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14. ABSTRACT This research project will build upon the Long Island Breast Cancer Study Project (LIBCSP), a large population-based, case-control study of the environment and breast cancer. Participants completed an in-person interviewer-administered interview, donated blood and urine samples and had home environment samples (dust, soil and water) collected. For this study, 200 cases and 200 controls who donated urine samples will be selected and their urine samples will be analyzed for a panel of EE biomarkers. In addition, these same women will be screened for polymorphisms in both the estrogen receptor alpha and beta genes. Breast cancer risk in relation to the combination of these multiple EE exposures and gene-environment interaction will be investigated using sophisticated statistical methods such as hierarchical regression models and factor analysis. Additionally, a pilot investigation of the correlation between EE levels in house dust and urinary biomarker levels will be conducted. Currently, samples for this study have been selected and the laboratory analyses are underway. Results of the proposed research project will be of enormous public health relevance since they may advance our knowledge of modifiable breast cancer risk factors and newly identified EEs, thereby providing information that is essential for primary prevention.

15. SUBJECT TERMS
epidemiology, environmental exposures, endocrine disruptors, estrogen receptor genes, gene-environment interaction, methodologic approaches to examining multiple exposures

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Introduction

The primary aim of the multidisciplinary postdoctoral award is to position Dr. Teitelbaum as an independent research scientist specializing in the environmental and molecular epidemiology of combined effects of multiple exposures. The research aims – relating multiple environmental estrogen exposure to breast cancer risk – will be examined in the Long Island Breast Cancer Study Project, a large population-based case-control study of breast cancer and the environment.

Specific Aims:

- To investigate whether women with higher combined exposure levels to multiple environmental estrogens are at increased risk of breast cancer.
- To investigate the possibility that women who carry adverse alleles in the estrogen receptor alpha and beta genes and have higher combined exposure levels to multiple environmental estrogens are at higher risk of breast cancer than women without these alleles.
- To evaluate the relationship between household dust and urinary levels of environmental estrogens.

Body

Below I have detailed the training and research that has been completed over the past year according to the tasks outlined in the Statement of Work.

Task 1. To undertake the proposed training program:

a. Complete graduate coursework in biostatistics, genetics, and pharmacology

• Completed advanced statistical methods courses: Applied Longitudinal Analysis, Graphs for Causal Inference.

• Completed several statistical programming courses to increase data analysis efficiency: SAS Macro Language, Base SAS to Microsoft Excel: Counting the Ways,

• Attended NIEHS Workshop on SNPs,

b. Conduct several epidemiologic analyses of multiple environmental exposures

• Author and co-author on multiple publications (see Reportable Outcomes)

• Continue to participate in departmental environmental and biometry journal clubs, attend monthly meetings of departmental projects, attend weekly Cancer Center seminars and Mount Sinai grand rounds on endocrinology, breast pathology, and oncology.

• Attended and presented at the departmental biometry and environmental epidemiology journal clubs.

• Attended monthly meeting of the NIEHS Center for Children's Environmental Health

• Attended Cancer Center seminars and grand rounds when topics were relevant to my research and training goals

c. Become a member of and alternately attend annual meetings of the International Society of Exposure Analysis (ISEA)/International Society for Environmental Epidemiology (ISEE) and Eastern North American Region of the International Biometric Society (ENAR).

• Current member of:
  - International Society for Environmental Epidemiology
  - Eastern North American Region of the International Biometric Society
  - American Association for Cancer Research (AACR) and Molecular Epidemiology Working Group of AACR
  - Society for Epidemiologic Research

• Attended professional scientific meetings:
  - International Society of Environmental Epidemiology (ISEE) and presented a poster on "How Representative is a Single Urine Sample of a Six-Month Average for Urinary Phthalate Metabolites and Bisphenol A?"
  - Emerging Topics in Breast Cancer and the Environment
  - Eastern North American Region of the International Biometric Society (ENAR)
  - Cornell University's Breast Cancer and Environment Research Factors Forum

d. Complete an internship in the lab conducting genetic screening to gain an appreciation for laboratory work commonly performed in molecular epidemiology studies.

• Completed in year 2.

e. Regularly meet with my mentors and advisors to oversee my progress and research development.

• Met both formally and informally with Dr. Wolff each week to review progress, discuss issues related to conducting research and professional development.

• Met with other mentors and advisors on an "as needed" basis depending on the specific research issue requiring discussion

Task 2. To conduct a case-control study of combined environmental estrogen exposure, the estrogen receptor alpha and estrogen receptor beta genes and breast cancer:

a. Conduct sample selection for urinary biomarkers (200 cases and 200 controls) and dust analysis (50 cases and 50 controls)

• Competed.

b. Conduct urinary biomarker environmental estrogen assays.

• Several in-person meetings, phone conferences and email communication with collaborators from the CDC (where biomarker assays will be conducted) have been held.

• Delayed CDC IRB approval and the extensive backlog for performing urinary biomarker analyses created delays in these analyses. The results for phthalate metabolite and phenol biomarkers from the LIBCSP urine samples were recently received. The urine samples are currently in the queue at the CDC for pyrethroid pesticide analysis. The head of the lab estimates that results for the remaining biomarkers from this study will not be available for another six months to a year. Communication with the CDC lab will continue throughout this period to monitor their progress.

• Creatinine concentrations to be used for correction of urine dilution have been received.

c. Conduct screening for estrogen receptor alpha and estrogen receptor beta genetic polymorphisms.

• Problems with the reliability of the genotyping methodology provided through the Mount Sinai DNA Core led us to redesign the approach used to screening for estrogen receptor alpha and beta genetic polymorphisms. Dr. Chen, it was decided to initially take a functional SNP approach for examining the association between estrogen receptor genes and breast cancer Genotyping results were received this month.

d. Conduct house dust environmental estrogen analyses.

• All selected dust samples were located in repository freezers.

• A backlog of work at the laboratory created delays in processing/analyses of the dust samples. The laboratory has completed the analysis of 30 samples – the data indicates that phenolic compounds are measurable in the dust samples that had been frozen for almost 10 years. The laboratory is continuing to work on the remaining 70 samples. Recent conversations with the lab indicate that the results will be available in June or July.

e. Conduct quality control and verification of data.

• Quality control for the phthalate and phenol biomarkers has been completed and the coefficient of variation for the phthalate metabolites and the phenol biomarkers were excellent.

• Quality control and verification of other data will occur when they become available.

• Review of the data delivered on the first 30 dust samples verified that phenolic compounds were present in the household dust samples selected from the Long Island Breast Cancer Study Project study participants.

Task 3. To conduct data analysis, manuscript preparation and dissemination of research results at conferences

• Concentration distribution of these biomarkers is being prepared so that they can be compared to National data from NHANES.

• Case-control analysis of the association between phthalate metabolites and breast cancer is underway.

• Case-control analysis of the association between phenol biomarkers and breast cancer will begin shortly.

• Case-control analysis of the association between these polymorphisms and breast cancer risk will begin shortly.
• As other laboratory results become available this task will be expanded.

Key Research Accomplishments

• Designed and oversaw field operations of a methodological study to assess the inter- and intra-person variability of urinary metabolites of the environmental estrogens, including bisphenol A, phthalates and pyrethroid pesticides.
  - o The collection of serial urine samples was completed.
  - o The CDC has competed sample analysis and has delivered the biomarker analyte results.
  - o Using this data, three abstracts have been presented at the Annual meeting of the International Society of Environmental Epidemiology (see appendices).
  - o The first of manuscript based on this data has been published (see appendices) and two more manuscripts are being submitted for publication.

• Abstract submitted to AACR special conference, Candidate Pathways, Whole Genome Scans: Reconciling Results, examining combined effect of multiple exposures and genotypes on breast cancer risk (see appendices).

Reportable Outcomes

• Publications

Susan Teitelbaum, Julie Britton, Nita Vangeepuram, Barbara Brenner, Manori Silva, Antonia Calafat, Mary Wolff. Phthalate Metabolites and Body Size Characteristics in Urban Minority Girls. Submitted for review to International Society of Environmental Epidemiology Annual Meeting 2008

Susan Teitelbaum, Julie Britton, Nita Vangeepuram, Rebecca Bausell, Barbara Brenner, Antonia Calafat, Mary Wolff. Phthalate Metabolites and Asthma in Urban Minority Girls. Submitted for review to International Society of Environmental Epidemiology Annual Meeting 2008.

Teitelbaum, SL, Britton, JA Calafat, AM, Silva, M J, Hsu, M, Brenner, B, Wolff, MS. Urinary Phthalate Metabolite Concentrations and Reported Use of Personal Care Products. Epidemiology. 2007 (ISEE Abstract)

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Terry MB, Perrin M, Salafia CM, Zhang FF, Neugut AI, Teitelbaum SL, Britton J, Gammon MD. Preeclampsia, pregnancy-related hypertension, and breast cancer risk. Am J Epidemiol. 2007 May 1;165(9):1007-14.

- Invited Presentation: Environmental Exposures and BPA: Questionnaire data and biomarkers. Breast Cancer and the Environment Research Centers' Integration Meeting: Practicing Transdisciplinary Science, June 26-28, 2007
- Principal Investigator of NIEHS funded Mentored Scientist Career Development Award.

- Co-Principal Investigator on Project 2 of NIEHS/EPA funded "Breast Cancer and the Environment Research Center" (MS Wolff, PI).
- New investigator award in our department's NIEHS/EPA funded Children's Environmental Health Center.
- Abstract reviewer for 3rd annual Breast Cancer and the Environment Research Symposia.
- Planning committee member for 4th annual Breast Cancer and the Environment Research Symposia.
- Peer reviewer for several prominent journals in epidemiology and environmental research
- Selected as a member of NIEHS study section for SuperFund Projects
- Invited for Board Membership of "The Open Epidemiology Journal"

Conclusions

I have made significant progress towards becoming an independent research scientist specializing in the environmental and molecular epidemiology of combined effects of multiple exposures. I have extended my multiple exposure study opportunities by obtaining 2 additional federally funded initiatives and increased my ability to conduct multiple exposure epidemiologic analyses through the training I have completed. The work accomplished during the third year of this grant has built a strong foundation for completing the proposed research in the additional year of this project provided by the no-cost extension of this award. The research I have conducted thus far is directly related to the goals of my postdoctoral award. All of these urinary metabolites measured in the temporal variability study will be measured in the urine samples of the case-control study analyses that will be completed in the coming year of this award. The results provide invaluable information for the data analysis of the case-control study and contribute to our understanding of how these biomarkers can be best used in future epidemiologic studies.

References

None

Appendices

Copies of recently published journal articles and submitted abstracts

Available online at www.sciencedirect.com
ScienceDirect
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Temporal variability in urinary concentrations of phthalate metabolites, phytoestrogens and phenols among minority children in the United States ☆

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Abstract

Background: Exposure to endocrine disruptors (EDs), including some phthalates, phytoestrogens and phenols can be quantified using biomarkers of exposure. However, reliability in the use of these biomarkers requires an understanding of the timeframe of exposure represented by one measurement. Data on the temporal variability of ED biomarkers are sparse, especially among children.
Objective: To evaluate intraindividual temporal variability in 19 individual urinary biomarkers (eight phthalate metabolites from six phthalate diesters, six phytoestrogens (two lignans and four isoflavones) and five phenols) among New York City children.
Methods: Healthy Hispanic and Black children ($N = 35$; 6–10 years old) donated several urine samples over 6 months. To assess temporal variability we used three statistical methods: intraclass correlation coefficient (ICC), Spearman correlation coefficients (SCC) between concentrations measured at different timepoints, and surrogate category analysis to determine how well the tertile categories based on a single measurement represented a 6-month average concentration.
Results: Surrogate category analysis indicated that a single sample provides reliable ranking for all analytes; at least three of four surrogate samples predicted the 6-month mean concentration. Of the 19 analytes, the ICC was >0.2 for 18 analytes and >0.3 for 10 analytes. Correlations among sample concentrations throughout the 6-month period were observed for all analytes; 14 analyte concentrations were correlated at 16 weeks.
Conclusions: The reasonable degree of temporal reliability and the wide range of concentrations of phthalate metabolites, phytoestrogens and phenols suggest that these biomarkers are appropriate for use in epidemiologic studies of environmental exposures in relation to health outcomes in children.

Keywords: Endocrine disruptor; Biomarker; Urine; Children; Reproducibility of results; Phthalates; Phytoestrogens; Phenols; Bisphenol A; Isoflavones; Ligans; Time factors

1. Introduction

Exposure to hormonally active agents, commonly referred to as endocrine disruptors (EDs), has been shown to be ubiquitous in the United States (Centers for Disease Control and Prevention, 2005). A number of EDs have been identified among the chemical families of phthalates, phytoestrogens, and phenols. Phthalates, a family of man-made chemicals, are used for a variety of purposes such as adding flexibility to plastics and making fragrances last

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longer (Centers for Disease Control and Prevention, 2005). Many, but not all, phthalates have been shown to have estrogenic, anti-estrogenic or anti-androgenic properties in animal studies (Lee and Koo, 2007; Takeuchi et al., 2005). Several phthalates have also been found to modulate hormonal activity and act in a dose additive manner (Gray et al., 2007; Howdeshell et al., 2007). Phytoestrogens, plant-derived chemicals with identified potential hormonal activity, can have beneficial health effects including lowering the risk of breast cancer and osteoporosis (Branca and Lorenzetti, 2005), but have also been shown to adversely interfere with thyroid function (Doerge and Chang, 2002). Of the phenol biomarkers that can be measured in urine, bisphenol A (BPA), used in the production of polycarbonate plastic, has received the most attention due to detrimental reproductive effects noted in animal studies (Maffini et al., 2006). Other phenols detectable in urine include benzophenone-3 (BP-3), a UV screen that has tested both positive and negative for hormonal activity (Gomez et al., 2005; Schlumpf et al., 2001; Schreurs et al., 2005) and triclosan (TCS), a microbicide used in many household and personal care products that may act as an anti-estrogen or androgen (Foran et al., 2000).

Given the hypothesized links among ED exposure, hormonal balance, and health outcomes it is of great interest to use biomarkers of exposure to assess EDs in epidemiologic studies. However, use of these biomarkers as reliable indicators of exposure requires an understanding of the timeframe of exposure represented by a single measurement. Data on the temporal variability of many of these biomarkers are sparse, especially among children. To address this gap in knowledge we conducted a study of intraindividual temporal variability in phthalate, phytoestrogen and phenol urinary metabolites among New York City (NYC) children.

2. Materials and methods

From June to October 2004, healthy Hispanic and Black children, between the ages of 6 and 10 years who were visiting an East Harlem, NYC community health clinic were invited to participate in this study. Thirty-five children were enrolled. Informed consent was obtained from each child's legal guardian and assent was obtained from each child. Interviews were conducted with the child's parent or legal guardian in Spanish or English. Children were asked to donate six serial urine samples over a period of 6 months, at initial interview (baseline), approximately 1, 2, 3, and 6 months after baseline as well as one additional sample donated approximately 2 weeks after any of the first four samples. At each time point, spot urine samples (~40 ml) were collected at the clinic or at home. Urine samples were aliquoted and frozen at −20 °C. Samples were analyzed at the National Center for Environmental Health of the Centers for Disease Control and Prevention (CDC) for EDs (10 phthalate metabolites, six phytoestrogens and six phenols; see Table 1 for the complete list of analytes). This study was approved by the Institutional Review Boards of Mount Sinai School of Medicine and the CDC.

2.1. Laboratory analysis

The analytical approaches have been published (Kato et al., 2005; Kuklenyik et al., 2004; Ye et al., 2005). Conjugated analytes are enzymatically hydrolyzed, and then concentrated and separated from other urine components by on-line solid phase extraction coupled to high performance liquid chromatography. Quantitation is achieved by isotope dilution tandem mass spectrometry. The method for phytoestrogens was modified from Kuklenyik et al. (2004) to use negative-ion TurboIonSpray ionization, a variant of electrospray ionization, instead of atmospheric pressure chemical ionization. For all analytical methods, standard quality control (QC) and reagent blank samples were included in each analytical batch along with the unknown samples. QC samples were evaluated according to standard statistical probability rules (www.westgard.com). If the QC samples failed the statistical evaluation, all samples in the batch were re-extracted. The CDC laboratory is certified by the Health Care Financing Administration to comply with the requirements set forth in the Clinical Laboratory Improvement Act of 1988 (CLIA '88) (Certification # 11D0668200) and is recertified biannually.

2.2. Statistical methods

For samples with values below the limit of detection (LOD), a value of the LOD divided by square root of two was substituted. Since interviews were conducted at various times of the day, the collected urine samples may vary in their water content. Differences in urinary analyte concentrations can be due to variations in urine water content. To account for this, all values were normalized to creatinine by dividing the analyte concentration by the creatinine concentration. Since there is continued discussion about the use of creatinine for correction of urine dilution, all analyses were performed on uncorrected (ng/ml) and creatinine-corrected (μg/g creatinine) values. Due to the non-normal distribution of the analyte concentrations, all analyses were conducted on natural-log-transformed values using SAS version 9.13 (Cary, NC).

A total of 159 urine samples donated by the 35 participants were analyzed. Six children donated a single sample at baseline and are only included in the descriptive statistics presented in Table 1. Four children contributed two to five samples, but did not provide a urine specimen at the end of the 6-month period. The remaining 25 participants completed the 6-month protocol donating between four and seven samples each for a total of 136 samples.

Four metabolites of di-2-ethylhexyl phthalate (DEHP) were measured in the urine samples: mono-2-ethyl-5-carboxypentyl phthalate (mECPP); mono-2-ethyl-5-hydroxyhexyl phthalate (mEHHP); mono-2-ethyl-5-oxo-hexyl phthalate (mEOHP); and mono-2-ethylhexyl phthalate (mEHP). We calculated the sum on a molar basis of the four metabolites (mECPP, mEHHP, mEOHP, mEHP; $\sum$(DEHP4)) as well as for three metabolites (mEHHP, mEOHP mEHP; $\sum$(DEHP3)). Similarly, we calculated the sum on a molar basis of daidzein (DAZ) and its two metabolites equol (EQU) and *o*-desmethyangolensin (DMA) ($\sum$(DAZ)). Two analytes were detected in less than 60% of the samples (mono-methyl phthalate and *ortho*-phenylphenol (*o*-PP)), and mECPP was measured in only 100 samples. These analytes and $\sum$(DEHP4) are included in the descriptive statistics presented in Table 1, but not considered in subsequent data analyses. To assess temporal variability of the remaining 19 analytes (eight phthalate metabolites from six phthalate diesters, six phytoestrogens (two lignans and four isoflavones) and five phenols) and the two sums, $\sum$(DEHP3) and $\sum$(DAZ), we used three statistical methods. For reproducibility we used the intraclass correlation coefficient (ICC) defined as the percent of total variance explained by between-person variance (Rosner, 2000). In addition, we computed Spearman correlation coefficients (SCC) between concentrations determined at different times within the 6-month period. Finally, we performed a surrogate category analysis to assess how well tertile ranking by a single biomarker measurement (surrogate sample) represented the average concentration over 6-months (Hauser et al., 2004; Willett, 1998).

Children providing at least two urine samples ($N = 29$ children; $N = 153$ samples) were included in the ICC and SCC calculations. The ICC was calculated from a one-way random-effects ANOVA model estimated independently for each analyte (SAS Proc Mixed, V9.13). An ICC of at least 0.40 is considered an indication of fair to good reproducibility and an ICC of 0.75 or greater is considered excellent (Rosner, 2000). The SCC was calculated for samples collected at

Table 1
Distribution of analyte concentrations (A. creatinine corrected and B. uncorrected) among 35 New York City children, 2004–2005

	Abbreviation	% Detect	*N*	Geo-metric mean	50th %tile	CDC 50th %tile[a]	Min	Max	95th %tile	CDC 95th %tile[a]
A. Analyte (μg/g creatinine)										
Phthalate metabolites										
Mono-ethyl	mEP	100.0	159	176.4	149.5	81.2	15.3	6410.4	1378.6	837
Mono-2-ethyl-5-carboxypentyl[b,c]	mECPP	100.0	100	124.4	104.2	NA	16.5	1806.9	726.4	NA
Mono-2-ethyl-5-hydroxyhexyl[c]	mEHHP	100.0	159	84.8	76.4	34.2	6.4	1101.0	592.1	211
Mono-2-ethyl-5-oxohexyl[c]	mEOHP	100.0	159	53.9	50.9	22.8	4.6	710.4	350.4	130
Mono-2-ethylhexyl[c]	mEHP	97.5	159	11.3	11.7	5.38	0.5	207.7	90.2	31.2
Sum (mEHHP, mEOHP, mEHP)[c]	Σ(DEHP3)	NC	159	521.0	481.2	NA	45.6	6901.3	3587.5	NA
Sum (mECPP, mEHHP, mEOHP, mEHP)[c]	Σ(DEHP4)	NC	100	940.8	789.7	NA	99.1	11,883.3	5640.0	NA
Mono-*n*-butyl	mBP	100.0	159	52.0	52.6	35.1	9.1	660.8	165.1	146
Mono-benzyl[d]	mBzP	100.0	158	40.3	36.6	37.2	2.8	823.8	287.5	195
Mono-iso-butyl	miBP	98.7	159	16.5	15.6	5.17	2.1	158.2	50.9	24.3
Mono-3-carboxypropyl	mCPP	94.3	159	6.3	6.1	7.07	0.1	94.2	53.6	26.4
Mono-methyl	mMP	57.9	159	NC	1.9	2.32	0.4	239.5	15.4	12.5
Phytoestrogens										
Enterolactone	ETL	100.0	159	314.2	366.2	349	16.5	5136.3	1529.0	1970
Enterodiol	ETD	94.3	159	26.7	28.8	39.3	0.5	809.2	201.8	304
Genistein	GNS	98.7	159	32.3	23.2	37.7	1.0	1723.0	601.7	487
Daidzein	DAZ	98.7	159	61.1	46.5	86.3	3.6	4453.4	805.5	930
Equol[e]	EQU	87.4	159	13.5	14.7	14	1.5	249.5	58.3	88.2
o-Desmethylangolensin[e]	DMA	66.7	159	2.4	2.0	6.49	0.1	582.3	114.4	305
Sum (DAZ, EQU, DMA)[e]	Σ(DAZ)	NC	159	393.2	327.3	NA	37.7	19802.7	3793.6	NA
Phenols										
2,5-Dichlorophenol	2,5-DCP	100.0	159	79.2	53.0	11.3	5.9	7598.8	1785.0	516
Triclosan	TCS	71.7	159	13.4	9.3	NA	0.7	1697.4	400.7	NA
Benzophenone-3	BP-3	96.2	159	10.9	10.6	NA	0.1	1113.9	296.1	NA
Bisphenol A	BPA	95.0	159	3.4	3.5	1.53	0.2	36.3	23.6	6.64
2,4-Dichlorophenol	2,4-DCP	98.1	159	2.7	2.2	0.966	0.2	94.7	29.3	25.3
ortho-Phenylphenol	o-PP	54.1	159	NC	0.2	<LOD	0.0	3.9	1.6	2.53
Creatinine (mg/dl)	C	100.0	159	100.8	112.8	NA	12.9	240.9	205.5	NA

	Abbreviation	% Detect	*N*	LOD	Geometric Mean	50th %tile	CDC 50th %tile[a]	Min	Max	95th %tile	CDC 95th %tile[a]
B. Analyte (ng/ml)											
Phthalate metabolites											
Mono-ethyl	mEP	100.0	159	0.4	177.7	165.8	74.7	7.4	3638.2	1723.2	756
Mono-2-ethyl-5-carboxypentyl[b,c]	mECPP	100.0	100	0.25	135.6	138.6	NA	10.4	1694.5	816.4	NA
Mono-2-ethyl-5-hydroxyhexyl[c]	mEHHP	100.0	159	0.32	85.5	83.1	32.9	1.6	1926.8	666.8	210
Mono-2-ethyl-5-oxohexyl[c]	mEOHP	100.0	159	0.45	54.3	54.1	22.6	1.2	1245.7	376.8	142
Mono-2-ethylhexyl[c]	mEHP	97.5	159	0.9	11.4	11.3	4.4	0.6	272.7	93.0	29.9
Sum (mEHHP, mEOHP, mEHP)[c]	Σ(DEHP3)	NC	159	NA	525.0	518.3	NA	13.5	11,800.8	3883.0	NA
Sum (mECPP, mEHHP, mEOHP, mEHP)[c]	Σ(DEHP4)	NC	100	NA	1025.9	1067.9	NA	78.4	13,438.1	6974.6	NA
Mono-*n*-butyl	mBP	100.0	159	0.4	52.4	55.7	32.4	2.8	1187.4	211.2	157

Table 1 (*continued*)

	Abbreviation	% Detect	*N*	LOD	Geometric Mean	50th %tile	CDC 50th %tile[a]	Min	Max	95th %tile	CDC 95th %tile[a]
Mono-benzyl[d]	mBzP	100.0	158	0.11	40.5	37.8	37	1.9	1038.6	314.3	226
Mono-iso-butyl	miBP	98.7	159	0.26	16.6	18.0	4.4	0.7	299.1	66.5	23.4
Mono-3-carboxypropyl	mCPP	94.3	159	0.16	6.4	6.4	6.6	0.1	129.2	61.4	24.7
Mono-methyl	mMP	57.9	159	1	NC	1.6	1.8	0.7	430.3	20.6	11.6
Phytoestrogens											
Enterolactone	ETL	100.0	159	1.9	316.6	365.0	329	15.9	3580.0	2100.0	2160
Enterodiol	ETD	94.3	159	1.5	26.9	30.0	35.4	1.1	528.0	184.0	201
Genistein	GNS	98.7	159	0.8	32.6	27.5	31.5	0.6	2570.0	665.0	502
Daidzein[e]	DAZ	98.7	159	1.6	61.5	53.9	72.7	1.1	7210.0	1160.0	1030
Equol[e]	EQU	87.4	159	3.3	13.6	15.0	13.6	2.3	228.0	65.1	85.4
o-Desmethylangolensin[e]	DMA	66.7	159	0.4	2.5	2.2	5.7	0.3	474.0	129.0	281
Sum (DAZ, EQU, DMA)[e]	Σ(DAZ)	NC	159	NA	396.2	392.4	NA	15.2	28,476.2	4703.1	NA
Phenols											
2,5-Dichlorophenol	2,5-DCP	100.0	159	0.12	79.8	60.4	9.00	1.6	10,000.0	1710.0	630
Triclosan	TCS	71.7	159	2.27	13.6	8.5	NA	1.6	1040.0	477.0	NA
Benzophenone-3	BP-3	96.2	159	0.34	10.9	10.2	NA	0.2	1340.0	250.0	NA
Bisphenol A	BPA	95.0	159	0.36	3.4	3.6	1.87	0.3	40.0	26.6	7.51
2,4-Dichlorophenol	2,4-DCP	98.1	159	0.17[a]	2.8	2.4	0.82	0.1	98.4	33.0	29
ortho-Phenylphenol	o-PP	54.1	159	0.1	NC	0.1	<LOD	0.1	3.8	1.3	2.28

NC: not calculated; NA: not available.

[a]Third National Report on Human Exposure to Environmental Chemicals, 2005; 6–11-year age group, 2001–2002 survey years; 1999–2000 survey years used for 2,4-DCP and 2,5-DCP; Calafat 2005 used for BPA reference values in adults.

[b]Fifty-nine samples were not analyzed for this phthalate metabolite.

[c]Di-2-ethylhexyl phthalate (DEHP) metabolites are mECPP, mEHHP, mEOHP, and mEHP. Sum is calculated on a molar basis (as nm/g creatinine or nm/l).

[d]One sample (<1%) did not fulfill QC requirements.

[e]Daidzein metabolites are EQU and DMA. Sum is calculated on a molar basis (as nm/g creatinine or nm/l).

approximate intervals determined by collection dates (2, 4, 6, 8, 12, 16, and 24 weeks apart). Depending on the timing of urine donation, between 15 and 59 sample pairs were included in each interval-based SCC calculation.

Finally, the surrogate category analysis was conducted for each analyte using data from 24 children who had urine samples collected at the following four timepoints: baseline; 8–10 weeks after baseline; 12–14 weeks after baseline; and 23–29 weeks after baseline. The "surrogate sample" is defined as the set of 24 samples collected at one of the four collection time points, e.g., baseline. The 6-month mean concentration (mean of the four repeated measures over the 6-month period) was calculated to represent the average analyte exposure for this time period. Tertile cutpoints were created using the concentration distribution of the 24 baseline samples (one per child) and were used to classify each child into low, medium, or high concentration groups. We then computed three tertile-specific 6-month grand means by averaging the 6-month mean concentrations of the children who were assigned to each tertile ($N \cong 8$). All calculations were performed on the natural-log transformed data and were then back transformed to obtain geometric means. A monotonic increase in the geometric means across tertiles indicates that the concentration measured in a single sample is predictive of the 6-month average concentration. Using each of the other three surrogate sample sets we calculated tertile cutpoints and tertile-specific grand means and examined each for a monotonic increasing trend. In the absence of a formal statistical test for these trends, we calculated SCCs using the four sets of surrogate ranks (1, 2, or 3) and tertile-specific geometric means. A positive SCC was considered an indicator of an overall monotonically increasing trend.

3. Results

The frequency of detection was high among the analytes; 18 of the 22 analytes were detected in over 94% of the samples. Percent detects range from 58% to 100% for phthalate metabolites, 67–100% for phytoestrogens and 54–100% for phenols (Table 1). The median concentrations of all phthalate metabolites and phytoestrogens were near or greater than 2.0 ng/ml (2.0 μg/g creatinine) as were the medians for five of the six phenols examined. The range of both creatinine-corrected and uncorrected concentrations was variable ranging from four-orders of magnitude for BP-3 and 2,5-dichlorophenol (2,5-DCP) to one-order of magnitude for *o*-PP.

3.1. Intraclass correlation coefficient

As shown in Table 2, the ICC measure of reproducibility was moderate (0.35–0.62) for two phthalate metabolites (mBzP and mBP), two phytoestrogens (ETL, DMA), and all five of the phenols. Reproducibility was fair for the remaining metabolites ($ICC > 0.2$), except for daidzein ($ICC < 0.2$). The ICCs were somewhat lower for mBP and MBzP when based on uncorrected concentrations.

3.2. Spearman correlation coefficients

SCCs between samples collected at selected intervals are presented in Table 2. In general, SCCs based on creatinine-corrected concentrations were similar or slightly stronger than the corresponding SCCs based on uncorrected concentrations.

Based on the creatinine-corrected concentrations, for the intervals of 16 weeks or shorter, a relatively constant exposure to sources of phthalates is suggested for three metabolites (mBP, mBzP, and mCPP) which exhibited SCC with moderate-to-high values (>0.4) for at least five of the seven possible intervals. The three metabolites of DEHP (mEHHP, mEOHP, and mEHP) had statistically significant or borderline-significant SCC for samples collected 16 and 24 weeks apart; mEHP also had a significant correlation for samples collected at 4- and 6-week intervals while mEHHP and mEOHP had moderate but not statistically significant SCC between samples collected 6 weeks apart. The SCCs for $\sum$(DEHP3) closely followed the pattern of mEHHP and mEHP. The SCCs between uncorrected concentrations were somewhat weaker for samples collected 6 and 16 weeks apart for most phthalate metabolites.

Among the phytoestrogens, the creatinine-corrected lignan (enterolactone and enterodiol) concentrations were correlated out to 8 and 12 weeks, respectively, but no SCCs were above 0.45. In contrast, the pattern of significant SCCs among the isoflavones (EQU, DAZ, GNS, and DMA) indicated more constant exposure over a longer-time interval; there were significant correlations at both short (2, 4, or 6 weeks) as well as long (16 and 24 weeks) intervals and SCCs were generally higher than those for the lignans. The pattern of SCCs for $\sum$(DAZ) followed that of the parent isoflavone. Across all of the phytoestrogens, several of the borderline-significant SCCs based on the creatinine-corrected concentrations were not significant for the uncorrected samples.

All of the phenols displayed similar correlation patterns. Significant, moderate to high SCCs were observed for the 2–12-week intervals. BP-3 concentrations were consistently correlated across every interval with moderate-to-high SCCs. 2,5-DCP was significantly and moderately correlated at all intervals through 16 weeks. BPA and triclosan had similar and moderate correlation patterns out to 12 weeks.

3.3. Surrogate category analysis

The surrogate category analysis showed consistent results for almost every surrogate tertile of the 76 presented (Table 3). Creatinine-corrected results are discussed since the patterns were so similar for creatinine-corrected and uncorrected concentrations. For each of the four surrogate phthalate sample tertiles (baseline, 8–10 weeks after baseline, 12–14 weeks after baseline, 23–29 weeks after baseline), a monotonic increasing geometric mean was observed for seven of the eight metabolites. For example, when the distribution based on samples collected at baseline was used to create the three surrogate categories, the 6-month geometric mean for mEHHP was 58.6 μg/g creatinine among the children in the lowest exposure group, 105.0 μg/g creatinine in the medium exposure group, and 142.9 μg/g creatinine in the highest exposure group.

Table 2
Measures of reliability by analyte:components of variance, intraclass correlation coefficients, and spearman correlation coefficients between samples collected at specific time intervals using analyte concentrations creatinine corrected (A) and uncorrected (B)

	Components of variance[a]		Intraclass correlation coefficient[a]	Spearman correlation coefficients[b]						
	Between-child Variance	Within-child Variance		Approximate # weeks between samples	2	4	6	8	12	16	24
				# Sample pairs	35	59	15	40	45	22	25
A. Creatinine corrected											
Phthalate metabolites											
mEP	0.25	0.70	0.26		0.56**	0.40**	0.66**			0.45*	
mEHHP	0.25	0.79	0.24				0.40			0.55**	0.44*
mEOHP	0.23	0.77	0.23				0.48#			0.53*	0.46*
mEHP	0.38	0.93	0.29			0.43**	0.55*			0.68**	0.36#
Σ(mEHHP, nEOHP, mEHP)	0.24	0.78	0.23				0.46*			0.53	0.40*
mBP	0.19	0.35	0.35		0.51**	0.34**	0.39	0.43**	0.29#	0.52*	
mBzP	0.69	0.42	0.62		0.51**	0.60**		0.66**	0.43**	0.77**	0.30
miBP	0.14	0.34	0.28		0.64**			0.38*			
mCPP	0.23	0.88	0.21		0.54**	0.38**	0.38		0.30*	0.61**	
Phytoestrogens											
ETL	0.45	0.63	0.42		0.36*	0.23#	0.44#	0.37*	0.32*		
ETD	0.28	0.98	0.22		0.30#			0.37*			
GNS	0.70	1.43	0.33		0.53**	0.32*	0.73**	0.57**	0.55**	0.53*	0.49*
DAZ	0.39	1.86	0.17		0.39*		0.42	0.28#	0.25#	0.44*	
EQU	0.23	0.79	0.23			0.48**	0.43	0.38*		0.48*	0.53**
DMA	1.59	2.42	0.40		0.39*	0.39**	0.80**	0.49**	0.45**	0.72**	
Σ(DAZ, EQU, DMA)	0.24	1.38	0.15		0.31#		0.58**	0.35*	0.27#	0.42#	
Phenols											
2,5-DCP	0.87	1.30	0.40		0.81**	0.52**	0.73**	0.50**	0.48**	0.45*	
TCS	1.50	2.39	0.39		0.61**	0.57**		0.38*	0.52**		
BP-3	1.38	1.60	0.46		0.65**	0.58**	0.83**	0.49**	0.45**	0.31	0.43*
BPA	0.35	0.65	0.35		0.41*	0.56**	0.39		0.29#	0.32	
2,4-DCP	0.55	0.98	0.36		0.65**	0.42**	0.36		0.25#		

	Components of variance[a]		Intraclass correlation coefficient[a]	Spearman correlation coefficients[b]						
	Between-child variance	Within-child variance		Approximate # weeks between samples	2	4	6	8	12	16	24
				# Sample pairs	35	59	15	40	45	22	25
B. Uncorrected											
Phthalate metabolites											
mEP	0.22	1.02	0.18		0.46**		0.54*				
mEHHP	0.28	1.01	0.22						0.30*	0.37#	0.51**
mEOHP	0.24	0.98	0.19			0.22#			0.32*	0.36#	0.53**
mEHP	0.36	1.03	0.26			0.38**	0.34			0.44*	0.41*
∑(mEHHP, nEOHP, mEHP)	0.26	0.98	0.21			0.22#			0.31*	0.37#	0.53**
mBP	0.12	0.73	0.14					0.27#			
mBzP	0.71	0.82	0.47			0.43**		0.55**	0.33*	0.57**	
miBP	0.19	0.73	0.21		0.30#			0.30#	0.37*		
mCPP	0.19	1.24	0.13		0.42*				0.27#		
Phytoestrogens											
ETL	0.42	0.90	0.32		0.35*		0.32	0.41**	0.31*		
ETD	0.37	1.30	0.22		0.31#	0.25#		0.40**		0.42#	
GNS	0.76	1.74	0.30		0.49**	0.43**	0.73**	0.44**	0.49**	0.41#	0.43*
DAZ	0.52	2.14	0.20				0.56*		0.29#		
EQU	0.17	0.89	0.16			0.27*					0.40*
DMA	1.63	2.56	0.39		0.36*	0.41**	0.79**	0.36*	0.40**	0.69**	
Σ(DAZ, EQU, DMA)	0.30	1.57	0.16				0.62*		0.33*		

Table 2 (*continued*)

Phenols										
2,5-DCP	0.89	1.53	0.37	0.72**	0.45**	0.56*	0.51**	0.31*	0.42$^{\#}$	
TCS	1.33	2.47	0.35	0.74**	0.57**	0.30	0.32*	0.44**		
BP-3	1.23	1.92	0.39	0.58**	0.54**	0.61*	0.50**	0.35*		0.34
BPA	0.28	1.00	0.22		0.41**		0.27$^{\#}$	0.39**		0.31
2,4-DCP	0.57	1.16	0.33	0.61**	0.33*	0.49$^{\#}$	0.32*			

$^{\#}p<0.10$; $^{*}p<0.05$; $^{**}p<0.01$.

[a]Variances are from a one-way random-effects ANOVA model using data from children with at least two samples over a 6-month period ($N=29$ children, 153 samples); calculations were performed on natural-log transformed data; Intraclass correlation coefficient = between-child variance/total variance.

[b]Values are presented if the Spearman correlation coefficient was at least 0.30 or the p-value for the statistic was <0.10.

Although miBP had a surrogate sample with a non-monotonic increasing geometric mean, the third tertile was higher than the other two. All surrogates for the phytoestrogens except two were predictive of the 6-month means. The exceptions were ETL and DMA which each had good predictive ability for all except the baseline surrogate samples. Among the phenols, BPA and TCS had all four surrogate samples with monotonically increasing geometric means while 2,5-DCP, 2,4-DCP and BP-3 had three predictive surrogate samples. Thus, of 19 analytes examined, only six did not have monotonically increasing geometric means for all surrogate samples. Even in these six analytes the trends were consistent with increasing concentrations, except that the mean of the second tertile was slightly higher than the third for four analytes or the mean of the second tertile was lower than the first in two. These observed trends were further supported by the SCCs calculated between the surrogate rank and the geometric means. For the 13 analytes with monotonically increasing means across all four surrogate samples SCCs were all greater than 0.92; the SCCs were between 0.84 and 0.92 for the six other analytes.

4. Discussion

There is a surprisingly reasonable degree of temporal reliability in the concentrations of these 19 urinary biomarkers. Based on creatinine-corrected concentrations, the surrogate category analysis indicates consistent ranking of a 6-month mean analyte concentration by a single sample collected at various times within the 6-month interval; for all analytes, at least three of the four surrogate samples predicted the ranking of the 6-month mean. The ICC was $\geqslant 0.2$ for 18 out of 19 analytes and >0.3 for more than half of the analytes, and every biomarker had a minimum of two time intervals with significant or borderline-significant correlations. Analyses of uncorrected biomarker concentrations confirmed the results based on creatinine-corrected concentrations, but were not as consistent. This may indicate that urine dilution may influence the assessment of temporal variability and creatinine correction provided a more representative biomarker concentration, an issue that has been widely discussed in the literature (Barr et al., 2005; Remer et al., 2002). This degree of intra-individual stability in biomarkers indicates that more-or-less constant exposure must exist to common environmental agents, as all three families of biomarkers and their parent compounds have relatively short half-lives (hours or days). By way of comparison, biomarkers that are widely accepted as long-term exposure metrics have long-term persistence in the body. Organochlorines have half-life of about 10 years, and show minimal intra-individual variability over 1–3 years, even in children (Karmaus et al., 2002). Blood lead is intermediate, having a half-life of about 36 days. We investigated phthalates and phytoestrogens based on limited knowledge of their potential stability over periods of days-to-months (Hauser et al., 2004; Hoppin et al., 2002; Nesbitt et al., 1999; Zeleniuch-Jacquotte et al., 1998; Zheng et al., 1999). Although, to our knowledge, there are no published data on the temporal variability of phenols in urine, these chemicals are widely detected and due to extensive use in consumer products, exposures are not expected to be highly variable over a year's time (Joskow et al., 2006; Matsumoto et al., 2003).

The predictive ability of a single sample to reliably rank individuals according to their 6-month average concentration using the surrogate category analysis for four of the phthalate metabolites (MEHP, MBzP, MEP, and MBP) confirms earlier findings of a study conducted in men over a 3-month period (Hauser et al., 2004). Comparisons for the other phthalate metabolites or the phytoestrogens and phenols do not exist. The results of the surrogate category analysis provide good support for the use of all of these biomarkers to rank study participants on their exposure levels in environmental epidemiology studies much the same way that food consumption levels are used in nutritional epidemiology studies. For 13 biomarkers the 6-month average concentration was predicted by a sample collected at the start of the interval as well as by a sample collected at the end of the interval. This suggests that an annual urine sample used for exposure assessment in longitudinal studies might be reflective of a participant's year-long exposure for these chemicals. However,

Table 3
Six-month geometric mean of analyte concentrations creatinine corrected (A) and uncorrected (B) within surrogate sample determined exposure tertiles[a]

Surrogate sample	Geometric mean (μg/g C)				Surrogate sample	Geometric mean (μg/g C)				Surrogate sample	Geometric mean (μg/g C)			
	Low	Medium	High			Low	Medium	High			Low	Medium	High	
A. Creatinine corrected					Phytoestrogens					Phenols				
Phthalate metabolites														
MEP					ETL					2,5-DCP				
Baseline	153.9	159.1	259.9	↑	Baseline	128.6	417.5	405.9		Baseline	33.4	142.3	110.8	
8–10 weeks	119.8	168.7	315.0	↑	8–10 weeks	194.1	243.2	461.6	↑	8–10 weeks	27.5	72.0	266.6	↑
12–14 weeks	139.0	148.6	308.2	↑	12–14 weeks	164.1	319.8	415.3	↑	12–14 weeks	29.7	68.3	259.9	↑
23–29 weeks	113.2	201.9	278.4	↑	23–29 weeks	149.0	343.5	425.7	↑	23–29 weeks	42.1	71.4	175.3	↑
mEHHP					ETD					TCS				
Baseline	58.6	105.0	142.9	↑	Baseline	14.4	24.7	38.9	↑	Baseline	3.8	10.7	44.8	↑
8–10 weeks	67.1	77.3	169.5	↑	8–10 weeks	17.0	21.6	37.7	↑	8–10 weeks	5.3	8.5	40.5	↑
12–14 weeks	71.9	72.0	169.7	↑	12–14 weeks	18.4	19.9	37.8	↑	12–14 weeks	3.0	14.2	43.0	↑
23–29 weeks	51.4	83.2	205.7	↑	23–29 weeks	18.2	23.2	32.7	↑	23–29 weeks	3.8	15.7	30.8	↑
mEOHP					GNS					BP-3				
Baseline	35.1	67.3	91.0	↑	Baseline	12.2	21.6	93.9	↑	Baseline	2.6	10.7	30.1	↑
8–10 weeks	34.3	62.0	101.0	↑	8–10 weeks	12.8	26.7	72.5	↑	8–10 weeks	3.1	10.3	26.5	↑
12–14 weeks	45.0	45.9	104.0	↑	12–14 weeks	11.7	27.6	76.9	↑	12–14 weeks	2.6	16.9	19.2	↑
23–29 weeks	32.0	55.3	121.5	↑	23–29 weeks	12.2	23.1	87.7	↑	23–29 weeks	3.5	16.5	14.3	
mEHP					DAZ					BPA				
Baseline	7.7	13.8	18.8	↑	Baseline	39.4	51.8	81.1	↑	Baseline	2.5	2.5	4.9	↑
8–10 weeks	5.3	17.5	21.6	↑	8–10 weeks	31.3	58.1	91.0	↑	8–10 weeks	1.6	3.8	5.3	↑
12–14 weeks	6.8	10.7	27.4	↑	12–14 weeks	30.0	55.6	99.1	↑	12–14 weeks	1.8	2.9	6.0	↑
23–29 weeks	5.1	15.9	24.4	↑	23–29 weeks	27.1	59.9	101.8	↑	23–29 weeks	1.9	3.0	5.7	↑
Σ(DEHP3)[b]					EQU					2,4-DCP				
Baseline	350.6	646.8	874.2	↑	Baseline	7.3	15.6	27.4	↑	Baseline	2.5	2.1	3.9	
8–10 weeks	325.6	600.4	1014.1	↑	8–10 weeks	8.3	12.4	30.3	↑	8–10 weeks	1.4	2.0	7.3	↑
12–14 weeks	439.3	446.4	1011.0	↑	12–14 weeks	10.4	11.4	26.2	↑	12–14 weeks	1.3	2.1	7.3	↑
23–29 weeks	313.9	520.1	1214.1	↑	23–29 weeks	7.9	15.3	25.7	↑	23–29 weeks	1.3	2.9	5.4	↑
mBP					DMA									
Baseline	39.2	48.7	81.1	↑	Baseline	0.6	5.5	4.7						
8–10 weeks	35.8	52.1	83.1	↑	8–10 weeks	0.6	2.8	9.7	↑					
12–14 weeks	40.3	49.8	77.1	↑	12–14 weeks	0.7	2.7	8.4	↑					
23–29 weeks	38.7	48.6	82.3	↑	23–29 weeks	0.6	2.5	11.2	↑					
mBzP					Σ(DAZ)[c]									
Baseline	23.4	33.4	76.8	↑	Baseline	230.6	469.8	491.4	↑					
8–10 weeks	18.1	33.8	74.6	↑	8–10 weeks	216.7	380.9	645.0	↑					
12–14 weeks	23.6	29.2	87.8	↑	12–14 weeks	213.2	393.5	634.6	↑					
23–29 weeks	17.9	38.6	87.4	↑	23–29 weeks	225.1	343.1	689.4	↑					
miBP														
Baseline	11.8	16.3	23.8	↑										
8–10 weeks	12.2	17.9	21.1	↑										
12–14 weeks	15.8	11.8	24.3											
23–29 weeks	12.1	16.6	22.7	↑										

mCPP				
Baseline	3.6	6.0	10.2	↑
8–10 weeks	3.4	6.4	10.3	↑
12–14 weeks	3.1	7.2	9.8	↑
23–29 weeks	3.8	5.9	9.8	↑

Surrogate sample	Geometric mean (ng/ml)			
	Low	Medium	High	
B. *Uncorrected*				
Phthalate metabolites				
MEP				
Baseline	153.0	174.7	256.1	↑
8–10 weeks	136.1	184.5	272.6	↑
12–14 weeks	140.2	146.1	334.0	↑
23–29 weeks	122.9	173.1	321.9	↑
mEHHP				
Baseline	51.0	97.6	189.8	↑
8–10 weeks	54.8	108.9	158.5	↑
12–14 weeks	62.9	64.8	232.1	↑
23–29 weeks	47.5	76.2	261.1	↑
mEOHP				
Baseline	32.2	66.7	107.4	↑
8–10 weeks	34.9	62.2	106.4	↑
12–14 weeks	39.7	40.9	142.3	↑
23–29 weeks	30.0	53.2	144.8	↑
MEHP				
Baseline	6.3	19.4	17.5	
8–10 weeks	6.6	14.2	22.9	↑
12–14 weeks	7.4	11.8	24.6	↑
23–29 weeks	6.3	10.3	33.2	↑
Σ(DEHP3)[b]				
Baseline	311.1	602.8	1136.9	↑
8–10 weeks	328.6	605.4	1071.7	↑
12–14 weeks	387.1	394.5	1396.2	↑
23–29 weeks	290.5	474.9	1545.3	↑
MBP				
Baseline	37.5	58.9	75.2	↑
8–10 weeks	46.6	41.9	85.2	
12–14 weeks	37.0	62.0	72.7	↑
23–29 weeks	39.2	52.5	81.0	↑
MBzP				
Baseline	23.7	33.6	74.7	↑
8–10 weeks	16.8	37.7	78.1	↑
12–14 weeks	27.0	26.5	84.9	
23–29 weeks	23.4	24.3	90.8	↑

Surrogate sample	Geometric mean (ng/ml)			
	Low	Medium	High	
Phytoestrogens				
ETL				
Baseline	137.6	391.1	435.3	↑
8–10 weeks	167.5	246.7	567.0	↑
12–14 weeks	165.6	317.0	446.3	↑
23–29 weeks	141.0	365.3	454.9	↑
ETD				
Baseline	12.4	36.2	33.0	
8–10 weeks	14.2	22.0	47.6	↑
12–14 weeks	17.4	27.9	30.5	↑
23–29 weeks	17.6	19.2	43.4	↑
GNS				
Baseline	15.5	15.2	112.7	
8–10 weeks	11.5	35.4	65.6	↑
12–14 weeks	12.2	18.3	120.0	↑
23–29 weeks	11.0	28.7	84.7	↑
DAZ				
Baseline	31.2	44.5	128.2	↑
8–10 weeks	30.1	60.7	97.4	↑
12–14 weeks	32.3	48.6	113.5	↑
23–29 weeks	24.6	83.8	86.3	↑
EQU				
Baseline	10.2	16.4	20.1	↑
8–10 weeks	9.8	12.5	30.8	↑
12–14 weeks	8.8	16.6	22.8	↑
23–29 weeks	9.4	15.2	23.5	↑
DMA				
Baseline	1.2	2.9	7.2	↑
8–10 weeks	0.6	2.7	10.3	↑
12–14 weeks	0.7	2.6	10.6	↑
23–29 weeks	0.6	2.9	11.9	↑
Σ(DAZ)[c]				
Baseline	260.8	307.6	713.7	↑
8–10 weeks	232.5	402.7	611.6	↑
12–14 weeks	241.0	370.4	641.4	↑
23–29 weeks	209.6	435.0	627.9	↑

Surrogate sample	Geometric mean (ng/ml)			
	Low	Medium	High	
Phenols				
2,5-DCP				
Baseline	42.2	96.5	139.2	↑
8–10 weeks	32.8	64.7	267.5	↑
12–14 weeks	30.6	73.0	253.9	↑
23–29 weeks	40.4	65.0	215.8	↑
TCS				
Baseline	5.1	11.6	36.8	↑
8–10 weeks	5.4	11.3	32.2	↑
12–14 weeks	3.9	14.7	34.7	↑
23–29 weeks	7.3	13.2	20.6	↑
BP-3				
Baseline	3.8	7.6	31.0	↑
8–10 weeks	4.6	7.6	25.6	↑
12–14 weeks	3.0	12.8	23.4	↑
23–29 weeks	4.5	11.9	16.6	↑
BPA				
Baseline	2.6	3.6	3.7	↑
8–10 weeks	1.7	3.9	5.3	↑
12–14 weeks	1.6	2.6	8.1	↑
23–29 weeks	1.6	3.2	7.3	↑
2,4-DCP				
Baseline	2.5	2.6	3.4	↑
8–10 weeks	1.8	1.7	7.3	
12–14 weeks	1.5	2.2	6.6	↑
23–29 weeks	1.3	2.7	6.3	↑

Table 3 (*continued*)

Surrogate sample	Geometric mean (ng/ml)				Surrogate sample	Geometric mean (ng/ml)				Surrogate sample	Geometric mean (ng/ml)			
	Low	Medium	High			Low	Medium	High			Low	Medium	High	
MiBP														
Baseline	10.3	15.5	30.7	↑										
8–10 weeks	11.8	15.1	27.5	↑										
12–14 weeks	12.2	15.0	26.8	↑										
23–29 weeks	10.7	15.2	30.1	↑										
MCPP														
Baseline	4.3	5.4	10.3	↑										
8–10 weeks	4.9	4.9	9.9											
12–14 weeks	3.2	7.1	10.5	↑										
23–29 weeks	3.7	7.0	9.1	↑										

↑Indicates a monotonic increase in geometric means across tertiles based on Spearman correlation coefficients (see methods for details).

[a]Analyses were conducted on data from 24 children with four samples collected at the surrogate sample timepoints. The concentration distribution of the 24 samples collected at the surrogate sample time points were used to create tertile cutpoints for assignment of each child into low, medium, or high exposure level. The three exposure category means were computed using the 6-month mean concentrations of the individual children assigned to each exposure group ($N \cong 8$). All calculations were performed on natural-log transformed data and were back transformed to obtain geometric means.

[b]Di-2-ethylhexyl phthalate (DEHP) metabolites are mECPP, mEHHP, mEOHP, and mEHP. Sum is calculated on a molar basis (as nm/g creatinine or nm/liter).

[c]Daidzein metabolites are EQU and DMA. Sum is calculated on a molar basis (as nm/g creatinine or nm/l).

confirmation in studies conducted over a longer-time period is needed. Categorization of study participants according to their biomarker level has the potential to determine, not only whether exposure–disease relationships exist, but also whether dose–response associations are evident.

Our most reproducible biomarkers had ICCs between 0.4 and 0.6 (Table 2). For comparison, in a 3-month study using spot urine samples from adult males ICCs for mEP, mEHP, mBP, and mBzP were 0.5, 0.2, 0.3, and 0.4, respectively (Hauser et al., 2004) while the ICCs for the same phthalate metabolites measured in samples collected from adult women over 2 consecutive days were 0.6, 0.7, 0.8, and 0.5, respectively (Hoppin et al., 2002). It is not surprising that the biomarker ICCs were higher for such a short sampling interval, but it is encouraging to observe that our study ICCs for samples collected over 6 months are quite similar to those calculated for intervals that are half as long. It would be expected that ICCs for long-term biomarkers would be much higher as was seen in a 2-year study of children where ICCs for the majority of organochlorine compounds examined were between 0.8 and 1.0 and the ICC for lead was 0.7 (calculated from (Sexton et al., 2006)). In contrast, metabolites of less persistent pesticides such as organophosphates are not very reproducible over time, as intra-individual coefficients of variance for TCPy, a metabolite of chlorpyrifos, are wide in children (55–74%) and in adults (50–200%) (Adgate et al., 2001; MacIntosh et al., 2001). However, among children living in an agricultural community, the alkyl phosphate metabolites (from organophosphate pesticides) were not as variable, although their levels were affected by pesticide spraying seasons (Koch et al., 2002).

These data confirm previous findings of the omni-present exposure to phthalates (Centers for Disease Control and Prevention, 2005; Koch et al., 2003) and phytoestrogens (Centers for Disease Control and Prevention, 2005) as well as BPA (Calafat et al., 2005). Additionally we report on some novel environmental chemicals (e.g., BP-3 and TCS) for which limited information exists on concentration distributions in large populations. Many of the analytes examined in this study were also included in the Third National Report on Human Exposure to Environmental Chemicals, which presents biomonitoring data from a random sample of participants from the National Health and Nutrition Examination Survey (NHANES) conducted between 1999 and 2002 (Centers for Disease Control and Prevention, 2005). NHANES is designed to collect data on the health and nutritional status of the US population. In general, analyte concentrations in our NYC study population were of the same magnitude as the United States concentrations reported for over 300 6–11-year old children (Centers for Disease Control and Prevention, 2005). However, relative to the US general population, urinary concentrations of phthalate metabolites and phenols in our NYC children tended to be higher. For example, the median and 95th percentile uncorrected concentration of the three DEHP metabolites (mEHHP, mEOHP, and mEHP) were two to three times greater than the NHANES concentrations. Phtyoestrogen levels were similar or higher in the NHANES population. The concentrations for all three analyte families were comparable to a recent report on young girls of the same age range (Wolff et al., 2006); the NYC girls who participated in that study were not the same as those included in this report, but they were from the same urban area. The wide range of concentrations measured for almost all of the analytes provides further support for the use of these biomarkers of exposure in epidemiologic studies. A sufficiently wide concentration range is required not only to identify relationships between exposures and health outcomes, but also to examine dose-response, a key criterion for establishing a causal association.

It is conceivable that gender and race/ethnicity could influence exposure patterns or metabolism. Additionally, exposure sources for some of these biomarkers may vary throughout the year, for example, sunscreen use (a potential source of BP-3 exposure) might be higher during the summer months. However, adjustment for these characteristics did not change the ICCs calculated for any of the analytes (data not shown). Although the sample size and the sample collection interval are greater than those used for several of the other investigations of this type, there are limitations that must be considered when evaluating our results. Only Black and Hispanic children participated in this study, which is representative of the racial/ethnic distribution in East Harlem, NY, but is not representative of the general US population. The participating children were between the ages of 6 and 10, an age range where behavior patterns, and thus exposure patterns, can substantially vary from the youngest to the oldest. The small number of children in each year of age prevented us from examining age-related temporal patterns. Future methodological studies in both children and adults are needed to increase our knowledge of racial/ethnic, gender as well as age-related differences in temporal variability of these analytes so that we can improve our ability to interpret epidemiologic findings associated with these biomarkers of exposure.

In summary, this study has provided information on intra-individual variability in three families of chemicals, phthalates, phytoestrogens, and phenols. Previous research has indicated that urinary concentrations of phthalate metabolites are reproducible, although these studies included only adults, and, compared to the present study, had fewer samples, and the intervals of sample collection were shorter and fewer. Children's habits and bodies change rapidly, so that their exposure may change with age; however, this is less likely to be so for adults. Although additional data are required, the reproducibility over 6 months for the majority of the biomarkers examined

implies that they are likely to be consistent over at least this time period. In conclusion, the reasonable degree of temporal reliability and the wide range of concentrations of the ED analytes suggest that these biomarkers are appropriate for use in epidemiologic studies of ED exposures in relation to health outcomes in children.

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Pilot Study of Urinary Biomarkers of Phytoestrogens, Phthalates, and Phenols in Girls

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Background: Hormonally active environmental agents have been measured among U.S. children using exposure biomarkers in urine. However, little is known about their variation by race, age, sex, and geography, and no data exist for newly developed biomarkers.

Objective: Our goal was to characterize relevant, prevalent exposures for a study of female pubertal development.

Methods: In a pilot study among 90 girls from New York City, New York, Cincinnati, Ohio, and northern California, we measured 25 urinary analytes representing 22 separate agents from three chemical families: phytoestrogens, phthalates, and phenols. Exposures occur chiefly from the diet and from household or personal care products.

Results: Participants represented four racial/ethnic groups (Asian, black, Hispanic, white), with mean age of 7.77 years. Most analytes were detectable in > 94% of samples. The highest median concentrations for individual analytes in each family were for enterolactone (298 μg/L), monoethylphthalate (MEP; 83.2 μg/L), and benzophenone-3 (BP3; 14.7 μg/L). Few or no data have been reported previously for four metabolites: mono(2-ethyl-5-carboxypentyl) phthalate, triclosan, bisphenol A (BPA), and BP3; these were detected in 67–100% of samples with medians of 1.8–53.2 μg/L. After multivariate adjustment, two analytes, enterolactone and BPA, were higher among girls with body mass index < 85th reference percentile than those at or above the 85th percentile. Three phthalate metabolites differed by race/ethnicity [MEP, mono(2-ethylhexyl) phthalate, and mono-3-carboxypropylphthalate].

Conclusions: A wide spectrum of hormonally active exposure biomarkers were detectable and variable among young girls, with high maximal concentrations (> 1,000 μg/L) found for several analytes. They varied by characteristics that may be relevant to development.

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Effects of hormonally active environmental agents on early child development have been of concern, as knowledge has become available about their biological activity and about widespread exposure. For agents that are short-lived in the body (i.e., rapidly metabolized and/or eliminated), assessment of exposure biomarkers in urine is usually preferred for several reasons: The metabolites are readily detectable in urine, urine is easy to collect, and urine generally has higher concentrations of polar metabolites than other biologic media. Although exposures to many of these agents have been characterized in children [Centers for Disease Control and Prevention (CDC) 2005], little is known about variation of these exposure biomarkers by race, age, body mass index (BMI), and sex.

The Breast Cancer and the Environment Research Centers (BCERC) are a consortium established by the National Institute of Environmental Health Sciences and the National Cancer Institute to elucidate influences of environmental factors on early pubertal development in girls, and thereby possible future risk for breast cancer and other chronic diseases among women. For this purpose, the study design employs biomarkers to assess a variety of environmental exposures. The highest-priority urinary exposure biomarkers identified by the BCERC consortium are phytoestrogens, phthalate acids, and phenols. Agents in these groups were selected because they possess hormonal activity that may be agonistic or antagonistic (Fenton 2006; Rajapakse et al. 2002; Sohoni and Sumpter 1998); they have been detected at sufficiently high concentrations to constitute a potential risk (CDC 2005); and they were known or expected to have adequate interindividual variability to serve as exposure markers. Exposures to these chemicals occur chiefly through the diet and use of household or personal care products (Table 1) (Calafat et al. 2005; CDC 2005; Duty et al. 2005). The CDC has previously reported concentrations in child participants in the National Health and Nutrition Examination Survey (NHANES) for some biomarkers (CDC 2005). However, no data are available in children for certain phenols, including bisphenol A (BPA), a chemical with hormonal activity relevant to pubertal development (vom Saal and Hughes 2005). In addition, prevalence and variability of these exposure biomarkers have not been described among young girls, and it is not known how these exposures may vary by race, geographic location, or age. In this report we

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provide initial information on levels of biomarkers for three chemical families of primary interest—phytoestrogens, phthalates, and phenols—and on their distribution by demographic factors among a subsample of the BCERC study population.

Methods

Participants in this pilot study were among the first children enrolled at three BCERC centers. We are recruiting approximately 1,200 girls 6–8 years of age into a longitudinal study, with the aim of following pubertal development from its earliest stages through menarche. Eligibility includes age 6–8 years, female sex, and no underlying endocrine medical conditions. All sites obtained informed consent from parent or guardian and child assent, approved by each institution's institutional review board. Study designs and methods were standardized for most but not all components, because each center retained some unique scientific aims, and their recruitment began at different dates. Mount Sinai School of Medicine (MSSM) is recruiting black and Latina girls from clinics, schools, and community centers in East Harlem in New York City; the sample population from the University of Cincinnati/Cincinnati Children's Hospital (Cincinnati) is recruited from and examined at schools or through a breast cancer registry; the Kaiser Permanente Northern California (Kaiser) study group is recruited from the Kaiser health maintenance organization (HMO) membership in the San Francisco Bay area. At all sites, a baseline questionnaire was completed by the girl's parent or guardian (usually the mother) that included a detailed medical history, product use and exposures, exercise, diet, and demographic variables. Self-reported race/ethnicity included white, black, American Indian, Pacific Islander, or Asian, as well as Hispanic ethnicity. There were 10 Hispanics of Mexican origin at MSSM who did not report a race but who were identified by interviewers as Native Americans by ascertaining child and parental birthplace and native language (Mexican Indians). For the purposes of this report, race/ethnicity was classified as black (including black Hispanic), non-black Hispanic, non-Hispanic white, or non-Hispanic Asian.

Height and weight were measured using calibrated scales and stadiometers by interviewers who had been trained and certified uniformly across all three sites. BMI was calculated as weight/height-squared (kilograms per square meter) and then classified as < 85th national percentile, age- and sex-specific, or ≥ 85th percentile (Himes and Dietz 1994), using CDC growth charts (CDC 2000). In this CDC data set, the 85th percentile BMI cut points for girls in the first month of 6, 7, and 8 years of age are 17.100, 17.626, and 18.317 kg/m^2, respectively. Urine specimens were collected at the time of the baseline examination or in a 6-month follow-up visit (Cincinnati). MSSM and Kaiser collected spot specimens at baseline, and Cincinnati collected early morning voids. Each center submitted 30 urine samples to CDC for determining the concentrations of phthalate metabolites, phenols, phytoestrogens, and creatinine (to normalize for urine dilution). Samples from MSSM and Cincinnati were selected randomly from the samples donated before December 2005 with at least 40 mL of urine. Kaiser sent the first 30 samples collected with sufficient volume. The study size was limited by budgetary constraints and by the need to conduct the pilot study at an early stage of recruitment.

Laboratory techniques used by CDC for measuring the selected exposure biomarkers in urine have been published. Briefly, metabolites are deconjugated enzymatically, because these agents are excreted almost entirely as conjugated metabolites. Matrix removal and analyte enrichment are accomplished by solid phase extraction, and instrumental analysis is done with high performance liquid chromatography–tandem mass spectrometry using isotope dilution quantification (Kato et al. 2005; Kuklenyik et al. 2004; Rybak et al. 2006; Ye et al. 2005a). The laboratory is certified according to the Clinical Laboratories Improvement Act, and procedures incorporate quality control (QC) measures to ensure accuracy and precision of results, including annual proficiency testing compliance (Norrgran et al. 2006). A laboratory batch must meet quality control criteria, including acceptable blanks, or the batch is entirely reanalyzed. Results are blank-corrected. Enterodiol data for two girls were not available because these results did not fulfill the quality assurance/quality control requirements. Creatinine was measured using an enzymatic reaction on a Roche Hitachi 912 chemistry analyzer (Roche Hitachi, Basel Switzerland).

We performed statistical analyses using SAS (version 9.1.3 for PC; SAS Institute Inc., Cary, NC). Because of the unequal distribution of characteristics among sites, we first used nonparametric methods to examine variation of exposure biomarker concentrations in relation to study characteristics. For analytes detected in > 60% of samples, we then performed multivariate analyses adjusting for age, race/ethnicity, site, BMI, and season of sample collection using the general linear model (GLM) procedure, which accommodates unbalanced designs. For age, we computed Spearman correlations with each biomarker. Using the Kruskal-Wallis test of rank sums

Table 1. Biomarkers, their parent compounds, and examples of their environmental sources.

Chemical class and examples	Abbreviation	Main parent compound (if applicable)	Exposure sources
Phytoestrogens			Dietary intake
Isoflavones (genistein, daidzein)			Soy products, including processed meats, meat substitutes, breads, and protein food bars
Lignans (enterolactone)			Flax, seeds, grains
Phthalate metabolites			Industrial and personal product additives
Mono(2-ethylhexyl) phthalate	MEHP	Di(2-ethylhexyl) phthalate (DEHP)	Soft plastic including tubing, especially polyvinyl chloride (PVC; e.g., sometimes present in clear food wrap)
Mono(3-carboxypropyl) phthalate	MCPP	Di-*n*-octyl phthalate	Soft plastic
Monobenzyl phthalate	MBzP	Butylbenzyl phthalate	Vinyl flooring, adhesives
Monoethyl phthalate	MEP	Diethyl phthalate (DEP)	Shampoo, scents, soap, lotion, cosmetics
Monomethyl phthalate	MMP	Dimethyl phthalate (DMP)	Insect repellant, plastic
Mono-*n*-butyl phthalate, mono-isobutyl phthalate	MBP, MiBP	Dibutyl phthalate (DBP), diisobutyl phthalate (DiBP)	Adhesives, caulk, cosmetics
Phenols			Commercial and personal products and additives
Bisphenol A	BPA		Polycarbonate containers and coatings (cans, cups), dental sealant
Benzophenone-3(2-hydroxy-4-methoxy-benzophenone)	BP3		Sunscreen
Triclosan [5-chloro-2-(2,4-dichlorophenoxy)phenol]	TRCS		Microbicide in cleaning fluids
2,4-Dichlorophenol and trichlorophenols	24DCP, 245TCP, 246TCP	Phenoxy- and other derivatives	Herbicides
2,5-Dichlorophenol	25DCP	4-dichlorobenzene	Mothballs
ortho-Phenylphenol	*o*-PP		Fungicide
4-*tert*-Octylphenol	4-*t*-OP		Detergent surfactant

(NPAR1WAY procedure with Wilcoxon option), we compared medians of the creatinine-corrected concentrations (micrograms per gram creatinine) by race/ethnicity, site, BMI, and season (coded as Summer = June, July, August vs. other seasons; use of several products listed in Table 1 was considered likely to vary by season). For exposure biomarkers whose medians exhibited differences with respect to these characteristics [see Supplemental Materials, Table 1 (http://www.ehponline.org/docs/2006/9488/suppl.pdf)], we then examined whether the multivariate geometric means were significantly different across characteristic levels using the LSMEANS option of the GLM procedure. Four Asians were retained in the multivariate analyses, with racial/ethnic differences reported only for blacks, Hispanics, and whites but not Asians. In parametric analyses, we used log-transformed values of the urinary metabolite concentrations to normalize the distribution and we substituted the value LOD/√2 for results below the limit of detection (LOD) following the CDC practice (Wolff et al. 2005).

Results

Participants represented four racial/ethnic groups (Asian, black, Hispanic, white) and three geographic locations (New York City, Cincinnati metropolitan area, and the San Francisco Bay area of California; Table 2). Mean age was 7.77 years at date of sample collection (range, 6.4–9.2). Samples collected at Kaiser were almost all from 7-year-old girls (29 of 30); there were two 6-year-old girls from Cincinnati and nine from MSSM. Race/ethnicity varied by site, with Kaiser and Cincinnati girls mainly white (> 60%) or black, and with no whites at MSSM. All four Asians were from Kaiser, and most Hispanics (18 of 22) were from MSSM. Compared with national data (CDC 2000), 32% of girls were ≥ 85th percentile of BMI, and the distributions of BMI within sites were similar. Samples from Kaiser were all collected in summer; no samples from Cincinnati and nine from MSSM were collected in summer.

Eighteen of the 25 analytes were detected in at least 94% of the samples (Table 3). Phytoestrogens as a group had the highest concentrations (e.g., median 298 µg/L for enterolactone), and all six phytoestrogens were detected in > 98% of samples. Phthalate metabolites were intermediate in concentration, with 9 of the 10 biomarkers detected in > 94% of samples. Phenols had the lowest concentrations and were least detected (only 3 of the 9 were detected in > 94% of samples). Seventeen exposure biomarkers had medians > 10 µg/L (10 ppb), and six had medians > 50 µg/L. Four phytoestrogens, four phthalates, and two phenols had maximum values > 1,000 µg/L (1 ppm). The ranges for 10 of 25 exposure biomarkers encompassed at least 3 orders of magnitude (e.g., 1–1,000 µg/L). The highest individual biomarker measurement was for benzophenone-3 (BP3; 26,700 µg/L). To verify that the very high BP3 urinary measurements reflected absorbed dose rather than surface contamination during sample collection, storage, or analysis, we measured free (i.e., unbound) BP3 in the nine samples having concentrations >1,000 µg/L. The concentrations of unbound BP3 were minimal, < 1–20 µg/L (data not shown), consistent with excretion of absorbed BP3 as conjugated species (Ye et al. 2005b).

Multivariate adjusted concentrations of creatinine-corrected exposure biomarkers (geometric means) are presented in Table 4 according to race/ethnicity, geographic site, BMI, and season of collection; included are the 20 analytes that were detected in at least 60% of samples. Differences in the medians (unadjusted) by characteristics are shown in the Supplemental Material, Table 1 (http://www.ehponline.org/docs/2006/9488/suppl.pdf). Compared with the unadjusted values, there were fewer significant associations in the multivariate models for race (5 vs. 8), site (3 vs. 9), and season (1 vs. 6). Enterolactone and BPA differed significantly with regard to BMI (< 85th percentile vs. ≥ 85th percentile). The adjusted geometric means of three phthalate metabolites varied by race/ethnicity, with whites having lower concentrations of mono(2-ethylhexyl) phthalate (MEHP) and monoethyl phthalate (MEP) but higher mono(3-carboxypropyl) phthalate (MCPP). Among the phenols, 2,5-dichlorophenol (25DCP) was higher in blacks than whites, and BP3 was higher in whites. *O*-Desmethylangolensin (*O*-DMA), 25DCP, and 2,4-dichlorophenol (24DCP) differed across the three study sites. BP3 was higher in samples collected in summer. Patterns by race and season for MEHP, MEP, MCPP, 25DCP, and 24DCP remained the same if the geometric means were adjusted for age, race, BMI, and season but not for site. When the correlation between age as a continuous variable and each analyte was examined, it was significantly associated only with equol (micrograms equol/grams creatinine; r_S –0.26, p = 0.013). In the multivariate model for equol [ln, micrograms per gram creatinine], the beta for age (years) was –0.44 (p = 0.029, adjusted for race/ethnicity, geographic site, BMI, and season of collection).

The strongest correlations between individual biomarkers within a family were seen among those arising from the same parent compound (e.g., r_S = 0.79–0.99 among four di(2-ethylhexyl) phthalate [DEHP] metabolites: mono(2-ethyl-5-carboxypentyl) phthalate [MECPP], mono(2-ethyl-5-hydroxyhexyl) phthalate [MEHHP], (2-ethyl-5-oxohexyl) phthalate [MEOHP], and MEHP; data not shown). We computed correlations between creatinine and the urinary exposure biomarkers to examine the appropriateness of creatinine-corrections for dilution. The lowest correlations were seen for BP3 (r_S = –0.03, p = 0.758) and *O*-DMA (r_S = 0.24, p = 0.022); correlations of creatinine with other biomarkers were fairly strong (r_S > 0.3, p < 0.01; data not shown). Associations of phthalate metabolites with creatinine were stronger (r_S = 0.50–0.72) than for phytoestrogens (r_S = 0.33–0.52, not including *O*-DMA) and phenols (r_S = 0.42–0.54 for triclosan, 25DCP, BPA, and 24DCP). Because BP3 was not related to urinary creatinine, it may be inappropriate to correct for dilution using creatinine (Hauser et al. 2004; Miller et al. 2004). When we examined

Table 2. Characteristics of the study population, BCERC pilot study, 2004–2005.

Characteristic	No. (%)	No. per site		
		MSSM	Cincinnati	Kaiser
Age at sample collection (years)[a]				
6.0–6.9	11 (12.2)	9	2	0
7.0–7.9	57 (63.3)	9	19	29
≥ 8.0	22 (24.4)	12	9	1
Race/ethnicity				
Asian	4 (4.4)	0	0	4
Black	26 (28.9)	12	9	5
Hispanic	22 (24.4)	18	1	3
White	38 (42.2)	0	20	18
Site				
Cincinnati	30 (33.3)			
MSSM	30 (33.3)			
Kaiser	30 (33.3)			
BMI for age[b]				
< 85th percentile	61 (67.8)	21	19	21
≥ 85th percentile	29 (32.2)	9	11	9
Collection time[c]				
June–August	39 (43.3)	9	0	30
Other months	51 (56.7)	21	30	0

[a]Some samples were collected at a visit 6 months after the baseline visit. [b]Available: http://www.cdc.gov/growthcharts/ (CDC 2000). [c]October 2004 to September 2005.

BP3 in relation to the characteristics in Table 4 using the concentration as micrograms per liter (uncorrected for creatinine), we obtained almost identical results. The range of creatinine was 7.6–255 mg/dL with 9 samples < 20 mg/dL, which could potentially influence the data in Tables 3 and 4 by overinflating the creatinine-corrected values. We examined the distribution of values for the nine low-creatinine samples in those exposure biomarkers that varied significantly by the factors in Table 4; they were fairly evenly distributed in terms of concentrations, and excluding them from the multivariate adjusted models did not alter the differences seen in the exposure biomarkers with regard to characteristics described above. In addition, in models where biomarkers were not creatinine-corrected (micrograms per liter), we observed results similar to those in Table 4, except that two associations were not significant (BPA with BMI, $p = 0.11$; and 24DCP by site, $p = 0.13$).

Discussion

This pilot study of peripubertal girls examined urinary biomarkers of exposures among three chemical families that possess known or likely hormonal activity. Biomarkers from these families appear to be ubiquitous, have wide variability, and show relatively high urinary concentrations in 6- to 9-year-old girls, suggesting that they are suitable for study of exposure–outcome associations related to puberty. Among the 25 exposure biomarkers measured in this study, we had initially identified eight compounds as high priority for the epidemiologic study, based on criteria of having prevalent, high exposure levels, toxicologic relevance, and exposure biomarker reliability. These included three phytoestrogens (enterolactone, daidzein, genistein), three phthalate metabolites (mono-*n*-butyl phthalate [MBP], monobenzyl phthalate [MBzP], MEP), and two phenols (BPA, nonylphenol). Seven of these biomarkers were detected in at least 94% of samples, and the ranges of concentrations were wide, from the LOD (< 1) to > 26,000 µg/L (minimum–maximum). Nonylphenol was not determined because CDC as yet has no optimal biomarker for this compound (Calafat et al. 2005).

Levels of phytoestrogen and phthalate metabolites in this study were similar to those reported in the NHANES 2001–2002 children (CDC 2005), although enterodiol appeared to be higher and MBzP and monomethyl phthalate (MMP) lower in our study population. MECPP and MEP had the highest levels of the 10 phthalate metabolites measured. MECPP, a DEHP metabolite, has not been previously reported in NHANES nor in school-age children. The relationship of equol with age could be of interest, but it was the only analyte related to age. This association could also be attributable to population characteristics that can be explored in the future, such as diet. Two biomarkers (enterolactone and BPA) varied by BMI, and three phthalates differed by race. Relationships of phthalates with race/ethnicity were quite similar to those reported for all ages in the NHANES 2001–2002—for example, MEP and MEHP were highest in blacks and MCPP highest in whites (CDC 2005) (the CDC report does not provide race-specific data for children). One biomarker varied by season (BP3) and three by site (*O*-DMA, 25DCP, 24DCP), differences that may reflect diverse exposures; alternatively, these observations may be attributed to the unequal distribution of characteristics by site, a notion supported by the finding that both BP3 and 25DCP also differed by race. Differences by race and BMI may also be attributed to other confounding factors, including socioeconomic status (SES), that were not available for consideration. SES may affect body size, dietary habits, and product use, for example.

Among the four DEHP metabolites measured, MEHP differed significantly by race after multivariate adjustment, whereas the trends for the three oxidative metabolites of DEHP were similar but not statistically significant (Table 4). It is possible that we did not detect significant racial/ethnic trends for the three DEHP oxidative metabolites because of limited sample

Table 3. Distribution of BCERC phytoestrogen, phthalate, and phenol and biomarkers for all sites combined, 2004–2005.

Analyte	No.	No. > LOD	LOD (µg/L)	Percent > LOD	Range (µg/L) Low	Range (µg/L) High	Median (µg/L)	Geometric mean [GSD (µg/L)]	Geometric mean [GSD (µg/gC)]	NHANES 50th percentile[a] (µg/L)
Phytoestrogens										
Enterolactone	90	90	0.3	100.0	4.6	6730.0	298.0	269.0 (4.1)	420.0 (3.7)	329.0
Daidzein	90	90	0.3	100.0	2.4	9690.0	98.0	112.0 (5.7)	175.0 (5.2)	72.7
Enterodiol	88	88	0.3	100.0	1.0	548.0	63.7	54.8 (3.3)	86.5 (2.8)	35.4
Genistein	90	90	0.3	100.0	1.2	5360.0	50.1	60.4 (5.4)	94.3 (4.7)	31.5
Equol	90	89	0.3	98.9	0.2	485.0	10.5	10.9 (4.1)	17.0 (3.5)	13.6
O-DMA	90	89	0.2	98.9	0.1	3210.0	5.7	5.7 (9.3)	8.9 (8.6)	5.7
Phthalates										
MECPP[b]	90	90	0.25	100.0	5.9	2260.0	53.2	50.3 (3.1)	78.5 (2.5)	
MEHHP[b]	90	90	0.32	100.0	1.4	1699.0	25.9	28.0 (3.4)	43.8 (2.7)	32.9
MEOHP[b]	90	90	0.45	100.0	1.3	1070.0	17.8	18.8 (3.3)	29.3 (2.6)	22.6
MEHP[b]	90	85	0.90	94.4	0.6	110.0	3.2	3.3 (3.0)	5.2 (2.7)	4.4
MEP	90	90	0.40	100.0	5.3	2580.0	83.2	75.7 (3.9)	118.0 (3.1)	71.9
MBP	90	88	0.40	97.8	0.3	363.0	37.4	28.2 (3.4)	44.1 (2.4)	32.4
MBZP	90	89	0.11	98.9	0.1	191.0	22.2	18.4 (4.0)	28.7 (2.8)	37.0
MIBP	90	87	0.26	96.7	0.2	144.0	7.7	7.1 (3.6)	11.1 (2.5)	4.4
MCPP	90	90	0.16	100.0	0.4	76.9	6.3	6.1 (2.9)	9.5 (2.1)	6.6
MMP	90	18	1.00	20.0	< LOD	15.6	< LOD	< LOD	< LOD	1.8
Phenols										
BP3	90	86	0.34	95.6	< 0.2	26700.0	14.7	19.7 (14.6)	30.8 (15.5)	82.3
Triclosan	90	61	2.27	67.8	< 1.6	956.0	7.2	10.9 (6.5)	17.1 (5.5)	12.5
25DCP	90	88	0.12	97.8	< 0.1	3120.0	7.1	9.0 (11.9)	14.0 (9.9)	3.1
BPA	90	85	0.36	94.4	< 0.3	54.3	1.8	2.0 (3.2)	3.0 (3.0)	2.4
24DCP	90	70	0.17	77.8	< 0.1	92.7	0.9	0.9 (5.3)	1.4 (4.3)	0.6
246TCP	90	22	0.50	24.4	< LOD	6.1	< LOD	< LOD	< LOD	0.3
245TCP	90	21	0.10	23.3	< LOD	1.2	< LOD	< LOD	< LOD	0.1
4-*t*-OP	90	5	0.17	5.6	< LOD	0.4	< LOD	< LOD	< LOD	0.5
o-PP	90	3	0.10	3.3	< LOD	2.5	< LOD	< LOD	< LOD	0.4
Creatinine (mg/dL)	90				7.6	254.8	76.2			

Abbreviations: GSD, geometric standard deviation; µg/gC, the urinary concentration (µg/L) corrected for creatinine (g/L).
[a]NHANES 50th percentile values for phthalates and phytoestrogens for 6- to 11-year-old children obtained from the Third National Report on Human Exposure to Environmental Chemicals (CDC 2005); 50th-percentile values for phenols obtained from Ye et al. (2005a). [b]Derived from DEHP.

size. It is also possible for MEHP to arise from sample contamination or hydrolysis, whereas the oxidative metabolites must be endogenous; however, this is unlikely to explain racial/ethnic variability. Alternatively, although overall exposures to DEHP may have been fairly uniform, recent ambient exposures to DEHP may have varied by race/ethnicity in this population. In another study, racial/ethnic differences in MEP levels were attributed to greater use of cologne by blacks and Hispanics (Duty et al. 2005). If recent exposures were responsible for the racial/ethnic differences in our data, a possibility would be that MEHP as the first metabolite formed might differ by race/ethnicity, reflecting mainly the most recent exposures. Another possible explanation is that there may be racial/ethnic variability in the primary but not the secondary DEHP metabolic routes. MEHP is the initial metabolite of DEHP, and it can undergo further oxidation through separate pathways, producing MECPP by one route and MEHHP and MEOHP by another. Racial/ethnic differences in the secondary pathways could be smaller than for MEHP formation. In addition, the oxidative metabolites have longer half-lives [10–15 hr vs. 5 hr for MEHP (Koch et al. 2006)], possibly constituting a more integrated measure of long-term exposures that vary less by race/ethnicity.

The high correlations observed among four DEHP metabolites signify a common source of exposure from the parent compound (Silva et al. 2006). The proportion of MECPP (> 50% of the total of four metabolites reported here) is consistent with previous research suggesting that infants have a much higher proportion of MECPP (66%) than adults (32%) (Silva et al. 2006). Among other phthalate metabolites, MCPP and MEP, which differed significantly by race but in different directions, were not highly correlated with each other (r_S = 0.33), supporting different product origins and environmental exposures for these agents.

The relationships of the urinary exposure biomarkers to creatinine levels are of interest. One site collected early-morning (but not first-morning) voids, which may reflect different accumulated exposures than daytime spot samples that creatinine correction cannot resolve entirely. However, collection time is not likely to bias our exposure measures, because the adjusted means differed by site for only three analytes in two chemical families, and specific exposures are as likely to explain these findings as collection time. As recognized by others, care must be taken in normalizing urinary analytes for dilution because the excretory mechanisms are not the same for creatinine and certain chemicals (Hauser et al. 2004; Miller et al. 2004). This possibility is evident in our study where the correlations between creatinine and endocrine disruptor biomarkers varied, from zero (BP3) to 0.72 (mono-isobutyl phthalate [MiBP]), suggesting that excretion or metabolic capacity may affect these associations, possibly related to the amount of bound (e.g., glucuronidated) versus unbound metabolites. However, in agreement with previous research (Ye et al. 2005b), our data show that even at very high concentrations, BP3 is excreted mostly conjugated in urine; in contrast, MEP is mostly unconjugated (Silva et al. 2003). Unlike creatinine, glucuronide conjugates are actively excreted by tubular secretion, which may explain in part the low correlation of BP3 with creatinine as well as the wide range of correlations between creatinine and these biomarkers (see Hauser et al. 2004 and references therein). An alternative to correcting for urinary concentration is urinary specific gravity (Hauser et al. 2004; Miller et al. 2004), but this measurement was not performed on our samples.

The differences in exposure biomarkers by race/ethnicity and BMI are potentially relevant to pubertal development which is known to be associated with these characteristics (Herman-Giddens et al. 1997). Furthermore, the associations of enterolactone and BPA with BMI and of phthalate metabolites with race are notable because these biomarkers did not vary by other factors in our data. Although the differences we observed are suggestive only, because the sample size is small and unbalanced with regard to some characteristics, we used a conservative approach, considering only a limited number of *a priori* comparisons. Furthermore, the findings for phthalate metabolites were consistent with earlier reports. In general, concentrations of the 25 urinary exposure biomarkers we measured are far higher than those of more widely studied environmental agents such as

Table 4. Geometric means (μg/g creatinine) of phytoestrogen, phthalate, and phenol biomarkers (those ≥ 60% LOD) adjusted for age, race, site, BMI, and season, 2004–2005.

		Race/ethnicity			Site			BMI for age		Season of sample collection	
Total (n = 90)	Asian (n = 4)	Black (n = 26)	Hispanic (n = 22)	White (n = 38)	Cincinnati (n = 30)	MSSM (n = 30)	Kaiser (n = 30)	< 85th reference percentile (n = 61)	≥ 85th reference percentile (n = 29)	Summer (n = 39)	Other season (n = 51)
Phytoestrogens											
Enterolactone	174.0	414.0	388.0	287.0	292.0	185.0	495.0	513.0	174.0*	236.0	378.0
Daidzein	53.4	166.0	188.0	205.0	154.0	69.8	234.0	115.0	161.0	97.1	190.0
Enterodiol	157.0	58.5	81.5	97.1	113.0	65.4	107.0	107.0	79.9	99.9	85.4
Genistein	52.2	83.7	96.8	111.0	81.8	56.3	123.0	68.4	100.0	55.5	123.0
Equol	12.1	10.7	20.2	17.9	17.9	20.1	8.9	16.7	12.9	10.7	20.2
O-DMA	0.9	12.0	6.6	7.5	7.1	1.6	10.1*	6.7	3.5	3.5	6.8
Phthalates											
MECPP[a]	119.0	80.7	98.1	69.2	91.5	111.0	71.5	86.6	93.4	100.0	80.9
MEHHP[a]	58.2	55.0	43.3	42.0	48.3	64.8	37.8	43.0	56.1	53.0	45.5
MEOHP[a]	37.3	33.8	29.0	29.0	31.6	41.5	25.2	28.8	35.8	33.6	30.6
MEHP[a]	8.3	7.9	4.5	4.3*	5.9	7.2	5.0	5.5	6.5	6.4	5.6
MEP	66.1	194.0	231.0	73.5*	161.0	100.0	111.0	102.0	144.0	140.0	105.0
MBP	48.2	39.0	50.9	44.4	46.7	49.7	40.3	43.3	47.6	42.1	48.9
MBZP	8.0	24.2	33.2	35.7	24.9	22.3	18.9	20.4	23.5	21.1	22.7
MiBP	15.3	10.1	11.3	11.8	10.4	14.6	11.3	10.8	13.3	10.2	14.1
MCPP	5.5	6.5	9.6	12.0*	9.3	7.8	7.2	8.8	7.3	8.5	7.6
Phenols											
BP3	42.2	21.7	14.9	92.7*	14.5	25.6	101.0	26.9	41.8	83.8	13.4*
Triclosan	6.7	22.0	16.3	14.3	21.2	14.8	8.0	15.8	11.7	13.3	13.9
25DCP	16.2	28.5	15.6	8.8*	6.7	85.0	7.1*	12.3	20.6	10.9	23.0
BPA	2.7	3.1	3.4	2.3	3.2	2.6	2.8	3.7	2.2*	2.9	2.8
24DCP	1.5	1.7	1.3	1.2	0.9	3.4	0.9*	1.3	1.6	1.1	1.8

[a]All derived from DEHP. *p-Value < 0.05 for one or more LSMEANS tests between characteristic levels (for race, significance is not indicated if it was found only for Asians).

1,1′-dichloro-2,2′-bis(4-chlorophenyl)ethylene (DDE) and elemental lead (CDC 2005). In addition, some of these agents have relatively potent hormonal activity. In yeast assays, for example, BPA and butylbenzyl phthalate (the parent compound of MBzP) have greater antiandrogenic and estrogenic activity than DDE (Sohoni and Sumpter 1998). However, the proportional biological effects of these exposures in humans are not known. Several exposure biomarkers reported here have not previously been measured in children nor in different parts of the United States. On the basis of this pilot study, we are considering the potential relevance to child development of additional exposures that were not originally planned for study, and we are exploring alternatives to creatinine correction. If we identify any of these biomarkers as either protective or detrimental in terms of child maturation, the levels of these chemicals in the body may be modifiable because they are derived from the diet or ambient environment or from personal product use. Additional studies are under way to identify sources of these agents in our population and to assess the temporal variation of urinary metabolites among children.

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EPIDEMIOLOGY

Urinary Phthalate Metabolite Concentrations and Reported Use of Personal Care Products

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Objective:

Public health concern about widespread phthalates exposure has grown due to reported adverse developmental outcomes and reproductive toxicity. Phthalates are ingredients in many personal care products. This study's objective was to identify whether self-reported use of products potentially containing phthalates is associated with urinary phthalate metabolite concentrations.

Material and Methods:

Healthy Hispanic and black children (age range: 6-10 years) were recruited from East Harlem, New York City. Spot urine samples were collected and the parent/guardian provided information on the child's personal care product use (N = 33). Urine samples were analyzed at the Centers for Disease Control and Prevention. The association between product use in the past week and phthalate metabolite concentrations (creatinine-corrected and natural log transformed) was examined using linear regression models or Wilcoxon 2-sample tests.

Results:

During the week before the interview, more than 40% of the children had used shampoo, moisturizer, chapstick, hair conditioner, or fragrance. More frequent shampoo use was significantly associated with increased concentration of 3 di-2-ethylhexyl phthalate (DEHP) metabolites, mono-2-ethyl-5-hydroxyhexyl phthalate (mEHHP), mono-2-ethyl-5-oxohexyl phthalate (mEOHP), and mono-2-ethylhexyl phthalate (mEHP) ($P < 0.05$). By using back-transformation on the regression parameters, it was found that the average concentration in ig/gC for children who did not use shampoo compared with those who used shampoo for 3 and 7 days were 48.6, 99.2, and 256.5 (mEHHP); 32.5, 63.6, and 156.2 (mEOHP); and 10.8, 18.7, and 39.3 (mEHP). Adjustment for age, gender, or race/ethnicity did not materially change the associations. Among girls, the 16% who used nail polish had significantly higher levels of these 3 metabolites than nonusers ($P < 0.05$).

Conclusions:

Preliminary examination identified associations between reported personal care product use and increased concentration of several urinary phthalate metabolites. These analyses will be expanded using additional data available from subsequent visits.

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Using Structural Equation Modeling to Examine the Role of One-Carbon Metabolism in Breast Cancer Risk

Teitelbaum SL, Gammon MD, Xu X, Neugut AI, Wetmur J, Bradshaw P, Wallenstein S, Santella RP, Zeisel S, Zhang Y, Chen J

One-carbon metabolism is a network of interrelated biological reactions in which a one-carbon moiety is transferred among a series of folate-derived compounds. It facilitates the cross-talk between DNA synthesis (genetics) and DNA methylation (epigenetics), both of which are essential processes in breast cancer etiology. Using the Long Island Breast Cancer Study Project (LIBCSP), a large population-based breast cancer case-control study, we have identified several genetic polymorphisms as well as dietary micronutrients involved in the one-carbon metabolism pathway that are associated with risk of breast cancer. For example, we observed a significantly reduced risk of breast cancer associated independently with the *MTHFR 677T* allele as well as high folate consumption among non-vitamin users; we also observed multiplicative interaction between these two factors.

Elements of this important pathway have been studied in relation to breast cancer risk on an individual basis or as an interaction between several factors; however, to the best of our knowledge, examination of the interrelationship among the entire group of potential risk factors has yet to be conducted. Therefore using the available information from the LIBCSP we are developing a pathway model to study the complex relationship among environmental as well as genetic variables involved in one-carbon metabolism. We have identified several genetic polymorphisms, dietary factors as well as breast cancer risk factors to be included in the modeling process. Our goal is to test and then refine this proposed pathway model using structural equation modeling.

As a first step in refining our proposed pathway model, we used multiple logistic regression modeling to identify the set of variables that significantly contribute to breast cancer risk. The resulting reduced set of variables included age, history of benign breast disease, education, family history of breast cancer, vitamin intake (B_1, B_2 and B_{12}), and 6 genetic polymorphisms [*MTHFR 677C>T, MTHFR 1298A>C, TYMS 5'UTR, DHFR19bp del, MTR 2756A>G, RFC1 80A>G, cSHMT 1420C>T*]. Next, we conducted a principal components analysis to identify underlying factors among the variables. Preliminary results of this analysis identified three factors that explained approximately 60% of the variance among these variables, providing additional support for the proposed model. The final step of building the regression models from the refined pathway model is currently ongoing.

Breast cancer is a complex disease that is not caused by a single risk factor. Examining individual components of the one-carbon metabolism pathway as breast cancer risk factors or even looking at interactions between two or more of these factors ignores the complex interrelationship of this biological process. The use of structural equation modeling that is based on a solid understanding of one-carbon metabolism may provide a way to examine this multifaceted process. Using this approach, we hope to better elucidate one-carbon metabolism's role in breast cancer etiology and provide encouragement for using this innovative method to examine other complex pathways that may play a role in breast cancer development.

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Phthalate Metabolites and Body Size Characteristics in Urban Minority Girls

Susan Teitelbaum, Julie Britton, Nita Vangeepuram, Barbara Brenner, Manori Silva, Antonia Calafat, Mary Wolff

According to the CDC, the prevalence of overweight among U.S. children aged 6-11 years has risen from 6.5% in 1976-1980 to 18.8% in 2003-2004. Overweight children are at increased risk of becoming overweight adults which in turn puts them at increased risk for many chronic diseases including diabetes, heart disease and cancer. Energy imbalance (more calories consumed than used) is considered the main contributor to becoming overweight. However, genetics and environmental factors may also play a role. Phthalates, chemicals found in many consumer products, flexible plastics and fragrances, have the potential to disturb endogenous hormonal levels and may influence the development of obesity by affecting insulin sensitivity. A recent report using the NHANES 2001-2002 cross-sectional data in men supports this hypothesis. Given the increasing rates of overweight and the ubiquitous exposure to phthalates, we are investigating the relationship between phthalate exposure and body size characteristics among young girls.

Methods: Growing Up Healthy (GUH) is a prospective cohort study of Hispanic and Black, New York City girls between 6 and 8 years old. GUH is part of a NIEHS/NCI funded consortium (Breast Cancer and the Environment Research Centers) of three centers across the U.S. At baseline, parents/guardians were interviewed in-person, in either English or Spanish, about the girls' environmental exposures, physical activity, medical history, and demographics. Anthropometric measurements (weight, height, waist (WC) and hip circumference (HC)), using a standardized protocol, were made by trained interviewers. Body mass index (BMI: weight/height^2) and BMI percentile (CDC age and gender specific) were calculated. Casual urine samples provided by each girl were analyzed by the Centers for Disease Control and Prevention for a panel of environmental exposure biomarkers, including 9 phthalate metabolites.

The molar sums of low and high molecular weight phthalate metabolites, (LoMWP:mEP, mBP, miBP and HiMWP:mBzP, mCPP, mEHP, mEHHP, mEOHP, mECPP, respectively) were calculated. Cross-sectional analysis using baseline data was conducted for this report. Age and race-ethnicity adjusted geometric means for each phthalate metabolite as well as LoMWP and HiMWP were compared between girls in the upper quartile of each body size characteristic (BSC) and those in the combined lower 3 quartiles using generalized linear models. Adjusted geometric means (ng/ml or nmoles/ml) and 95%CI are presented.

Results: Data from 398 girls were available for analysis from 412 girls enrolled at baseline. Adjusted mEP geometric means (BSC Q4 vs. BSC Q1-3) were significantly higher for BMI [271.2(176.2-417.5) vs. 155.7(127.2-190.7); p= 0.05], HC [275.6(186.4-407.6) vs. 154.3(127.0-187.5); p=0.02] and WC [291.5(196.8-431.8) vs. 151.9(125.3-184.3); p=0.01]. Similar differences were observed for the LoMWP, primarly due to mEP's contribution to this sum. Adjusted HiMWP geometric means were elevated for girls in the upper quartile of HC and WC [1.198 (0.833-1.723) vs. 0.764 (0.638-0.916) p=0.05; 1.342 (0.932-1.932) vs. 0.737 (0.616-0.882) p=0.01, respectively].

Discussion: These findings suggest that phthalate exposure may contribute to increased overweight in children. However given the cross-sectional design of this analysis, we expect that our longitudinal data, when it becomes available, will help to clarify this association.

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Phthalate Metabolites and Asthma in Urban Minority Girls

Susan Teitelbaum, Julie Britton, Nita Vangeepuram, Rebecca Bausell, Barbara Brenner, Antonia Calafat, Mary Wolff

Asthma is a chronic respiratory condition that affects more than six-million U.S. children, making it one of the most common diseases of childhood. Epidemiological data show that the prevalence of asthma has increased markedly since the 1980s and while its etiology is not completely understood, environmental factors such as second hand smoke, dust mites, and chemical toxins have been associated with the frequency and severity of symptoms. Phthalates, which are respiratory toxins, are present in many consumer products as well as many common household materials such as plastics and fragrances. Given the rising rates of asthma and the universality of human exposure to phthalates, we are investigating the relationship between phthalate exposure and asthma among young girls.

Methods: Growing Up Healthy (GUH) is a prospective cohort study of Hispanic and Black, New York City girls between 6 and 8 years old. GUH is part of a NIEHS/NCI funded consortium (Breast Cancer and the Environment Research Centers) of three centers across the U.S. At baseline, parents/guardians were interviewed in-person, in either English or Spanish, about the girls' environmental exposures, physical activity, medical history, and demographics. Doctor diagnosed asthma and asthma-related symptoms were ascertained. Anthropometric measurements, using a standardized protocol, were made by trained interviewers. Casual urine samples provided by each girl were analyzed by the Centers for Disease Control and Prevention for a panel of environmental exposure biomarkers, including 9 phthalate metabolites.

The molar sums of low and high molecular weight phthalate metabolites, (LoMWP:mEP, mBP, miBP and HiMWP:mBzP, mCPP, mEHP, mEHHP, mEOHP, mECPP, respectively) were calculated. Cross-sectional analysis using baseline data was conducted for this report. Age/race-ethnicity/BMI adjusted geometric means for each phthalate metabolite as well as LoMWP and HiMWP were compared between girls with and without reported asthma or asthma-related symptoms using generalized linear models. Adjusted geometric means (nmoles/ml) and 95%CI are presented.

Results: Data from 398 girls were available for analysis from 412 girls enrolled at baseline. Adjusted geometric means for HiMWP were significantly different for doctor diagnosed asthma [Yes:1.058 (0.821-1.362 vs. No:0.801 (0.686-0.935)); p=0.05], cough at night not associated with a cold [Yes:1.031 (0.824-1.290) vs. No:0.784 (0.667-0.923);p=0.04], and at least one of four reported asthma-related symptoms [Yes:0.976 (0.811-1.174) vs. No:0.757 (0.630-0.908);p=0.04]. There were no LoMWP geometric mean differences for any of the asthma-related symptoms or doctor diagnosed asthma.

Discussion: Our results are supported by a reported relationship between DEHP in household dust and asthma or other respiratory problems as well as a suggested a link between phthalate containing PVC flooring/plastic wall material and respiratory problems, both among children. Public concern about phthalate exposure and child health has been growing mainly due to detrimental reproductive effects observed in animals. These results highlight another possible heath effect that has not yet been well studied.

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