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Cardiopulmonary consequences of gestational toxicant exposure: symposium overview at the 56th Annual SOT Meeting, Baltimore, MD

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Abstract

Xenobiotic exposures affect the maternal and/or *in utero* environment resulting in impairments in fetal development. During the period of rapid fetal growth, developing cardiovascular systems are especially vulnerable to their environment. Furthermore, fetal exposures can evoke changes in epigenetic signatures that result in permanent modifications in gene expression. This symposium focused on the intersection between maternal and fetal exposure and the developing cardiovascular system. The impact of maternal exposures on prenatal development is of major concern for regulatory agencies given the unique vulnerability of the embryo/fetus to environmental factors, the importance of vascular biology to maternal-fetal interactions, and the adverse consequences of vascular disruption to children's health. Speakers provided data from diverse exposures: nanomaterials, particulate matter or air pollution (PM_{2.5}), nicotine, and environmental chemicals. The current findings related to susceptible gestational windows for cardiovascular development

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and epigenetic, transcriptomic, toxicokinetic, and toxicodynamic changes in vascular physiology and cardiac function. In response to these concerns, new concepts in predictive modeling and risk assessment associated with *in utero* exposures were presented as future avenues of research within developmental toxicology. Finally, current applications using an Adverse Outcome Pathway framework for developmental toxicity were presented to integrate data from *in vitro* profiling of chemical libraries (e.g. ToxCast™) with computational models for *in silico* toxicology. In summary, this symposium addressed the significant threats to cardiovascular health that are associated with fetal/perinatal exposures, and offered new insights into the predictive, mechanistic, and risk assessment strategies in developmental toxicology.

Keywords

maternal exposures; cardiovascular; in utero toxicity

INTRODUCTION

Cardiovascular disease is the leading cause of death worldwide [1, 2]. One important, yet under-emphasized risk factor is the impact of genetic-environmental-lifestyle factors on maternal-fetal interactions during gestation. Concern over these interactions is in part, motivated by the epidemiological association between maternal metabolic conditions during pregnancy and cardiovascular disease rates and risk in their adult children [3]. These associations highlight the vulnerability of the cardiovascular system during fetal development.

Xenobiotic exposures during pregnancy can have significant effects on the developing fetus. Disruption of vascular development was identified as one mode of action for medical drugs associated with teratological outcomes among women of childbearing potential [4]. Exposures to developmental toxicants during early gestation may lead to lethal teratogenic outcomes or structural malformations [5], reviewed in [6]. Conditions initiated by toxicant exposure or a change in the uterine environment associated with maternal compensation may manifest after birth thus contributing to adult cardiovascular diseases [7, 8].

While studies of maternal pulmonary xenobiotic exposures on adverse birth outcomes and developing fetal systems (e.g. immune, reproductive, respiratory) are being conducted, pointed studies evaluating cardiovascular outcomes are few overall and proportionally underrepresented within developmental toxicology literature [9–11].

This symposium assembled experts in the areas of cardiovascular and developmental toxicology to discuss the impacts of gestational windows of exposure, routes of maternal exposure, predictive modeling, and risk assessment. Xenobiotic exposure focused on maternal and fetal outcomes of the developing cardiovascular system evaluating mechanistically diverse agents such as particulate matter, nanomaterials, environmental chemicals, and nicotine. Adverse outcomes included cardiac anatomical irregularities, vascular disruption, metabolic disorders, and the use of compensatory molecular signaling pathways.

After a brief Introduction by Dr. Lynette Rogers, Dr. Christopher Wingard's presentation addressed directed studies of vascular responsiveness in the maternal and umbilical vasculature, identifying maternal vascular impairments after engineered nanomaterial pulmonary exposure which may influence the maternal-fetal environment and impair fetal growth. Dr. Judith Zelikoff presented evidence of fetal cardiac dysfunction after particle exposure (PM_{2.5}), as identified via ultrasound and persisting through PND 21. Dr. Alison Holloway followed with evidence that cardiovascular disturbances can persist in adult progeny after maternal nicotine exposure and include metabolic disorders further increasing cardiovascular risk in these offspring. Dr. Timothy Nurkiewicz spoke on the microvascular impairments in the maternal and fetal circulations after nanomaterial exposure and the implications of transcriptomic and epigenetic alterations identified through analysis of fetal cardiomyocytes. Dr. Thomas Knudsen presented EPA's work in the creation of a virtual embryo modeling framework which may be used to identify toxicants having the potential to disrupt developmental angiogenesis. Reports of their symposium presentations may be found below.

Altered maternal and placental vascular responses following acute nanomaterial administration.

Christopher J. Wingard, M.S., Ph.D., FACVS, Bellarmine University, Louisville, KY.

Nanomaterials (NM) are continually being developed and utilized in a variety of consumer and biomedical applications. There is an ongoing discussion as to the possibility of adverse biological outcomes following a purposeful or non-purposeful exposure to these materials [12, 13]. Pregnancy and lactation are physiological conditions that may increase the susceptibility of mothers and their offspring to the effects of nanoparticles [14]. We hypothesize that changes in the vasculature induced by nanomaterial exposure may alter blood vessel responses redistributing organ blood flow and the distribution of nanomaterials to the mother, fetus, and neonates. We have examined and reported on the impact of gestational exposure to the fullerene C60, multi-walled carbon nanotubes (MWCNT), and forms of nanosilver (AgNP) on their tissue distribution, cytokine generation, and vascular function [15–19]. Here we reported only on C60 and MWCNT procured and characterized through the NIEHS U19 Nano consortium. Maternal and fetal outcomes were variably impacted dependent on route and type of nanomaterial administration resulting in widely differing tissue distributions dependent on life stage. Our data contrasts the rapid clearance for AgNPs, compared with long persistence for C60 in maternal, fetal or neonatal tissues. Results illustrate cardiovascular effects on non-pregnant and pregnant females, 1 day and 7 day post exposure. Whether by intratracheal instillation or intravenous administration, C60 or MWCNT could augment the contractile strength of isolated umbilical arteries and uterine veins. We observed as much as a 30% increase in the stress generation of uterine arteries, from pregnant dams, following these material exposures and the responses waned by only 10% between 1 and 7 day post-exposure. These contractile responses were of greater magnitude with thromboxane mimetic than adrenergic stimulation. Additionally, a reduced relaxation response to Rho-Kinase inhibition (> 15% difference from naïve or control) of uterine vessels was observed; while minimal changes in nitric oxide donor's relaxation responses were detected (< 10% difference from control or naïve) in those same tissues.

Finally, with regard to MWCNT aspect ratio there was a negative impact on umbilical artery or uterine vein responses with longer or wider dimensioned nanotubes; increasing the agonist induced contractile response by as much as 20% and impairing endothelial dependent relaxation by as much as 15%. Those contractile response patterns were partially mitigated (reduced by 1/3 towards controls) by use of an artificial surfactant rather than a clinical grade surfactant to disperse the nanomaterial prior their administration. A summary of the results suggests there is complex relationship of endothelial dysfunction and smooth contraction in these vascular preparations that is related to the form of the nanomaterial administered and the stage of pregnancy. These variables may impact maternal, fetal or neonatal tissues narrowing their homeostatic responses and contributing to life stage vulnerabilities.

Alterations in placental vascular function and the fetal epigenome after maternal nanomaterial inhalation during gestation.

Timothy R. Nurkiewicz, West Virginia University, Morgantown, WV.

Engineered nanomaterials (ENM) are firmly established in diverse human activities from foods, and surface coatings, to biomedical applications. The scope of these activities increases the potential for ENM exposures that may result from intentional or unintentional introduction to the body. We and others have consistently observed that such exposures (typically via the lung) negatively impact cardiovascular development and function through both direct and indirect mechanisms. We have reported that maternal ENM inhalation (nano-titanium dioxide, count mode aerodynamic diameter ~150 nm, 10–12 mg/m³, 4–6 hrs/day) during pregnancy (days 6–20) impairs uterine microvascular function and creates a hostile gestational environment. The impact of this toxicity on placental vascular function remains poorly studied. Therefore, we developed the isolated, perfused placenta technique to assess fetal blood flow impairments. Recent studies have also revealed that the fetal transcriptome and epigenome are significantly altered. In addition to cardiovascular dysregulation, markers of immune adaptation, organismal growth, renal and hepatic function were altered. Collectively, these microvascular consequences impact cardiovascular dysfunctions that persist into adulthood.

Fetal Cardiac Function and related health parameters are altered by maternal exposure to ambient particulate matter during specific gestational exposure windows in a mouse model.

Judith T. Zelikoff, New York University School of Medicine, Tuxedo, NY.

Over the past decade, research has shown a causal link between air pollution exposure and cardiovascular disease [20, 21]. Ambient air pollution is a heterogeneous mixture of gases and particles, with fine-sized particulate matter <2.5 µm (PM_{2.5}) possibly posing the greatest threat to cardiovascular health [22]. The composition of PM varies greatly from organic to inorganic components and is classified by size. The major components of PM are transition metals, ions (sulfate, nitrate), organic compound, stable radicals of carbonaceous material, minerals, reactive gases, and materials of biologic origin [23]. Data from toxicological studies demonstrate that PM has several mechanisms of action that result in adverse cellular

effects, such as oxidative stress, damaging proteins, DNA and lipids, mutagenicity, and release proinflammatory mediators.

Epidemiologic research shows a causal link between exposure to fine-sized ambient particulate matter (PM_{2.5}) and cardiovascular disease in adults. However, effects of inhaled PM_{2.5} during pregnancy on fetal cardiovascular parameters are relatively unknown. The goal of this study was to determine if maternal PM exposure during different gestational periods affects fetal growth and cardiac development and function. Timed-pregnant mice were exposed (6hr/d) to either particle-free filtered air (FA; 4 µg/m³) or concentrated ambient PM (CAPs; average = 113µg PM_{2.5}/m³) collected from Tuxedo, NY. Mice were exposed either throughout gestation (GD 0.5–16.5) or only during fetal organogenesis, particularly that gestational window associated with cardiac development (GD6.5–14.5). At GD 12.5, cardiac fractional area change (FAC), intra-cardiac, and umbilical cord Doppler waveforms, were measured in all treatment groups by ultrasound-biomicroscopy (UBM). Dams exposed throughout gestation were euthanized on GD17.5, CRL was measured and placentas were collected for stereology; at PND21 offspring body weight was assessed and hearts collected for stereology. Building on data that revealed PM-induced decreases in fetal FAC and crown-to-rump length on GD12.5 and reduced offspring BW on GD17.5 and PND21, more recent studies demonstrate that maternal CAPs exposure (compared to controls): prolongs fetal isovolumic contraction time; decreases umbilical artery peak velocity; and alters labyrinth zone morphology of GD17.5 placentas. Cardiac dysfunction observed in the fetus persisted until 3-wk-of-age and at post-natal day 21 cardiac stereology revealed a 12% increase in left ventricle volume fraction, a 38% decrease in left atrium total volume and a 30% increase in cardiomyocyte volume in PM-exposed offspring compared to control. Overall, these findings suggest a developmental origin for PM-induced cardiovascular disease in adults by, as of yet, unknown mechanisms. Moreover, that effects on cardiac function and related fetal/placental parameters are dependent upon the gestational window of PM exposure.

Cardiovascular risk factors following fetal and neonatal exposure to nicotine.

Alison Holloway, McMaster University, Hamilton, ON, Canada.

Despite evidence that maternal smoking is associated with numerous adverse obstetrical and neonatal outcomes, recent data reports that 10–35% of women worldwide still smoke during pregnancy, with prevalence rates as high as 59% reported in some subpopulations [24, 25]. Maternal smoking represents a significant modifiable risk factor for the development of adverse cardio-metabolic disturbances in the offspring as it has been consistently linked to an adverse cardiovascular risk profile in the offspring [26–29]. Although smoking cessation is beneficial for both the woman and fetus, for many women, nicotine addiction remains a significant barrier to smoking cessation [30, 31]. Therefore, nicotine replacement therapy (NRT; i.e. nicotine containing gum and transdermal patches) has been widely developed as a pharmacotherapy of smoking cessation, and is considered to be of benefit for pregnant women who are highly dependent and have been unable to quit smoking by other means. However, the long-term cardiovascular health implications of nicotine exposure during early life have not been fully elucidated. Our group and others have developed animal models to examine the consequences of early life exposures to nicotine on postnatal cardiometabolic

outcomes. These studies have shown that perinatal exposure to nicotine resulted in cardiac dysfunction and significantly higher blood pressure in association with increases in adiposity, hypertriglyceridemia and insulin resistance [32–36].

Perinatal nicotine exposure results in a number of adverse cardiac outcomes encompassing a wide range of functional (e.g. increased blood pressure and heart rate, decreased ejection volume) and structural (e.g thickened septum, ventricular-arterial stiffening and increased collagen content) changes [36, 37]. Studies have suggested that some of the functional deficits may be explained, in part, due to increased myocardial fibrosis, mitochondrial defects and/or impaired expression of tissue inhibitor of metalloproteinase-4, a critical matrix metalloproteinase for cardiac contractility and health [38, 39]. Taken together these studies demonstrate that nicotine exposure can have profound effects on cardiac remodeling in postnatal life and result in significant functional deficits.

A common feature of early-life exposure to nicotine is increased adiposity and dyslipidemia in postnatal life, a well-established risk factor for cardiovascular disease. Interestingly this observation has been reported in a wide range of studies over a wide range of doses and exposure windows (reviewed in [34]). Molecular analyses have identified that the observed nicotine-induced dyslipidemia results from a combination of adipose tissue dysfunction [40, 41] and increased *de novo* hepatic lipid synthesis [33]. Our group has demonstrated that fetal and neonatal exposure to nicotine alters the epigenetic regulation of the Liver X Receptor (LXR α), a nuclear receptor that increases the expression of critical enzymes (e.g. FAS, ACC α) involved in triglyceride synthesis [33]. Importantly, increased hepatic lipid accumulation is associated with insulin resistance, another cardiovascular disease risk factor [42].

Taken together these results suggest that maternal nicotine exposure may have long-lasting impacts on the cardiovascular health of the offspring. This is particularly worrisome given that e-cigarette use during pregnancy has been rapidly increasing [43] and that the use of e-cigarettes during pregnancy is highly likely to result in nicotine exposure for the fetus.

Adverse Outcome Pathway (AOP) framework for embryonic vascular disruption and developmental defects.

Thomas B. Knudsen, US EPA, Research Triangle Park, NC.

The unique vulnerability of fetuses, infants, and children has a critical role in setting health and environmental policy [44]. Traditional animal-based methods for assessing prenatal developmental toxicity (e.g. OECD TG 414) in litters of pregnant rats / rabbits exposed during organogenesis are resource-intensive [45]. Furthermore, the traditional paradigm does not scale to testing the large number of environmental chemicals in the human exposome [46]. High-throughput screening (HTS) assays that harness data on many chemicals (e.g. ToxCast/Tox21) now provides an opportunity for profiling the *in vitro* bioactivity of chemical-biological interactions that may disrupt critical developmental processes such as blood vessel formation (e.g. vasculogenesis and angiogenesis). Challenges remain to demonstrate the predictive value of new approach methods for developmental toxicity and

defining how risk assessors can apply the new HTS data streams to hazard identification and regulatory decisions.

Considerable mechanistic data exists in the biomedical literature with regards to establishing an integrated strategy using systems toxicology to essentially “*decode the blueprint of active substances*” [47] that interact with the developing vasculature to invoke a potential developmental hazard [6]. The Adverse Outcome Pathway (AOP) framework is an approach to compile the relevant information and organize it in way that is useful for regulatory toxicology [48]. An AOP points to a biological perturbation leading to a specific adverse outcome and outline one potential route from a molecular initiating event to an adverse outcome. The AOP knowledgebase maintained at the Organisation for Economic Cooperation and Development (OECD) provides a public resource for the growing compendium of AOPs with key events that are chemically agnostic but can be integrated with mode-of-action information for specific chemicals (if such information is known). [<https://aopkb.oecd.org/>]. Dr. Knudsen presented progress from an interdisciplinary team of collaborators working to build and test an AOP with OECD status (Aop:43), entitled “Disruption of VEGFR Signaling Leading to Developmental Defects”.

The flow of information in Aop:43 starts with perturbation of the master switch for angiogenic sprouting (activation of the VEGFR2 receptor) during early development. The molecular initiating event (MIE) of VEGFR2 inhibition may be invoked via altered VEGFA (production, mobility, liganding) or direct effects on the receptor itself (density, function, transduction). How we think this might lead to adverse outcomes such as a birth defects entails downstream key events on endothelial tip cells (exploratory behavior, cell migration) and endothelial stalk cells (cell proliferation, apoptosis). Interactions with other cells (stromal cells, macrophages), extracellular matrix (ECM) and micro-physiology (hemodynamics, metabolism) are potential key events in an AOP network that ultimately determines adverse outcomes by anatomical region, organ system, gestational stage and state of the embryo, fetus or placenta. Collaborators are working with diverse empirical models such as engineered human cell-based assays for angiogenic sprouting [49], vascular toxicity screening and stem cell expansion during endothelial network formation [50], transgenic reporter zebrafish models to assess immediate and long-term consequences of embryonic vascular disruption [51, 52] and their translation into tiered-testing strategies using diverse platforms including mammalian embryo culture [53, 54]. These *in vitro* assays support AOP elucidation and *in silico* models for predictive toxicology and computer simulation to quantitatively evaluate a chemical’s potential to impact blood vessel development.

Subsequent mapping HTS signatures for predicted vascular disrupting chemicals (pVDCs) in ToxCast to an AOP framework for embryonic vascular disruption (Aop:43) enables a rank order potency for predicted vascular disruption across thousands of chemicals. In this regard, virtual tissue models have become increasingly important in simulating exposures and toxicities thereby translating *in vitro* predictions into an *in vivo* biological context. For example, a computational Agent-Based Model has been constructed for angiogenesis based on extant knowledge of cellular dynamics. This heuristic approach reconstructs multicellular dynamics from the bottom-up, cell-by-cell and interaction-by-interaction and was shown to accurately predict the quantitative impairment of *in vitro* blood vessel formation solely

based on HTS data from ToxCast [55]. Computer simulation of mammalian embryology made it possible to recapitulate a morphogenetic series of events, and this is used both analytically to understand and theoretically to predict developmental toxicity.

CONCLUSIONS

Our speakers all addressed the importance of maternal and *in utero* exposures in cardiovascular health; however, the overwhelming conclusion is that we still have much to learn and understand in this area of research. Evidence for correlations between maternal particulate matter exposures and metabolic dysregulation resulting in obesity are currently being evaluated and impact the overall population of the world. New chemicals and additives are constantly being developed and the toxicities of these xenobiotics alone or in combination with other pollutants need to be assessed in the context of fetal exposures. Nanoparticles have gained widespread usage in pharmaceuticals and cosmetics but the potential harm remains underexplored. Even though we have made significant strides in understanding toxicities and modeling many of the newer compounds or mixtures, much more is needed to adequately protect future generations. An overall outcome of the symposium was the continued need for research to elucidate the molecular pathways and cellular processes underlying the prenatal risks for cardiovascular disease and related outcomes associated with xenobiotic exposure(s) in women of reproductive potential and during pregnancy (and lactation). The organizers would like to thank the authors for their time and effort both as speakers for the SOT symposium and their efforts in compiling this symposium report. We would also like to acknowledge support from the Cardiovascular Toxicology and Reproductive and Developmental Toxicology specialty sections, along with the Women in Toxicology special interest group for this symposium.

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