NCS Formative Research Template for OIRA Clearance

TO BE COMPLETED BY STUDY CENTER:

LOI #: LOI3-RT-01-N
Title of Formative Research: GC/MS methods to determine environmental factors on fetal and newborn gene expression
Participating Institutions: **Michigan Alliance for the National Children's Study (MANCS) Study Center**
Recruitment Study Arms:
SME: **Carol Kasten**
COTR: **Eric Lorenzo**

Purpose of the Study: The goal of this project is to determine if a combination of gas chromatography / mass spectrometry (GC/MS) and high-throughput microassay gene technology can be used to establish whether exposure of the human fetus to phthalates alters androgen-receptor gene expression in the newborn.

Benefit to NCS Vanguard or Main Study: Phthalates have been detected in medicines, nutritional supplements, emulsifying agents, adhesives, agricultural products, building materials, personal-care products, detergents, and packaging. More importantly, for the NCS, phthalates are found in children's toys and alimentation products. Diet is believed to be a common source of some of the more widely-used phthalates, with foods such as milk, butter, and meats being major contributors. However, absorption through the skin and via inhalation of low-molecular-weight phthalates may occur. Some reports in the literature have indicated that endocrine disruptors, i.e. phthalates, can have an effect on normal bone growth and the ratio lengths of specific fingers. In this regard, this study may provide important insights.

Study Design: Pregnant women who plan to deliver at Hutzel Women's Hospital will be recruited in the University Health Center Prenatal Clinic. A study staff member (usually Dr. Meghan Dwaihy or Dr. William Lyman as Dr. Dwaihy's back-up) will be in the labor and delivery service area when the potential study participant arrives (note: Providers and hospital/clinic staff will not be involved). After written consent is obtained, each woman will be asked to provide a urine sample (into a sample container). It is important to obtain the maternal urine before the time of labor, not only to reduce stress on the mother, but also because IV tubes and other instruments used at the time of delivery may contain phthalates that could affect the specimen. The urine sample will be stored in a lab at Children's Hospital of Michigan, and later sent for analysis of phthalate concentration.

Once the participant gives birth, study staff will be in the newborn nursery and collect the diapers after the male baby has urinated. These diapers will be transported to the laboratory where they will be centrifuged and newborn urine will be collected. That sample will be stored and sent for analysis of phthalate concentration. At the time of the infant urine collection, the study staff member will photocopy the baby's hand in the newborn nursery using a dedicated portable photocopy device. Alternatively, the baby's hand will be photographed using a smartphone, photographic camera, or similar device. We will use the photocopy of the baby's hand to measure and record the finger length ratio. We will also measure anogenital distance of the infant, as previous studies have shown that phthalate exposure can affect these measurements. If the mother chooses to have the male baby circumcised by Dr. Cepeda (study staff member as well as hospital attending), a second study staff member will be at his side to collect the foreskin after the circumcision is complete and place it in liquid nitrogen. This foreskin sample will then be analyzed for androgen gene expression using real-time PCR techniques combined with functional genomic analysis. Note: All specimens, urine foreskin, and photocopy/photograph, will be transported to the laboratory in Children's Hospital of Michigan, which is contiguous with Hutzel Women's Hospital (birthing hospital). Only Dr. Cepeda or other hospital-authorized personnel will touch the baby and take the samples and measurements.

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Summary of involvement
Involvement of the mother consists of one maternal urine sample prior to delivery. Involvement of the infant consists of one urine sample, two physical measurements (finger length ratio and anogenital distance), and one foreskin sample (if male infant is circumcised) after birth.

Target Respondents: A convenience sample of 400 pregnant women from the University Health Center Prenatal Clinic will be selected. Respondents will include non-NCS participants. Target respondents include the 400 pregnant women and their 400 male infants, once delivered. Women who have had a previous baby with severe endocrine abnormalities will be excluded. Women who know the gender of their baby to be female will be excluded.

Sample Size Calculation:

Between 500 and 600 total women will need to be approached to achieve the desired sample of 400 women. This estimate is predicated on previous other study recruitment experience both at Hutzel Women's Hospital and our experience for multiple similar-type studies conducted. The estimate is based on assumptions about the willingness of women to be study participants and potential attrition of women previously consented but who change their mind. We think it is also important to note that, at Hutzel Hospital, there are between 4,000 and 5,000 births per year. That equates to between, approximately, 80 to 100 births per week or, once again approximately, 10 to 15 births per day. As we will be in the Labor and Delivery area 5 days per week for 8 to 10 hours per day, we will have access to, between, 5 and 10 potential study participants per day. Calculating an 80% participation willingness, we will enroll between 25 and 40 women per week. Thus, we will approach the, up to 500 women, in 5 months – maximum time.

Thus, twenty-five matched pairs of samples in each of the low versus high concentration phthalate groups will be obtained. We have set 400 maternal-newborn dyads as the upper limit because, statistically, we need to dichotomize the study population into high and low phthalate concentration groups. As we do not/cannot know a priori the concentrations of phthalate in their urines, we may be able to recruit fewer than 400 matched dyads. We will only know this after we start collecting and analyzing samples.

This balanced design, paired with a continuously scaled dependent variable, will provide more precision and power to conduct the analyses of percent gene expression between phthalate study groups. Phthalate metabolite distributions, if skewed, will be $\log_{10}$ transformed before groupings. We hypothesize that the mean percent change in gene expression at high levels of exposure will be approximately 55%±5% in comparison to 25%±5% in low levels of exposure. This 30% mean difference, with a balanced design of 25 matched pairs in each study group, will provide power exceeding 99.9% to yield a statistically significant result, with a 95% confidence interval of 27.18 to 32.82%.

Method of Recruiting: Pregnant women who plan to deliver at Hutzel Women's Hospital will be recruited in the University Health Center Prenatal Clinic. Study staff will review the medical record of women who arrive in the Labor and Delivery service at Hutzel Women's Hospital to give birth. Study staff will determine if the sex of their fetus is known. Study staff will approach women who either know the gender of their unborn baby to be male, or those who have chosen not to reveal the baby's gender.

When a suitable candidate is identified, a hospital staff member will ask the woman if she is willing to be approached by a study staff member, i.e. Dr. Dwaihy, to inform the woman of the study and solicit informed consent to participate (see attached script). Study staff will inform that we are conducting a study that examines the potential for factors that negatively affect their male infants. Study staff will then ask if they are willing to hear about the study. If the woman agrees to speak with the study staff member, she will receive a copy of the consent form for her use. The consent form will be read and discussed with the potential participant. They will then be asked if they and their male infants would like to potentially participate. If the mother says yes, then informed consent will be administered.

Women who know the gender of their baby to be female will not be approached.

***Confidentiality:** Study Centers must abide by the terms of their Data Use Agreement, which should reference all formative research efforts involving the collection or management of NCS restricted-use data. The participating Study Center has an approved Data Use Agreement and Security Plan.

Each paired set of samples (maternal and newborn urine plus the foreskin and handprint) will receive a unique identifier that is matched and recorded for this sample set. However, this sample set will not be linked to any hospital record or other source that could link it to a particular patient. Hence, these samples will all be de-identified.

All original signed informed consent will be kept in the principal investigator's office. The file cabinet will be locked at all times.

***IRB Approval:** Local IRB clearance for this activity has been obtained by the participating Study Center. Please see the attached IRB approval letter and the IRB continuation approval letter.

Incentives: Participants will receive a $25 gift card as appreciation for their time at consent.

Sensitive Questions: We will not ask sensitive questions as a component of this study.

Proposed Project Schedule: We will begin this project upon receipt of all regulatory approvals.

Data Collection Burden:

Estimates of Annual Hour Burden – Formative Research Project: LOI3-RT-01-N, "GC/MS methods to determine environmental factors on fetal and newborn gene expression"

Data Collection Activity	Type of Respondent	Estimated Number of Respondents	Estimated Number of Responses per Respondent	Average Burden Per Response (in hours)	Estimated Total Annual Burden Hours
Recruitment Script	Mother	500	1	2/60	17
Urine sample	Mother	400	1	5/60	33
Measurements, urine sample, foreskin sample	Infant	400	1	15/60	100
TOTAL		900			150

* To be completed before project proposal is submitted for OIRA clearance.

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Annualized Cost to Respondents – Formative Research Project: LOI3-RT-01-N, "GC/MS methods to determine environmental factors on fetal and newborn gene expression"

Data Collection Activity	Type of Respondent	Estimated Total Annual Burden Hours	Hourly Wage Rate	Respondent Cost
Recruitment Script	Mother	17	$10.00	$167
Urine sample	Mother	33	$10.00	$333
Measurements, urine sample, foreskin sample	Infant	100	$10.00*	$1,000
TOTAL		150		$1,500

*The allotted hourly wage rate accounts for the mother's time associated with the data collection activity.

☒ **Please check here after ensuring that all calculations have been verified**

Estimated Costs: Staff Hours: 300 hours
Supervisor Hours: 75 hours

Attachments: IRB Approval Memo, IRB Continuation Approval Memo, Consent Form, Recruitment Script

X Please check here after ensuring that the OMB #: 0925-0647 and Expiration Date: 1/31/2015 have been inserted as first-page headers on each proposed instrument.

X Please check here after ensuring that the following OMB burden statement has been inserted as a first-page footer on each proposed instrument.

Public reporting burden for this collection of information is estimated to average X minutes per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to: NIH, Project Clearance Branch, 6705 Rockledge Drive, MSC 7974, Bethesda, MD 20892-7974, ATTN: PRA (0925-0647). Do not return the completed form to this address.

Appendix 1. Maximum NCS Incentives, by Study Activity and Impact on Participants (Approved by OMB 1/5/12)

Data Collection Activity Characteristics	Initial NCS Vanguard Study	NCS Recruitment Substudy and Formative Research		
		Phase 1	Phase 2	Formative Research
Time for encounter	3 hours	0.5 to 1 hour	0.5 to 1 hour	0.5 to 1 hour
Sensitivity of questions	Sensitive, including sexual activity	Few sensitive questions	Few sensitive questions	Few sensitive questions
Physical measures	Yes	No	No	Yes*
Environmental specimens	Yes	No	Yes	Yes*
Biospecimens	Yes	No	Yes	Yes*
Participant observation	Yes	No	No	No
Monetary incentive, per visit	$100	$25	$25 for the group of study questionnaires, plus $25, in total, for any bio-specimens collected during a contact and, where appropriate for environmental specimens	$25, in total, for any bio-specimens collected during a contact. For questionnaires, or any environmental specimens – up to $25 when deemed necessary
Non-monetary incentives (tote bags, post its, key chains, etc.)	In addition to the monetary incentive, non-monetary incentives valued at $25 or less may be offered to participants	As an alternative to the monetary incentive, NCS logo gifts valued at $25 or less may be offered to the participants in lieu of cash or local incentives not exceeding $25 in value and deemed non-coercive by local IRBs	In addition to the monetary incentive, NCS logo gifts valued at $25 or less may be offered to the participants if these are deemed acceptable by local IRBs	Instead of monetary incentives, NCS logo gifts valued at $25 or less may be offered to the participants if these are deemed acceptable by local IRBs