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Connor Valenzuela 4/25/2018

Prenatal Phthalate Exposure and Performance on the Neonatal Behavioral Assessment Scale in a Thai, Agricultural Birth Cohort

By

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Abstract

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By Connor Valenzuela

Background: Phthalates are endocrine disrupting chemicals (EDC) derived from phthalic acid and found in human tissues either as phthalate diesters or their monoester metabolites. Studies have demonstrated that phthalate exposure is associated with a number of adverse health outcomes, particularly in highly susceptible populations including pregnant women and children. Of interest is the effect that high phthalate exposure can have on neurodevelopment during critical development periods (i.e. infancy or prenatal exposure). Our objective was to evaluate prenatal environmental exposure to phthalates by measuring their monoester and oxidative metabolites in longitudinally collected urine samples and characterize their relation to infant neurodevelopment in a Thai farmworker population.

Methods: We performed analysis of de-identified pilot cohort data using linear regression models to evaluate the effect of prenatal phthalate exposure on infant neurodevelopment using the Brazelton Neonatal Behavioral Assessment Scale (NBAS) among 10 Thai agricultural workers.

Results: In our limited cohort, we found inconclusive effects of prenatal phthalate exposure on infant development. Using linear regression, we observed a single significant association between average high molecular weight (HMW) phthalate exposure and decreased range of state average score ($p=0.0346$, $R^2 = 0.5683$). The results do not agree with current research, as no associations were found with low molecular weight (LMW) or high molecular weight (HMW) phthalates and any other domain average scores.

Conclusions: No conclusive protective or harmful effect of either LMW or HMW phthalates on NBAS outcomes was found in our analyses. Additional research is needed with larger samples a more power to further examine these relationships and investigate both mechanisms by which phthalates can cause deficits in neurobehavioral assessment and which trimester of exposure has the greatest impact on neurodevelopment.

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Table of Contents

1 Introduction

Phthalates are endocrine disrupting chemicals (EDC) derived from phthalic acid and found in human tissues either as phthalate diesters or their monoester metabolites (**Figure 1, Figure 2**) (1). They were first introduced in the 1920's and are used in a variety of products including personal care products, plastics, flooring and medical tubing (2,3). Every year, approximately 4.99 million metric tons of phthalates are produced worldwide, making them a prevalent and harmful environmental exposure (2).

Studies have demonstrated that phthalate exposure is associated with a number of health effects in human populations including: changes in insulin resistance, adverse effects on sperm and semen quality, renal and hepatic effects, varying effects on infant and toddler physical development, as well as neurodevelopmental effects (4,5). Recently, focus of these adverse effects has shifted to highly susceptible populations, including pregnant women and children, as detectable levels of phthalate can be found in amniotic fluid, demonstrating passage from mother to fetus (2,6). Through a number of potential biological pathways demonstrated in rodent and human studies, such as reduction of circulation T4 Thyroid hormone and other anti-androgenic effects, phthalates can have ranging effects on the development of the central nervous system, especially during critical developmental periods (2,6,3,7).

Epidemiological studies on phthalate exposure and neurodevelopment have had varied results, but generally suggest that higher prenatal and childhood exposure to both low molecular weight (LMW) and high molecular weight (HMW) phthalates is associated with a broad array of outcomes on several different neurodevelopmental measurement scales (2). Studies indicate deficits in masculine composite scores, reduction in normal reflexes and response to stimuli, withdrawn social behaviors and learning differences, as well as reduction in executive function (2,4,6,8–10). Exposure windows and outcome measurements are widely varied in previous studies and few have examined the effects of longitudinal prenatal phthalate exposure on

newborn neurodevelopment assessed by Brazelton Neonatal Behavioral Assessment Scale (NBAS) (2,4).

Our objective was to evaluate prenatal environmental exposure to phthalates by measuring their monoester and oxidative metabolites in longitudinally collected urine samples and characterize their relation to infant neurodevelopment in a Thai pregnant farmworker population. We expect that higher average maternal urinary phthalate metabolite levels are associated with lower newborn scores in one or more domains assessed on the Brazelton Neonatal Behavioral Assessment Scale (NBAS).

2 Materials and Methods

2.1 SAWASEE Pilot Cohort

De-identified pilot cohort data from the **S**tudy of **A**sian **W**omen **A**nd their off**S**pring's **D**evelopment and **E**nvironmental **E**xposures (SAWASDEE) pilot study was utilized to examine the relationship between prenatal phthalate exposure and neurodevelopment. The SAWASDEE study was conducted in Fang District of Chiang Mai Province in northern Thailand. Pregnant women (n=56) who had their first prenatal clinic visit at Fang Hospital were recruited in the pilot cohort from March 2011 to February 2012 and were followed longitudinally until birth. In order to be eligible for the study, the pregnant women were required to fit the following inclusion criteria: (11)

1) Aged 18-40 years
2) Pregnancy at enrollment and in first trimester or early second trimester (16 weeks' gestation or less)
3) Occupation as farmworker
4) No serious medical problems in the mother including hypertension, diabetes, thyroid disease, or HIV
5) Possession of Thai identification card permitting hospital and antenatal clinic access with at least one monthly prenatal visit allowed
6) Spoke Thai as primary language at home
7) Current Residence in their Fang district for at least 6 months including planned residence at least 1 month after delivery

The pilot birth cohort was followed longitudinally through the pregnancy until 3 days post-partum. All study protocols were reviewed and approved by the Ethic Boards of Chiang Mai University, the Thai Ministry of Health and the Institutional Review Board of Emory University.

2.2 Exposure Assessment : Phthalate – Maternal Urine Samples

Phthalate metabolite levels are measured in blood, breast milk, and meconium; however, urine is the most common, reliable, and non-invasive means of measuring phthalate metabolites in human populations (2). Maternal urine samples were collected monthly from each mother and analyses were performed for ten mothers to measure exposure to phthalates by measuring eight metabolites: monoethylphthalate (MEP), mono-n-butylphthalate (MBP), mono-isobutylphthalate (MiBP), monobenzylbutyl phthalate (MBzP), mono (2-ethylhexyl) phthalate (MEHP), mono (2-ethyl-5-oxohexyl) phthalate (MEOHP), mono (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), and mono (2-ethyl-5-carboxypentyl) phthalate (MECPP). The phthalate metabolites were measured in 500 μL aliquots of urine by spiking with isotopically labeled internal standards and subjecting the samples to an enzyme hydrolysis to liberate glucuronide-bound metabolites. The hydrolysates were extracted using solid phase extraction and were then concentrated for analysis using liquid chromatography-tandem mass spectrometry with isotope dilution calibration. Quality control materials, blanks, NIST standard reference materials and calibrants were analyzed concurrently with the samples. These phthalate metabolites were summed as low molecular weight phthalate metabolites (LMW, <250 dalton; MEP, MBP, MiBP) or high molecular weight phthalate metabolites (HMW, >250 dalton; MBzP, MEHP, MEOHP, MEHHP, MECPP) for analyses according to similar biologic activity and sources (Table 2) (4,12) .

2.3 NBAS Outcomes

The NBAS was administered to 56 infants 0-3 days after birth. The assessment consisted of 28 behavioral components scored on a 9-point scale and 18 reflex components scored on a 4-point scale. This system is most commonly analyzed in the context of seven clusters or domains based on the natural organization of behavior and skill development in newborns (13). The seven

domains, habituation, orientation, motor performance, range of state, regulation of state, autonomic stability, and abnormal reflexes, each contain three to six testing items and have a single summary score for each domain (Table 9) (4,13–15). It is suggested that poor outcomes on certain domains, such as motor, state regulation, and orientation in neonates could assess risk of behavioral and neurodevelopmental issues later in life (16). In our analyses, we examined the effect of prenatal phthalate exposure on all domains.

2.4 Statistical Analyses

Statistical analyses were performed using SAS, v. 9.4 (SAS Institute, Inc., Car, North Carolina). Univariate analyses were performed for descriptive statistics of cohort, exposure, and outcomes. Multilinear regression models were fit for 3 of the NBAS clusters (Orientation, Motor, Regulation of State) for each time point (1^{st} trimester, 2^{nd} trimester, 3^{rd} trimester) as well as Average LMW and HMW phthalates. We fit both logged as well as unlogged LMW phthalate models as sometimes logged fit better than unlogged and vice versa. For HMW phthalate models, the unlogged exposure was always a better fit, and only unlogged models were included. Due to limited data from 1^{st} trimester, averages of low and high molecular weight phthalates over all trimesters were considered for final models.

Covariates considered for the full model included maternal education (categorical), maternal income (categorical), ethnicity (categorical), maternal age (categorical), as well as birth weight (continuous). Gestational age was excluded for consideration due to correlation with birth weight. Covariate selection for consideration was based on their associations with NBAS outcomes (4).

3 Results

3.1 Demographic Characteristics

Of the initial 59 participants in the study, 56 were retained through follow up, and one was excluded due to a spontaneous abortion (95% retention). Among those participants, ten had measurements of phthalate metabolites from maternal urine samples and were used in the final analysis for this study.

Table 1 demonstrates the demographic characteristics of the sub-population (n=10) used for this study. The mean maternal age for this sub-population at enrollment was 26.4 years, with an even split in ethnicity between Thai and Thai Yai, and in country of birth as well, with half born in Thailand and half in Burma. The majority were living as married (80%), were enrolled in their second trimester (60%), and half had some high school education with no diploma (50%), with the remainder only attending primary school (30%) or never attended school (20%).

3.2 Exposure

Tables 3-5 show the distribution of average LMW, log LMW (natural log) and HMW phthalate metabolites, respectively, measured from urine samples. Urine samples were taken roughly monthly from enrollment with increased frequency of sampling toward the end of pregnancy, resulting in more measurements overall for third trimester. Due to this variation and because of limited data for first trimester values (n=4 for first trimester enrollment), overall phthalate averages were used for final analyses. Means for LMW phthalates were higher for first and second trimester than for third, with greater standard deviation and range than HMW phthalates. Natural log transforming LMW values helped to normalize the results and reduce the size of standard deviation and range. Trends for phthalate exposure by trimester remained the same as unlogged LMW. Means for HMW phthalates steadily increased by trimester and had less

variation overall than LMW. LMW and HMW phthalates were kept separate for analyses as indicated in the literature (4,6,17).

3.3 Outcome

Table 5 shows the distribution of average scores from testing items in a cluster across all seven NBAS clusters. NBAS outcomes were treated as continuous variables. It should be of note that each domain has a different maximum score and a different number of behavioral items in each, with higher scores typically indicative of better development aside from the abnormal reflex domain in which a lower score represents a lower number of abnormal reflexes. (**See Table 17**).

3.4 Linear Regression Models

To assess covariates for final models, potential covariates were assessed by their correlation with the exposures (average LMW and HMW phthalates) as well as correlation with other covariates. Birth weight had the highest correlation with LMW phthalates (Correlation Coeff. = 0.4436, p=1991) and was tied with Age for highest correlation with HMW phthalates (Birth weight: Correlation Coeff. =.4523, p=0.1894 ; Age: Correlation Coeff. = 0.4595, p=0.1815). Due to small sample size (n=10) we attempted to limit models to a single covariate, and because birth weight and age are relatively well correlated (Correlation Coeff. = 0.3842), birth weight was established as the sole covariate for both LMW phthalate and HMW phthalate models. Including birth weight in the model also controls for potential confounding and we included correlations between exposures and outcomes to demonstrate the effect on the relationship between phthalate exposure and NBAS scores when removing birth weight from our models (See Table 9). Results of linear regression models for all domains by average LMW and HMW phthalates are presented with regression coefficients, standard error, studentized t-test values, p-value, partial correlation coefficients, and R^2 values for each model **(Table 10-16).** We

observed a single significant association between increased average HMW phthalate exposure and decreased range of state average score ($p=0.0346$, $R^2 = 0.5683$). No associations between models with other domain average scores and either LMW phthalate or HMW phthalate were statistically significant.

4 Discussion

In our study, we find an inverse relationship in the association between higher HMW phthalate exposure and a decrease in range of state average score. From our model, we find a harmful effect of HMW phthalates on neurodevelopmental outcome, such that for every 0.3 ng/ mL increase in detectable HMW phthalate in maternal urine, we find a 1unit score decrease in infant range of state average score. Range of state is a measure of infant arousal and emotional lability that includes the test items of peak of excitement, rapidity of build-up, irritability and lability of states (18) and an association between phthalate exposure and effect on this cluster has not been well explored. While past studies have typically focused on orientation, motor, and state regulation domains (2,4,14), we did not find any significant associations in these domains, potentially as a result of small sample size. In general, our models predicted very minute, statistically insignificant harmful effects of phthalates on neurodevelopment, however the few protective effects found in our models are likely attributed to lack of predictive power of our models.

We included birth weight as a potential confounder as it is positively associated with both phthalates and with NBAS outcomes (See **Figure 3**). If birth weight is a confounder and removed from the model, it could bias the results either toward or away from the null, providing us with an inaccurate estimation of the association. See **Table 9** for correlations between exposure and outcome without birth weight included as a confounder. Additionally, there is the concern that birth weight could act as a potential intermediate (See **Figure 4**). The proposed mechanism would suggest that increased phthalate exposure could result in lower birth weight (19), which in turn could produce lower scores within NBAS domains (20,21). If birth weight is an intermediate, removing it from the model should show an unbiased estimate of the association between phthalate and NBAS outcomes, displaying a stronger association if one is truly present. At

present, we do not have sufficient data to determine whether birth weight is acting either as a confounder or an intermediate.

4.1 Limitations

Our study was a preliminary analysis from a pilot study, with a larger cohort (n=300) to come from the SAWASDEE population. The analysis of phthalate exposure among these ten subjects was meant to inform future longitudinal analysis and areas of study going forward around both phthalates and other environmental exposures in this population. Sample size for this portion of the study was small (n=10) leading us to believe that the predictive power of our models and the statistical significance of our associations would be limited. In assessing fourteen different relationships, we expected to find at least one significant association, yet cannot say with certainty the effect of prenatal phthalate exposure on infant neurodevelopment.

As the study focuses on farmworkers in Thailand who were exposed to organochlorine pesticides which have been shown to be associated with adverse birth outcomes (11) , there is also the potential of confounding via alternative environmental exposures. Future analyses on a larger cohort may investigate organochlorine pesticides as a potential confounder, and any relationship between phthalate and pesticide exposures.

5 Conclusions and Future Directions

In our analyses of a pilot study of Thai, agricultural women, we have found inconclusive effects of prenatal phthalate exposure on infant neurodevelopment. Neurobehavioral assessment clusters were affected differently by LMW and HMW phthalates, with no conclusive protective or harmful effect. Using linear regression, we observed a single significant association between increased average HMW phthalate exposure and decreased range of state average score. Current research suggests negative associations between increased LMW and HMW phthalates and

several domains, most notably orientation, motor, and regulation of state domains for which we found no significant associations. Additional research with larger samples is needed to further examine these relationships and investigate the mechanisms by which phthalates can cause deficits in neurobehavioral assessment. Defining the trimester of exposure with the greatest effect on neurobehavioral assessment should also be a concern for future study.

The next steps for this study are to examine a longitudinal data analysis approach for the larger main birth cohort. Additionally, because our results were limited for the first trimester (n=4 subjects), future study on this population should examine the effects of phthalate by trimester of exposure on neurodevelopment. For the main cohort, a larger population (n=300) will be examined for acute and chronic effects of environmental exposures, particularly pesticides, and will involve two different study cites in Thailand. Analyses of a larger population will help to mitigate the limitations of this analysis and provide more insight into the effect of prenatal phthalate exposure on neurodevelopment.

References

1. Council NR. Phthalates and Cumulative Risk Assessment: The Tasks Ahead. Washington, DC: The National Academies Press; 2008.(https://www.nap.edu/catalog/12528/phthalates-and-cumulative-risk-assessment-the-tasks-ahead)

2. Ejaredar M, Nyanza EC, Ten Eycke K, et al. Phthalate exposure and childrens neurodevelopment: A systematic review. *Environ. Res.* [electronic article]. 2015;142:51–60. (https://www-sciencedirect-com.proxy.library.emory.edu/science/article/pii/S0013935115001899). (Accessed April 20, 2018)

3. Boas M, Feldt-Rasmussen U, Skakkebaek NE, et al. Environmental chemicals and thyroid function. *Eur. J. Endocrinol.* [electronic article]. 2006;154(5):599–611. (http://www.ncbi.nlm.nih.gov/pubmed/16645005). (Accessed April 21, 2018)

4. Engel SM, Zhu C, Berkowitz GS, et al. Prenatal phthalate exposure and performance on the Neonatal Behavioral Assessment Scale in a multiethnic birth cohort. *Neurotoxicology*. 2009;30(4):522–528.

5. Hauser R, Calafat AM. Education: Phthalates and Human Health. *Occup. Environ. Med.* 62:806–818. (http://www.jstor.org.proxy.library.emory.edu/stable/27732628). (Accessed April 20, 2018)

6. Téllez-Rojo MM, Cantoral A, Cantonwine DE, et al. Prenatal urinary phthalate metabolites levels and neurodevelopment in children at two and three years of age. *Sci. Total Environ.* [electronic article]. 2013;461–462:386–390. (https://www-sciencedirect-com.proxy.library.emory.edu/science/article/pii/S0048969713005640). (Accessed April 21, 2018)

7. Borch J, Metzdorff SB, Vinggaard AM, et al. Mechanisms underlying the anti-androgenic effects of diethylhexyl phthalate in fetal rat testis. *Toxicology*. 2006;223(1–2):144–155.

8. Whyatt RM, Liu X, Rauh VA, et al. Maternal prenatal urinary phthalate metabolite concentrations and child mental, psychomotor, and behavioral development at 3 years of age. *Environ. Health Perspect.* 2012;120(2):290–295.

9. Yolton K, Xu Y, Strauss D, et al. Prenatal exposure to bisphenol A and phthalates and infant neurobehavior. *Neurotoxicol. Teratol.* 2011;33(5):558–566.

10. Factor-Litvak P, Insel B, Calafat AM, et al. Persistent associations between maternal prenatal exposure to phthalates on child IQ at age 7 years. *PLoS One*. 2014;9(12).

11. Naksen W, Prapamontol T, Mangklabruks A, et al. Associations of maternal organophosphate pesticide exposure and PON1 activity with birth outcomes in SAWASDEE birth cohort, Thailand. *Environ. Res.* [electronic article]. 2015;142:288–296. (http://www.sciencedirect.com/science/article/pii/S0013935115300116?via%3Dihub). (Accessed September 14, 2017)

12. Wolff MS, Teitelbaum SL, McGovern K, et al. Phthalate exposure and pubertal development in a longitudinal study of US girls. *Hum. Reprod.* [electronic article]. 2014;29(7):1558–1566. (https://academic.oup.com/humrep/article-lookup/doi/10.1093/humrep/deu081). (Accessed April 19, 2018)

13. Costa R, Figueiredo B, Tendais I, et al. Brazelton Neonatal Behavioral Assessment Scale: a psychometric study in a Portuguese sample. *Infant Behav. Dev.* [electronic article]. 2010;33(4):510–7. (http://www.ncbi.nlm.nih.gov/pubmed/20800286)

14. Engel SM, Berkowitz GS, Barr DB, et al. Prenatal Organophosphate Metabolite and Organochlorine Levels and Performance on the Brazelton Neonatal Behavioral

Assessment Scale in a Multiethnic Pregnancy Cohort. *Am. J. Epidemiol.* [electronic article]. 2007;165(12):1397–1404. (https://academic.oup.com/aje/article-lookup/doi/10.1093/aje/kwm029). (Accessed April 19, 2018)

15. Brazelton TB. Neonatal Behavioral Assessment Scale. (http://nidcap.org/wp-content/uploads/2013/12/Brazelton-1973-BNBAS.pdf). (Accessed April 19, 2018)

16. Ohgi S, Takahashi T, Nugent JK, et al. Neonatal Behavioral Characteristics and Later Behavioral Problems. *Clin. Pediatr. (Phila).* [electronic article]. 2003;42(8):679–686. (http://journals.sagepub.com/doi/10.1177/000992280304200803). (Accessed April 21, 2018)

17. Ejaredar M, Nyanza EC, Eycke K Ten, et al. Phthalate exposure and childrens neurodevelopment: A systematic review. *Environ. Res.* [electronic article]. 2015;142:51–60. (http://www.sciencedirect.com/science/article/pii/S0013935115001899?via%3Dihub). (Accessed September 14, 2017)

18. Lundqvist C, Sabel K-G. The Brazelton Neonatal Behavioral Assessment Scale Detects Differences Among Newborn Infants of Optimal Health. *J. Pediatr. Psychol.* [electronic article]. 2000;25(8):577–582. (https://academic.oup.com/jpepsy/article-lookup/doi/10.1093/jpepsy/25.8.577). (Accessed April 21, 2018)

19. Zhang Y, Lin L, Cao Y, et al. Phthalate levels and low birth weight: a nested case-control study of Chinese newborns. *J. Pediatr*. [electronic article]. 2009;155(4):500–4. (http://www.ncbi.nlm.nih.gov/pubmed/19555962). (Accessed April 21, 2018)

20. Als H, Tronick E, Adamson L, et al. The Behavior of the Full-term but Underweight Newborn Infant. *Dev. Med. Child Neurol.* [electronic article]. 2008;18(5):590–602. (http://doi.wiley.com/10.1111/j.1469-8749.1976.tb04205.x). (Accessed April 21, 2018)

21. Lester BM, Emory EK, Hoffman SL, et al. A Multivariate Study of the Effects of High-Risk Factors on Performance on the Brazelton Neonatal Assessment Scale. *Child Dev.* [electronic article]. 1976;47(2):515. (https://www.jstor.org/stable/1128811?origin=crossref). (Accessed April 21, 2018)

Table 1

Phthalate Study Cohort Characteristics (n=10)

Characteristic	n	%
Household Income		
1500 Baht or less	0	0
1501-3000 Baht	0	0
3001 - 6000 Baht	3	30
6001-9000 Baht	3	30
9001-12000 Baht	2	20
12000 + Baht	2	20
*1000 Baht ≈ 31 USD		
Maternal Education (Highest Attended Level)		
Never Attended School	2	20
Primary School	3	30
Junior High/ High School	0	0
High School - No Diploma	5	50
Ethnicity		
Thai	5	50
Thai Yai	5	50
Marriage Status		
Married	2	20
Living As Married	8	80
Country of Birth		
Thailand	5	50
Burma	5	50
Trimester of Enrollment		
1	4	40
2	6	60
Season of Enrollment		
Dry	0	0
Hot	10	100
Rainy	0	0
Gender (Child)		

Male	5	50
Female	5	50

Continuous Characteristics (n=10)	
Variable	Mean(SD)
Birthweight(g)	2,701 (405.15)
Height (cm)	50.1 (3.75)
Head Circumference (cm)	32.9 (1.85)
Gestational Age(Wks)	38.2 (1.03)
Maternal Age (Yr)	26.4 (4.70)
Maternal Creatinine	65.13 (27.46)

Table 2
Summary Statistics for Phthalate Concentration in Urine, Urine ng/mL

Phthalate Metabolite	n	Mean	Median	SD	IQR
Low-molecular weight(LMW)					
MEP	86	114.58	12.28	365.19	61.68
MBP	86	14.99	6.62	20.58	15.74
MiBP	73	5.90	2.80	8.14	6.42
High-molecular weight (HMW)					
MBzP	79	2.29	0.71	6.03	1.62
MEHP	74	5.35	2.41	13.89	3.29
MEOHP	86	5.73	4.08	4.97	5.49
MEHHP	86	7.76	4.92	7.23	8.50
MECPP	86	13.75	10.25	12.18	12.64

Monoethyl phthalate (**MEP**), mono-butyl phthalate (**MBP**), mono-isobutyl phthalate (**MiBP**), monobenzyl phthalate (**MBzP**), mono (2-ethylhexyl) phthalate (**MEHP**), mono (2-ethyl-5-oxohexyl) phthalate (**MEOHP**), mono (2-ethyl-5-hydroxyhexyl) phthalate (**MEHHP**), mono (2-ethyl-5-carboxypentyl) phthalate (**MECPP**)

Table 3
Summary Statistics for Low Molecular Weight (LMW) Phthalate Concentration in Urine, Urine ng/mL

Collection Time (Trimester)	n	Mean	Median	SD	Range	IQR
T1	4	70.66	38.82	89.50	196.10	117.38
T2	10	86.91	34.13	104.64	316.72	120.72
T3	10	17.54	16.09	12.99	32.69	25.28
Average	10	47.45	42.99	39.82	133.41	50.76

Table 4
Summary Statistics for Log Low Molecular Weight (LMW) Phthalate Concentration in Urine (**Natural Log Transformed**), Urine ng/mL

Collection Time (Trimester)	n	Mean	Median	SD	Range	IQR
T1	4	3.46	3.52	1.62	3.81	2.45
T2	10	3.69	3.45	1.40	4.08	2.29
T3	10	2.48	2.77	1.04	2.98	1.81
Average	10	3.46	3.76	1.05	3.33	1.35

Table 5
Summary Statistics for High Molecular Weight (HMW) Phthalate Concentration in Urine, Urine ng/mL

Collection Time (Trimester)	n	Mean	Median	SD	Range	IQR
T1	4	4.45	4.83	2.02	4.67	2.98
T2	10	7.35	8.61	3.32	8.79	6.01
T3	10	6.79	6.61	3.66	9.25	7.09
Average	10	6.76	7.49	3.08	7.84	6.74

Table 6
Summary Statistics - NBAS Outcomes

Cluster (Avg. Score)	n	Mean	Median	SD	Range	IQR
Habituation	10	7.33	7.25	0.31	1.00	0.50
Orientation	10	6.10	6.71	1.28	3.57	2.14
Motor	10	6.58	6.60	0.63	2.00	0.80
Range of State	10	4.42	4.25	1.10	3.75	1.50
Regulation of State	10	4.63	4.25	1.31	4.25	2.00
Autonomic Stability	10	2.97	3.00	0.19	0.67	0.00
Abnormal Reflexes	10	1.66	1.67	0.13	0.44	0.11

Table 7
Correlation Coefficients for Exposures with Covariates, (n=10)

	Avg LMW		Avg HMW	
	Correlation Coeff.	P value	Correlation Coeff.	P value
Birthweight	0.4436	0.1991	0.4523	0.1894
Education	-0.1381	0.7037	-0.1760	0.6268
Income	-0.2415	0.5014	-0.4310	0.2137
Age	0.1543	0.6704	0.4595	0.1815
Ethnicity	0.1313	0.7176	0.2268	0.5287

Table 8
Spearman Correlation Coefficients for Covariates, (n=10)

	Birthweight	Education	Income	Age	Ethnicity
Birthweight	1.0000	0.0461	0.3439	0.3842	-0.1045
Education	0.0461	1.0000	0.6585	-0.1721	-0.9449
Income	0.3439	0.6585	1.0000	0.0189	-0.8262
Age	0.3842	-0.1721	0.0189	1.0000	0.1401
Ethnicity	-0.1045	-0.9449	-0.8262	0.1401	1.0000

Table 9
Spearman Correlation for Exposures with NBAS Outcomes

	Avg LMW	Avg HMW	Habituation	Orientation	Motor	Range of State	Regulation of State	Autonomic Stability	Abnormal Reflexes
Avg LMW	1	0.4909	0.2581	-0.1216	0.5994	-0.1702	0.0122	0.0375	0.6233
Avg HMW	0.4909	1	0.4469	0.2918	0.2569	-0.3344	-0.0427	0.1348	0.0125
Habituation	0.2581	0.4469	1	0.1578	0.3049	-0.0505	-0.3609	0.2256	-0.1877
Orientation	-0.1216	0.2918	0.1578	1	-0.1503	-0.4878	-0.0122	0.1428	0
Motor	0.5994	0.2569	0.3049	-0.1503	1	0.2117	0.5231	0.5557	0.6636
Range of State	0.1702	-0.3344	-0.0505	-0.4878	0.2117	1	0.0214	0.5297	0.1813
Regulation of State	0.0122	-0.0427	-0.3609	-0.0122	0.5231	0.0214	1	0.3015	0.4076
Autonomic Stability	0.0375	0.1348	0.2256	0.1428	0.5557	0.5297	0.3015	1	0.3082
Abnormal Reflexes	0.6233	0.0125	-0.1877	0	0.6636	0.1813	0.4076	0.3082	1

Table 10
Habituation Models (LMW, log (LMW), HMW)

LMW

Model : HABITUATIONFU = B0 + B1 (AVG_LOWMW_TOTAL) + B2 (WEIGHT) + e

Variable	β	SE	t Value	PR > |t|	Partial Correlation Coeff.	R^2
Low MW Phthalate	0.0008	0.0033	0.2500	0.8092	0.0065	0.0090
Weight	0.0000	0.0003	-0.1300	0.8974	0.0026	

log (LMW)

Model : HABITUATIONFU = B0 + B1 (LOGAVG_LOWMW_TOTAL) + B2 (WEIGHT) + e

Variable	β	SE	t Value	PR > |t|	Partial Correlation Coeff.	R^2
Log Low MW Phthalate	0.1032	0.1243	0.8300	0.4335	0.0648	0.0898
Weight	-0.0001	0.0003	-0.4400	0.6738	0.0268	

HMW

Model : HABITUATIONFU = B0 + B1 (AVG_HIGHMW_TOTAL) + B2 (WEIGHT) + e

Variable	β	SE	t Value	PR > |t|	Partial Correlation Coeff.	R^2
High MW Phthalate	0.0439	0.0398	1.1000	0.3061	0.1151	0.1484
Weight	-0.0002	0.0003	-0.5200	0.6168	0.0377	

Table 11
Orientation Models (LMW, log (LMW), HMW)

LMW

Model: ORIENTATIONFU = B0 + B1 (AVG_LOWMW_TOTAL) + B2 (WEIGHT) + e

Variable	β	SE	t Value	PR > |t|	Partial Correlation Coeff.	R^2
Low MW Phthalate	0.0046	0.0134	0.3400	0.7414	0.0064	0.0227
Weight	-0.0005	0.0013	-0.3400	0.7429	0.0164	

log (LMW)

Model : ORIENTATIONFU = B0 + B1 (LOGAVG_LOWMW_TOTAL) + B2 (WEIGHT) + e

Variable	β	SE	t Value	PR > |t|	Partial Correlation Coeff.	R^2
Log Low MW Phthalate	0.0523	0.5314	0.1000	0.9243	0.0001	0.0076
Weight	-0.0003	0.0014	-0.2300	0.8243	0.0075	

HMW

Model : ORIENTATIONFU = B0 + B1 (AVG_HIGHMW_TOTAL) + B2 (WEIGHT) + e

Variable	β	SE	t Value	PR > |t|	Partial Correlation Coeff.	R^2
High MW Phthalate	0.1557	0.1659	0.9400	0.3792	0.0685	0.1173
Weight	-0.0008	0.0013	-0.6200	0.5534	0.0524	

Table 12
Motor Models (LMW, log (LMW), HMW)

LMW

Model: MOTORFU = B0 + B1 (LOWMW_TOTAL) + B2 (WEIGHT) + e

Variable	β	SE	t Value	PR > |t|	Partial Correlation Coeff.	R^2
Low MW Phthalate	0.0049	0.0043	1.1500	0.2885	0.3211	0.5884
Weight		0.0009	0.0004	2.1300	0.0704	

log (LMW)

Model : MOTORFU = B0 + B1 (LOGAVG_LOWMW_TOTAL) + B2 (WEIGHT) + e

Variable	β	SE	t Value	PR > |t|	Partial Correlation Coeff.	R^2
Log Low MW Phthalate	0.1940	0.1677	1.1600	0.2851	0.3611	0.5894
Weight	0.0009	0.0004	1.9700	0.0891	0.3574	

HMW

Model : MOTORFU = B0 + B1 (AVG_HIGHMW_TOTAL) + B2 (WEIGHT) + e

Variable	β	SE	t Value	PR > |t|	Partial Correlation Coeff.	R^2
High MW Phthalate	0.0016	0.0606	0.0300	0.9799	0.1085	0.5109
Weight	0.0011	0.0005	2.4000	0.0475	0.4514	

Table 13
Range of State Models (LMW, log (LMW), HMW)

LMW

Model : RANGEFU = B0 + B1 (AVG_LOWMW_TOTAL) + B2 (WEIGHT) + e

Variable	β	SE	t Value	PR > |t|	Partial Correlation Coeff.	R^2
Low MW Phthalate	-0.0109	0.0100	-1.0900	0.3117	0.0214	0.2699
Weight	0.0015	0.0010	1.5400	0.1666	0.2539	

log (LMW)

Model : RANGEFU = B0 + B1 (LOGAVG_LOWMW_TOTAL) + B2 (WEIGHT) + e

Variable	β	SE	t Value	PR > |t|	Partial Correlation Coeff.	R^2
Log Low MW Phthalate	-0.3849	0.3971	-0.9700	0.3647	0.0070	0.2469
Weight	0.0015	0.0010	1.4900	0.1790	0.2416	

HMW

Model : RANGEFU = B0 + B1 (AVG_HIGHMW_TOTAL) + B2 (WEIGHT) + e

Variable	β	SE	t Value	PR > |t|	Partial Correlation Coeff.	R^2
High MW Phthalate	-0.2604	0.0995	-2.6200	0.0346**	0.1656	0.5683
Weight	0.0019	0.0008	2.5600	0.0378	0.4826	

** Denotes statistically significant result

Table 14
Regulation of State Models (LMW, log (LMW), HMW)

LMW

Model : REGULATIONFU = B0 + B1 (AVG_LOWMW_TOTAL) + B2 (WEIGHT) + e

Variable	β	SE	t Value	PR > |t|	Partial Correlation Coeff.	R^2
Low MW Phthalate	0.0045	0.0105	0.4300	0.6825	0.1557	0.4286
Weight	0.0019	0.0010	1.8300	0.1102	0.3233	

log (LMW)

Model : REGULATIONFU = B0 + B1 (LOGAVG_LOWMW_TOTAL) + B2 (WEIGHT) + e

Variable	β	SE	t Value	PR > |t|	Partial Correlation Coeff.	R^2
Log Low MW Phthalate	-0.3275	0.3982	-0.8200	0.4380	0.0159	0.4654
Weight	0.0025	0.0010	2.4300	0.0457	0.4568	

HMW

Model : REGULATIONFU = B0 + B1 (AVG_HIGHMW_TOTAL) + B2 (WEIGHT) + e

Variable	β	SE	t Value	PR > |t|	Partial Correlation Coeff.	R^2
High MW Phthalate	-0.0974	0.1330	-0.7300	0.4878	0.0118	0.4555
Weight	0.0024	0.0010	2.3900	0.0483	0.4490	

Table 15
Autonomic Stability Models (LMW, log (LMW), HMW)

LMW

Model : AUTONOMICFU = B0 + B1 (AVG_LOWMW_TOTAL) + B2 (WEIGHT) + e

Variable	β	SE	t Value	PR > |t|	Partial Correlation Coeff.	R^2
Low MW Phthalate	-0.0005	0.0018	-0.2700	0.7916	0.0111	0.1899
Weight	0.0002	0.0002	1.2400	0.2538	0.1808	

log (LMW)

Model : AUTONOMICFU = B0 + B1 (LOGAVG_LOWMW_TOTAL) + B2 (WEIGHT) + e

Variable	β	SE	t Value	PR > |t|	Partial Correlation Coeff.	R^2
Log Low MW Phthalate	-0.0408	0.0696	-0.5900	0.5762	0.0019	0.2195
Weight	0.0003	0.0002	1.4000	0.2052	0.2180	

HMW

Model : AUTONOMICFU = B0 + B1 (AVG_HIGHMW_TOTAL) + B2 (WEIGHT)+ e

Variable	β	SE	t Value	PR > |t|	Partial Correlation Coeff.	R^2
High MW Phthalate	-0.0097	0.0233	-0.4100	0.6908	0.0046	0.2008
Weight	0.0002	0.0002	1.3100	0.2312	0.1971	

Table 16
Abnormal Reflex Models (LMW, log (LMW), HMW)

LMW

Model : ABNORMALFU = B0 + B1 (AVG_LOWMW_TOTAL) + B2 (WEIGHT) + e

Variable	β	SE	t Value	PR > |t|	Partial Correlation Coeff.	R^2
Low MW Phthalate	0.0017	0.0010	1.7700	0.1208	0.3171	0.3344
Weight	0.0610	0.0722	0.8500	0.4260	0.0926	

log (LMW)

Model : ABNORMALFU = B0 + B1 (LOGAVG_LOWMW_TOTAL) + B2 (WEIGHT) + e

Variable	β	SE	t Value	PR > |t|	Partial Correlation Coeff.	R^2
Log Low MW Phthalate	0.0016	0.0011	1.4500	0.1910	0.3171	0.2018
Weight	0.0000	0.0001	0.4300	0.6824	0.0427	

HMW

Model : ABNORMALFU = B0 + B1 (AVG_HIGHMW_TOTAL) + B2 (WEIGHT) + e

Variable	β	SE	t Value	PR > |t|	Partial Correlation Coeff.	R^2
High MW Phthalate	-0.0133	0.0155	-0.8600	0.4166	0.0083	0.2184
Weight	0.0002	0.0001	1.3700	0.2124	0.2119	

Table 17
NBAS Clusters (Higher cluster score indicates better development in all domains, except abnormal reflexes where a lower score indicates fewer abnormalities)

Cluster	Measurement of Cluster Score	Behavioral Items Included	Max Score
Orientation	Sum / Average	Animate visual Animate visual and auditory Inanimate visual Inanimate visual and auditory Animate auditory Inanimate auditory Alertness	63
Motor	Sum / Average	General tone Motor maturity Pull to sit Defensive movements Activity level	45
Regulation of state	Sum / Average	Cuddliness Consolability Self=quieting Hand to mouth	36
Range of State	Sum / Average	Peak of excitement Rapidity of build-up Irritability Lability of States	36
Habituation	Sum / Average	Response decrement to light Response decrement to rattle Response decrement to bell Response decrement to foot stimulation	36
Autonomic Stability	Sum / Average	Tremulousness Startles Lability of skin color	27
Supplementary Items	Sum / Average	Examiner facilitation Examiner's emotional response	18
Abnormal Reflexes	Count of # Abnormal Reflexes	NA	# of Abnormalities

Figures

Figure 1. Generic structure of phthalate diesters and their monoester metabolites (1)

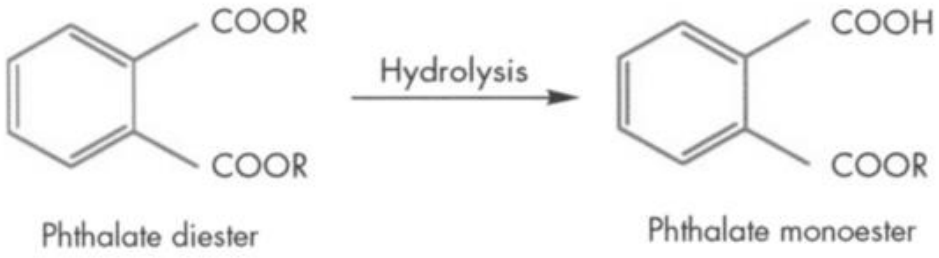

Figure 2. General phthalate metabolism (1)

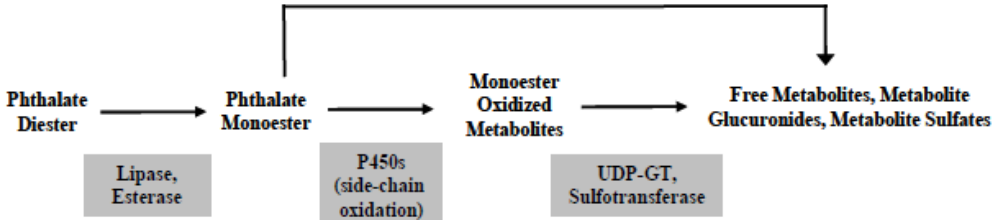

Figure 3. Potential mechanism for weight as a confounder for the relationship between phthalate exposure and NBAS scores

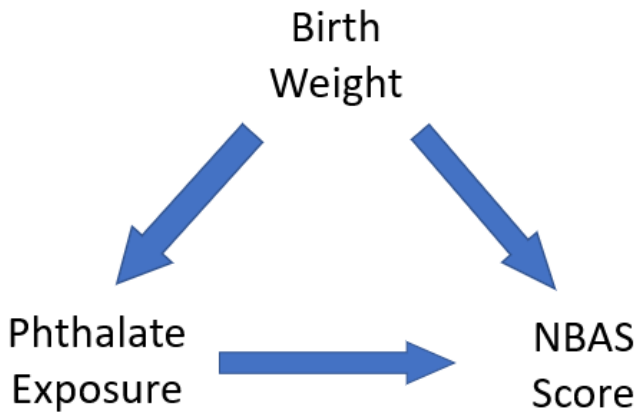

Figure 4. Potential mechanism for birth weight as an intermediate between phthalate exposure and NBAS scores.