Bisphenol-A and phthalates contamination of urine samples by catheters in the Elfe pilot study: implications for large-scale biomonitoring studies.

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HighLights Research

- First assessment of Di-(2-ethylhexyl)-phthalate metabolites and bisphenol A exposures in french pregnant women
- High levels of Di-(2-ethylhexyl)-phthalate metabolites and Bisphenol A suggesting recent exposure
- Women having a caesarean section (or forceps) had higher levels of Bisphenol A and Di-(2-ethylhexyl)-phthalate metabolites than those giving birth naturally.
- Contamination can occur from medical devices either from catheterization or urine probes when biomonitoring at delivery.
- This significant limitation for large-scale biomonitoring studies when including women who have just given birth and who have been put on drips.

Reports from the field

Bisphenol-A and phthalates contamination of urine samples by catheters in the Elfe pilot study: implications for large-scale biomonitoring studies.

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Abstract

Exposure to phthalates and Bisphenol A could cause developmental and reproductive toxicity. This study provides a first assessment of these exposures for more than 250 French pregnant women. The median concentrations of total and free Bisphenol A in urine were similar to those in other studies except the highest concentrations (5% of women had total and free Bisphenol A >50 µg/L). Our study highlights high levels of Di-(2-ethylhexyl)-phthalate metabolites in pregnant women, suggesting recent exposure, probably in hospital. Differences between types of delivery (caesarean vs. natural) support this hypothesis. This is a significant implication for large-scale biomonitoring studies among this population.

Keys words: Bisphenol A – Phtalates – Biomonitoring – Pregnancy – Maternal exposure – Epidemiology

Introduction

Bisphenol A [BPA: 4,4'-dihydroxy-2,2-diphénylpropane] is present in a variety of common products including water bottles, sports equipment, medical and dental devices, dental fillings and sealants and household electronics (Amanti-Kandarakis, 2009). Di-(2-ethylhexyl)-phthalate is the primary phthalate typically found in plasticizers, solvents, lubricants, addictives in the textile industry, and in cosmetic and medical device components (Meeker, 2009).

Endocrine disrupting Bisphenol A and Di-(2-ethylhexyl)-phthalate can mimic the body's own hormones and lead to adverse health effects (Booker 2001;Vom Saal, 2008). Exposure to these compounds has been shown to cause developmental and reproductive toxicity, including diabetes (Vom Saal *et al.*, 2008) and sexual dysfunction (Kortenkamp, 2010; Li, 2010). Previous studies have suggested adverse health effects associated with prenatal exposure to monoethyl phthalate in male infants, such as reduced anogenital distance (Swan, 2005) and shortened gestational age (Latini, 2003). Exposure during fetus development (in-utero) and first years of childhood appears to be the period of greatest sensitivity to its effects (Vandenberg, 2007).

Once incorporated in the body, Bisphenol A and phthalates are rapidly metabolized and excreted in urine. Studies on human metabolism have shown that phthalates are first metabolized by hydolysis to produce primary monoester metabolites and then by oxidation to produce secondary metabolites. Due to the rapid excretion of these compounds, urine samples are considered an appropriate body fluid to assess Bisphenol A and phthalates' exposure. However, because Di-(2-ethylhexyl)-phthalate and Bisphenol A are already present in so many products used for assessment, urinary sample contamination is a general problem in biomonitoring studies. In fact, phthalates (diesters) are omnipresent during the analytical procedure and consequently monoester phthalate metabolites are generated by various processes other than human metabolism (Koch et al., 2003). Secondary metabolites are thus preferred to monoester phthalates for Di-(2-ethylhexyl)-phthalate studies, because of the major advantage that external contamination during analyses is avoided. The impact of post-sampling contamination for phthalates could be greatly minimized through the measurements of phase II oxidative metabolites, such as mono (2-ethyl-5 hydroxyhexyl) phthalate and mono (2-ethyl-5-oxo-hexyl) phthalate metabolites (Koch, 2003). It is important that both total and free Bisphenol A are measured to elucidate whether contamination has occurred or not, as free Bisphenol A is more subject to sampling contamination than the conjugated form, this latter occurring in the liver (Volkel, 2011;Ye, 2008).

The French Longitudinal Study of Children (Etude Longitudinale Française depuis l'Enfance; Elfe) is a national cohort study examining the effects of environmental exposure to children's health. Prior to this study, a pilot survey was conducted in two regions to validate the protocol. It included using questionnaires, sampling protocols and analytical methods. During this pilot study, urine samples were collected before birth. Here, we present the impact of post-sampling contamination from catheters on Bisphenol A and Di-(2-ethylhexyl)-phthalate measurements in pregnant women.

Methods

The pilot survey was carried out in thirty hospital maternity units between the 1st and 4th of October 2007, in the Seine Saint Denis district of Paris and Rhone-Alpes region in southeast France. The survey was restricted to single and twin births. A total of 279 urine samples were collected in high-density polyethylene vials of 250 mL in the delivery room from ninety per cent of mothers who had agreed to both participate in the study and provide biological samples. Samples were collected by midwives for 80% (10% could not be collected because staff were too busy during child delivery). The following data were obtained through medical records and self-administered questionnaires: diet; exposure to environmental pollutants; mother's health during pregnancy and prenatal and postnatal periods; growth and health of the fetus during pregnancy.

Urinary concentrations of the total Bisphenol A (conjugated and unconjugated forms) and free Bisphenol A were measured in 258 samples. Several aliquots of 10 mL were drawn and stored in polypropylene vials at –80°C until analysis. The analyses were performed by Idhesa Bretagne Oceane (Plouzané, France) on Hewlett Packard gas chromatographic system HP 7890A coupled to a mass spectrometer HP 5975C after a liquid-liquid extraction. Reference standards for Bisphenol A and the internal standard D4-Bisphenol A were purchased from CIL-Cluzeau, France. The internal standard was added directly to the sample before extraction. For the extraction, a solvent mix (dichloromethane and ethyl acetate) was used, the samples were shaken and the aqueous phase was removed. For the analysis of total Bisphenol A a solution of helix pomatia (beta glucuronidase) was used to hydrolyze Bisphenol A conjugates, the samples were kept at 37°C in a drying oven during 1h30, before the extraction. A purification phase on a Florisil column, and a derivatization with acetic anhydride were realized. The analyses were performed with GC-MS using SIM (Single Ion Monitoring mode with oven) measuring mass-to-charge ratios (213, 228 (quantifier), 270,

312). This method was validated according to the standard XPT 90-210 (Dekant, 2008;Matsumoto, 2003;Volkel, 2008).

The limits of detection and quantification were 0.10 µg/L and 0.30 µg/L respectively. All validation procedures were performed with fresh samples of herbicide-free human urine. The linearity of the method is controlled for each sequence. A number of blanks and control samples were inserted into each batch of samples to verify the accuracy and precision of the measurements (0.3; 2.5; 4.5; 10; 44.6 and 89.2 µg/L). The coefficient of variation of concentrations in control samples was less than 20%.

The urinary concentrations of metabolites of phthalates (monoethyl phthalate; mono (2-ethyl-5 hydroxyhexyl) phthalate; mono (2-ethyl-5-oxo-hexyl) phthalate) were measured in 279 samples. Solid Phase Extraction was realized on LiChrospher® RP-8 (Sorbent characteristics: particles of silica with Octyl derivative; specific surface area: 350 m^2/g; pH range: 2-7.5; shipping eluent: acetonitrile/water). The analyses were performed using Liquid Chromatography coupled to tandem Mass Spectrometry after enzymatic hydrolysis (with beta-glucuronidase at 37°C). The limit of quantification was 0.5 µg/L. In a similar way to the Bisphenol A measurements, a number of blanks and control samples (LGC Standarts) were inserted into each batch of samples to verify the accuracy and precision of the measurements (3.07 and 25.8 µg/L for monoethyl phthalate; 10.41 and 68.26 µg/L for mono (2-ethyl-5 hydroxyhexyl) phthalate; 7.79 and 55.64 µg/L for mono (2-ethyl-5-oxo-hexyl) phthalate). The coefficient of variation of concentrations in control samples was less than 20%. The analyses of creatinine were performed using the kinetic Jaffe method on a Roche Cobas Integra 700 system.

Several studies (Adibi *et al*., 2008; Huang et al., 2007; Perrone et al., 1992) suggested that creatinine adjustment might not be the optimal method of urinary dilution adjustment for pregnant women (as urinary creatinine levels may be unusually diluted or concentrated during pregnancy) Therefore we chose to present our results in µg/L (data in µg/g creatinine not shown).

Statistical analyses were performed using the Wilcoxon test to compare the distribution of Bisphenol A and metabolites of Di-(2-ethylhexyl)-phthalate according to the type of delivery (caesarean vs natural). We also performed a complementary analysis on urine probes to estimate the influence of the contact period between the probe and urine for 0, 12 hours and 24 hours at ambient temperature.

Results

Table 1 shows the distribution of concentrations of free and total Bisphenol A (in µg/L), monoethyl phthalate, mono (2-ethyl-5 hydroxyhexyl) phthalate and mono (2-ethyl-5-oxo-hexyl) phthalate (µg/L) in all urine samples [data in µg/g of creatinine not shown].

Total and free Bisphenol A were detected in more than 90% and 74% of the urines samples respectively. Total Bisphenol A levels ranged from 0.3 to 598 µg/L, with a median concentration was 2.5 µg/L, its 25th percentile was 1.0 µg/L, the 75th was 5.6 µg/L and its 95th percentile was 115.4 µg/L. Free Bisphenol A levels ranged from 0.3 to 512.8 µg/L, with a median concentration of 0.4 µg/L, its 25th percentile was 0.1 µg/L, the 75th was 1.4 µg/L and its 95th percentile was 35.1 µg/L. Di-(2-ethylhexyl)-phthalate metabolites (monoethyl phthalate, mono (2-ethyl-5 hydroxyhexyl) phthalate and mono (2-ethyl-5-oxo-hexyl) phthalate) were detected in more than 95% of the urine samples. Monoethyl phthalate levels ranged from 0.5 to 768.1 µg/L; the median concentration was 13.7 µg/L, its 25th percentile was 4.4 µg/L, the 75th was 51.3 µg/L and its 95th percentile was 256.5 µg/L. mono (2-ethyl-5 hydroxyhexyl) phthalate and 5oxo- monoethyl phthalate were detected in all the samples. Their levels ranged from 0.5 to 1587.9 µg/L, and from 0.5 to 924.5 µg/L respectively. Median values were respectively 50.7 and 28.3 µg/L, 25th percentiles 7.3 and 12.4 µg/L, 75th percentiles 222.7 and 146.9 µg/L and 95th percentiles 635.1 and 435.5 µg/L.

Analysis was then performed separately for two types of delivery: caesarean section or forceps versus natural delivery. Women having a caesarean section (or forceps) had higher levels of both total and free Bisphenol A than those giving birth naturally. Figure 1 shows the density of probabilities of Bisphenol A and metabolites of phthalates' concentrations in urine samples collected from women having natural and caesarean or forceps deliveries. The latter group (caesarean or forceps) had much higher free Bisphenol A values (median values 0.7 µg/L) than those who delivered naturally, p=0.036 (median values 0.3 µg/L).

The complementary analysis performed on two urine probes in latex showed a release of Bisphenol A over time: 8.1 µg/L at 0 hours, 149 µg/L at 9 hours and 281 µg/L at 24 hours for the first sample and 4.8 µg/L at 0 hours and 193 µg/L at 9 hours for the second one.

Women having caesarean sections or delivery with forceps had higher levels of monoethyl phthalate, mono (2-ethyl-5 hydroxyhexyl) phthalate and mono (2-ethyl-5-oxo-hexyl) phthalate than those who gave birth naturally (p=0.002, p=0.16 and p=0.19 respectively). Median values were respectively: 22.9 µg/L in caesarean section vs 10.2 in natural delivery for monoethyl phthalate; 50.7 vs. 32.3 µg/L for mono (2-ethyl-5 hydroxyhexyl) phthalate and 33.9 vs. 23.7 µg/L for mono (2-ethyl-5-oxo-hexyl) phthalate.

Discussion

We found the median, 25th and 75th percentiles of total Bisphenol A were similar to the 2517 subjects of the National Health and Nutrition Examination Survey, which is a national program designed to assess health of children in the United States: respectively 2.8 µg/L; 1.3 µg/L and 5.5 µg/L, but the 95th percentile was much higher than expected (115.4 µg/L vs 16 µg/L in the National Health and Nutrition Examination Survey). These higher values were found in women who had caesarean sections and highlight a potential problem of contamination. The higher levels of free BisphenolA also found in women who had caesarean sections could elucidate this contamination: the 95th percentile being 273.9 µg/L for this group versus 4.2 µg/L for those giving birth naturally. Contamination of human urine samples with BisphenolA from exogenous sources during specimen collection may have contributed to the concentrations of free BisphenolA reported, as has been shown in other studies (Markham, 2010;Twaddle, 2010;Ye, 2011). The differences in free BisphenolA urinary concentrations according to the type of delivery, suggested that the source of contamination may be hospital-based. The release of BisphenolA from catheterization probes further supported the hypothesis that post-sampling contamination occurred, primarily occurring in caesarean deliveries. The complementary analyses confirmed the release of BisphenolA from urine probes over time, suggesting that contamination of urine samples was effectively possible.

The median concentrations of urinary Di-(2-ethylhexyl)-phthalate metabolites (monoethyl phthalate, mono (2-ethyl-5 hydroxyhexyl) phthalate and mono (2-ethyl-5-oxo-hexyl) phthalate) were higher than those previously reported in the literature for pregnant women (Adibi, 2009;Wolff, 2008). However, they were similar to concentrations measured in a study by Yan et al. (2009), where pregnant women had had intravenous injection of glucose, water and electrolyte balance support after admission to hospital (Yan, 2009). They observed median concentrations of 114.7 µg/L monoethyl phthalate, mono (2-ethyl-5 hydroxyhexyl) phthalate 108.9 µg/L, and 95.1 µg/L mono (2-ethyl-5-oxo-hexyl) phthalate. Our study highlighted the high exposure to metabolites of Di-(2-ethylhexyl)-phthalate (monoethyl phthalate, mono (2-ethyl-5 hydroxyhexyl) phthalate and mono (2-ethyl-5-oxo-hexyl) phthalate) for women who had just given birth. The very high values of mono (2-ethyl-5 hydroxyhexyl) phthalate and mono (2-ethyl-5-oxo-hexyl) phthalate observed suggested recent exposure, probably in the hospital. The very high values of monoethyl phthalate also suggested recent exposure, but these may have been due to contamination of urinary samples.

Our results suggest that contamination can occur from medical devices either from catheterization or urine probes when biomonitoring at delivery. This is a significant limitation to be taken into account for large-scale biomonitoring studies when including women who have just given birth and who have been put on drips. Considering the high Di-(2-ethylhexyl)-phthalate metabolites concentrations found in such women, there is a strong potential that other patients, especially infants in neonatal and pediatric intensive care units, will be exposed to these substances in countries where catheters containing phthalates have not yet been banned.

References

Adibi, J.J., Hauser, R., Williams, P.L., Whyatt, R.M., Calafat, A.M., Nelson, H., Herrick, R., Swan, S.H., 2009. Maternal urinary metabolites of Di-(2-Ethylhexyl) phthalate in relation to the timing of labor in a US multicenter pregnancy cohort study. Am J Epidemiol 169**,** 1015-1024.

Amanti-Kandarakis, E., Bourguignon, J.P., Giudice, L.C., Hauser, R., Prins, G.S., Soto, A.M., Zoeller, R.T., Gore, A.C., 2009. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocr Rev 30**,** 293-342.

Booker, S.M., 2001. NTP center reports on phthalate concerns. Environ Health Perspect 109**,** A260-A261.

Dekant, W., Volkel, W., 2008. Human exposure to bisphenol A by biomonitoring: methods, results and assessment of environmental exposures. Toxicol Appl Pharmacol 228**,** 114-134.

Koch, H.M., Rossbach, B., Drexler, H., Angerer, J., 2003. Internal exposure of the general population to DEHP and other phthalates--determination of secondary and primary phthalate monoester metabolites in urine. Environ Res 93**,** 177-185.

Kortenkamp, A., Faust, M., 2010. Combined exposures to anti-androgenic chemicals: steps towards cumulative risk assessment. Int J Androl 33**,** 463-474.

Latini, G., De, F.C., Presta, G., Del, V.A., Paris, I., Ruggieri, F., Mazzeo, P., 2003. In utero exposure to di-(2-ethylhexyl)phthalate and duration of human pregnancy. Environ Health Perspect 111**,** 1783-1785.

Li, D.K., Zhou, Z., Miao, M., He, Y., Qing, D., Wu, T., Wang, J., Weng, X., Ferber, J., Herrinton, L., Zhu, Q., Gao, E., Yuan, W., 2010. Relationship between Urine Bisphenol-A (BISPHENOL A) Level and Declining Male Sexual Function. J Androl.

Markham, D.A., Waechter, J.M., Jr., Wimber, M., Rao, N., Connolly, P., Chuang, J.C., Hentges, S., Shiotsuka, R.N., Dimond, S., Chappelle, A.H., 2010. Development of a method for the determination of bisphenol A at trace concentrations in human blood and urine and elucidation of factors influencing method accuracy and sensitivity. J Anal Toxicol 34**,** 293-303.

Matsumoto, A., Kunugita, N., Kitagawa, K., Isse, T., Oyama, T., Foureman, G.L., Morita, M., Kawamoto, T., 2003. Bisphenol A levels in human urine. Environ Health Perspect 111**,** 101-104.

Meeker, J.D., Sathyanarayana, S., Swan, S.H., 2009. Phthalates and other additives in plastics: human exposure and associated health outcomes. Philos. Trans R Soc Lond B Biol Sci 364**,** 2097-2113.

Swan, S.H., Main, K.M., Liu, F., Stewart, S.L., Kruse, R.L., Calafat, A.M., Mao, C.S., Redmon, J.B., Ternand, C.L., Sullivan, S., Teague, J.L., 2005. Decrease in anogenital distance among male infants with prenatal phthalate exposure. Environ Health Perspect 113**,** 1056-1061.

Twaddle, N.C., Churchwell, M.I., Vanlandingham, M., Doerge, D.R., 2010. Quantification of deuterated bisphenol A in serum, tissues, and excreta from adult Sprague-Dawley rats using liquid chromatography with tandem mass spectrometry. Rapid Commun Mass Spectrom. 24**,** 3011-3020.

Vandenberg, L.N., Hauser, R., Marcus, M., Olea, N., Welshons, W.V., 2007. Human exposure to bisphenol A (BISPHENOL A). Reprod. Toxicol 24**,** 139-177.

Volkel, W., Kiranoglu, M., Fromme, H., 2008. Determination of free and total bisphenol A in human urine to assess daily uptake as a basis for a valid risk assessment. Toxicol Lett 179**,** 155-162.

Volkel, W., Kiranoglu, M., Fromme, H., 2011. Determination of free and total bisphenol A in urine of infants. Environ Res 111**,** 143-148.

Vom Saal, F.S., Myers, J.P., 2008. Bisphenol A and risk of metabolic disorders. JAMA 300**,** 1353-1355.

Wolff, M.S., Engel, S.M., Berkowitz, G.S., Ye, X., Silva, M.J., Zhu, C., Wetmur, J., Calafat, A.M., 2008. Prenatal phenol and phthalate exposures and birth outcomes. Environ Health Perspect 116**,** 1092-1097.

Yan, X., Calafat, A., Lashley, S., Smulian, J., Ananth, C., Barr, D., Silva, M., Ledoux, T., Hore, P., Robson, M.G., 2009. Phthalates Biomarker Identification and Exposure Estimates in a Population of Pregnant Women. Hum Ecol Risk Assess 15**,** 565-578.

Ye, X., Tao, L.J., Needham, L.L., Calafat, A.M., 2008. Automated on-line column-switching HPLC-MS/MS method for measuring environmental phenols and parabens in serum. Talanta. 76**,** 865-871.

Ye, X., Zhou, X., Needham, L.L., Calafat, A.M., 2011. In-vitro oxidation of bisphenol A: is bisphenol A catechol a suitable biomarker for human exposure to bisphenol A? Anal Bioanal. Chem. 399**,** 1071-1079.

Tables

Table 1: GM with CIs, GSD and distribution (25th, 50th and 75th percentiles) of free and total Bisphenol A (BPA) and Di-(2-ethylhexyl)-phthalate (DEHP) metabolites : Monoethyl phthalate (MEHP), mono (2-ethyl-5 hydroxyhexyl) phthalate (5-OH-MEHP) and mono (2-ethyl-5-oxo-hexyl) phthalate (5-oxo-MEHP) for all births, with natural and caesarean/forceps deliveries in the ELFE pilot survey.

	% non detected		*All births (n= 254)*					*Natural delivery (n=164)*					*Ceaserean / forceps delivery (n=79)*				
	< LOD	<LOQ	GM [CIs]	GSD	P25	P50	P75	GM	GSD	P25	P50	P75	GM	GSD	P25	P50	P75
BPA (N=254)																	
BPA Total (µg/L)	8.1	3.1	2.6 [2.1–3.2]	71.0	1.0	2.5	5.6	2.0 [1.6-2.5]	22.9	0.9	2.2	5.1	4.5 [2.8-7.1]	118.8	1.2	3.3	7.5
BPA Free (µg/L)	25.6	9.4	0.4 [0.3–0.5]	62.8	0.1	0.4	1.3	0.2 [0.2-0.3]	22.0	0.0	0.3	1.0	0.7 [0.3-1.4]	104.5	0.1	0.7	2.3
DEHP metabolits (N=279)																	
MEHP (µg/L)		9.3	12.9 [10.4–16.2]	100.5	4.4	13.7	51.3	10.4 [7.8-13.7]	97.3	3.6	10.2	37.7	21.1 [14.3-31.1]	110.6	6.9	22.9	83.6
5-OH-MEHP (µg/L)	0.0	0.3	51.5 [42.6–62.3]	256.8	15.3	41.9	222.7	48.2 [38.3-60.8]	247.0	13.8	32.3	210.7	61.3 [43.0-87.4]	279.8	18.2	50.7	299.6
5-oxo-MEHP (µg/L)	0.0	0.3	38.2 [31.9–45.7]	159.5	12.4	28.3	146.9	35.9 [28.9-44.8]	152.5	12.0	23.7	142.1	44.2 [31.8-61.5]	172.8	15.3	33.9	168.9

Figure

Figure 1: distribution of Bisphenol A (BPA), Monoethyl phthalate (MEHP), mono (2-ethyl-5 hydroxyhexyl) phthalate (5-OH-MEHP) and mono (2-ethyl-5-oxo-hexyl) phthalate (5-oxo-MEHP) by type of delivery (the leftmost part of the distribution show a sort of pseudo-node/mode, due to random fluctuation below the LOD)