Exposure to plasticizers in German daycare centers: the LUPE 3 study

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Abstract

Plasticizers have been widely used for decades in diverse applications. Therefore indoor air and settled dust samples from 63 daycare centers in Germany were analyzed for the presence of 11 phthalate diesters, 4 adipates, di-isononyl cyclohexane-1,2-dicarboxylate (DINCH), di(2-ethylhexyl) terephthalate (DEHT), acetyl tri-*n*-butyl citrate (ATBC), and trioctyl trimellitate (TOTM). 10 primary and secondary phthalate metabolites were quantified in urine samples of 663 children (1.7 to 6.7 years old) after they attended the facilities. Moreover, 4 metabolites of DINCH were quantified in urine samples of 208 children.

Di-isobutyl phthalate (DiBP), dibutyl phthalate (DnBP), di-2-ethylhexyl phthalate (DEHP), and DINCH were present in the highest concentrations in the indoor air, with median values of 468, 227, 194, and 108 ng/m³, respectively. In dust, median values of 888 mg/kg for DEHP, 302 mg/kg for DINCH, and 302 mg/kg for di-isononyl phthalate (DiNP) were detected.

The highest median values observed in urine were 44.7 µg/l for the monoester of DiBP, 32.4 µg/l for that of DnBP, and 16.5 µg/l and 17.9 µg/l for the two secondary DEHP metabolites. The three secondary metabolites of DINCH were observed with median values between 1.1 and 1.7 µg/l.

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Compared with recommended tolerable daily intake (TDI) values, especially DiBP and DnBP reached a higher proportion of TDI.
Keywords: phthalates, DEHP, DINCH, DEHT, adipates, indoor, human biomonitoring.

1 Introduction

Phthalate esters have been widespread in technical applications for decades and are a class of substances consisting of a phthalic acid combined with variable-length alkyl side chains. Due to their physicochemical properties, dialkylated phthalates have been used as plasticizers to soften consumer products and make them more flexible and resilient. In particular, the high-volume chemicals di-2-ethylhexyl phthalate (DEHP), dibutyl phthalate (DnBP), benzyl butyl phthalate (BzBP), di-isononyl phthalate (DiNP), and di-isodecyl phthalate (DiDP) can be found in diverse applications, including pharmaceutical products, cosmetics, construction materials, wood finishers, adhesives, floorings, paints, medical devices, and, primarily, as plasticizers in PVC production. Meanwhile, DEHP, DnBP and BzBP are subject to restriction by the REACH Annex XIV, which places restrictions on the marketing of and prohibits the use of these substances. Particularly due to the ongoing scientific debate of the health risks and subsequent regulation of short-chain phthalates these phthalates have been continuously replaced by longer chain phthalates, such as DiNP and DiDP. Additionally, "new" substances, such as di-isononyl cyclohexane-1,2-dicarboxylate (DINCH), di(2-ethylhexyl) terephthalate (DEHT) and di(2-ethylhexyl) adipate (DEHA), which exhibit a considerably more favorable toxicological profile, have been substituted for the phthalates.

The aim of this study was to investigate the exposure of certain phthalates and non-phthalate plasticizers in daycare centers in Germany (see Table 1). Overall, 19 plasticizers were quantified in the indoor air and settled dust and 14 primary and secondary metabolites of these substances were measured in the urine of children attending daycare centers. The total exposure of the children was quantified using a back-calculation approach from the excreted metabolites and was compared with toxicological values.

This study is one sub-project performed by health authorities of some German Bundesländer (Länderuntersuchungsprogramme, LUPE) to characterize the exposure of a vulnerable subset of the population and to develop strategies to minimize the current exposure to these substances. Results of organophosphates in indoor air and dust as well as their metabolites have been previously reported [1, 2].

2 Methods

The indoor air samples were collected on a cartridge with a glass fiber filter and on polyurethane foam using a sampler with a constant air flow of 3.5 l/min over approximately 6 hours. Settled dust samples were collected by slowly vacuuming

Table 1: Plasticizers and metabolites measured in this study.

Substance	Abbr.	Metabolite	Abbr.
Dimethyl phthalate	DMP	-	-
Diethyl phthalate	DEP	Mono-ethyl phthalate	MEP
Di-n-butyl phthalate	DnBP	Mono-n-butyl phthalate	MnBP
Di-isobutyl phthalate	DiBP	Mono-isobutyl phthalate	MiBP
Di-n-pentyl phthalate	DnPP	Mono-4-hydroxypentyl phthalate	MHPP
Di-isopentyl phthalate	DiPP	Mono-isopentyl phthalate	MiPP
Dicyclohexyl phthalate	DcHP	-	-
Benzylbutyl phthalate	BzBP	Mono-benzyl phthalate	MBzP
Di-2-ethylhexyl phthalate	DEHP	Mono-(2-ethyl-5-oxohexyl) phthalate Mono-(2-ethyl-5-hydroxyhexyl) phthalate	5oxo-MEHP 5OH-MEHP
Di-isononyl phthalate	DiNP	Mono-4-methyl-7-oxo-octyl phthalate	7oxo-MiNP
Di-isodecyl phthalate	DiDP	Mono(4,5-dimethyl-7-hydroxyoctyl) phthalate	OH-MiDP
Di-iso-nonyl-1,2-cyclohexanedi-carboxylate	DINCH	cyclohexane-1,2-dicarboxylic acid monoisononyl ester cyclohexane-1,2-dicarboxylic acid-mono(hydroxy-isononyl) ester cyclohexane-1,2-dicarboxylic acid-mono(carboxy iso-octyl) ester cyclohexane-1,2-dicarboxylic acid mono(oxo-isononyl) ester	MINCH OH-MINCH cx-MINCH oxo-MINCH
Acetyltri-n-butyl citrate	ATBC	-	
Di(2-ethylhexyl) adipate	DEHA	-	
Di-n-butyl adipate	DnBA	-	
Di-iso-butyl adipate	DiBA	-	
Di-iso-nonyl adipate	DiNA	-	
Di(2-ethylhexyl) terephthalate	DEHT	-	
Tri(2-ethylhexyl) trimellitate	TOTM	-	

the floor of the room during a fixed time period using an ALK dust filter holder mounted on a sampler connected to a vacuum cleaner. After extraction the analysis of air and dust samples was performed on a gas chromatographic system with mass selective detector (GC-MS). For urine samples the analytical method consisted of an offline protein precipitation followed by HPLC and MS/MS detection using a column switching LC–MS/MS unit.

3 Results

3.1 Indoor air

The results are given in Table 2. We were able to detect DMP, DEP, DBP, DiBP, and DEHP in 95% to 98% of the samples above the LOQ, as well as DiNP in 92%, DiDP in 38%, BzBP in 10%, and DcHP in 6% of the samples above the LOQ. DnPP could not be quantified in any sample. DEHA, DEHT, and DINCH were quantified in 85%, 85%, and 76% of the samples, respectively. The median values were 468 ng/m³ for DiBP, 227 ng/m³ for DBP, 194 ng/m³ for DEHP, 183 ng/m³ for DEP, 102 ng/m³ for DiNP, and 76 ng/m³ for DMP. The highest maximum values were seen for DiBP, DMP, and DnBP, with concentrations of 2600, 1400, and 1300, respectively. For the three substitutes, the median values were 20 (DEHT), 34 (DEHA), and 108 ng/m³ (DINCH).

3.2 House dust

The statistical parameters for the phthalate measurements in dust from daycare centers are provided in Table 2. DEP, DiBP, DnBP, DEHP, DiNP, and DiDP were detected in 100%, DMP and BzBP in 95%, DPhP and DcHP in 79%, and DnOP in 65% of the house dust samples. The median values were 888 mg/kg (DEHP), 302 mg/kg (DiNP), 34 mg/kg (DiDP), 21 mg/kg (DBP), 21 mg/kg (DnBP), 20 mg/kg (DiBP), 6 mg/kg (BzBP), 1.3 mg/kg (DEP), 0.7 mg/kg (DMP), and 0.3 mg/kg (DcHP). In particular, DEHP and DiNP varied widely, and high maximum values of 10086 mg/kg and 7091 mg/kg were observed for DEHP and DiNP, respectively. DnPP and DPrP were detected in only 13 and 9 samples with maximum values of 0.7 mg/kg and 0.6 mg/kg, respectively. The three other plasticizers analyzed were detected in all samples above the LOQ except DEHA (only in 62 samples), and median concentrations of 302 mg/kg for DINCH, 40 mg/kg for DEHT, and 49 mg/kg were found. Again, the results, especially for DINCH, varied over a wide range.

3.3 Metabolites in urine samples

In Table 3 the results are given. MnBP, MiBP, 5OH-MEHP, and 5oxo-MEHP were detected in 100%, 7oxo-MiNP in 98%, MBzP in 82%, MEP in 67%, and OH-MiDP in 30% of all 663 samples collected after daycare attendance. MnOP and MiPP were found in only 6 and 2 samples, with maximum values of 1.8 and 6.1 µg/l, respectively. MHPP, a secondary metabolite of DnPP, was not detected in any sample. As can be seen in Table 3, the highest median values were observed for MiBP (44.7 µg/l), MnBP (32.4 µg/l), and the two secondary DEHP metabolites (16.5 µg/l for 5OH-MEHP and 17.9 µg/l for 5oxo-MEHP). Overall, the readings ranged over several orders of magnitude, with the highest maximum values of 5225 µg/l for MEP and 2063 µg/l for MnBP. In addition, 5OH-MEHP, 5oxo-MEHP, 7oxo-MiNP, and MBzP were found in significantly higher concentrations in males. No significant differences were observed among the three age groups.

Table 2: Plasticizers in indoor air and house dust of 63 daycare centers.

Compound	N>LOQ	Mean	Min–Max	Median	95th percentile
Dust (mg/kg)					
DMP	60	0.7	<0.04–11	0.3	2.4
DEP	63	3.4	0.4–101	1.4	7.4
DiBP	63	39	7–335	20	174
DnBP	63	30	2–266	21	95
DnPP	10		<0.1–0.69		
BzBP	60	21	<1.0–348	6	93
DEHP	63	1973	99–10086	888	7616
DcHP	50	5.4	<0.03–239	0.3	12
DiNP	63	745	30–7091	302	2955
DiDP	63	60	5–571	34	170
DINCH	63	504	32–2732	302	1622
DEHT	63	74	9–312	40	256
DEHA	63	80	1.0–724	49	307
DiBA	25		<0.1–6.0		
DnBA	0				
DiNA	3		<1–34		
ATBC	40	146	<8–3314	24	407
TOTM	63		<7–107		
Indoor air (ng/m³)					
DMP	60	163	<0.01–1431	76	833
DEP	62	208	<0.01–781	183	383
DiBP	60	516	<0.01–2613	468	916
DnBP	60	283	<0.01–1276	227	884
DnPP	0				
BzBP	6		<0.01–215		
DEHP	61	276	<0.01–889	194	765
DcHP	2		<0.01–64		
DiNP	58	133	<0.05–763	102	341
DiDP	24	49	<0.05–226	25	165
DINCH	48	159	<0.05–781	108	547
DEHT	54	31	<0.01–226	20	120
DEHA	54	49	<0.01–206	34	139
DiBA	37*	17	<0.01–89	35	53
DnBA	41*	45	<0.01–134	91	136
DiNA	0*				
ATBC	27*	19	<0.01–122	45	72
TOTM	0*				

Values below the LOQ were assigned half of the LOQ;
*: in a subset of 43 daycare centers.

Table 3: Phthalate and DICH metabolites in 663 and 208 urine samples in µg/l.

	N> LOQ	Mean	Median	95th percentile	Min–Max
Phthalate metabolites					
MEP	443	34.9	14.5	112	<2.5–5225
MnBP	663	42.6	32.4	124	2.3–221
MiBP	663	64.4	44.7	155	2.1–2063
MBzP	543	20.2	11.6	80.7	<2.5–311
5OH-MEHP	663	22.5	16.5	60.2	0.8–225
5oxo-MEHP	663	23.1	17.9	58.2	0.8–168
7oxo-MiNP	648	10.5	4.6	29.9	<0.5–486
OH-MiDP	199	2.8	0.8	8.6	<1.3–155
DINCH metabolites					
MINCH	47	<0.1	<0.1	0.41	<0.10–2.0
OH-MINCH	208	2.87	1.66	9.95	0.14–34.9
cx-MINCH	208	1.89	1.14	6.11	0.11–19.1
oxo-MINCH	206	2.54	1.54	7.98	<0.05–34.8

Values below the LOQ were assigned half of the LOQ.

Secondary DINCH metabolites OH-MINCH and cx-MINCH were quantified in all samples, while oxo-MINCH and monoester MINCH were respectively quantified in 99% and 23% of the samples above the LOQ. The highest readings were observed at 34.9 µg/l for OH-MINCH and at 34.8 µg/l for oxo-MINCH.

3.4 Intake calculated from biomonitoring data

Using medians and 95th percentiles of the metabolite concentrations in urine, we back-calculated a "typical" and "high" total daily intake for our study population. The methods were described in detail elsewhere [3–5]. These estimates are given in Table 4. Regarding the total intake calculated from biomonitoring data, "high" intake levels were seen for DiNP (14.1 µg/kg b.w.) and DEHP (11.9 µg/kg b.w.).

3.5 Comparison with toxicological values

The "typical" and "high" total daily intake and daily inhaled and ingested (house dust) amounts reported in our study were compared with the estimated tolerable daily intake (TDI) recommended by scientific institutions.

With regard to phthalates, TDI values were determined by the European Food Safety Agency and by the World Health Organization. A TDI for DINCH was determined from the no observed adverse effect level (NOAEL) of 100 mg/kg b.w. for renal effects and the application of an uncertainty factor of 100, resulting in a TDI of 1,000 µg/kg b.w. Additionally, Bhat *et al.* (2014) [9] derived an oral reference dose of 700 µg/kg b.w. for thyroid hypertrophy/hyperplasia in adults rats exposed in utero.

Table 4: Overall intake of phthalates and DINCH in μg/kg b.w.

Substance	Total daily intake		TDI-value	Reference of TDI-value
	"typical"	"high"		
DEP	0.9	6.7	500	WHO 2003 [6]
DnBP	1.3	4.9	10	EFSA 2005 [7]
DiBP	1.8	6.2	10	EFSA 2005 [7]*
BzBP	0.4	3.0	500	EFSA 2005 [7]
DEHP	3.3	11.9	50	EFSA 2005 [7]
DiNP	2.2	14.1	150	EFSA 2005 [7]
DINCH	0.5	2.8	1000	EFSA 2006 [8]

*DiBP has the same toxicity as DnBP; therefore, a similar TDI was assumed.

Compared with the above-mentioned TDI values recommended by scientific institutions, the share of the "high" total intake scenario was 62% for DiBP, 49% for DnBP, 24% for DEHP, 9% for DiNP, and 0.3% for DINCH. For BzBP and DEP, only 0.6% and 1.3% of the TDI was reached.

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