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Kinetics of di(2-ethylhexyl) phthalate (DEHP) and mono(2-ethylhexyl) phthalate in blood and of DEHP metabolites in urine of male volunteers after single ingestion of ring-deuterated DEHP

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ABSTRACT

The plasticizer di(2-ethylhexyl) phthalate (DEHP) is suspected to induce antiandrogenic effects in men via its metabolite mono(2-ethylhexyl) phthalate (MEHP). However, there is only little information on the kinetic behavior of DEHP and its metabolites in humans. The toxikokinetics of DEHP was investigated in four male volunteers (28–61 y) who ingested a single dose (645 ± 20 μg/kg body weight) of ring-deuterated DEHP (DEHP-D_4). Concentrations of DEHP-D_4, of free ring-deuterated MEHP (MEHP-D_4), and the sum of free and glucuronidated MEHP-D_4 were measured in blood for up to 24 h; amounts of the monoesters MEHP-D_4, ring-deuterated mono(2-ethyl-5-hydroxyhexyl) phthalate and ring-deuterated mono(2-ethyl-5-oxohexyl) phthalate were determined in urine for up to 46 h after ingestion. The bioavailability of DEHP-D_4 was surprisingly high with an area under the concentration-time curve until 24 h (AUC) amounting to 50% of that of free MEHP-D_4. The AUC of free MEHP-D_4 normalized to DEHP-D_4 dose and body weight (AUC/D) was 2.1 and 8.1 times, that of DEHP-D_4 even 50 and 100 times higher than the corresponding AUC/D values obtained earlier in rat and marmoset, respectively. Time courses of the compounds in blood and urine of the volunteers oscillated widely. Terminal elimination half-lives were short (4.3–6.6 h). Total amounts of metabolites in 22-h urine are correlated linearly with the AUC of free MEHP-D_4 in blood, the parameter regarded as relevant for risk assessment.

Introduction

The plasticizer di(2-ethylhexyl) phthalate (DEHP) is an antiandrogenic toxicant, the rat being the most sensitive species (for a detailed review, see ECB, 2008). In contrast, marmosets, a primate species, showed no DEHP dependent effects (Kurata et al., 1998; Tomonari et al., 2006). DEHP is hydrolyzed in the first metabolic step to mono(2-ethylhexyl) phthalate (MEHP), which is further metabolized via oxidation at the side chain, conjugation with glucuronic acid, and hydrolysis to phthalic acid (Albro, 1989). DEHP dependent antiandrogenic effects in rats have been attributed to free MEHP which affects Sertoli cells (Li and Kim, 2003), disturbs the testicular testosterone production (Chauvigné et al., 2009), and antagonizes the testosterone action in the testes (Laguë and Tremblay, 2008). Therefore, the blood burden of free MEHP is regarded as relevant for risk assessment (Gentry et al., 2011). In an earlier study, oral administration of 30 mg/kg body weight of ring-deuterated DEHP (DEHP-D_4) to rats and marmosets led to a systemic burden of the parent compound given as the area under the concentration-time curve (AUC) in blood normalized to the DEHP-D_4 dose (AUC/D [nmol*h/ml per mmol DEHP-D_4/kg body weight]) of 8.5 - 21 and 6.5 - 8.9, respectively. Corresponding AUC/D values of free MEHP-D_4 were 695 and 181 (Kessler et al., 2004), consistent with the low sensitivity of marmosets. In DEHP exposed humans, no data on the systemic burden of DEHP and only limited data on that of MEHP is available. In a single volunteer who ingested a DEHP-D_4 dose of 0.65 mg/kg body weight, the AUC/D of free MEHP-D_4 in blood accounted for 11,000 (Koch et al., 2005).

Abbreviations: AUC, area under the concentration-time curve in blood until 24 h; AUC/D, AUC normalized to dose and body weight; DEHP, di(2-ethylhexyl) phthalate; DEHP-D_4, ring-deuterated DEHP; MEHP, mono(2-ethylhexyl) phthalate; MEHP-D_4, ring-deuterated MEHP; MEHP-$^{13}C_4$, $^{13}C_2$-1,2-ring-$^{13}C_2$-dicarboxyl MEHP; 5OH-MEHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; 5OH-MEHP-D_4, ring-deuterated 5OH-MEHP; 5oxo-MEHP, mono(2-ethyl-5-oxohexyl) phthalate; 5oxo-MEHP-D_4, ring-deuterated 5oxo-MEHP; 5oxo-MEHP-$^{13}C_4$, $^{13}C_2$-1,2-ring-$^{13}C_2$-dicarboxyl 5oxo-MEHP.

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According to the AUC/D values of free MEHP, humans should be 16 and 90 times more sensitive than rats and marmosets, respectively.

The general population is exposed to DEHP primarily via food (Fromme et al., 2007; Wittassek et al., 2011). Since biomonitoring of DEHP and MEHP in blood is impaired by analytical disturbances due to the ubiquitous presence of both compounds (Kessler et al., 2001; Takatori et al., 2004), the individual daily DEHP intake has been estimated from biomonitoring of DEHP metabolites in spot urine samples (Fromme et al., 2007; Guo et al., 2011a, 2011b; Hines et al., 2011; Koch et al., 2006, 2011; Lin et al., 2011; Marsee et al., 2006; Wittassek et al., 2007) according to the procedure of Koch et al. (2006): Concentrations of MEHP, mono(2-ethyl-5-hydroxyhexyl) phthalate (5OH-MEHP), and mono(2-ethyl-5-oxohexyl)phthalate (5oxo-MEHP) in a single spot urine sample were extrapolated to the 24-h urine by means of a standardized creatinine excretion. The relationship between the 24-h urine excretion data and the DEHP intake was obtained from a study with a male volunteer who ingested 3 different doses of DEHP-D_4 (Koch et al., 2005). The estimation of the DEHP intake via this procedure is, however, linked with an uncertainty since the concentrations of the metabolites in a spot urine sample are not necessarily the same as in the 24-h urine. Furthermore, the relationship between excretion data and DEHP intake is based on data obtained from a single individual.

Considering the lacking or limited data on the kinetics of DEHP in humans, the goal of the present controlled dosing study was to investigate time courses of DEHP and MEHP in blood and of the monoesters MEHP, 5OH-MEHP, and 5oxo-MEHP in urine of four volunteers after a single oral dose of ring-deuterated DEHP.

Methods

Chemicals and reagents. DEHP-D_4 (min. 98 at.% $^2H_4$) was purchased from ISOTEC (Miamisburg, USA). $^{13}C_2$-1,2-ring-$^{13}C_2$-dicarboxyl MEHP (MEHP-$^{13}C_4$, ≥98%), 5oxo-MEHP (≥95%), $^{13}C_2$-1,2-ring-$^{13}C_2$-dicarboxyl 5oxo-MEHP (5oxo-MEHP-$^{13}C_4$, ≥98%) were from Cambridge Isotope Laboratories (Andover, USA). MEHP-D_4 (>99%), 5OH-MEHP-D_4 (≥97%), and MEHP-D_4-β-glucuronide (>99%) were obtained from Biochemisches Institut für Umweltcarcinogene Prof. Dr. Gernot Grimmer Stiftung (Großhansdorf, Germany). Di-n-octyl phthalate (≥99%), and mono-n-octyl phthalate (95%) were gifts from BASF (Ludwigshafen, Germany). β-Glucuronidase of E. coli K12 (140 U/ml) was from Roche Diagnostics (Mannheim, Germany). Organic solvents of highest available purity (Promochem picograde) were from LGC Standards (Wesel, Germany).

Volunteers. Four healthy male adult volunteers (Table 1) gave written informed consent to participate in the study, which was reviewed by the Bavarian Chamber of Physicians Ethical Committee (decision Nr. 08104 from 16.2.2009) and by the data protection administrator of the Bavarian Health and Food Safety Authority.

The volunteers took breakfast between 5 min and 2 h before DEHP ingestion in order to stimulate intestinal DEHP hydrolysis (Albro and Thomas, 1973; Kessler et al., 2004). The substance was given by means of a disposable syringe as a single DEHP-D_4 dose of 618–665 μg/kg body weight in an emulsion (60 mg DEHP-D_4/ml) consisting of an aqueous saccharose solution (70% w/v). The exact dose was determined by reweighing the syringe. Blood was collected from the first volunteer investigated (volunteer 3) at 0.25 h before ingestion, at 0.00 h, and at 0.25, 0.5, 0.75, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10 and 24 h after ingestion. Because of the complex time courses of DEHP-D_4 and MEHP-D_4 obtained from this volunteer, additional blood samples were taken from the other volunteers at 5.0, 7.0 and 9.0 h after ingestion. Blood samples (11–12 ml at each time point) were taken from the forearm vein via an indwelling catheter using heparinized disposable syringes. Total urine was collected using screw-capped polypropylene bottles at the following time points: 0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10, 12, 14, 18, 22, 26, 30, 34, 38, 42 and 46 h after ingestion. Urine samples collected overnight were stored at 4-8 °C until the next morning. Volumes and pH values of all samples were determined as quickly as possible and aliquots of 1.0, 10 and 100 ml (if available) were stored at -80 °C until analysis. At this temperature, the metabolites have been proven to be stable for at least one year (Samandar et al., 2009; Silva et al., 2008).

Treatment of blood samples. MEHP-D_4-β-glucuronide was determined indirectly from the measurement of free MEHP-D_4 and of total MEHP-D_4 (the sum of MEHP-D_4-β-glucuronide and free MEHP-D_4). Total MEHP-D_4 was determined in blood samples of 2.0 ml that were acidified to pH 6.45 with 50 μl of ammonium acetate buffer (1 mol/l, pH 5.0) and incubated with 5 μl β-glucuronidase (0.7 units) at 37 °C for 1 h. The incubation period had been verified in advance using MEHP-D_4-β-glucuronide as substrate in blood (20 nmol/ml) and determining the concentration-time course of the reaction product MEHP-D_4. Because Kato et al. (2003, 2004) reported enzymatic hydrolysis of DEHP to MEHP in human serum at 37 °C, it was investigated whether this could influence the determination of MEHP-D_4-β-glucuronide. Aliquots of 2 ml from the blood samples taken 3 to 8 h after DEHP-D_4 intake were incubated at 37 °C for 1.0 h after acidification with ammonium acetate buffer (see above). In those of volunteers 2 and 4, DEHP-D_4 was determined before and after incubation with β-glucuronidase. In those of volunteers 1 and 3, MEHP-D_4 was determined before and after incubation in absence of β-glucuronidase. During the 1-h incubations, DEHP-D_4 did not hydrolyze to MEHP-D_4 (Fig. 1). This is in contrast with a high hydrolytic rate shown by Kato et al. (2004), but is in agreement with a very low one reported by Kato et al. (2003).

Analysis of DEHP-D_4 and MEHP-D_4 in blood. DEHP-D_4 and MEHP-D_4 in blood were analyzed by means of gas chromatography with mass selective detection (GC/MS) in the electron ionization mode using di-n-octyl

Table 1
Relevant data on the volunteers.

Volunteer	Body weight	Age	DEHP-D_4 dose	Time of ingestion	Breakfast		Lunch	
	[kg]	[y]	[μg/kg body weight]	[h:min]	Time	Food	Time	Food
1	76	28	653	9:20 am	9:05 am	Sandwich with cheese	00:30 pm	Fish with potato salad
2	105	39	618	9:00 am	8:50 am	2 toasts with butter and cheese	12:00 am	3 sandwiches with cheese
3	59	49	644	9:05 am	7:00 am	Slice of bread with cheese and sausage	11:00 am	Sandwich with cheese, buttermilk
4	88	61	665	9:02 am	8:50 am	Bread and butter with cheese and sausage	11:00 am	Green salad with plenty of oil, buttermilk