3558 Fetal Growth Restriction after Repeated Micro- and Nanoplastic Inhalation during Pregnancy and Uterine Nitric Oxide Bioavailability

C. M. Cary, T. N. Seymore, S. Adams, and P. A. Stapleton. *Rutgers, The State University of New Jersey, Piscataway, NJ.*

Micro and nanoplastics (MNPs) are an emerging environmental pollutant that is increasingly being detected in human tissues. We have shown in virgin female rats that a single inhalation of MNPs blunts endothelial function in the uterine vasculature. Under conditions like pregnancy, xenobiotics impairing vascular function have detrimental effects but the consequences of maternal MNP exposure are unknown. Here, we assessed fetal health, placental efficiency, uterine vascular function, and nitric oxide (NO) bioavailability after MNP inhalation throughout pregnancy. Pregnant rats were exposed to aerosolized polyamide-12 MNPs [10.5 ± 0.73 mg/m^3; geo. mean particle size 138 ± 8.72nm] for 4-5h daily, 5d/week from gestational day (GD) 5-19 and experimental analyses were performed on GD 20. MNP exposure significantly reduced fetal weight (4.17 ± 0.09g vs 4.40 ± 0.05g) and placental efficiency (8.31 ± 0.30 vs 9.89 ± 0.40). Uterine artery responses to increasing concentrations of methacholine (MCh), sodium nitroprusside (SNP), and phenylephrine (PE) were assessed by wire myography. Exposure resulted in trending impairments of endothelium-dependent dilation stimulated by MCh at 10^{-9} M (3.87 ± 3.55 vs -11.9 ± 7.65% max. tension) as well as endothelium-independent dilation stimulated by SNP at 10^{-9} M (1.19 ± 3.23 vs -12.6 ± 6.96% max. tension). The contribution of eNOS to endothelium-dependent dilation was determined by measuring endothelium-dependent dilation after inhibition with L-NAME. Concentration response curves after stimulation with MCh, SNP, and PE as well as reliance on eNOS for endothelium-dependent dilation, revealed no significant differences between groups. NO bioavailability in the uterine vasculature was measured by quantification of nitrites and western blot of tyrosine nitration (a product of peroxynitrite) after stimulation of NO release. Nitrites were decreased in exposed dams (5.05 ± 4.01 picomol/g vs 8.11 ± 2.46 picomol/g; p = 0.31). Preliminary western blot of uterine vasculature suggests elevated tyrosine nitration in exposed dams. These data show that MNP inhalation induces fetal growth restriction and may decrease NO release from the uterine vasculature without causing functional deficits in the uterine macrocirculation. Studies are ongoing to examine uterine microvascular function as well as mechanistic cause of decreased NO bioavailability in the uterine vasculature. *Supported by: R01-ES-031285-S1, R01-ES-031285, P30-ES-005022, and T32-ES-007148.*

3559 Development of a Study Quality Tool for Use in a Systematic Review of Literature Reporting Microplastic Exposure and Reproductive and Developmental Toxicity

S. Fitch[1], J. Rogers[1], S. Marty[2], R. Ellis-Hutchings[2], R. Becker[3], and D. Wikoff[4]. *[1]ToxStrategies Inc., Houston, TX; [2]Dow Chemical Company, Midland, MI; [3]American Chemistry Council, Washington, DC; and [4]ToxStrategies Inc., Asheville, NC.*

Microplastics (MPs) have been detected in air, water, soil and food, but understanding these exposures in the context of potential human health risks requires both hazard characterization and exposure evaluation. Initial reviews of hazard studies identified developmental and reproductive toxicity (DART) as potentially "critical effects" (effects exhibiting the lowest NOAEL/BMD). However, these reviews also highlighted that aspects of study reliability relating to these experimental investigations - particularly test article identity, exposure, dose-response, and relevance of mechanistic endpoints to adverse effects - impact confidence in hazard characterization. To better understand such impacts and to systematically gather, appraise, and integrate the relevant empirical evidence, a systematic review of the potential effects of MPs on DART outcomes in both epidemiological and experimental animal studies was conducted using a stepwise and highly refined approach to critical appraisal. This approach combines explicit and transparent determinations of risk of bias (systematic error), test article attributes and characterization information, based on the published Nano- and Microplastic Particle Toxicity Assessment Tool (PMID: 35098152), with internal and construct validity evaluations for DART study conduct and reporting (based on subject matter expertise). The protocol was developed *a priori* and published online. The systematic literature search performed on December 17, 2021, resulted in identification of 9 publications that met the inclusion criteria; no relevant epidemiological studies were identified. These included studies that evaluated MP exposure in mice (ICR, BALB/C, or C57BL/6 strains) via drinking water, gavage, or intratracheal instillation with exposure periods ranging from 18 days (gestational exposure) to 90-days. Studies used a variety of MP test materials, varying in shape (microspheres or irregular), polymer (polyethylene or polystyrene), and size (ranging from 5 to 45µm). Four studies assessed female reproductive effects, 6 studies assessed male reproductive effects, and 4 studies assessed developmental toxicity. The measured parameters in the developmental toxicity studies were limited to outcomes such as offspring organ weight and body weight. None of the identified studies followed published standardized and harmonized OECD/EPA test guidelines. Following application of the internal and construct validity tools, only 4 of 9 studies were shown to achieve sufficient internal construct validity to proceed to the second stage of critical appraisal — the evaluation of sufficiency for risk assessment. The appraisal for applicability in risk assessment showed that none of these studies meet all the criteria to be considered sufficient for hazard characterization; in each study one or more key criteria were not met (statistical analysis, dose-response relationship, concentration range, reporting of an effect threshold or adequate data to derive one, test particle relevance). Therefore, the available body of literature did not meet the minimum standards of validity and confidence for use in hazard characterization of MPs for potential human health risks. This systematic review and evidence appraisal methodology enables more precise understanding of the current state of the science and illustrates opportunities for developing the additional information needed for improving the scientific basis of MPs hazard characterization, exposure evaluations, and risk assessments.

3560 Sexually Dimorphic Modifications in Placental Cyclooxygeanse Metabolites after Maternal Nanomaterial Inhalation Exposure

J. A. Griffith[1,2], R. D. King[1], A. C. Dunn[1,2], S. E. Lewis[1], B. A. Maxwell[1], W. T. Goldsmith[1,2], K. Wix[1,2], E. C. Bowdridge[1,2], S. Hussain[1], and T. R. Nurkiewicz[1,2]. *[1]West Virginia University, Morgantown, WV; and [2]NIOSH, Morgantown, WV.*

Maternal nano-titanium dioxide (nano-TiO_2) inhalation exposure during gestation results in decreased fetal female mass, maternal estrogen production, and placental mass. Placental function is critical during gestation due to its roles in nutrient-waste exchange and endocrine production. Adverse fetoplacental environments profoundly impact fetal growth and development. Additionally, the impacts of toxicant exposure can occur in a sexually dimorphic manner, which has been shown after maternal diesel exhaust exposure during gestation. Therefore, we hypothesized that *maternal nano-TiO_2 inhalation exposure during gestation alters placental function in a sexually dimorphic manner.* Pregnant Sprague-Dawley rats were exposed to nano-TiO_2 aerosols (12.17 ± 1.69 mg/m^3) or HEPA-filtered air (sham-control) from gestational day (GD) 10-19. Dams were euthanized on GD20, and placental junctional zone (JZ), labyrinth zone (LZ), and fetal serum were collected and separated based on fetal sex. Fetoplacental units, also based on fetal sex, nearest the cervix were removed, the umbilical vein and artery were separated and cut close to the pup. The placentas were then mounted in an isolated, perfused vessel chamber to assess outflow pressure and flow rate through the placenta in response to vasoactive drugs. The stable thromboxane and prostacyclin metabolites, TXB_2 and 6-keto-$PGF_{1\alpha}$, respectively, were assessed in the JZ and LZ, and fetal serum using ELISA's. Nano-TiO_2 exposed fetal females demonstrated significantly increased TXB_2 production (11186.48 ± 189.92 pg/ml) from LZ tissue compared to female sham-control (483.77 ± 86.09 pg/ml) and male exposed (598.39 ± 135.69 pg/ml). The JZ also had a significantly increased TXB_2 levels in the exposed fetal females compared to exposed fetal males (1188.09 ± 130.38 pg/ml vs 670.63 ± 94.50 pg/ml, respectively) and sham-control females (649.25 ± 143.27 pg/ml). Fetal exposed female serum 6-keto-$PGF_{1\alpha}$ levels were significantly increased compared to exposed fetal males (97.56 ± 25.86 pg/ml vs 16.33 ± 4.44 pg/ml, respectively) and sham-control females (37.62 ± 5.18 pg/ml). Placental outflow pressure was measured to assess perfused vascular resistance, in which, exposed fetal females demonstrated a significantly decreased outflow pressure (3.97 ± 1.30 mm Hg) in the presence of the thromboxane mimetic, U46619, compared to sham-control fetal females (9.10 ± 1.07 mm Hg) and nano-TiO_2 exposed fetal males (9.96 ± 0.66 mm Hg). Immunohistochemical staining was utilized to evaluate cytotrophoblast invasion, using the pan-cytokeratin marker, between placental zones. The JZ had significantly increased pan-cytokeratin intensity for sham-control males compared to females (67.24 ± 5.21 AU vs 44.43 ± 2.59 AU). The LZ pan-cytokeratin staining was decreased in sham-control females compared to males (64.65 ± 7.47 AU vs 91.55 ± 10.23 AU). The changes in placental hemodynamics and production of cyclooxygenase metabolites reflect a functional change that is occurring in the nano-TiO_2 exposed fetal females. Modifications to cellular composition, as seen in staining, provides evidence there may also be changes in cellular composition leading to these functional changes. These results demonstrate that maternal nano-TiO_2 inhalation exposure during gestation has greater impacts on fetal females and their placental units. *Support: WV-CTSI U54 GM104942-05; K01 10029010 (ECB), P20GM103434 (WV-INBRE), R01 ES015022 (TRN), T32 AG 52375 (JAG), P20 GM103434 (WV-INBRE), T32 ES032920 (JAG).*

3561 Phthalate-Induced Decreases in Ovulatory Prostaglandin Levels Are Restored with Supplementation of cAMP in Human Granulosa Cells *In Vitro*

K. Land, H. Xu, G. Ruschman, M. Wilson, and P. Hannon. *University of Kentucky, Lexington, KY.*

Phthalates are endocrine-disrupting chemicals that are found in common consumer products due to their use as solvents and plasticizers. Phthalates are reproductive toxicants that directly target the ovary, meaning exposure may have negative effects on ovulation, fertility, and reproductive health. Due to their ubiquitous use, humans are exposed daily to a mixture of phthalates; therefore, this study investigated the effects of an environmentally relevant phthalate mixture (PHTmix) on the ovulatory process. Ovulation is triggered by the surge of luteinizing hormone (LH), which binds to its receptor on granulosa cells and upregulates signaling molecules, including cyclic adenosine monophosphate (cAMP). Increased cAMP levels lead to the upregulation of progesterone receptor (PGR), which is a transcription factor

62nd Annual Meeting & ToxExpo
Nashville, TN • March 19–23, 2023

The Toxicologist

Supplement to *Toxicological Sciences*

SOT | Society of Toxicology

Toxicological Sciences

The Official Journal of the Society of Toxicology

OXFORD UNIVERSITY PRESS

ISSN 1096-6080 Volume 192, Issue S1 March 2023
www.academic.oup.com/toxsci

Publication Date: March 14, 2023