Published in final edited form as:
Endocr Disruptors (Austin). ; 1(1): . doi:10.4161/endo.25066.

Urinary phthalate metabolite concentrations among men with inflammatory bowel disease on mesalamine therapy

Elizabeth J Hait, MD, MPH[1], **Antonia M. Calafat, PhD**[2], and **Russ Hauser, MD, ScD**[3,4]

[1]Division of Gastroenterology, Children's Hospital Boston, Harvard Medical School, Boston, MA, USA

[2]National Center for Environmental Health, Centers for Disease Control and Prevention, Boston, MA, USA

[3]Department of Environmental Health, Harvard School of Public Health, Boston, MA, USA

[4]The Fertility Center, Vincent Memorial Obstetrics and Gynecology, Massachusetts General Hospital, Boston, MA, USA

Abstract

Background—Phthalates, a family of compounds used in a variety of consumer products, are reproductive and developmental toxicants in experimental animals. One of these phthalates, dibutyl phthalate (DBP), is an inactive ingredient in the coating of Asacol.

Aim—To determine if men with inflammatory bowel disease taking Asacol have higher urinary concentrations of monobutyl phthalate (MBP), a metabolite of DBP, compared to the general population in the United States.

Methods—Five patients at the Massachusetts General Hospital Crohn's and Colitis Center, taking at least 800 mg of Asacol three times a day, provided one spot urine sample. Urinary MBP and other phthalate metabolite concentrations were measured by using online solid phase extraction coupled with isotope dilution high-performance liquid chromatography tandem mass spectrometry.

Results—In four of the five men, the urinary concentrations of MBP (9888 ng/mL, 12,308 ng/mL, 10,124 ng/mL, and 41,590 ng/mL) and of a minor DBP metabolite, mono(3-carboxypropyl) phthalate (MCPP, 116.4 ng/mL, 163.4 ng/mL 72.6 ng/mL, 5604 ng/mL) were orders of magnitude higher than the background concentrations among the US general population. One subject missed his morning Asacol dose and had urinary MBP concentrations (17.5 ng/mL) similar to background levels.

Conclusion—We confirmed that men with inflammatory bowel disease taking Asacol have urinary concentrations of MBP and MCPP much higher than background levels.

Corresponding Author: Russ Hauser, Professor of Environmental and Occupational Epidemiology, Department of Environmental Health, Department of Epidemiology, 665 Huntington Avenue, Building I 14th Floor, Boston, MA 02115, 617.432.3326, rhauser@hohp.harvard.edu.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the CDC.

Keywords

Asacol; phthalate; endocrine disruptor

Introduction

Phthalates are a family of compounds that are widely used in consumer products, medical devices, certain medications, and plastics. Some phthalates, primarily diethyl phthalate and dibutyl phthalate (DBP), can be used to hold color and scent in products such as soap, shampoo, hairspray, nail polish, cosmetics, and perfume[1]. They can also be used as solvents in glue, paint, and insect repellents. Di(2-ethylhexyl) phthalate (DEHP) is used to soften flexible plastics like polyvinyl chloride[2].

Recent scientific and public concern has arisen about the potential reproductive toxicity of some phthalates[3]. In adult rodents, DBP and DEHP can cause testicular toxicity through effects on Leydig and Sertoli cells[4]. It has also been shown in rat studies that *in utero* exposure to these same phthalates impaired Leydig cell steroidogenesis and induced Leydig cell hyperplasia[5]. *In vitro* studies have shown that these phthalates disturb the interaction of follicle stimulating hormone with its receptor[6]. Male offspring of female rats exposed to high levels of some phthalates during pregnancy have a higher incidence of congenital urogenital anomalies, such as cryptorchidism, hypospadias, nipple retention, and agenesis of the prostate, seminal vesicles, and epididymus[4]. Despite the abundant animal data, there are limited human studies to date.

Asacol is a medication that is commonly used as maintenance therapy for patients with inflammatory bowel disease (IBD). Asacol has been a popular choice for both adults and children with IBD largely due to its excellent safety profile. It contains the active ingredient mesalamine (5-amino-2-hydroxybenzoic acid) and is encapsulated in a delayed release coating for targeted delivery of the drug to the terminal ileum and colon. The delayed release coating contains several inactive ingredients including DBP[7].

We previously reported that a thirty year old man with ulcerative colitis was incidentally found to have a urinary concentration of the major metabolite of DBP, monobutyl phthalate (MBP), of 16,868 ng/mL[8]. He was participating in an environmental health study evaluating the relationship between phthalates and reproductive health[9]. Based on the National Health and Nutrition Examination Survey (NHANES) data, this man's urinary concentration of MBP was two orders of magnitude higher than the US population 95th percentile[10]. In our environmental health study, we collected detailed information on lifestyle, occupation and medical history and concluded that the source of his phthalate exposure was likely from the medication Asacol (Proctor & Gamble, Cincinnati, OH).

The objective of the present study was to confirm our previous case report and to determine if men with inflammatory bowel disease taking Asacol have urinary concentrations of MBP higher than those reported for the general US population.

Methods

Institutional Review Board approval from the Massachusetts General Hospital (MGH), Harvard School of Public Health and the Centers for Disease Control and Prevention (CDC) was obtained. In 2005, five patients at the MGH Crohn's and Colitis Center who were taking at least 800 mg of Asacol three times a day were approached at their routine health visit. None of the subjects were employed in a potential phthalate exposed occupation (e.g., painter, plastic worker, electrician, salon worker, or printer) and none had hepatic or renal disease.

After informed consent was obtained, each subject provided one sample of urine in a standard specimen cup. The urine was a spot urine sample and not a first morning void. The urine was immediately aliquotted and frozen at −80°C. Deidentified samples were shipped on dry ice to the CDC laboratory at the National Center for Environmental Health and stored at or below −40°C until analysis. The samples were analyzed by online solid phase extraction coupled with isotope dilution high-performance liquid chromatography tandem mass spectrometry as previously described[11]. Phthalate urinary concentrations were reported both in micrograms per liter of urine and in micrograms per gram of urinary creatinine. Creatinine adjustment was used to correct for urine dilution[11].

Results

Three men had ulcerative colitis and two had Crohn's disease. Disease severity ranged from mild to severe (Table 1). Four of the subjects were on other medications in addition to Asacol, but none of these other medications were known to contain DBP as either an active or inactive ingredient.

In four of the five men, the urinary concentrations of MBP were much higher than those reported for the US general population (Table 2) ranging from 9888 ng/mL to 41,590 ng/mL, which was three times higher than the concentration reported in our case study[8]. These concentrations are strikingly higher than the 95th percentile reported from NHANES 2003–2004 which was 108 ng/mL for individuals 20 years of age and older [12]. Urinary concentrations of mono(3-carboxypropyl) phthalate (MCPP), a minor metabolite of DBP, in these four men were also higher than background levels. However, in participant 2, MBP and MCPP urinary concentrations were similar to those in the general US population. This man reported that he had missed his morning Asacol dose before providing the urine sample for analysis. His last dose of Asacol was taken the prior evening. The elimination half life of MBP is short (i.e., a few hours)[13]. The urinary concentrations of all other metabolites were within the ranges reported for the general US population[12].

Discussion

In this study, we confirmed that men with inflammatory bowel disease taking Asacol have urinary concentrations of two of the metabolites of DBP, MBP and MCPP, much higher than those reported among the US general population. This is likely attributable to exposure to DBP from the enteric coating of Asacol. We do not believe that the higher than background concentrations of MBP and MCPP were related to underlying intestinal disease

affecting absorption of DBP from other sources, such as personal care products and diet. The urinary results on the man who missed his morning dose of Asacol confirmed that the source of exposure to DBP was the medication. He had concentrations of MBP not different from background concentrations twenty-two hours after his last Asacol dose despite having moderate disease activity. DBP has a half life of hours, and so it is expected that twenty-two hours after his last dose of Asacol, this patient had excreted most of the DBP metabolites[14].

The data from this pilot study are in agreement with the results of a recent publication from our group, where we explored the contribution of medications to human exposure to phthalates[15]. We used publicly available files from NHANES for the years 1999–2004. For certain survey periods, participants were asked to recall use of prescription medication during the past 30 days, and for a subsample of individuals, the urinary concentrations of phthalate metabolites were measured. We *a priori* identified medications potentially containing phthalates as inactive ingredients and then compared the mean urinary concentration of phthalate metabolites between users and nonusers of those medications. Six persons reported use of mesalamine; their individual MBP concentrations were 4,691, 4,358, 3,191, 1,055, 185, and 59 μg/L, and their corresponding creatinine-adjusted concentrations were 6,426, 4,150, 1,707, 1,160, 110, and 29.4 μg/g creatinine. The corresponding estimated doses of DBP for these six persons were 233, 151, 62, 42, 4, and 1 μg/kg/day. Thus, two of the six individuals exceeded the U.S. EPA RfD for DBP of 100 μg/kg/day (U.S. EPA 2008), including a woman of childbearing age. The mean MBP concentration for mesalamine users was 2,257 μg/L, 50 times higher than the mean for nonusers ($p < 0.0001$); the mean concentrations of MCPP, a minor metabolite of DBP and also a metabolite of some other high-molecular-weight phthalates[16], was about 10 times higher. One limitation of the NHANES dataset is that we did not have specific information on the brand of medication taken, but only the active ingredient. Therefore, the wide range of urinary MBP concentrations among mesalamine users may be explained by the use of a medication containing mesalamine but not DBP (i.e., not Asacol but another mesalamine formulation).

The clinical significance of the much higher exposure to DBP from the use of Asacol than background exposure remains unclear. Very few studies have been conducted to investigate the potential reproductive toxicity of phthalates in humans. Several studies have investigated the association between the urinary concentrations of phthalate metabolites and human semen parameters[17,18,19]. These studies examined the semen of male partners of subfertile couples, and then correlated semen quality with urinary concentrations of phthalate metabolites. It was concluded that exposure to some phthalates was associated with altered sperm quality. In particular, urinary concentrations of MBP were associated with increased risk of poor semen quality, including low sperm concentration and motility. However, a study in Sweden of 234 men did not report similar associations[20].

Other human studies have explored associations between phthalate exposure and endocrine markers, including serum hormone levels. Pan et al. studied 74 male workers at a factory producing unfoamed polyvinyl chloride flooring exposed to DBP and DEHP and compared them with samples from 63 male workers from a construction company, matched for age and smoking status[21]. They observed a significant reduction of serum free testosterone in workers with higher levels of the urinary metabolites of DBP and DEHP, MBP and mono(2-

ethylhexyl) phthalate, respectively, compared with unexposed workers. Meeker, along with members of our group, recently studied the relationship between DEHP metabolites and reproductive hormones in 425 men. They showed urinary metabolites of DEHP were inversely associated with circulating steroid hormone levels[22].

Swan et al studied the relationship between anogenital distance (AGD) in baby boys and their mother's prenatal urinary concentration of phthalate metabolites[23]. They reported that urinary concentrations of four metabolites [MBP, monobenzyl phthalate, monoethyl phthalate, and mono-isobutyl phthalate] were inversely and significantly related to AGD.

Asacol was approved by the FDA for the treatment of inflammatory bowel disease in August 1997 and its side effects profile has been extensively studied[24,25]. While sulfasalazine, another mesalamine preparation, has been linked to male infertility and abnormal semen quality, this has been attributed to the sulfa component of the drug[26,27]. As far as we are aware, there have been no publications in the medical literature describing male infertility associated with Asacol use. Interestingly, birth outcome studies have shown that women taking Asacol during pregnancy do not have any increase risk of congenital malformations in their offspring[28].

Our study is important for two reasons. First, it has confirmed that patients taking Asacol have a significant exposure to a potential human reproductive and developmental toxicant. While most studies evaluate the active ingredients of medications, this study emphasizes the need to consider the effects of the "inactive" components of drugs. Second, this study has identified an important cohort of individuals with a potentially high level of phthalate exposure. Therefore, patients exposed to Asacol are an ideal population to study the developmental and reproductive health effects of phthalates in humans.

In conclusion, based on the present results, our earlier case report, and our recent publication using NHANES data, we have shown that Asacol contributes to exposure to DBP. Although DBP is a reproductive and developmental toxicant in experimental animals, at this time there is limited human evidence and no evidence we are aware of to suggest that Asacol is associated with altered reproductive health in humans. It is an effective medication that has had a long history of safety. However, these results may raise concern and necessitate the need for human studies to determine whether exposure to DBP from Asacol is associated with health effects, allowing patients and clinicians to weigh the risks.

Acknowledgments

We thank Manori Silva, Ella Samandar, Jim Preau, Jack Reidy and others at CDC for the measurements of the phthalate metabolites.

Funding Sources: Not applicable.

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Endocr Disruptors (Austin). Author manuscript; available in PMC 2014 November 10.

Table 1

Description of disease location, severity and treatment in the 5 study participants

	Participant 1	**Participant 2**	**Participant 3**	**Participant 4**	**Participant 5**
Dose of Asacol	800 mg three times daily	2 g twice daily	1.6 g twice daily	1.2 g three times daily	2.4 g twice daily
Time since last dose (in hr)	6.5	22	9	3.5	4.5
Disease location:	pancolitis	ileitis	pancolitis	ileocolonic	pancolitis
Disease severity:	mild	moderate	moderate	severe	moderate
Other medications:	none	Paxil	Prednisone	Prednisone Ciprofloxacin Imuran Prilosec Remeron Celexa Rowasa Enemas	Prednisone 6 Mercaptopurine

Paxil (paroxetine – antidepressant), prednisone (anti-inflammatory), Ciprofloxacin (antibiotic), Immuran (immunomodulator), Prilosec (omeprazole – antacid), remeron (mirtazapine – antidepressant), Celexa (citalopram – antidepressant), Rowasa (mesalamine – anti-inflammatory), 6 Mercaptopurine (immunomodulator)

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Table 2

Comparison of participants' urinary phthalate metabolite concentrations (ng/mL) to concentrations detected in the general population (based on NHANES data from 2003–2004).

Subject#	MBP	MCPP	MBzP	MEHP	MEOHP	MEHHP	MECPP	MEP	MiBP
Population Geometric Mean**	21.1	2.91	13.7	2.34	14.5	21.7	34.7	193	3.8
Population 95%ile**	122	15.3	101	31	157	266	339	2700	21.3
Participant 1	9888	116	1.1	<LOD	12.1	20.9	39.4	36.2	19.3
Participant 2	17.5	5.8	14.8	3.4	28.3	52.8	92.7	2739	7
Participant 3	12308	163	19	5	35.5	64.4	92.7	300	30.7
Participant 4	10124	72.6	9.3	4.8	35	61.1	90.3	412	21.1
Participant 5	41590	560	10.8	10.6	34.4	59.2	67.2	350	59.3

Abbreviations: MBP = Mono-n-butyl phthalate; MBzP = Monobenzyl phthalate; MCPP = Mono-3-carboxypropyl phthalate; MECPP = Mono-2-ethyl-5-carboxypentyl phthalate; MEHHP = Mono-2-ethyl-5-hydroxyhexyl phthalate; MEHP = Mono-2-ethylhexyl phthalate; MEOHP = Mono-2-ethyl-5-oxohexyl phthalate; MEP = Monoethyl phthalate; MiBP = Mono-isobutyl phthalate LOD = limit of detection. LOD (in ng/mL): mECPP = 0.25; mEHHP = 0.32; MEOHP = 0.45; mEHP = 0.9; mCPP = 0.16; miBP = 0.26; mBP = 0.4; mBzP = 0.11; mEP = 0.4;

** www.cdc.gov/exposurereport (NHANES 2003–2004) - accessed 05/10/10