Reproducibility of Urinary Phthalate Metabolites in First Morning Urine Samples

Jane A. Hoppin,[1] John W. Brock,[2] Barbara J. Davis,[3] and Donna D. Baird[1]

[1]Epidemiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA; [2]Centers for Disease Control and Prevention, Atlanta, Georgia, USA; [3]Laboratory of Women's Health, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA

Phthalates are ubiquitous in our modern environment because of their use in plastics and cosmetic products. Phthalate monoesters—primarily monoethylhexyl phthalate and monobutyl phthalate—are reproductive and developmental toxicants in animals. Accurate measures of phthalate exposure are needed to assess their human health effects. Phthalate monoesters have a biologic half-life of approximately 12 hr, and little is known about the temporal variability and daily reproducibility of urinary measures in humans. To explore these aspects, we measured seven phthalate monoesters and creatinine concentration in two consecutive first-morning urine specimens from 46 African-American women, ages 35–49 years, residing in the Washington, DC, area in 1996–1997. We measured phthalate monoesters using high-pressure liquid chromatography followed by tandem mass spectrometry on a triple quadrupole instrument using atmospheric pressure chemical ionization. We detected four phthalate monoesters in all subjects, with median levels of 31 ng/mL for monobenzyl phthalate (mBzP), 53 ng/mL for monobutyl phthalate (mBP), 211 ng/mL for monoethyl phthalate (mEP), and 7.3 ng/mL for monoethylhexyl phthalate (mEHP). These were similar to concentrations reported for other populations using spot urine specimens. Phthalate levels did not differ between the two sampling days. The Pearson correlation coefficient between the concentrations on the 2 days was 0.8 for mBP, 0.7 for mEHP, 0.6 for mEP, and 0.5 for mBzP. These results suggest that even with the short half-lives of phthalates, women's patterns of exposure may be sufficiently stable to assign an exposure level based on a single first morning void urine measurement. *Key words*: biologic markers, environmental exposure, phthalates, reliability, urine, women's health. *Environ Health Perspect* 110:515–518 (2002). [Online 3 April 2002]
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Phthalates represent a large class of chemicals that are widely used in commercial products. The potential for human exposure is high given their use in a vast range of products including medical packaging, cosmetics, children's toys, wood finishes, paints, and upholstery (*1–5*). Although phthalate exposures are widespread, characterization of human exposure has been limited because of the difficulty of chemical analysis for phthalates.

Animal data suggest a broad spectrum of health outcomes associated with phthalate exposure including developmental toxicity (cleft palate, decreased pup weight, testicular damage), endocrine disruption (testicular toxicity, decreased fertility, decreased sperm motility, decreased milk synthesis), and carcinogenicity (*1–3, 6–8*). Phthalates differ with respect to their toxicity. Of the phthalates most commonly used, diethylhexyl phthalate (DEHP) and dibutyl phthalate (DBP) and their metabolites have the greatest potential toxicity. DEHP suppresses estradiol and ovulation in cycling rats (*9*), is a reproductive toxicant, and is carcinogenic in animal models (*10,11*). DBP causes testicular toxicity in rats through an antiandrogen mechanism (*12*) and impairs uterine function, leading to fetal death and resorption in rats (*13*). Butyl benzyl phthalate is weakly estrogenic *in vitro* (*14*) and is teratogenic in rodents (*15,16*). In 2000, the National Toxicology Program's Center for the Evaluation of the Risks to Human Reproduction evaluated seven phthalates for their potential to cause reproductive harm to humans. One of the main conclusions from these evaluations was that more data regarding human exposure were necessary (*6–8*). The National Institute of Occupational Safety and Health (*17*) has classified dibutyl phthalate as a high-priority chemical for study because of its widespread use and its reproductive toxicity in animals. Accurate and reliable biologic markers of phthalate exposure are necessary to assess and appropriately classify exposures in humans.

Until recently, chemical analysis for human exposure to phthalates was limited to highly exposed populations (*18,19*) because contamination of samples by laboratory materials hampered measurements in environmentally exposed subjects. A new assay is available that measures the phthalate monoester metabolites in urine, reducing the potential for sample contamination from laboratory materials (*20*). Commercial products contain primarily phthalate diesters; metabolism in biologic systems cleaves one of the ester groups, producing a phthalate monoester metabolite (*21*). The metabolite is subsequently glucoronidated and excreted in the urine. In addition to reducing the potential for contamination, measurement of monoester metabolites has the added advantage of having longer biologic half-lives (~12 hr) than the diester phthalate (< 3 hr) (*18,19,21*). Furthermore, most of the toxicity is associated with the phthalate monoester.

Population-based samples of U.S. residents indicate that human exposure to phthalates is common, particularly to monobutyl phthalate and monoethyl phthalate (*22,23*). Although the new analytic method allows accurate determination of phthalate levels in urine, little is known about the temporal variability and reproducibility of these measures that affect their application in epidemiologic studies. To explore these aspects, we analyzed phthalate levels in two consecutive first morning void samples from women ages 35–49 years and assessed their reproducibility.

Materials and Methods

Subjects. Participants were 46 African-American women from a randomly selected sample of members of a prepaid health plan in Washington, DC. They were participants in a study to screen for uterine fibroids in 1996–1997. The women ranged in age from 35 to 49 years, with a mean age of 42.3 years (SD = 3.9). The women selected for this analysis were not anticipated to have unique phthalate exposures and were selected to represent low- and high-income strata of the parent study population. All subjects provided informed consent prior to participating.

Urine samples. Women provided two consecutive first morning void urine samples on the second and third days of their menstrual cycles. Samples were shipped to the storage laboratory by overnight mail in a specially designed styrofoam kit that included a frozen cold pack. Samples were aliquoted and frozen at –20°C for future analyses. Urine samples were collected and stored in plastic containers. These containers were screened for phthalate monoesters before the pilot study began, and none contained

Address correspondence to J.A. Hoppin, Epidemiology Branch, MD A3-05, NIEHS, PO Box 12233, Research Triangle Park, NC 27709-2233. Telephone: (919) 541-7622. Fax: (919) 541-2511. E-mail: hoppin1@niehs.nih.gov

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detectable levels. This analysis was restricted to urine samples collected on Tuesdays through Fridays, to minimize variation associated with potential differential exposures on weekends as compared to weekdays. First morning voids were used because we expected these samples to vary less than other types of urine samples and because they are generally more concentrated (*24*).

Laboratory analyses. One 1.2-mL aliquot of each day's urine was analyzed. We included both urine samples from an individual in the same laboratory batch to eliminate interset variation. To assess interset variation in the analysis, at least one quality assurance sample was included in each batch. Interset variation (variation across the analytic batches) was evaluated using all 12 quality assurance samples. Two batches included three quality assurance samples to assess intraset variation—that is, variation within an analytic run. The quality assurance samples were from a pooled first morning void sample from women of similar age as the study population. These quality assurance samples were labeled and handled in an identical manner to other samples, and the analytic laboratory staff were unaware of sample status. All samples were shipped frozen to the analytic laboratory and remained frozen until chemical analysis.

We analyzed urine samples for seven phthalate monoesters using methods described previously (*20,25*). The phthalate monoesters analyzed were monobenzyl phthalate (mBzP), monobutyl phthalate (mBP), monocyclohexyl phthalate (mCHP), monoethyl phthalate (mEP), monoethylhexyl phthalate (mEHP), monoisononyl phthalate (mINP), and monooctyl phthalate (mOP). Samples were spiked with $^{13}C_4$-labeled phthalate monoesters and 4-methylumbelliferone glucuronide. The samples were then treated with β-glucuronidase to release the phthalate monoesters from their conjugated forms. Deconjugated urine samples were extracted twice with Oasis hydrophobic lipophilic balance (Waters Corp, Milford, MA) solid-phase extraction and resuspended in mobile phase. Chromatographic separation by high-pressure liquid chromatography was followed with tandem mass spectrometry on a triple quadrupole instrument using atmospheric pressure chemical ionization (Finnigan Inc., San Jose, CA). We monitored levels of 4-methylumbelliferone as quality control for the deconjugation step. We analyzed method blanks, laboratory quality control samples (spiked human urine), and standards along with the study urine samples. We measured urinary creatinine using an ASTRA analzyer (Beckman Inc., Brea, CA) based on a Jaffe rate reaction (*25*). Samples that failed to meet the laboratory quality assurance criteria were not included the analysis; as a result, mEP measurements on 11 subjects and mINP and creatinine in one sample were unavailable.

Statistical analyses. We reported phthalate results on the basis of concentration in urine (nanograms per milliliter) and concentration based on creatinine concentration in urine (nanograms per gram creatinine). We used creatinine adjustment to correct for variation in urine concentration. We conducted statistical analyses on both unadjusted and creatinine-adjusted phthalate levels. Nondetected values were assigned a value of 0 and excluded from analyses of log-transformed data. Because we were interested in reproducibility in population samples, we did not exclude outliers from any analyses.

Statistical analyses consisted of summary and descriptive statistics, paired *t*-tests comparing the difference in phthalate levels between the 2 days, Pearson and Spearman correlation analyses between the 2 days, and random effects models to estimate the intraclass correlation coefficient (ICC) between the two samples. We calculated the ICC using random effects models to estimate the within- and between-subject variance (*26,27*). Because the distributions were log-normally distributed, all analyses requiring a normality assumption were conducted using log-transformed data. We estimated reliability using the ICC and the number of samples necessary to obtain 80% reliability was estimated using the method described by Fleiss (*26*). We calculated the percent of variability associated with the individual as opposed to the laboratory measurement by subtracting the observed variability in the method from the total within-subject variability and then calculating the percentage of the total variability explained. All analyses were conducted using SAS software (SAS Institute, Cary, NC).

Results

We detected mEP, mBP, mEHP, and mBzP in urine samples from all subjects. We found no significant differences in the mean phthalate levels between day 1 and day 2 using paired *t*-tests (data not shown). Table 1 presents the average concentrations of the two

Table 1. Average phthalate and creatinine levels in urine.

Analyte	No.[a]	Mean	SD	Median	Minimum	Maximum
Unadjusted phthalates (ng/mL urine)						
mBzP	46	39.4	30.0	31.5	5.6	135.2
mBP	46	78.1	58.0	53.0	0.7	251.3
mCHP	46	1.0	3.8	0.3	0.0	26.1
mEP	35	259.8	212.8	211.4	57.9	1042.8
mEHP	46	16.5	30.1	7.3	1.0	143.9
mINP	46	8.1	45.2	0.0	0.0	306.8
mOP	46	4.4	25.5	0.5	0.0	173.7
Creatinine-adjusted phthalates (μg/g creatinine)						
mBzP	46	26.5	18.5	21.6	8.3	119.7
mBP	46	52.7	35.8	43.4	0.4	157.3
mCHP	46	0.6	1.8	0.2	0.0	12.5
mEP	35	183.0	135.3	134.8	32.1	611.1
mEHP	46	12.3	17.4	6.4	0.4	77.3
mINP	45	4.6	25.1	0.0	0.0	169.0
mOP	46	2.1	11.4	0.3	0.0	77.8
Creatinine (mg/dL)	46	150.6	64.7	133.2	66.4	304.9

Based on two consecutive first morning void samples from 46 African-American women, Washington, DC, 1996–1997.
[a]Average of two measures per subject. If both measures were missing, then they were not included here. Sample results outside the laboratory quality assurance range were not reported (11 mEP and 1 mINP/creatinine).

Table 2. Performance of the phthalate analytic method in a general population sample.

		Study samples			Quality assurance samples		
	Samples	DL	Total < DL[b]		Mean conc	Intraset	Interset
Analyte	analyzed[a]	(ng/mL)	No.	Percent	(ng/mL)	CV (%)[c]	CV (%)[d]
mBzP	92	0.8	1	1	31.2	19	16
mBP	92	0.6	1	1	45.0	11	15
mCHP	92	0.7	70	76	0.5	60	69
mEP	70	1.0	0	0	116.4	16	15
mEHP	92	1.2	5	5	10.2	10	24
mINP	91	0.8	69	76	ND	ND	ND
mOP	92	0.9	70	76	0.7	62	122

Abbreviations: conc, concentration; DL, detection limit; ND, not determined. Sample: 46 African-American women, Washington, DC, 1996–1997.
[a]Two samples per subject; 22 mEP and 1 mINP samples were outside laboratory quality assurance limits and are not reported. [b]Total number of samples below the DL. [c]Intraset coefficient of variation, variation within an analytic batch. [d]Interset coefficient of variation, variation across the eight analytic batches.

samples for each phthalate measured. Subjects' mean phthalate concentrations ranged up to three orders of magnitude, with monoethyl phthalate detected at the highest levels of all phthalates analyzed.

The performance of the analytic method in this population can be described by the number of samples below the detection limit and the performance of quality assurance samples (Table 2). More than 75% of the samples analyzed for mCHP, mINP, and mOP were below the analytic detection limit. The coefficients of variation (CVs) for the quality assurance samples were very low for the common phthalates (mEP, mBP, mEHP, mBzP) and were high among analytes that were present at very low concentrations. These CVs were based on our pooled urine sample, which had phthalate concentrations comparable to the population medians. There were no differences between intraset and interset CVs, suggesting no important variation between laboratory batches. The observed CVs for the quality assurance samples represent a small portion of the total variation associated with an individual (e.g., within-person error). Even for chemicals present at low concentration, 96–100% of the observed within-person variation was associated with the individual and not with the measurement. Because of the infrequency of detection of mCHP, mINP, and mOP, we conducted no further analysis of these chemicals.

Correlations between the two measures of phthalates were good and generally improved when the results were adjusted for creatinine concentration (Table 3). The Spearman correlation coefficients and the Pearson correlation coefficients for the log-transformed data yielded similar results. Figure 1 presents scatter plots between the two measures for creatinine-adjusted phthalate concentrations for all phthalates. We calculated the ICCs as a measure of reliability; these values were similar to those for the Pearson correlation coefficients because only two measures per individual were available. Monobutyl phthalate had the highest estimate of reliability, with an ICC of 0.80 [95% confidence interval (CI), 0.69–0.88]. mEP and mEHP had ICCs ≥ 0.6. Given the observed variability in the urinary phthalate measurements, to achieve a reliability of 80%, one sample would be needed to classify mBP exposure accurately, two samples for mEHP, three samples for mEP, and four samples for mBzP.

Table 3. Correlation coefficients and reliability estimates for urinary phthalate measurements.

	Correlation coefficients		Reliability	
Analyte	Spearman	Pearson[a]	ICC[a]	95% CI
Unadjusted phthalate concentrations				
mBzP	0.27	0.34	0.34	0.11–0.54
mBP	0.42	0.62	0.61	0.43–0.74
mEP	0.49	0.47	0.48	0.23–0.67
mEHP	0.59	0.52	0.52	0.32–0.68
Creatinine-adjusted phthalate concentrations				
mBzP	0.50	0.53	0.53	0.31–0.69
mBP	0.67	0.83	0.80	0.69–0.88
mEP	0.66	0.59	0.60	0.36–0.76
mEHP	0.71	0.67	0.67	0.49–0.79

Sample: 46 African-American women, Washington, DC, 1996–1997. Nondetected samples were excluded for analyses using log-transformed data ($n = 1$ for mBzP, mEHP).
[a]Using log-transformed data.

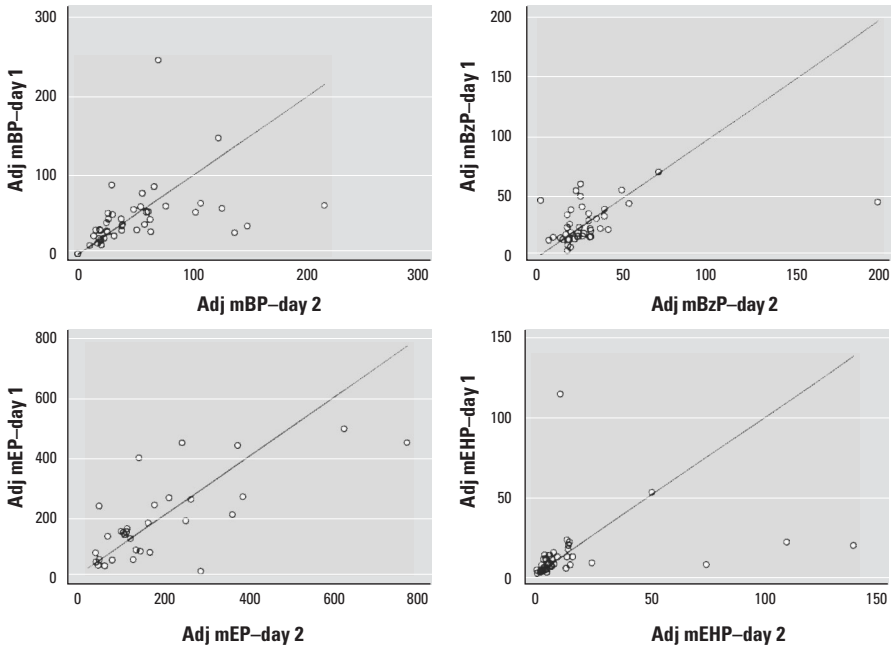

Figure 1. Scatter plots of phthalate levels for 2 consecutive days. Adj, adjusted. 1:1 line indicated on each plot.

Discussion

We observed a high degree of reliability for the four phthalates most commonly detected in urine. The ICCs for these four creatinine-adjusted phthalates ranged from 0.53 for mBzP to 0.80 for mBP. Given that these metabolites have biologic half-lives of approximately 12 hr or less in humans and that exposures can vary from day to day, the observed ICCs are surprisingly high. We designed our experiment for the most similar conditions from one day to the next; thus, these ICCs may represent the upper bound for reproducibility as a long-term measure of phthalate exposure.

The high ICCs indicate that between-subject variability is much greater than within-subject variability. The observed CVs for the quality assurance samples represent a small portion of the total variation associated with an individual's measurement (e.g., within-person error). Laboratory variation explained less than 4% of the within-subject variability, suggesting that most intraindividual variation results from temporal differences in exposure. Although the ICCs were not as high as those observed for other environmental exposures such as organochlorine pesticides (*28*), they were similar to or better than those observed for nutritional components of diet (*29*). Because dietary nutrients have substantial daily variation whereas organochlorines exposure does not, comparisons to nutritional data are more relevant. The success observed for studies of nutritional elements indicates that the ICCs for phthalates should be sufficient for epidemiologic studies.

The phthalate concentrations observed in first morning void urine samples collected from this group of African-American women in 1996–1997 were similar to those reported in two large population samples of U.S. residents (*22,23*). With the same analytic technique, phthalate levels were measured in spot urine samples from 289 adult participants of the National Health and Nutritional Examination Survey (NHANES) III collected from 1988 through 1994 and from 1,029 adult participants of NHANES IV collected in 1999 (*22,23*). Table 4 lists the

Table 4. Median phthalate levels in U.S. population-based samples, 1988–1999.

Phthalate	Concentration (ng/mL urine) NHANES III (*n* = 289)	NHANES IV (*n* = 1,029)	Present study (*n* = 46)
mBP	41.0	27.5	53.0
mBzP	21.2	18.5	31.5
mEP	305.0	171.0	211.4
mEHP	2.7	3.3	7.3

NHANES III: 289 adult participants, 1988–1994, spot urines (*22*); NHANES IV: 1,029 adult participants, 1999, spot urines (*23*); present study: 46 women, DC, 1996–1997, first morning voids. All samples measured using same analytic technique.

median values observed in each population. As with our population, detection of mCHP, mINP, and mOP was rare. Our group had moderately higher levels of mEHP, mBzP, and mBP, but the values were within the ranges reported for these other groups. In the NHANES III sample, women of reproductive age had higher levels of mBP, which is consistent with our data (*22*). Although we restricted our population to minimize variation, our sample appears comparable to other populations.

Phthalate exposure is believed to be ubiquitous given the widespread use of phthalates in plastics and cosmetic products; however, little is known about the sources and patterns of human exposure. Although exposures are common, these data, along with the NHANES data, indicate extensive variation in the level of exposure among individuals. Reliable biologic markers will enable application in epidemiologic studies as well as development of questionnaires that may identify key predictors of phthalates. Because the toxicologic properties of phthalates vary, biologic markers may be key to assessing health effects because the commercial uses of some phthalates may be sufficiently similar that assessing exposure using questionnaires may be difficult. The ICCs for the two phthalates of greatest toxicologic importance, mBP and mEHP, are sufficiently high to warrant application of these measures in population-based studies.

Our study, designed as a best-case example to explore reproducibility of phthalate levels in urine samples, showed a surprisingly high degree of reliability in urinary phthalate levels from one day to the next, suggesting that women's phthalate exposure patterns were relatively stable. We saw no significant differences in phthalate monoester levels from one day to the next. The levels observed were similar to concentrations reported in spot urine samples collected from the U.S. general population from 1988 through 1999. Although the distributions of phthalate concentrations in the general population and our sample appear similar, whether the reproducibility of random spot samples is comparable to that in first morning void samples is not known. Additionally, no data were available to determine how well either of these types of samples predicts an individual's exposure over time. Repeated-measures studies are needed to address the temporal variation of phthalate exposure, both over the course of a day, a week, and throughout the year. However, given the good reliability for the four most common phthalates in this study, these biomarkers could be useful tools to estimate human exposure to phthalates, to determine the sources of phthalate exposure, and to evaluate potential health effects associated with exposure.

References and Notes

1. ATSDR. Toxicological Profile for Di(2-ethylhexyl)phthalate, Update. Atlanta, GA:Agency for Toxic Substances and Disease Registry, 1993.
2. ATSDR. Toxicological Profile for Diethyl Phthalate. Atlanta, GA:Agency for Toxic Substances and Disease Registry, 1995.
3. ATSDR. Toxicological Profile for Di-*n*-butylphthalate (Update). Atlanta, GA:Agency for Toxic Substances and Disease Registry, 1999.
4. Cadogan DF. Plasticizers. In: Kirk-Othmer Concise Encyclopedia of Chemical Technology (Kroschwitz JI, ed). New York:Wiley, 1999;1577–1582.
5. Api AM. Toxicological profile of diethyl phthalate: a vehicle for fragrance and cosmetic ingredients. Food Chem Toxicol 39:97–108 (2001).
6. NTP-CERHR. NTP-CERHR Expert Panel Report on Butyl Benzyl Phthalate. NTP-CERHR-BBP-00. Research Triangle Park,NC:National Toxicology Program, 2000.
7. NTP-CERHR. NTP-CERHR Expert Panel Report on Di-*n*-butyl phthalate. NTP-CERHR-DBP-00. Research Triangle Park, NC:National Toxicology Program, 2000.
8. NTP-CERHR. NTP-CERHR Expert Panel Report on Di(2-ethylhexyl)phthalate. NTP-CERHR-DEHP-00. Research Triangle Park, NC:National Toxicology Program, 2000.
9. Davis BJ, Maronpot RR, Heindel JJ. Di-(2-ethylhexyl) phthalate suppresses estradiol and ovulation in cycling rats. Toxicol Appl Pharmacol 128:216–223 (1994).
10. Lamb JC, Chapin RE, Teague J, Lawton AD, Reel JR. Reproductive effects of 4 phthalic-acid esters in the mouse. Toxicol Appl Pharmacol 88:255–269 (1987).
11. Tickner JA, Schettler T, Guidotti T, McCally M, Rossi M. Health risks posed by use of di-2-ethylhexyl phthalate (DEHP) in PVC medical devices: a critical review. Am J Ind Med 39:100–111 (2001).
12. Heindel JJ, Powell CJ. Phthalate ester effects on rat Sertoli cell function in vitro: effects of phthalate side chain and age of animal. Toxicol Appl Pharmacol 115:116–123 (1992).
13. Ema M, Miyawaki E, Kawashima K. Effects of dibutyl phthalate on reproductive function in pregnant and pseudopregnant rats. Reprod Toxicol 14:13–19 (2000).
14. Zacharewski TR, Meek MD, Clemons JH, Wu ZF, Fielden MR, Matthews JB. Examination of the *in vitro* and *in vivo* estrogenic activities of eight commercial phthalate esters. Toxicol Sci 46:282–293 (1998).
15. Uriu-Adams JY, Kevin Reece C, Nguyen LK, Horvath BJ, Nair R, Barter RA, Keen CL. Effect of butyl benzyl phthalate on reproduction and zinc metabolism. Toxicology 159:55–68 (2001).
16. Ema M, Itami T, Kawasaki H. Embryolethality and teratogenicity of butyl benzyl phthalate in rats. J Appl Toxicol 12:179–183 (1992).
17. Moorman WJ, Ahlers HW, Chapin RE, Daston GP, Foster PM, Kavlock RJ, Morawetz JS, Schnorr TM, Schrader SM. Prioritization of NTP reproductive toxicants for field studies. Reprod Toxicol 14:293–301 (2000).
18. Liss GM, Albro PW, Hartle RW, Stringer WT. Urine phthalate determinations as an index of occupational exposure to phthalic-anhydride and di(2-ethylhexyl)phthalate. Scand J Work Environ Health 11:381–387 (1985).
19. Dirven H, Vandenbroek PHH, Arends AMM, Nordkamp HH, Delepper A, Henderson PT, Jongeneelen FJ. Metabolites of the plasticizer di(2-ethylhexyl)phthalate in urine samples of workers in polyvinylchloride processing industries. Int Arch Occup Environ Health 64:549–554 (1993).
20. Blount BC, Milgram KE, Silva MJ, Malek NA, Reidy JA, Needham LL, Brock JW. Quantitative detection of eight phthalate metabolites in human urine using HPLC-APCI-MS/MS. Anal Chem 72:4127–4134 (2000).
21. Albro PW. Absorption, metabolism, and excretion of di(2-ethylhexyl) phthalate by rats and mice. Environ Health Perspect 65:293–298 (1986).
22. Blount BC, Silva MJ, Caudill SP, Needham LL, Pirkle JL, Sampson EJ, Lucier GW, Jackson RJ, Brock JW. Levels of seven urinary phthalate metabolites in a human reference population. Environ Health Perspect 108:979–982 (2000).
23. CDC. National Report on Human Exposure to Environmental Chemicals. Atlanta, GA:Centers for Disease Control and Prevention, 2001.
24. Que Hee SS. Excretion and the media for biological monitoring. In: Biological Monitoring: An Introduction. New York:Van Nostrand Reinhold, 1993;139–148.
25. Gunter EW, Lewis BL, Koncikowski SM. Laboratory Methods used for the Third National Health and Nutrition Examination Survey (NHANES III), 1988–1994. Hyattsville, MD:Centers for Disease Control and Prevention, 1996.
26. Fleiss JL. The Design and Analysis of Clinical Experiments. New York:Wiley-Interscience, 1986.
27. Hankinson SE, Manson JE, Spiegelman D, Willett WC, Longcope C, Speizer FE. Reproducibility of plasma hormone levels in postmenopausal women over a 2–3-year period. Cancer Epidemiol Biomarkers Prev 4:649–654 (1995).
28. Gammon MD, Wolff MS, Neugut AI, Terry MB, Papadopoulos K, Levin B, Wang Q, Santella RM. Temporal variation in chlorinated hydrocarbons in healthy women. Cancer Epidemiol Biomark Prev 6:327–332 (1997).
29. Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, Hennekens CH, Speizer FE. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 122:51–65 (1985).