normal use. These additives may include carcinogens or endocrine disrupting factors (e.g., vinyl chloride, phthalates) [32, 33]. Historical studies identify and refer to the potential for chemical leakage from polystyrene products after the addition of hot, cold, or boiling water [34]. Withey also reported elevated blood styrene in rats after exposures to vapor-phase and intragastric styrene [34]. Together, these outcomes indicate the likelihood of chemical release from particles within an organism. Fetal nanoplastic deposition could lead to life-long localized low-level exposure to these additives or adsorbed chemicals. Future studies are planned to examine chemical release from plastic nanomaterials within a biological environment.

In this study, we identified nanopolystyrene translocation from the maternal lungs to the fetal compartment and deposition in the fetal lungs, liver, heart, kidney, and brain within 24 hours of maternal exposure. It is likely that these particles would remain in the fetus after birth. Endothelial cell exposure to engineered nanomaterials enhances endothelial barrier permeability [35, 36, 37], which offers accessibility to the interstitial space between cells within systemic tissues. Reports pertaining to the development and function of the blood brain barrier in a fetus are inconclusive [38, 39]. Therefore, the blood-brain barrier may not yet be fully formed, rendering the fetal brain susceptible to particle sedimentation. We, and others, have identified that maternal exposures to ENM during gestation can initiate developmental onset of disease within the maturing fetus. In laboratory studies, young and adult offspring have been reported to exhibit coronary dysfunction [40, 41, 42, 43, 44, 45], vascular perturbations [27, 45], reproductive consequences [46, 47, 48, 49, 50], and neurodevelopmental delays [51, 52] after maternal inhalation of engineered nanomaterials during pregnancy. Therefore, particle deposition, and likely particle accumulation and retention, impact offspring health after birth and into adulthood.

Plastic particles are so prevalent in the natural environment that human exposure to microplastics is inevitable. The majority of plastic materials produced end up in landfills [1] and have been estimated to take hundreds of years to breakdown from a larger size through mechanical, ultraviolet, and frictional forces to smaller microplastics and nanosized plastic particles [53]. These particles can enter water and food sources, as identified in tap water, bottled water, and beer [54, 55], become airborne as a constituent of indoor air particulate [56], be identified in planting soils and root structures [57], or contribute to environmental contamination by atmospheric fallout [3, 4, 7].

Conclusion

Collectively, these results identified the impact of a pulmonary exposure to an environmentally relevant dose of nanopolystyrene and examined maternal and fetal parameters and the translocation of plastic

particles to, and deposition within fetal tissues. These data are vital to the understanding of plastic particulate toxicology and the developmental onset of health and disease. Future studies are required to provide a more detailed exploration of organ-specific toxicity and the implications of nanoplastic exposure on reproductive potential and fetal development.

Declarations

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CONTRIBUTIONS

SF conducted the animal exposures, dissections, and was a major contributor in data analysis and writing the manuscript. JD conducted the animal exposures, placental perfusion experiments, and was a major contributor in data analysis and writing the manuscript. DS conducted the optical imaging studies and was a major contributor in data analysis. SK completed the nanoparticle characterizations. MG completed the histological reviews. LF completed the nanoparticle characterizations. EY contributed to data analysis of the optical imaging studies. PS developed the experimental design, oversaw the exposures and experiments, and was a major contributor to the data analysis and writing the manuscript. All authors read and approved the final manuscript.

ETHICS DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All animal experiments were approved by the Institutional Animal Care and Use Committee of Rutgers University.

CONSENT FOR PUBLICATION

Not Applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analysed during this study are included in this published article.

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Figures

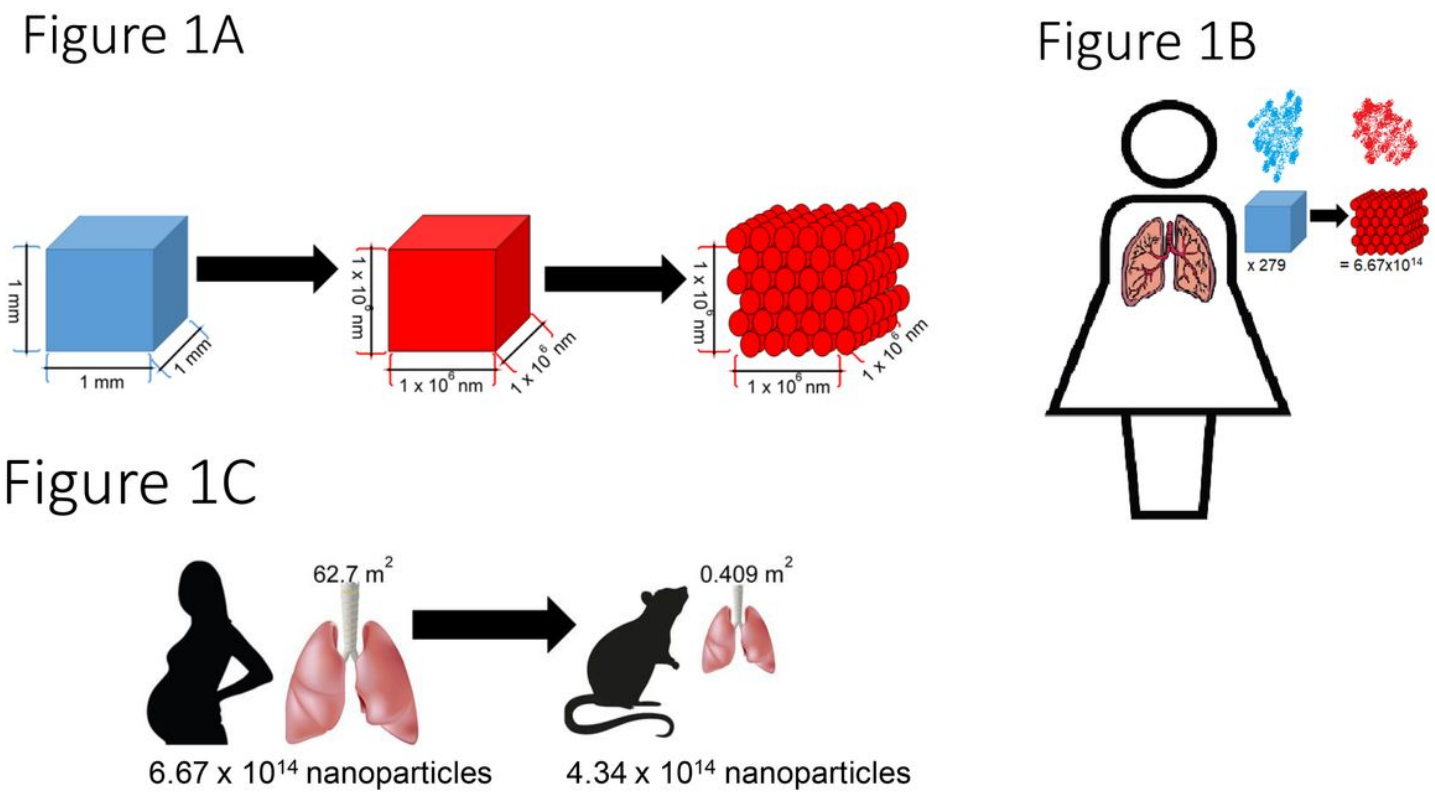
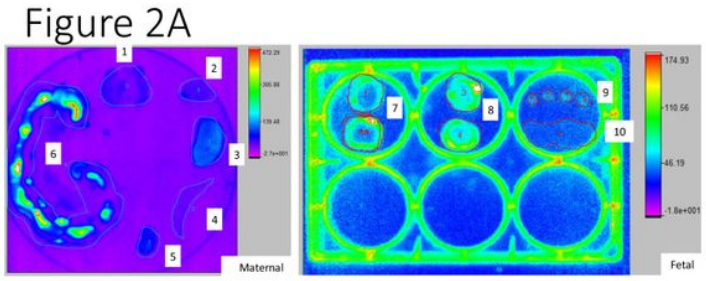
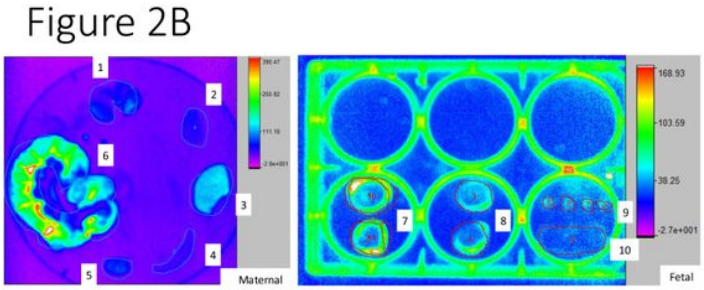
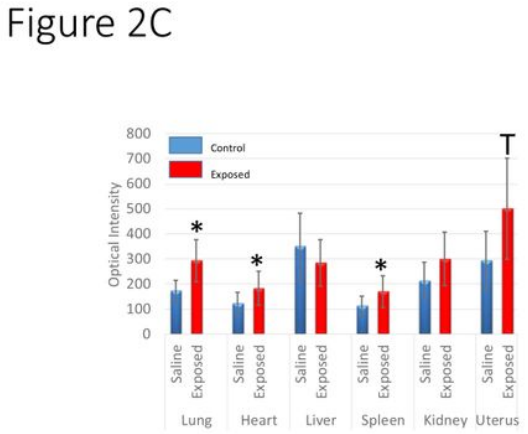


Figure 1

Schematic of nanoplastic exposure and dosimetry. (A) We utilized a 1 mm2 microparticle as a representative microplastic (blue). The extrapolation of this microplastic microparticle to a nanoparticle is 1 x 106. Further, our representative nanopolystyrene nanobeads are spherical and 21 nm in diameter (red). Therefore, there would be 2.39 x 1014 nanopolystyrene beads in a single plastic microparticle. (B) Cox et al. identified that women inhale an average of 132 microplastics. The upper bound of this measurement (279 microplastics), is more representative of exposure for pregnant women. The calculated dosage is 6.67 x 1016 nanopolystyrene beads. (C) The surface area of the Sprague Dawley rat lung is significantly smaller (0.409 m2) compared with the human lung (62.7 m2). The calculated dose for a laboratory rat is 4.34 x 1014. The exposure dose used in these studies was 2.64 x 1014 nanopolystyrene beads.







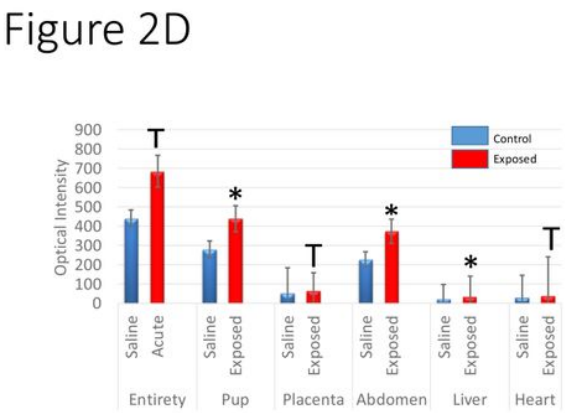


Figure 2

Optical images of maternal and fetal tissues. Representative imaging of (A) control and (B) exposed tissues: (1) lungs, (2) heart, (3) liver, (4) spleen, (5) kidney, (6) gravid uterus, (7) maternal surface of placenta, (8) fetal surface of placenta, (9) isolated fetal hearts, and (10) isolated fetal livers. Graphical representation of the optical intensities between (C) maternal and (D) fetal control and exposed tissues. n=6-8 pregnant rats. Values are shown as mean ± SEM. Statistics were analyzed with a one-way analysis of variance (*$p \leq 0.05$; T ≤ 0.10).

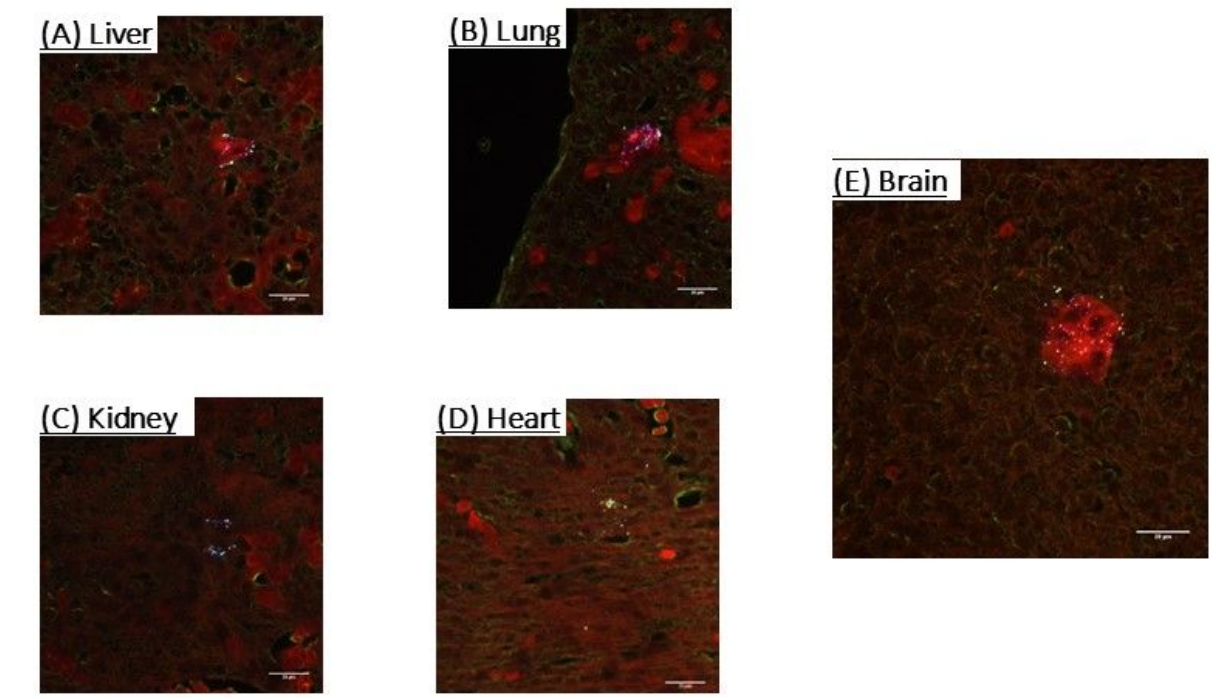


Figure 3

Identification and visualization of nanopolystyrene particle deposition within the fetal tissues placenta after material pulmonary exposure using enhanced hyperspectral microscopy (CytoViva, Inc.). These tissues include fetal (A) liver, (B) lung, (C) kidney, (D) heart, and (E) brain. n=3 fetal pups from 3 different pregnant rats. Polystyrene nanoparticles are identified as white specs within the images.

Figure 4A

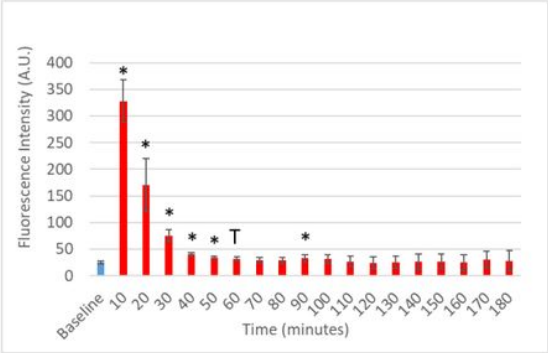


Figure 4B

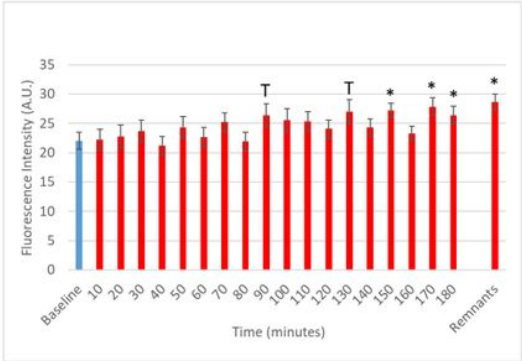


Figure 4C

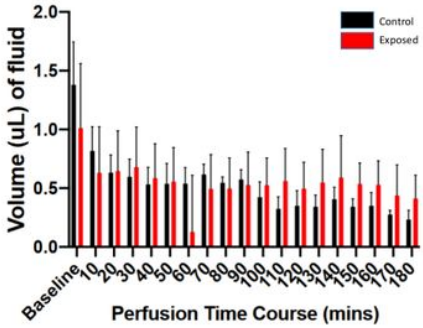


Figure 4

Identification of rhodamine-labeled nanopolystyrene bead translocation based on increased fluorescence through the (A) distal uterine effluent and (B) umbilical vein effluent over time. n=9-24. (C) Time-course of fluid flow through the umbilical vein between saline (black) and nanopolystyrene (red) infused placenta. n=6-8. Values are shown as mean ± SEM and presented as percent above baseline. Statistics were analyzed with a one-way analysis of variance (*$p \leq 0.05$; T ≤ 0.10).