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Estimated Daily Intake and Hazard Quotients and Indices of Phthtalate Diesters for Young Danish Men

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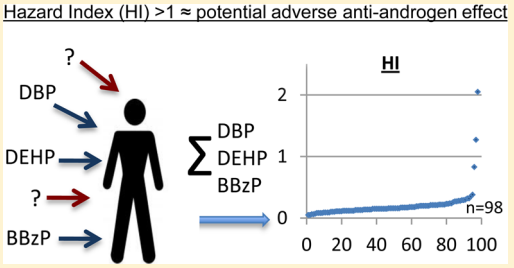
ABSTRACT: Because of wide exposure to phthalates, we investigated whether simultaneous exposure to several phthalates reached levels that might cause adverse antiandrogenic effects. Thirty three healthy young Danish men each delivered three 24-h urine samples during a three months period. The daily intakes of the sum of di-*n*-butyl and di-iso-butyl phthalate, di(2-ethylhexyl) phthalate, di-iso-nonyl phthalate, and butylbenzyl phthalate were estimated based on urinary excretion of the metabolites. Based on a hazard quotient (HQ) of the individual phthalate (i.e., the ratio between the daily intake and an acceptable level of exposure), a hazard index (HI) for each man was calculated as the sum of HQs for the individual phthalates. All men were exposed to all phthalates during the urine collection periods. Median HIs were all below 1 (i.e., below an acceptable cumulative threshold) ranging from 0.11 to 0.17 over the three different sample collections. Of the 33 men, 2 men had HIs above 1 in one of their three samples, indicating that occasionally the combined exposure to the investigated phthalates reached a level that may not be considered safe. Besides the phthalates investigated here, humans are exposed to numerous other chemicals that also may contribute to a cumulative antiandrogenic exposure.



INTRODUCTION

Experimental animal studies have shown antiandrogenic effect of di(*n*)butyl phthalate (DnBP), di-iso-nonyl phthalate (DiNP), and di(2-ethylhexyl) phthalate (DEHP),[6−11] and human studies have also suggested an antiandrogenic effect on the male reproductive hormone system of some phthalates.[12−15] Phthalates are found in personal care products, toys, food storage, packaging film, medical devices, lubricating oils, etc. Humans are exposed to phthalates through ingestion, inhalation, and dermal contact with products.[16−19]

The general approach in risk assessment has been to evaluate a single chemical at a time, without considering potential cumulative effects of multiexposure. However, a significant dose-additive, probably even synergistic, antiandrogenic effect after exposure of multiple chemicals, including several phthalates (DEHP, DBP, and benzylbutyl phthalate (BBzP)) have been described.[10,20] Because of this potential "cocktail effect", a cumulative risk assessment, which focuses on the effects of multiexposure, have been suggested.[1,21]

The aim of this study was to perform a cumulative risk assessment of four phthalates (DEHP, DBP, BBzP, and DiNP), with known capability to induce antiandrogenic effects, by estimations of a hazard index (HI). An HI is a cumulated ratio between the *actual* exposure level, (estimated from the excreted amount of phthalates in 24-h urine samples), and the *acceptable* exposure level.[22] For this, the phthalate exposure of a group of young Danish men from the general population was here investigated. Other research groups have conducted cumulative risk assessments prior to this study: Benson (2009) performed a cumulative antiandrogenic risk assessment based on urine samples from a US (NHANES) and a German population.[23] However, the risk assessment was based on spot urine samples and not 24-h urine samples and might thus lead to misclassification due to diurnal variation in urinary excretion. Others have focused on cumulative risk assessment in children.[24,25] However, children may have different exposure patterns and as risk is considered in relation to intake per kg body weight per day children may be relatively more exposed because of their size. Results from children can therefore not be directly extrapolated to adults.

METHODS AND MATERIALS

Study population. From April to June 2008 33 young men were consecutively included in this present study (participation rate 55%; 60 men in total were invited) from an ongoing study of male reproductive health, where men representative of the young general population are invited to participate at the time of their compulsory medical conscript examination.[26,27] The participants gave their written consent after having received written and oral information. The study had been approved by the ethical committee for the Copenhagen municipality (ref. nos.: H-KF-289429 with amendment of March 18, 2008). The participants were financially compensated (approximately 130 Euro) upon completion. Their median anthropometric data (range) were the following: age, 19.0 years (18.3−22.3); height, 181 cm (171−197), weight, 77.1 kg (53.9−93.2); and body mass index (BMI), 22.7 kg/m^2 (16.5−31.9). All were Caucasian-Danes.

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Table 1. Excretion Fractions (EF) and Reference Values (RfV-AA and RfV-EFSA)

		DnBP	DiBP	DEHP	BBzP	DiNP
excretion fractions	Anderson 2001	69%	(69%)[a]		73%	
	Anderson 2011			45.3%		30.5%
ref. values	RfD-EFSA (TDI EFSA 2005)	10[b]	[c]	50[b]	500[d]	150[e]
	RfD-AA (Kortenkamp 2010)	100[f]	200[f]	30[g]	330[f]	1500[h]

[a]No excretion fractions for DiBP; therefore DnBP was used. [b]Based on germ cell development. [c]EFSA has no reference value for DiBP. [d]Based on spermatozoa concentration. [e]Based on liver toxicity. [f]Based on suppression of fetal testosterone prouction. [g]Based on nipple retention. [h]Based on nipple retention and testes malformation.

The 33 men were instructed to deliver three 24-h urine samples with intervals of 40−46 days. Altogether, 98 24-h urine samples were collected (one man did not deliver the second sample). The samples were collected in polyethylene containers during the period April to September 2008 and the individual samples were collected on Mondays−Thursdays. All men received thorough verbal and written instructions in how to perform the collections in order to eliminate sampling biases. The study were designed to investigate intraindividual variation in phthalate excretion, see Frederiksen et al. 2012, where also details of the outline of the study have previously been described.[28]

Analysis of Urinary Phthalates. Urine samples were analyzed for the content of monobenzyl phthalate (MBzP), the sum of mono-*n*-butyl and monoiso-butyl phthalate ($\sum$MBP$_{(i+n)}$), mono(2-ethylhexyl) phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono(2-ethyl-5-carboxypentyl) phthalate (MECPP), monoiso-nonyl phthalate (MiNP), mono(hydroxy-iso-nonyl) phthalate (MHiNP), mono(oxo-iso-nonyl) phthalate (MOiNP), and mono(carboxy-iso-octyl) phthalate (MOiCP) by liquid chromatography-tandem mass spectrometry (LC-MS/MS) with preceding enzymatic deconjugation and solid phase extraction. The methods for preparation of samples, standard solutions, and quality controls as well as the instrumental analysis have previously been described[29] and were used with modification.[28] Since the chromatographic method used was only 17 min long, MnBP and MiBP were measured as one compound and expressed as $\sum$MBP$_{(i+n)}$ (and the parent phthalate expressed $\sum$DBP$_{(i+n)}$). However, subsequent analysis of spot urine samples collected in 2007−2009 from 881 men comparable to the participants in this study showed that the median ratio of MnBP:MiBP of the total $\sum$DBP$_{(i+n)}$ was 35:65 (not published). The presented estimated exposure for DnBP and DiBP therefore corresponds to respectively 35% and 65% of the calculated $\sum$DBP$_{(i+n)}$, which was based on the measurement of both isoforms together.

The metabolites of DEHP and DiNP were expressed combined as the sum of the assessed DEHP metabolites ($\sum$DEHPm) and the sum of the DiNP metabolites ($\sum$DiNPm). In order to combine the metabolites, the amount of each metabolite was converted into the corresponding amount of its parent compound by correcting for differences in molecular weight.

Data Analysis/Statistics. The 24-h urinary excretion of the phthalate metabolites was calculated as the 24-h urine volume multiplied with urinary phthalate metabolite concentration (ng/mL). The estimated daily intake of the parent phthalates for each subject was calculated as

$$\mathrm{DI}\ (\mu\mathrm{g/kg\,bw/24\,h}) = \frac{(\mathrm{UC}_m(\mu\mathrm{g/L})\cdot \mathrm{mw_p/mw_m})\cdot \mathrm{UV(L)}}{\mathrm{EF_u}\cdot \mathrm{bw(kg)}} \quad (1)$$

The model is based on the urinary metabolite concentration (UC_m), molecular weights for metabolites (mw_m) and their respective parent phthalate (mw_p), the volume of the 24 h urinary collection (UV), an excretion fraction (EF_u) for each of the metabolites, and body weight (bw).[30] The excretion fractions (EF), that is, how much of a given dose that is excreted trough urine, were based on human studies of oral intake of the parent phthalates: The excretion fractions of the major metabolites of DBP and BBzP have been estimated to 69% and 73%, respectively, by Anderson et al. (2001).[31] The excretion fractions for DEHP have been estimated to 45.3% (the sum of the four metabolites, which constitutes the majority of metabolized DEHP; MEHP, MEOHP, MEHHP, and MECPP) and 30.6% for DiNP (the sum of MiNP, MHiNP, MOiNP, and MCiOP).[32]

Hazard Quotients (HQ). Based on the daily intake, the HQs were calculated for each participant as:

$$\mathrm{HQ} = \frac{\text{exposure}}{\text{acceptable exposure(RfD)}} \quad (2)$$

European Food and Drug Administration (EFSA) have proposed antiandrogenic reference values for DEHP, DnBP, and BBzP based on estimated Tolerable Daily Intake (TDI).[2−5] Kortenkamp and Faust recently proposed reference values, based specifically on antiandrogenic end points, which differed from the reference values used by EFSA.[1] We therefore calculated the HQs based on the reference values of 1) EFSA (denoted RfV-EFSA) and 2) Kortenkamp (denoted RfV-AA) (Table 1). EFSA, however, offers no official reference value for DiBP, and the reference value for DINP was not based on an antiandrogenic end point. HQs for DiBP and DINP were therefore only estimated based on RfV-AA.

Hazard Index (HI). An HI for each participant was calculated as the summarization of the HQs:

$$\mathrm{HI} = \mathrm{HQ}_1 + \mathrm{HQ}_2 + \ldots + \mathrm{HQ}_n \quad (3)$$

The HI based on the RfV-EFSA approach was created from the sum of the HQs for DnBP, $\sum$DEHPm, and BBzP but did not include DiBP and $\sum$DiNPm due to the above-mentioned reason. Oppositely, DiBP and $\sum$DiNPm *were* included in the HI based on RfV-AA approach. Thus, two different HIs were estimated based on two approaches for the reference values.

RESULTS

All metabolites were measured in concentrations above limits of detection (LOD) except for MBzP in a single sample and MiNP in 54 out of the total 98 24-h urine samples (i.e., detectable in 45% of the samples). An overview of the

Table 2. Estimated Daily Intake (μg/kg bw/day) of Four Phthalates Based on 24-h Urine Samples from 33 Danish Men

	first 24 h urine (n = 33)				second 24 h urine (n = 32)				third 24 h urine (n = 33)			
	min	median	95p	max	min	median	95p	max	min	median	95p	max
DnBP[â]	0.33	0.90	2.86	3.40	0.31	0.83	4.22	7.40	0.29	0.76	5.11	12.00
DiBP[â]	0.62	1.66	5.30	6.31	0.57	1.54	7.84	13.75	0.55	1.41	9.49	22.28
$\sum DBP_{(i+n)}$	0.95	2.6	8.2	9.7	0.87	2.4	12.1	21.1	0.84	2.2	14.6	34.3
BBzP	0.05	0.7	2.9	3.7	0.00	0.5	8.8	9.5	0.06	0.6	1.8	2.4
DEHPm	0.65	2.9	35.2	97.7	1.1	3.6	8.0	10.0	0.73	2.9	8.3	12.1
DiNPm	0.29	1.2	5.6	6.0	0.3	1.2	22,0	52.5	0.42	1.3	38.9	50.3

[â]Daily intake of DnBP and DiBP was estimated from the measured level of $\sum DBP(i+n)$ based on a ratio of 35:65. Previous results (not published) have shown distribution of the two isomeres in this ratio.

Table 3. Hazard Quotients (HQ) and Hazard Index (HI) in Young Danish Men Based on Reference Values from EFSA

	first 24 h urine (n = 33)				second 24 h urine (n = 32)				third 24 h urine (n = 33)			
	min	median	95p	max	min	median	95p	max	min	median	95p	max
DnBP[a]	0.03	0.09	0.29	0.34	0.03	0.08	0.42	0.74	0.03	0.08	0.51	1.20
DiBP[a]												
BBzP	0.000	0.001	0.006	0.007	0.000	0.001	0.018	0.019	0.000	0.001	0.004	0.005
DEHPm	0.01	0.06	0.7	1.95	0.02	0.07	0.16	0.2	0.01	0.06	0.17	0.24
DINPm[b]												
HI*	0.06	0.17	0.88	2.05	0.05	0.16	0.50	0.83	0.05	0.15	0.58	1.27

[a]Previous results (not published) have shown distribution of DnBP:DiBP of 35:65% of $\sum DBP(i+n)$. Hazard qoutients of DnBP calculated based on this ratio. DiBP was not included in HI due to absence of an official reference value from EFSA. [b]No antiandrogenic end point from EFSA exists for DiNP; HQs therefore not performed for DiNP.

Table 4. Hazard Quotients (HQ) and Hazard Index (HI) Calculated for the Three 24 h Urine Samples Based on Reference Values Proposed by Kortenkamp et al.

	first 24 h urine (n = 33)				second 24 h urine (n = 32)				third 24 h urine (n = 33)			
	min	median	95p	max	min	median	95p	max	min	median	95p	max
DnBP[a]	0.003	0.009	0.03	0.03	0.003	0.008	0.04	0.07	0.003	0.008	0.05	0.12
DiBP[a]	0.003	0.008	0.027	0.03	0.003	0.008	0.040	0.07	0.003	0.007	0.047	0.11
BBzP	0.000	0.002	0.009	0.01	0.000	0.002	0.027	0.03	0.000	0.002	0.005	0.007
DEHPm	0.02	0.1	1.17	3.26	0.03	0.11	0.27	0.33	0.02	0.1	0.28	0.4
DINPm	0.000	0.0008	0.004	0.004	0.000	0.0008	0.015	0.035	0.00	0.009	0.026	0.034
HI	0.03	0.13	1.19	3.28	0.04	0.14	0.32	0.38	0.03	0.11	0.37	0.44

[a]Previous results (not published) have shown distribution of DnBP:DiBP of 35:65% of $\sum DBP(i+n)$. Hazard qoutients of DnBP and DiBP were calculated based on this ratio of the measured sum of both.

concentrations of each metabolite has been published previously.[28]

An overview of the estimated daily intakes based on three 24-h urine samples per participant is given in Table 2. Median $\sum DBP_{(n+i)}$ and $\sum$DEHPm levels were in general 2−3 fold higher than median BBzP and $\sum$DiNPm levels. Although the median daily intakes of the four phthalates were similar for the three different sample collections, the 95th percentile and maximum levels varied considerably due to occasional samples with a significantly higher content.

Hazard Quotients (HQ) and Hazard Indexes (HI). Calculated HQs for the individual phthalates as well as HI calculated based on respectively RfV-EFSA and RfV-AA are summarized for the three different sample collections in Tables 3 and 4. Median HIs of the two approaches were similar in the range 0.11−0.17 even though the RfV-AA approach included two more phthalates.

Using the RfV-EFSA approach, the HQs of DnBP and DEHPm contributed more or less equally to the HI of DnBP, BBzP, and DEHPm combined. Together, HQs of DnBP and DEHP contributed to ∼98% of the combined HI (data not shown). Using the RfV-AA approach DEHPm was the single dominant phthalate contributing >80% of the HI of DnBP, DinBP, BBzP, DEHPm, and DiNPm combined, followed by DnBP (∼7%) and DiBP (∼6%) (data not shown).

Each participant's individual HI in each of the three 24-h urine samples can be seen in Figures 1 and 2. Based on the RfV-

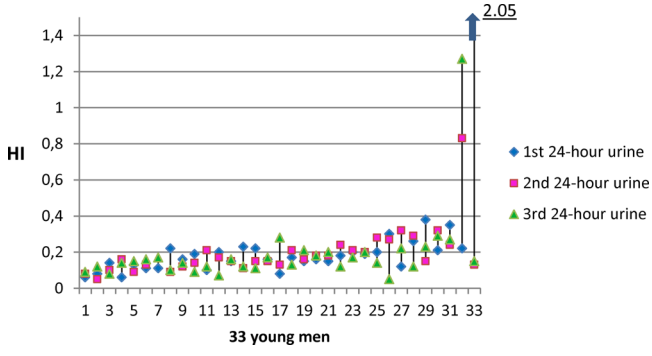

Figure 1. Hazard index (HI) for each of the three urine samples from each participant based on the reference values from EFSA. HI includes DnBP, BBzP, and DEHP.

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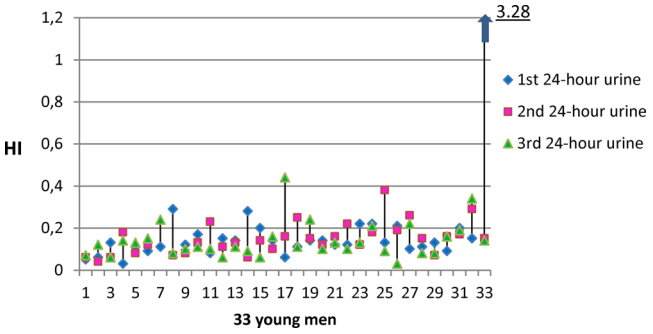

Figure 2. Hazard Index (HI) for each of the three urine samples from each participant based on the reference values from proposed by Kortenkamp et al (2010). HI includes DnBP, DiBP, BBzP, DEHP, and DiNP.

EFSA approach two out of 33 participants had HIs above 1 in one of three 24-h urine samples. The RfV-AA approach led to one HI above 1 due to a single extremely high ∑DEHPm exposure, which resulted in a single very high HI of 3.28.

DISCUSSION

The main finding in this study was that *independent* of which of two different approaches that were used, four phthalates alone accounted for 10−20% of the median acceptable daily level of antiandrogenic exposure in young men from the general Danish population. 97 of 98 samples showed exposure to all of the four phthalates: Thus, the men seemed to be regularly exposed. 2 out of 33 men had an HI exceeding 1 in one of three samples based on the RfV-EFSA approach, and based on the RfV-AA approach the maximum HI was 3.28. This indicates that occasional "spikes" of exposure may occur in some individuals. Other prevalent chemicals than phthalates also possess antiandrogenic effects, such as some pesticides, nonylphenol, polychlorinated biphenyls, linuron, etc.[20,33,34] and may thus also contribute to a *cumulative* antiandrogenic effect, which was not accounted for in our study of "only" four phthalates.

More and more studies indicate an antiandrogenic effect of phthalates in humans at all life stages. An association between decreased anogenital distance and the phthalate metabolites MnBP, MiBP, and MBzP in newborn boys has been suggested,[35] implying that testicular function *in utero* were compromised. In our previous study of 129 children, a median HI of 0.5 was estimated (based on the same phthalates and the same RfV-EFSA values).[25] Furthermore, phthalates seem also to be harmful toward adult men as well: Hauser et al. (2007) showed that human spermatozoa DNA damage was correlated to MEHP.[14] Recently, Joensen et al. (2012) showed a negative association between the percentage of total ∑DEHPm excreted as MEHP (%MEHP) and testosterone in a study population (n = 881) comparable to the young men in this study.[15]

It should be noted, that HI-values below 1 represent an exposure level likely to represent a minimal antiandrogenic risk over a lifetime. Furthermore, the reference values used for calculating HQs are based on effects seen after repeated daily exposures over a period and/or at sensitive developmental periods and safety factors of 100 or 200 are included in the values. Thus, an occasional HI-value above 1 might not necessarily imply a significant risk for an adult man. However, our knowledge on the effects of occasional "spikes" of exposure to phthalates compared to a less varying daily exposure pattern is limited as experimental animal studies most often have been conducted with repeated daily dosing over a period. Our calculations of daily intakes were based on 24-h urine samples and therefore not affected by an intraday variation in the excretion pattern of phthalate metabolites. Collecting multiple samples on the same men allowed us to see if the HI of individuals were more or less constant or varied over time.

One participant had on one occasion an extremely high estimated daily intake of ∑DEHPm (97.7 μg/(kg day)). The urinary levels of the primary metabolite (MEHP) as well as the three secondary metabolites (MEHHP, MEOHP, and MECPP) were approximately 10-fold higher in this sample compared to the other samples. Only the metabolism from DEHP to MEHP (but no further metabolism) can occur ex vivo,[36] and since the secondary metabolites were also found in very high levels, the high ∑DEHPm level cannot be explained by contamination during sampling or in the laboratory. Thus, it most likely represents a true exposure, again suggesting that a rather spike-like exposure pattern for especially DEHP may occur.

Estimation of daily intakes of the same phthalates as we analyzed in this study but, based on concentrations in spot urine samples from the German and American general population published by different research groups from 2000 to 2007, were recently performed in a review by Koch & Calafat. They obtained median estimated daily intake level for DnBP ranging from 0.84 to 5.22 μg/(kg day), for BBzP ranging from 0.26 to 0.88 μg/(kg day), for DEHP ranging from 0.71 to 4.6 μg/(kg day), and for DiNP based on a single study an estimated daily intake of 0.29 μg/(kg day).[37] These estimated daily intakes correspond well to our estimates although our estimated daily intakes of DnBP were in the lower end and estimated daily intakes of DiNP were higher. These slight differences could be due to country differences in use of phthalates but more likely reflects changes in the use of phthalates over time in relation to time of sample collection; for examples, samples in our study were collected in 2008—three years after the use of DBP in cosmetics were banned in EU. Cosmetics are, however, far from the only source of DBP exposure (e.g., exposure through ingestion and inhalation).[16] Some surveys of urinary level of DnBP and DiBP have found an opposite internal ratio between the two isoforms compared to the ratio used in this study (MnBP:MiBP = 35:65): Results from National Health and Nutrition Examination Survey (NHANES) 2001−2004 based on spot urine samples report median levels of MnBP of 17.0−19.0 μg/L and MiBP of 2.4−3.9 μg/L from American adults; a factor 5 overweight of MnBP compared to MiBP[18] and thus very different from our ratio. However, several German studies have found ratios of MnBP:MiBP more identical to the one we used: Koch and Calafat reported levels of MnBP of 12.6 μg/L and MiBP of 22.4 μg/L based on the first morning urine of 45 German adults.[37] The ratio of 35:65 based on the 881 men was not based on first morning urines, so the analogous ratio cannot be explained by a similar sampling protocol. Wittasek et al. found a decline in the ratio of MnBP:MiBP from 1988 to 2003 from 178:29 (μg/L) to 51:30 (μg/L),[38] which also shows a general trend of declining exposure. The discrepancy in the ratios between the countries to a large extend still remains unexplained.

Another approach to estimate daily intakes is the scenario-based estimations of exposure. This indirect method involves description of exposure to various products containing phthalates, assigning a concentration of the phthalate from each product, and for each product to estimate an intake

rate.[39,40] However, great uncertainty follows this approach; that is, unknown or only partially known absorption rates from the different organ systems, a considerable variance and uncertainty in the estimations of the consumed amount of a given product, uncertainty about the exact concentrations in the given product, etc. We therefore prefer the back-calculated method of daily intake estimations based on actual biomonitoring level although this approach also has some uncertainty mainly associated with the excretion factors (EF) used in the back-calculation.

Reference Values. Calculation of HQ can only be done if a reference value has been defined below which a daily exposure is considered to be safe. The US Environmental Protection Agency (EPA) defines a reference dose as "*an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure of a chemical to the human population including sensitive subpopulations that is likely to be without risk of deleterious non-cancer effects during a lifetime*".[41] The chosen level of acceptable exposure, that is, a reference value, will influence the outcome, depending on results based on TDI or No Observed Adverse Effect Level (NOAEL), etc. The RfV-EFSA approach generally led to slightly higher HIs than the RfV-AA approach, mainly explained by a 10-fold lower RfV-EFSA for DnBP compared to RfV-AA (10 μg/kg bw/24h versus 100 μg/kg bw/24h). The RfV-EFSA approach is thus more conservative than the RfV-AA. Since EFSA have only assessed DnBP, we excluded the contribution from DiBP in our HI estimates when using the RfV-EFSA approach. However, lack of a defined reference level does not necessarily mean that the compound is safe. The two isoforms of DBP have recently been shown to have similar adverse effect at a comparable potency.[8] Therefore, the EFSA approach is likely to underestimate the antiandrogenic risk. The RfV-AA scheme also has them comparable, although DiBP is evaluated as half as potent with an RfV of 200 compared to 100 μg/(kg day) for DnBP.[1] The EFSA value for DiNP was based on liver toxicity estimation.[5]

Considerations of Uncertainty Regarding Exposure, Metabolism, and Excretion. The procedures to estimate daily intakes from the 24-h urine samples have several uncertainty points. First, as mentioned, we chose 24-h urine samples in order to eliminate within day variation which can occur in spot urine samples. It has been shown by Anderson et al. (2011) that more than 90% of the amount of ingested phthalates, which were excreted in urine, were excreted within 24 h after oral intake,[32] and therefore, we assume that the content measured in 24-h urine pools correlates well to the intake over 24 h.

Second, the fractional excretion parameter is based on a limited number of human tests. The EFs of DEHP of 45.3% and DiNP of 30.5% applied in this study were based on the estimations from a toxicokinetic study of oral exposure of DEHP and DiNP, respectively, by Anderson et al. (2011) including 20 adults (10 women and 10 men).[32] Two previous pioneer studies by Koch et al. in 2005 and 2007, respectively, based on exposure of phthalates of a single man, showed somewhat higher EFs of 62.7% for DEHP and 39.6% for DiNP.[42,43] Due to the larger study size we chose to use the EFs estimated by Anderson et al. Regarding $\sum DBP_{(i+n)}$, Koch et al. recently estimated EFs of 84% (MnBP) and 71% (MiBP) for DnBP and DiBP, respectively.[44] These estimations were likewise based on only one single participant versus the estimations of Anderson et al. (2001) based on 24 participants, and we therefore chose to use the excretion fractions by Anderson et al. Some interindividual variation in the metabolism and subsequent urinary excretion exists, and using an average EF for all individuals is a simplification that might either under- or overestimate the "true" intake, but for time being it is our best suggestion for estimations of daily intake based on urinary excretion.

Third, another issue with this fractional excretion parameter is associated with the pathway of exposure. The EFs were developed from oral exposures, but also other routes of exposure have been identified. For example, Janjua et al. found that diethyl phthalate and DnBP from a skin lotion were systemically absorbed, metabolized, and excreted in urine 8−12 h postapplication.[19] Other exposure routes could lead to different metabolism. Furthermore, an EF of approximately 50% implies that 50% of the exposure is not taken into account. Part of it may either be excreted through alternative routes (evaporation, perspiration, faecal excretion), bioaccumulated, or excreted as yet unidentified metabolites.

The approach for a cumulative risk assessment used in this study is based on an assumption of dose additivity of chemicals with common adverse out-comes (in the case of phthalates possibly even common mode of action). Several experimental animal studies on mixtures' effects have indicated that this may be a fair assumption regarding antiandrogenic effects on male reproductive out-come.[9,10,20] Whether it is also a fair assumption in a broader context remains to be seen and has been questioned by some.[45] We chose to base our calculations on 24-h urine samples rather than spot urine samples to eliminate within-day variation in urinary phthalate excretion. Furthermore, this study is the first of its kind, which to our knowledge included three consecutive 24-h samples. This approach provides a better estimate about the true general exposures as it reduces the impact of an occasional high or low exposure. The weaknesses of this study included some uncertainty relating to the estimations of the daily intakes as discussed above. Furthermore, using standard equations do not account for the potential individual differences in metabolism of the phthalates, which most likely exists.

The participants were included from a larger study of reproductive health of young men representative of the general population.[26,27] Therefore, we believe our presented results are representative for young Danish men. There were no defining differences between the 33 men, who accepted participation, and those who did not (data not shown).

This study has shown that young Danish men from the general population were regularly exposed to four phthalates with known antiandrogenic effect and that some men occasionally experience combined exposures very close to or exceeding the threshold level for acceptable exposure for an adverse antiandrogenic effect. This is even without consideration of possible concurrent exposure to other antiandrogenic chemicals. Our results indicate that the human exposure pattern of DEHP include occasional high daily exposures. More knowledge on the effects of this exposure pattern compared to a more even exposure level seems warranted. Knowledge about exposure, metabolism, and excretion of phthalates and other endocrine disrupting chemicals is far from fully elucidated. Hence, studies about alternative routes of exposure and excretion apart from urinary excretion would establish a more complete knowledge about the total burden of phthalates.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

%MEHP	the percentage of DEHP metabolites excreted as the primary metabolite MEHP
∑DEHPm	sum of DEHP metabolites
∑DiNPm	sum of DiNP metabolites
$\sum MBP_{(i+n)}$	sum of MnBP and MiBP metabolites
BBzP	butylbenzyl phthalate
DEHP	di(2-ethylhexyl) phthalate
DiBP	di-iso-butyl phthalate
DiNP	di-iso-nonyl phthalate
DnBP	di-*n*-butyl phthalate
LOD	limit of detection
MBzP	monobenzyl phthalate (metabolite of BBzP)
MCiOP	mono(carboxy-iso-octyl) phthalate (metabolite of DiNP)
MECPP	mono(2-ethyl-5-carboxypentyl) phthalate (metabolite of DEHP)
MEHHP	mono(2-ethyl-5-hydroxyhexyl) phthalate (metabolite of DEHP)
MEHP	mono(2-ethylhexyl) phthalate (metabolite of DEHP)
MEOHP	mono(2-ethyl-5-oxohexyl) phthalate (metabolite of DEHP)
MHiNP	mono(hydroxy-iso-nonyl) phthalate (metabolite of DiNP)
MiBP	monoiso-butyl phthalate (metabolite of DiBP)
MiNP	monoiso-nonyl phthalate (metabolite of DiNP)
MnBP	mono-*n*-butyl phthalate (metabolite of DnBP)
MOiNP	mono(oxo-iso-nonyl) phthalate (metabolite of DiNP)
EF	(urinary) excretion fraction
RfV	reference value
HQ	hazard quotient
HI	hazard index
TDI	tolerable daily intake
EFSA	European Food and Drug Administration
RfV-AA	Reference values proposed by Kortenkamp et al.[1]
RfV-EFSA	Reference values proposed by European Food and Drug Administration (EFSA)[2−5]

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