Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 847 (2007) 114–125
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Determination of secondary, oxidised di-*iso*-nonylphthalate (DINP) metabolites in human urine representative for the exposure to commercial DINP plasticizers

Holger M. Koch [a,b,*], Johannes Müller [a], Jürgen Angerer [a]

[a] *Institut und Poliklinik für Arbeits-, Sozial-und Umweltmedizin, Schillerstrasse 25/29, 91054 Erlangen, Germany*
[b] *Berufsgenossenschaftliches Forschungsinstitut für Arbeitsmedizin (BGFA), Bürkle-de-la-Camp Platz 1, 44789 Bochum, Germany*

Received 19 June 2006; accepted 22 September 2006
Available online 20 October 2006

Abstract

Di-*iso*-nonylphthalate (DINP) is the major plasticizer for polyvinylchloride (PVC) polymers. Two DINP products are currently produced: DINP 1 and DINP 2. We analyzed the isononyl alcohol mixtures (INA) used for the synthesis of these two DINP plasticizer products and thus identified 4-methyloctanol-1 as one of the major constituents of the alkyl side chains of DINP 1 (8.7%) and DINP 2 (20.7%). Based on this isomer, we postulated the major DINP metabolites renally excreted by humans: mono-(4-methyl-7-hydroxy-octyl)phthalate (7OH-MMeOP), mono-(4-methyl-7-oxo-octyl)phthalate (7oxo-MMeOP) and mono-(4-methyl-7-carboxy-heptyl)phthalate (7carboxy-MMeHP). We present a fast and reliable on-line clean-up HPLC method for the simultaneous determination of these three DINP metabolites in human urine. We used ESI-tandem mass spectrometry for detection and isotope dilution for quantification (limit of quantification 0.5 μg/l). Via these three oxidised DINP isomer standards, we quantified the excretion of all oxidised DINP isomers with hydroxy (OH-MINP), oxo (oxo-MINP) and carboxy (carboxy-MINP) functional groups. With this approach, we can for the first time reliably quantify the internal burden of the general population to DINP. Mean urinary metabolite concentrations in random samples from the general German population ($n = 25$) were 14.9 μg/l OH-MINP, 8.9 μg/l oxo-MINP and 16.4 μg/l carboxy-MINP. Metabolites strongly correlated with each other over all samples analyzed ($R > 0.99$, $p < 0.0001$).

Keywords: DINP; Phthalate; Urine; Plasticizer; Metabolites; Biological monitoring

1. Introduction

Di-*iso*-nonylphthalate (DINP) has replaced di(2-ethylhexyl) phthalate (DEHP) as the major plasticizer of polyvinylchloride (PVC) polymers. Annually around 500,000 tonnes of DINP are being produced in Europe [1,2]. Ninety five percent of DINP is used in PVC applications [3]. Presently, two different DINP types are on the market. Their chemical composition is only insufficiently described in scientific literature. DINP 1 (CAS 68515-48-0) is a mixture of esters of *o*-phthalic acid with C8–C10 alkyl alcohols of different chain lengths and branching distributions. DINP 2 (CAS 28553-12-0) consists solely of isomeric C9 alcohols in the ester chain. A third DINP type has in the meantime vanished from the market [3,4].

Previous risk assessments in the USA [4] and in Europe [3,5] evaluated the different DINP products as one. In rodents, DINP exhibits toxic effects on liver and kidney [3–6]. Based on the critical endpoint, spongiosis hepatis the U.S. Consumer Product Safety Commission (CPSC) derived an Acceptable Daily Intake (ADI) for DINP of 120 μg/kg body-weight/day [7]. The European Food Safety Authority (EFSA) derived a Tolerable Daily Intake (TDI) of 150 μg/kg body-weight/day [5]. We also have to regard DINP as an endocrine disruptor/modulator. Effects like nipple retention and testis atrophy are comparable to DEHP and di-*n*-butylphthalate (DnBP) [8].

DINP is a large volume workplace chemical and a ubiquitous environmental contaminant. Exposure to DINP can occur both through occupation and environment [3,4]. Therefore, for health protection the determination of DINP exposure is of utmost importance. Biological monitoring of internal DINP

* Corresponding author at: Berufsgenossenschaftliches Forschungsinstitut für Arbeitsmedizin (BGFA), Bürkle-de-la-Camp Platz 1, 44789 Bochum, Germany. Tel.: +49 234 302 4647; fax: +49 234 302 4505.
E-mail address: koch@bgfa.ruhr-uni-bochum.de (H.M. Koch).

1570-0232/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.09.044

exposure through specific oxidised metabolites is especially eligible because it is not influenced by external DINP contamination and because it measures each individuals DINP burden over all routes of exposure [9–11]. We therefore saw the need to develop an analytical method to determine oxidised DINP metabolites in urine of the general population as has previously been done for DEHP [12–15]. The simple monoester mono-*iso*-nonylphthalate (MINP), which has been used in some studies [16–18] is not a reliable parameter for DINP exposure because it is extensively further oxidised by ω-, ω-1- and β-oxidation before renal excretion [19–21].

Analysis of exposure to commercial DINP is especially challenging compared to DEHP, because it is a complex mixture of alkyl chain isomers and homologues.

Therefore, first, it was necessary to identify the alkyl chain isomer representatively indicating the exposure to the commercial DINP mixtures. For this purpose, we analyzed different intermediate *iso*-nonyl-alcohol (INA) mixtures used for esterification with phthalic acid in DINP production. Based on the identified major alkyl chain isomer of DINP together with known metabolism of DEHP [22–26], we postulated the major ω- and ω-1-oxidation products with hydroxy, oxo and carboxy functional groups: mono-(4-methyl-7-hydroxy-octyl)phthalate (7OH-MMeOP), mono-(4-methyl-7-oxo-octyl)phthalate (7oxo-MMeOP) and mono-(4-methyl-7-carboxy-heptyl)phthalate (7carboxy-MMeHP). We developed an analytical method (LC/LC–MS/MS) to determine these oxidised metabolites in urine. Based on the calibration curves of these three specific metabolites of the most abundant alky chain isomer, we quantified the sum of all other C9 alky chain isomers with hydroxy, oxo and carboxy functional groups: carboxy-MINP, OH-MINP and oxo-MINP. We applied the method on urine samples of a small occupationally non-DINP exposed population.

2. Experimental

2.1. Anaylsis of commercial isononylalcohols used for DINP production

Both currently available commercial DINP plasticizer types are produced by esterification of phthalic acid anhydride (PAN) with so-called C9 oxo alcohols (isononyl alcohol, INA). The different composition of the INA used for esterification is directly related to the different compositions of the alkyl chains of the final DINP 1 and DINP 2 products. DINP 1 is manufactured by the "polygas" process, which generates INA from butenes and propylenes resulting in C8–C10 (C9-rich) branched alkylalcohols (CAS 68526-84-1). The INA used for DINP 2 is *n*-butene based and solely consists of C9 alcohol isomers (CAS 27458-94-2). Table 1 summarizes what is presently known about the composition of the two INA types used for synthesis of the two different DINP products.

Obviously, compositions described for INA type 2 differ considerably but detailed information on their chemical structure is very limited [3,27–29]. For INA type 1, we only found one rudimentary reference [29]. Therefore, to further elucidate the composition of the DINP alkyl chains and with the intention to identify the major alkyl sidechain we obtained two INA type 2 intermediates and one INA type 1 intermediate from three major DINP producers. *N*-nonanol as a straight chain reference standard was commercially available. 4-Methyloctanol-1 described by Hellwig and Jackh [27] and Otter [28] as a major constituent of INA 2 (29%, respectively <20%) has recently been synthesized by Rochat et al. [30].

2.1.1. Chemicals

Isononylalcohols (INA):

INA Type 1 (Cas No. 68626-84-1) used for the synthesis of DINP 1: Exxal 9, Batch No. 30505 SB 18, Lot No. 10006936, was obtained from Exxon Mobil (Machelen, Belgium).
INA Type 2 (Cas No. 27458-94-2) used for the synthesis of DINP 2: Nonanol N, Art. No. 50004039, was obtained from BASF Aktiengesellschaft (Ludwigshafen, Germany). Isononanol, with no further specification, was obtained from OXENO Olefinchemie GmbH (Marl, Germany).

1-Nonanol (*n*-nonyl alcohol, puriss., ≥99.5%) was obtained from Fluka (Deisenhofen, Germany). 4-Methyloctanol-1 was a gift from Dr. Didier Rochat (UMR 1272, INRA, Centre de Versailles-Grignon).

N-tert-butyldimethylsilyl-*N*-methyltrifluoro-acetatamid (MTBSTFA, 98%) and acetonitrile SeccoSolv^{TM} (max 0.005% H_2O) were obtained from Merck (Darmstadt, Germany).

2.1.2. Sample preparation

The INA's were diluted with acetonitrile to 50 mg/l solutions. The *n*-nonanol and 4-methyloctanol-1 standards were diluted with acetonitrile to 10 mg/l solutions. These solutions were subject to GC–MS analysis as such and after derivatisation with MTBSTFA. For derivatisation, 20 μl of MTBSTFA was added to 100 μl of the alcohol dilutions directly in the GC vials, the vials were heated at 70 °C for 45 min in an oven. A 1-μl volume of both the derivatised and non-derivatised sample was then analyzed by GC–MS.

2.1.3. Gas chromatography

Analysis was carried out on a Hewlett-Packard HP 5890 Series II plus gas chromatograph equipped with a Hewlett-Packard HP 7673 autosampler and a split/splitless injector operating in splitless mode. The inlet purge off time was 1.0 min. The operating temperature of the injector was 280 °C. Chromatographic separation was performed using a HP-35 capillary column (crosslinked 35% diphenyl-dimethylpolysiloxane, 60 m × 0.25 mm internal diameter, 0.25 μm film thickness) purchased from Hewlett-Packard (Waldbronn, Germany). Helium 5.0 was used as the carrier gas at a constant flow of 1.1 ml/min. The initial column temperature of 60 °C was held for 1 min, and then raised at a rate of 7.5 °C/min to 160 °C, held at this temperature for 2 min and finally raised at 25 °C/min to 290 °C, remaining at this temperature for 4 min.

Table 1
Content (in %) of the different chain structures (INA-isomers) in DINP 1 and DINP 2 according to the literature

	INA type 2			INA type 1
	Hellwig and Jackh [27]	Otter [28]	ECPI [29]	ECPI [29]
3-Ethyl-4-methylhexanol-1	1	n.s.	5–10[a]	5–10[a]
4,5-Dimethylheptanol-1	23	n.s.	40–45[b]	45–55[b]
2,5-Dimethylheptanol-1	n.s.	12		
2-Methyloctanol-1	n.s.	6	35–40[c]	5–20[c]
4-Methyloctanol-1	29	<20		
6-Methyloctanol-1	15	<20		
3-Ethylheptanol-1	3	8	n.s.	n.s.
n-Nonanol-1	n.s.	<10	0–10	0–1
Isodecanol	0	0	0	15–25

n.s., Not specified.
[a] Sum of methyl ethyl hexanols.
[b] Sum of dimethyl heptanols.
[c] Sum of methyl octanols.

2.1.4. Mass spectrometry

We used a Hewlett-Packard HP MSD 5972 Series mass spectrometer fitted with a quadrupole mass filter in electron impact (EI) mode. EI mass spectra were obtained at an energy level of 70 eV and the electron multiplier voltage was 2300 V. The MSD transfer line temperature was maintained at 300 °C. For the analysis of the alcohols, we performed a total ion scan over the mass range *m/z* 50–300. We identified 4-methyloctanol-1 and *n*-nonanol in the INA samples both by retention times and characteristic fragmentation patterns both with and without derivatisation. The amount of these two alcohols within the INA mixtures was estimated by the percentage of the total ion chromatogram (TIC) response of their individual peaks in the total response recorded for the whole INA mixtures.

2.2. Biological monitoring of oxidised DINP/MINP metabolites in urine

2.2.1. Chemicals

Mono-(4-methyl-7-hydroxy-octyl)phthalate (7OH-MMeOP), mono-(4-methyl-7-oxo-octyl)phthalate (7oxo-MMeOP) and mono-(4-methyl-7-carboxy-heptyl)phthalate (7carboxy-MMeHP) and their D4-ringlabelled analogues D4-7OH-MMeOP, D4-7oxo-MMeOP and D4-7carboxy-MMeHP were synthesized in cooperation with the Institut für Dünnschichttechnologie und Mikrosensorik e.V. (IDM), Teltow, Germany. All six compounds had a chemical purity > 95%. The isotopic purity of each labelled internal standard was tested by LC–MS/MS and contained no measurable unlabelled or partially labelled (D3 or D2) compound. Acetonitrile, water, methanol (all of HPLC-grade), acetic acid (glacial, extra pure) and ammonium acetate p.a. were purchased from Merck, Darmstadt, Germany. β-Glucuronidase from *Escherichia coli* K12 was purchased from Roche Biomedical, Mannheim, Germany.

2.2.2. Standard preparation

The initial stock solution for the standards was prepared by dissolving approximately 10 mg of 7OH-MMeOP, 7oxo-MMeOP and 7carboxy-MMeHP in 25 ml of acetonitrile (400 mg/l). This stock solution was stored at −18 °C in a Teflon-capped glass vial until further use. In order to prepare six calibration standards during routine operation of the method, the initial stock solution was gradually diluted with water to achieve standard concentrations in the range from 0.5 to 500 μg/l.

The stock solution for the internal standards was prepared by dissolving approximately 10 mg of D4-7OH-MMeOP, D4-7oxo-MMeOP and D4-7carboxy-MMeHP in 25 ml of acetonitrile (400 mg/l). This stock solution was stored at −18 °C in a Teflon-capped glass vial until further use. An aliquot of 375 μl was placed in a 100 ml glass volumetric flask and diluted to the mark with water (1.5 mg/l). This solution served as the working solution.

2.2.3. Sample collection and preparation

Urine samples were collected in 250 ml polyethylene containers and frozen immediately until analysis. In preparation for the analysis, urine samples were thawed and equilibrated to room temperature. The samples were vortex mixed and aliquots of 1 ml were then transferred to 1.8 ml glass screw-cap vials. Two hundred microliters of ammonium acetate buffer solution (1 M, pH 6.5), 50 μl of the D4-internal standard solution (1.5 mg/l) and 10 μl β-glucuronidase were added to the samples. The samples were incubated for 1.5 h at 37 °C in a drying oven. After hydrolysis, each sample was thoroughly mixed and centrifuged at 1500 × *g* for 10 min. The supernatant was transferred into another 1.8 ml glass screw-cap vial and 500 μl were then injected into the LC–MS/MS system for quantitative analysis. Urinary creatinine concentrations were determined according to Larsen [31].

2.2.4. Calibration procedure and quantification

An aliquot of 1 ml of the standard solutions was used and processed in the same way as described in Section 2.2.3. Pure water, which was processed in the same way, was used as a blank. Linear calibration curves were obtained by plotting the quotients of the peak areas of the three specific DINP metabolites and the respective D4-internal standards as a function of the concentrations. The coefficients of correlation for all calibration

curves were higher than 0.99. These graphs from the specific metabolites were used to ascertain the unknown concentration of the oxidised DINP isomer metabolites in urine samples.

Using the calibration curve for 7carboxy-MMeHP, we derived the sum of the DINP metabolites with a carboxylic acid functional group, using the calibration curve for 7OH-MMeOP we derived the sum of the DINP metabolites with a hydroxy functional group and using the calibration curve for 7oxo-MMeOP we derived the sum of the DINP metabolites with a keto functional group. Metabolite concentrations in the following are therefore depicted as carboxy-MINP (based on 7carboxy-MMeHP), OH-MINP (based on 7OH-MMeOP) and oxo-MINP (based on 7oxo-MMeOP).

2.2.5. Quality control

As there was no quality control material commercially available it had to be prepared in the laboratory. During method development, it became obvious that almost all urine samples contained oxidised MINP metabolites over a wide concentration range. We therefore chose to pool different urine samples to obtain a low concentration quality control material (Q_{low}, metabolite concentration approximately 15 μg/l) and a high concentration quality control material (Q_{high}, metabolite concentration approximately 50 μg/l). These pooled urine samples were frozen, thawed and filtered before being divided into aliquots and stored at −18 °C. Creatinine content for Q_{low} was 1.1 g/l, for Q_{high} 0.75 g/l.

Within-day repeatability was determined by analysing Q_{low} 10-times in a row. Between-day repeatability was determined by analysing both quality control samples on eight different days.

Inaccuracy which might be due to the influence of the urinary matrix was determined using 10 different individual urine samples with creatinine contents ranging from low (0.30 μg/l) to very high (3.8 g/l). These urine samples were spiked with each metabolite (55.9 μg/l 7OH-MMeOP, 58.4 μg/l 7oxo-MMeOP and 81.3 μg/l 7carboxy-MMeHP) and analyzed. The same specimens without the addition of the metabolites were analyzed to obtain the native metabolite concentration and the recovery of the spiked amount of each metabolite was determined by subtraction. Individual recovery values were also used to calculate imprecision within these 10 very different individual urine samples.

To test the stability of the metabolites, we also determined the phthalate metabolites after the quality control samples have been left at room temperature (approximately 20 °C) for 3 days, and after quality control samples have been frozen and thawed two times.

2.2.6. High-performance liquid chromatography

Liquid chromatography was carried out on a Hewlett-Packard HP 1100 Series HPLC apparatus (auto sampler, quaternary pump, vacuum degasser) and an additional isocratic Merck-Hitachi L6000A pump from Merck (Darmstadt, Germany).

The latter was used to load the sample (500-μl aliquot) on the restricted access material (RAM) phase, a LiChrospher RP-8 ADS, 25 μm, 25 mm × 4 mm from Merck (Darmstadt, Germany) using a 1% aqueous solution of acetic acid and methanol

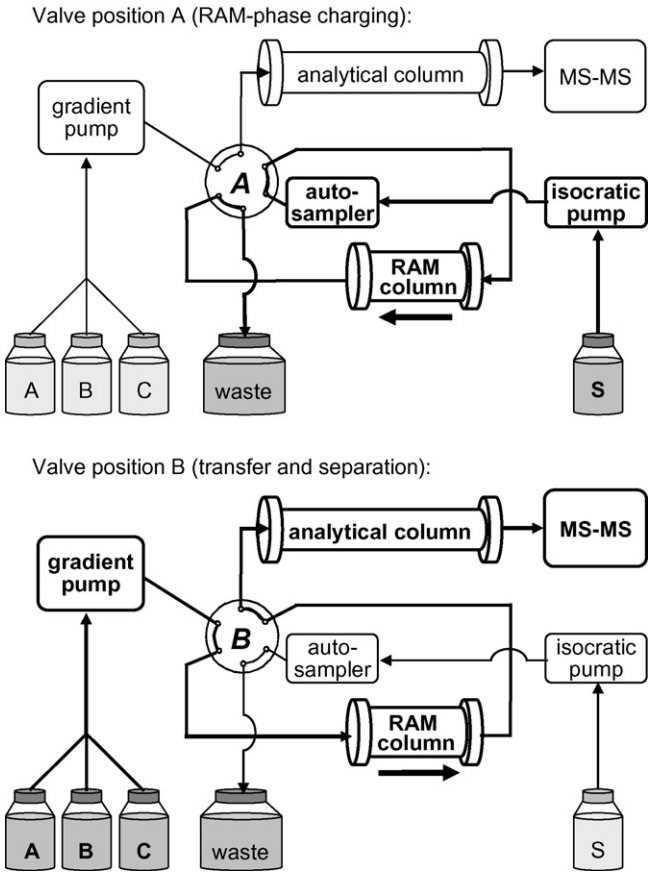

Fig. 1. Two-column HPLC system for direct on-line enrichment and clean-up of the samples on the RAM phase (valve position A) and analyte transfer onto the analytical column for chromatographic separation (valve position B). The system is automatically controlled via a six-port switching valve.

(95:5, v/v) as the mobile phase and a flow rate of 1 ml/min. The isocratic pump was solely operated with this mobile phase.

After this clean-up and enrichment step, the analytes were transferred to a reversed-phase HPLC column (Synergi™ Fusion-RP, 4 μm, 250 mm × 3 mm from Phenomonex, Aschaffenburg, Germany) in backflush mode through a time controlled 10-port valve on the API 2000 LC/MS/MS system (PE Biosystems, Langen, Germany) and an LC pump gradient. Fig. 1A and B shows a schematic diagram of the whole assembly. The timetables of the analysis program of the gradient HPLC pump and the automated switching procedure are given in Tables 2 and 3. All steps were controlled by the Analyst 1.2 Software from Perkin-Elmer except the isocratic pump, which was operated in isocratic mode with a constant flow rate.

2.2.7. Mass spectrometry

The MS/MS detection was performed on a Sciex API 2000 LC/MS/MS system (PE Biosystems, Langen, Germany) equipped with a software controlled 10-port valve. The source specific parameters were optimized manually for the LC conditions used during analysis depending on flow rate and eluent composition. The ion source-dependent (turbo ion spray) conditions were the same for all of the analytes with an ion spray (IS) voltage of −4250 V in the negative ionization mode. Nitrogen

Table 2
Analysis program of the gradient pump

Time (min)	Flow rate (μl/min)	Solvent A (%)	Solvent B (%)	Solvent C (%)
0.0–9.9	500	65	35	0
10.5–12.0	500	50	50	0
13.0–15.0	500	40	60	0
20.1–22.0	600	25	75	0
24.5–28.0	600	0	100	0
28.1–33.0	600	0	25	75
33.1–35.0	500	65	35	0

Solvent A: 10% methanol in a 0.25% aqueous solution of acetic acid; solvent B: 90% methanol in a 0.25% aqueous solution of acetic acid; solvent C: 100% methanol.

Table 3
Timetable for the automatic column switching valve

Time (min)	Valve position	Event
0–9.9	A	RAM charging
10.0–13.9	B	Analyte transfer
14.0–29.9	A	Analyte separation
30.0–31.4	B	Washing
31.5–35.0	A	Re-equilibration

as nebulizer and turbo heater gas (temperature TEM: 475 °C) was set to a pressure of 35 and 70 psi (ion source gas GS1/GS2), respectively. The curtain gas (CUR) was set to 40 psi. The collision gas (N_2) for the MS/MS mode at quadrupole Q_2 was set to a flow of four instrument units (CAD). Compound specific mass spectrometer parameters were optimized for each compound by the Quantitative Optimization Wizard of the Sciex Analyst software. Continuous flow injections with the syringe pump system of the API 2000 of standard solutions were performed for all analytes to establish their MS/MS operating conditions. For each analyte, we monitored two specific parent and daughter ion combinations with one combination being used for quantification ("quantifier") and the other for verification ("qualifier"). MS/MS operating conditions in the MRM-mode for all parameters were as follows: resolution Q_1: low; resolution Q_3: unit; settling time: 10 ms; MR pause: 10 ms; scan time: 50–75 ms. Analyte specific parameters are shown in Table 4.

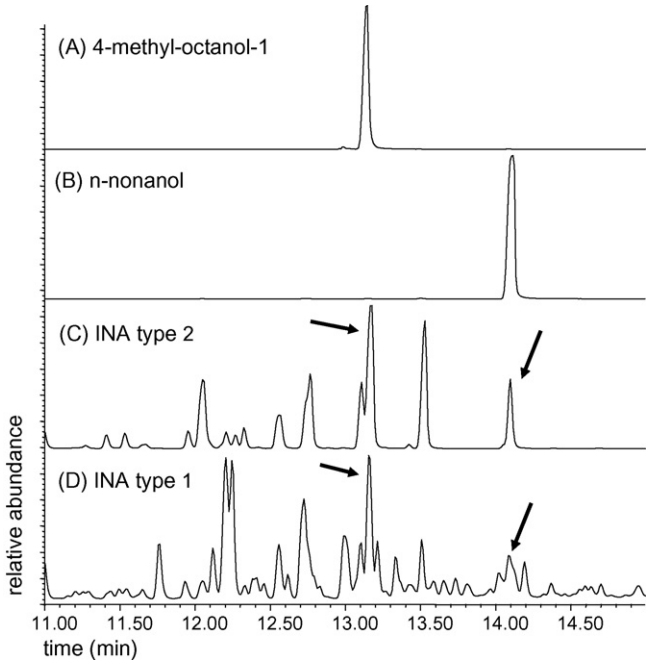

Fig. 2. GC–EI-MS chromatogram (total ion chromatogram, TIC) of 4-methyl-octanol-1 (A), *n*-nonanol (B), *iso*-nonylalcohol type 2 (Nonanol N) of BASF (C) and *iso*-nonylalcohol type 1 (Exxal 9) of ExxonMobil (D) after silylation with MTBSTFA. The respective peaks of 4-methyl-octanol-1 and *n*-nonanol in the industrial mixtures are marked with arrows.

2.2.8. Study subjects

For a pilot study, we investigated 25 spot urine samples randomly taken from the general population in Southern Germany (12 females, 13 males, ages between 6 and 73 years, median 32 years). All of them were not exposed to phthalates occupationally. Creatinine levels were in the range from 0.58 to 2.76 g/l (median 0.95 g/l).

3. Results and discussion

3.1. The major alcohol in commercial INA mixtures

Fig. 2 shows the total ion chromatograms of 4-methyloctanol-1 (A), *n*-nonanol (B), INA type 2 (C) and INA type 1 (D)

Table 4
MRM-parameters for each specific parent daughter combination

Analyte	Parent ion (Q_1)	Daughter ion (Q_3)	Retention time (min)	DP	FP	EP	CE
7oxo-MMeOP	305	77	23.66	−26	−340	−8.5	−40
		121[a]		−26	−340	−8.5	−32
D4-oxo-MMeOP	309	125	23.59	−21	−320	−9.5	−26
7carboxy-MMeHP	321	127	24.33	−31	−350	−5.0	−38
		173[a]		−31	−340	−6.5	−26
D4-7carboxy-MMeHP	325	173	24.29	−31	−350	−5.0	−26
7OH-MMeOP	307	77	24.70	−21	−320	−12	−44
		121[a]		−21	−320	−12	−22
D4-7OH-MMeOP	311	125	24.60	−31	−340	−10	−22

DP, declustering potential [V]; FP, focussing potential [V]; EP, entrance potential [V]; CE, collision energy [V].
[a] Used for quantification.

after derivatisation with MTBSTFA. The two INA type 2 products of the two different suppliers (BASF and OXENO) produced almost identical chromatograms (not shown). We therefore regarded these to two INA type 2 mixtures to be of the same composition. The identity of the 4-methyloctanol-1 and the *n*-nonanol peak in the chromatograms of both INA types (C and D) was confirmed by retention time (13.16 min for 4-methyloctanol-1 and 14.09 min for *n*-nonanol) and characteristic mass fragmentation patterns (not shown). For INA type 2, the chromatogram indicated that around 15 INA isomers were present within the mixture. For INA type 1, the chromatogram indicated to considerably more isomers, probably above 35. Based on the responses of the total ion chromatograms, we estimated a 4-methyloctanol-1-content in INA type 2 of 20.7% (for the products of both suppliers) and in INA type 1 of 8.7%. In both INA types (INA types 1 and 2), we identified 4-methyloctanol-1 as the primary component. For *n*-nonanol, we estimated a content of 7.8% in INA type 2 and of about 1.4% in INA type 1. In the chromatogram of INA type 1, various peaks (with retention times higher than 13.5 min) had mass fragments with an *m*/*z* that had to origin from a C10-alkylchain. We roughly estimated a C10-content in INA type 1 of 15–20%. All estimates were confirmed also by measurements of the non-derivatised alcohols. Chromatography and peak resolution, however, was better after derivatisation with MTBSTFA.

The chromatograms of the two different INA types, although indicating to some common constituents, illustrate their considerable difference and therefore also the considerable differences in chemical composition of the resulting DINP types. According to the American Chemistry Council (ACC), DINP composition (of each DINP type) is not variable due to the stability of the manufacturing process of the INA [4,32]. The fact that the two INA type 2 mixtures we analyzed were almost identical supports this view. However, slight changes in the manufacturing process of INA over time or manufacturing site specific features consequently lead to changes of the DINP composition. This aspect is possibly reflected by qualitative and quantitative descriptions of DINP compositions in literature that differ considerably from each other (see Table 1). Therefore, we have to point out, that our findings concerning the composition of the different INAs only apply to the INA intermediates of the three producers we investigated and might not apply to INA intermediates of other producers, of other production sites or of older origin. Yet, we have unambiguously identified and quantified 4-methyloctanol-1 as the major side chain constituent being representative for both currently produced DINP types.

3.2. Oxidised MINP metabolites (4-methyloctyl-sidechain)

Metabolism of DINP in humans has not been investigated yet. In rodents, DINP is metabolised to MINP in a first step, which in a second step is completely metabolised to oxidised MINP metabolites before excretion in urine. These oxidised MINP metabolites have not been unambiguously identified in respect of their chemical structure [19]. DEHP – a phthalate of one carbon atom less in its side chain compared to DINP – is also known to be extensively oxidised before renal excretion. More than 25 oxidised monoester metabolites are known to be generated by ω-, ω-1 and β-oxidation. The three major oxidised metabolites (mono(2-ethyl-5-hydroxyhexyl)phthalate (5OH-MEHP), mono(2-ethyl-5-oxo-hexyl)phthalate (5oxo-MEHP) and mono(2-ethyl-5-carboxypentyl)phthalate (5carboxy-MEPP)) represent more than 50% of the original oral DEHP dose [22,23]. While DEHP consists of only one side chain derived from 2-ethylhexanol, we have shown that DINP 2 consists of at least 15 and DINP 1 of at least 35 different side chains. Each of these side chains can probably be metabolised by ω-, ω-1- and β-oxidation like the single side chain of DEHP. Therefore, the number of possible oxidised monoester metabolites of DINP can be estimated to be well above 100. However, with the major side chain identified for both DINP types and under assumption that this side chain is metabolised like the side chain of DEHP we can single out the structures of the most important metabolites representing the internal exposure to DINP of both types.

So, based on the major alcohol of both INA mixtures identified in Section 3.1 (4-methyloctanol-1), we postulated the following ω- and ω-1-oxidation products of DINP: mono-(4-methyl-7-hydroxy-octyl)phthalate (7OH-MMeOP), mono-(4-methyl-7-oxo-octyl)phthalate (7oxo-MMeOP) and mono-(4-methyl-7-carboxy-heptyl)phthalate (7carboxy-MMeHP). A scheme of the proposed DINP metabolism starting from the 4-methyloctyl-isomer is shown in Fig. 3. We synthesized these three oxidised metabolites both non-labelled and isotopically labelled with four deuterium atoms on the benzene ring (publications in preparation) the same way we synthesized the major DEHP metabolites before [33–35]. These metabolites served as standard and internal standard substances for the determination of the major oxidised DINP metabolites in urine.

3.3. HPLC-MS/MS analysis of oxidised MINP metabolites in urine

3.3.1. General considerations

The analytical method presented here is in line with two previously published analytical methods for the determination of primary and secondary phthalate metabolites in urine [12,13]. The fast and robust on-line LC/LC–MS/MS method has proven its reliability in a continuous laboratory intercomparison program (www.gequas.de) and in a number of medium and large scale biomonitoring studies [10,37–42]. Minor modifications were made in the use of the analytical HPLC column (now a Synergi^{TM} Fusion-RP from Phenomenex) and the solvent gradient. The Fusion-RP column proved to have better separation power for the isomers mono-*n*-butylphthalate (MnBP) and mono-*iso*-butylphthalate (MiBP) as well as for the oxidised isomers of both DEHP and DINP. The switch from acetonitrile to methanol as the organic solvent also led to a considerable reduction of the running costs. The major advancement of this method, however, is the implementation of the three oxidised DINP metabolites 7OH-MMeOP, 7oxo-MMeOP and 7carboxy-MMeHP as standard substances for the quantification of the sum of DINP metabolites with carboxy, hydroxy and oxo functional groups, respectively.

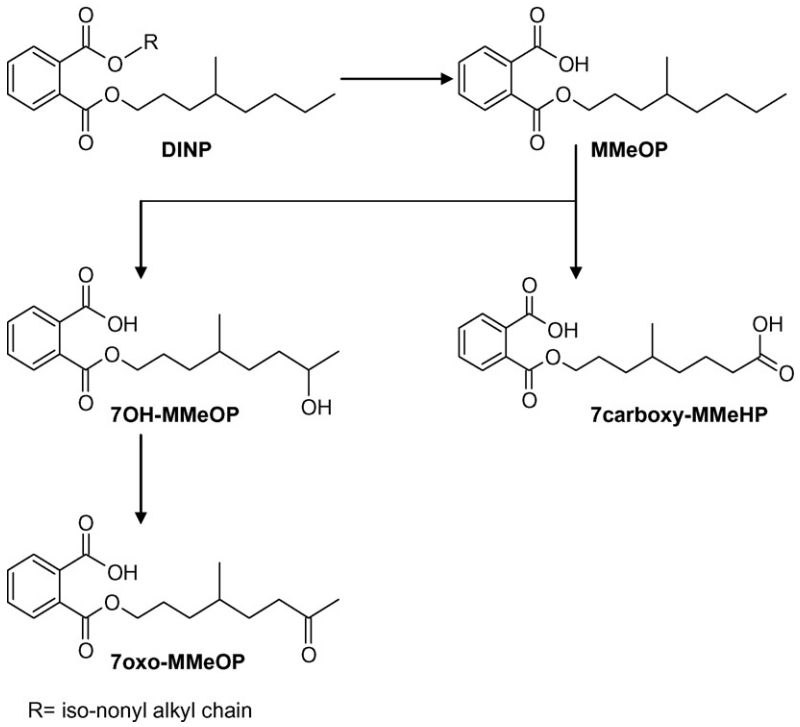

Fig. 3. Suggested DINP metabolism in humans, illustrated for the 4-methyl-1-octyl-sidechain.

3.3.2. Mass spectrometry

Q_1 ESI negative ion mass spectra with tentative fragment structures for 7oxo-MMeOP, 7OH-MMeOP, 7carboxy-MMeHP and D4-7carboxy-MMeHP are shown in Fig. 4. Fragmentation characteristics are very similar to the ones previously described for the oxidised DEHP metabolites [12,13]. As parent ions for the MS–MS fragmentation, we chose the $[M-H]^-$ ion for all analytes. Based on the parent ion, the Quantitative

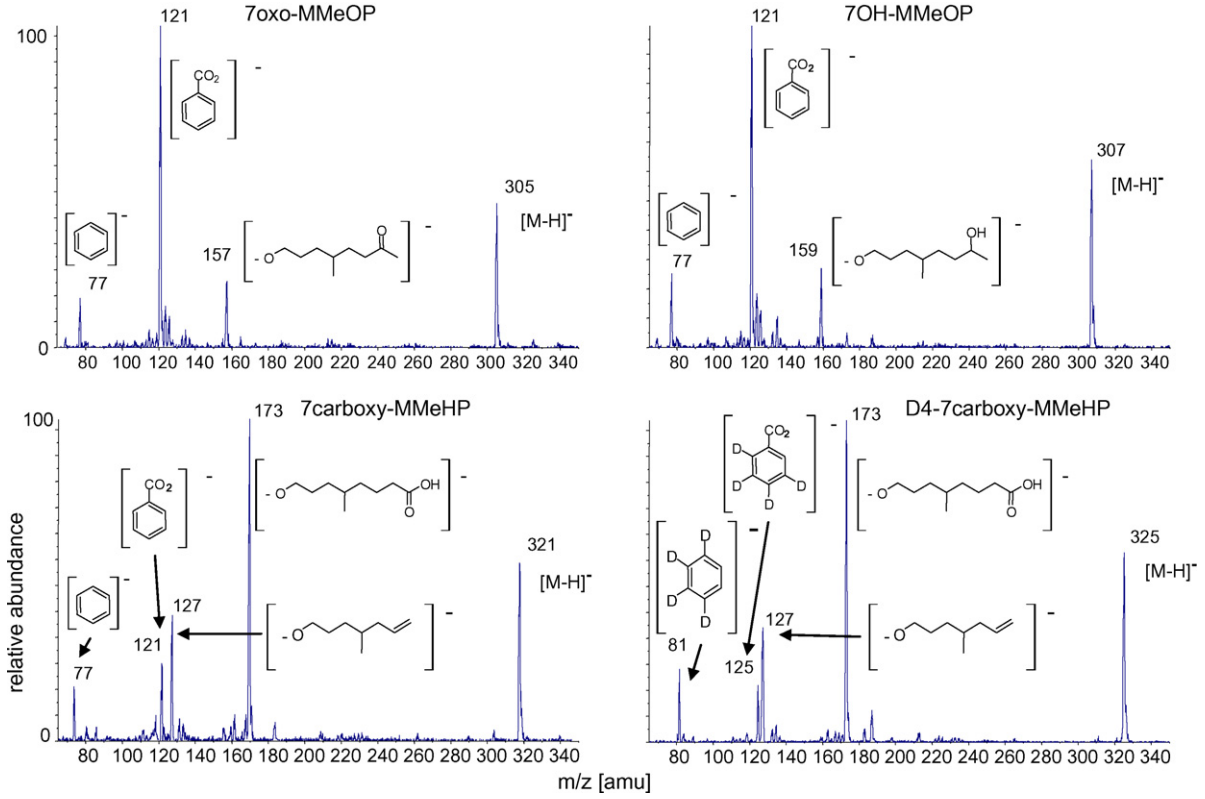

Fig. 4. ESI-negative Q_1 mass spectra of 7oxo-MMeOP, 7OH-MMeOP, 7carboxy-MMeHP and D4-7carboxy-MMeHP.

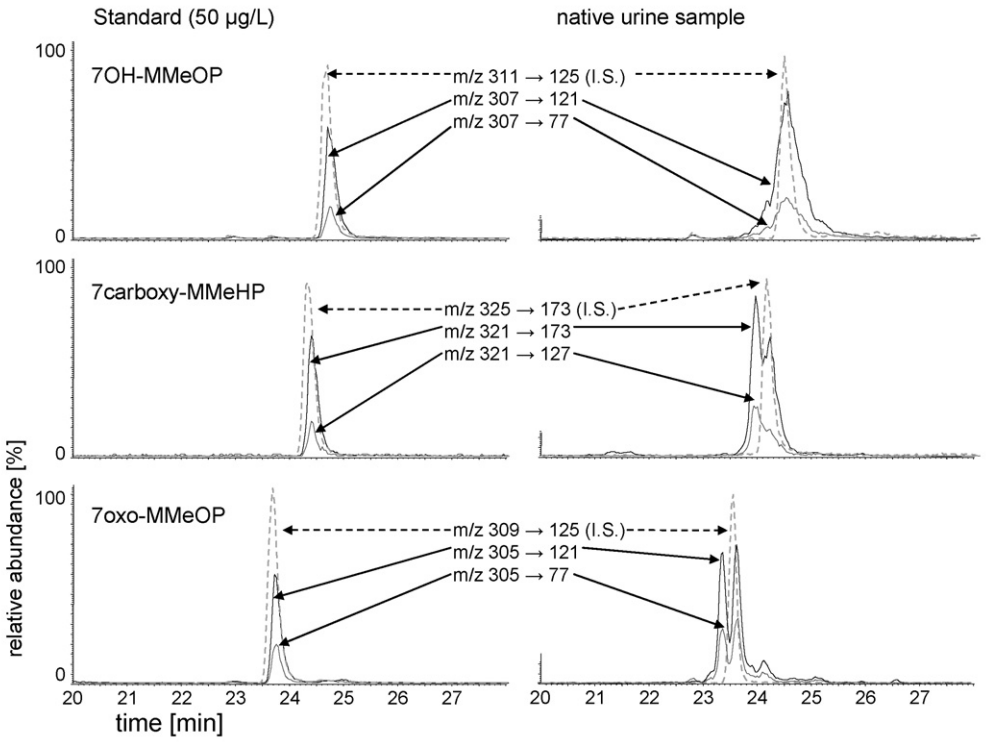

Fig. 5. Chromatogram of a processed 50 µg/l standard in water (left) and a native urine sample (right). Metabolite concentrations in the native urine were as follows: 10.0 µg/l oxo-MINP, 22.4 µg/l carboxy-MINP and 16.8 µg/l OH-MINP. The creatinine content of the urine sample was 0.70 g/l.
I.S.: Internal standard.

Optimization Wizard of the Sciex Analyst™ software selected the most intense parent daughter ion pairs and optimized mass spectrometer parameters for each compound automatically (see Table 4).

For 7oxo-MMeOP and 7OH-MMeOP, the most intense mass fragments after optimization were *m/z* 77 for benzene and *m/z* 121 for benzoic acid. We also observed a distinct *m/z* of 157 for 7oxo-MMeOP and of *m/z* of 159 for 7OH-MMeOP representing their oxidised alkyl side chains. The D4-labelled analogues of 7oxo-MMeOP and 7OH-MMeOP produced distinct fragments with an *m/z* 81 for D4-benzene and an *m/z* 125 for D4-benzoic acid. Again, we also observed an *m/z* 157 for D4-7oxo-MMeOP and an *m/z* 159 for D4-7OH-MMeOP representing their (non-labelled) oxidised alkyl side chains, however, this time originating from the D4-containing parent ions *m/z* 309 and 311 (not shown).

For 7carboxy-MMeHP the fragmentation pattern was slightly different. Here, the alkyl side chain represented the most intense fragment with *m/z* 173. This fragment can further break down into *m/z* 127 through the loss of carbon dioxide. A similar fragmentation pattern (*m/z* 311 → 159 → 113) has already been observed for 5carboxy-MEPP [13] and other possibly carboxylated DEHP and DINP metabolites [20,21,25,26]. Probably, the carboxylic acid group in the side chain stabilizes the negatively charged fragment and thus explains the high relative abundance of the alkyl chain fragments compared to the benzene containing fragments.

3.3.3. LC/LC–MS/MS-quantification

Fig. 5 shows the chromatogram of a processed 50 µg/l standard in water (left) and a processed urine sample (right). The specific mass transitions recorded are depicted within the figure. The bold lines represent the signals received for the oxidised DINP metabolites (one quantifier and one qualifier signal); the dotted lines represent the deuterium labelled internal standards.

Signals for the deuterium labelled internal standards were symmetrically shaped in both chromatograms (standard and urine) showing no influence of the urinary matrix and indicating to no other compounds eluting with the same fragmentation pattern.

For the ion chromatograms of the oxidised metabolites indicating internal exposure to DINP the picture is more complicated, though not unexpected. In the chromatogram of the processed urine sample multiple, respectively misshaped peaks indicate to the presence of a number of substances with elution characteristics and fragmentation patters similar to the standard substances. We assume that these fragmentations bordering and/or superimposing the peak of the 4-methyloctyl-isomer standards originate both from oxidised metabolites of other alky chain isomers of DINP and/or from the 4-methyloctyl-isomer which has been oxidised in other than the ω- and ω-1-positions of the alkyl chain backbone. However, the chromatograms clearly show the metabolite standards based on the 4-methyloctyl-isomer to be highly representative for all other possible DINP metabolites eluting with the major DINP metabolite peaks found

Table 5
Quality control data for the HPLC-MS/MS method

		OH-MINP		oxo-MINP		carboxy-MINP	
		Q_{low}[a]	Q_{high}	Q_{low}[a]	Q_{high}	Q_{low}[a]	Q_{high}
Within-series ($n = 10$)							
Measured conc. (μg/l)	Mean	16.5	n.d.	12.5	n.d.	17.7	n.d.
	Range	14.6–18.0	n.d.	12.1–13.1	n.d.	15.6–19.0	n.d.
R.S.D. (%)		5.8	n.d.	3.1	n.d.	5.1	n.d.
Between-day ($n = 8$)							
Measured conc. (μg/l)	Mean	17.2	69.9	13.8	47.3	18.6	52.4
	Range	15.4–19.5	58.9–80.2	12.2–15.5	44.5–50.2	16.9–19.8	49.6–56.5
R.S.D. (%)		8.5	11.5	8.6	4.3	5.1	4.5

n.d., Not determined; instead of the Q_{high} within-series experiment, we performed recovery and precision experiments with 10 different native urine samples (see Table 6); R.S.D.: relative standard deviation; conc.: concentration.
[a] Different Q_{low} quality control samples were used for the within-series and the between-day measurements.

Table 6
Accuracy and precision data for the HPLC-MS/MS method calculated from 10 different urine samples of varying creatinine content (0.3–3.8 g/l) and varying native DINP metabolite burden after spiking with a known amount of the three DINP metabolites (approximately 50–100 μg/l, exact concentrations given in the table)

	7OH-MINP	7oxo-MINP	7carboxy-MINP
Spiked concentration (μg/l)	55.9[a]	58.4[b]	81.3[c]
Native concentration, measured (μg/l)	<LOQ–23.0	<LOQ–8.2	<LOQ–18.9
Native + spiked concentration, measured (μg/l)	50.1–78.5	61.9–73.7	77.9–108.6
Spiked concentration, calculated (μg/l)			
Mean	55.7	65.8	84.1
Range	49.9–60.8	58.8–71.3	75.4–95.2
R.S.D. (%)	5.3	6.2	8.9
Recovery (%)	99.6 (89.1–108.6)	109.2 (100.5–121.9)	103.5 (82.8–117.5)

R.S.D.: Relative standard deviation.
[a] Spiked as 7OH-MMeOP.
[b] Spiked as 7oxo-MMeOP.
[c] Spiked as 7carboxy MMeHP.

in the native urine samples. In Section 3.3.2, we have shown that the MS/MS fragmentation characteristics do not seem to depend on the branching position but on the type of the oxidative modification of the alkyl chain. The carboxy metabolite favours the intact alkyl chain fragment, the hydroxy and oxo modified metabolites favour benzene containing fragments. Therefore, we assume that the other oxidised isomers of DINP have the same mass fragments, which can be used for quantification.

Table 7
Urinary levels (μg/l) of metabolites of DINP and other phthalates in 25 samples randomly taken from the general German population

Parent phthalate	Metabolite	Median	Mean	Range	% Above LOQ
DINP	OH-MINP	2.5	14.9	<LOQ–287.1	96
	oxo-MINP	1.3	8.9	<LOQ–174.1	80
	carboxy-MINP	5.0	16.4	<LOQ–260.2	96
DEHP[a]	5OH-MEHP	14.7	32.9	3.0–258.0	100
	5oxo-MEHP	13.5	25.0	1.3–173.7	100
	5carboxy-MEPP	25.1	47.5	3.3–375.7	100
	2carboxy-MMHP	6.5	12.4	1.4–98.0	100
	MEHP	4.7	12.2	<LOQ–141.8	92
DnBP[a]	MnBP	50.8	67.2	12.6–366.9	100
DiBP[a]	MiBP	48.4	53.1	8.1–132.6	100
BBzP[a]	MBzP	5.6	8.6	1.7–27.4	100

LOQ: Limit of quantification (0.5 μg/l); 5OH-MEHP: mono-(2-ethyl-5-hydroxyhexyl)phthalate; 5oxo-MEHP: mono-(2-ethyl-5-oxohexyl)phthalate; 5carboxy-MEPP: mono-(2-ethyl-5-carboxypentyl)phthalate; 2carboxy-MMHP: mono-[2-(carboxymethyl)hexyl]phthalate; MEHP: mono-(2-ethylhexyl)phthalate; MnBP: mono-*n*-butylphthalate; MiBP: mono-*iso*-butylphthalate; MBzP: mono-benzylphthalate.
[a] Determined according to Koch et al. [12] and Preuss et al. [13].

To unambiguously distinguish between all possible different oxidised DINP isomers solely by means of LC/LC–MS/MS is hard to accomplish; first, because of the limited separation power of liquid chromatography, second, because of the sheer number of expected isomeric metabolites. Consequently, we chose to integrate each *m/z* signal over the whole time range of elution of the oxidised DINP metabolites. We are confident, based on the points mentioned above, that this approach represents the best possible estimate for the sum of the renally excreted oxidised metabolites of DINP.

3.3.4. Reliability of the LC/LC–MS–MS method

Analyzing Q_{low}, 10-times in a row, we determined the relative standard deviations (R.S.D.) for the three parameters, which were between 3.1% and 5.8%. Between-day imprecision was determined by analysing two quality control samples (Q_{low} and Q_{high}) on 8 different days. For Q_{low}, relative standard deviations for the three parameters were between 5.1% and 8.6%, for Q_{high} the relative standard deviations were between 4.3% and 11.5% demonstrating good reproducibility of this method over the concentration range these metabolites can be found in the general population. All data are presented in Table 5.

As no certified reference material was commercially available, accuracy had to be checked by special recovery experiments with 10 different urine specimens, which were spiked with the oxidised DINP metabolite standards. The urine specimens were selected to reflect a composition as different as possible. As an indicator, we use the creatinine content. This way we can also check for a possible influence of the complex biological matrix on the analytical result. We determined relative recoveries in the range from 82.8% to 121.9% over all parameters and all specimens (see Table 6). Mean relative recoveries were 99.6% for 7OH-MINP, 103.5% for 7carboxy-MINP and 109.2% for 7oxo-MINP. We are highly satisfied with these relative recoveries because they document good recoveries over the whole range of urinary matrix. Determination of the relative recoveries was especially difficult because we had to take account of considerable differences in native DINP metabolite burdens (<LOQ–23.0 μg/l). Based on these recovery experiments, we were also able to calculate the imprecision of the method for these 10 individual urine samples. The relative standard deviations for the spiked concentrations were 5.3–8.9% and therefore comparable to the standard deviations from the within-series and between-day experiments. We regard these relative standard deviations as highly acceptable bearing in mind that matrices of the urine samples were highly different.

Leaving the urine samples at room temperature for 3 days as well as freezing and thawing the urine samples two times in a row did not influence the analytical result (data not shown). However, to avoid any negative influence on the analytical result, e.g. by microbial degradation, we generally advise urine samples to be stored in the freezer at −18 °C and only be thawed once before analysis.

3.3.5. Detection limit and quantification limit

The limits of detection (LOD), defined as a signal to noise ratio of three for the quantifier fragment ions were estimated to be 0.25 μg/l. These estimations were based on the response of the three oxidised DINP metabolites (4-methyloctyl-isomer) in the standard solutions in water. The limits of quantification (LOQ), defined as a signal-to-noise ratio of 6, were estimated to be 0.5 μg/l.

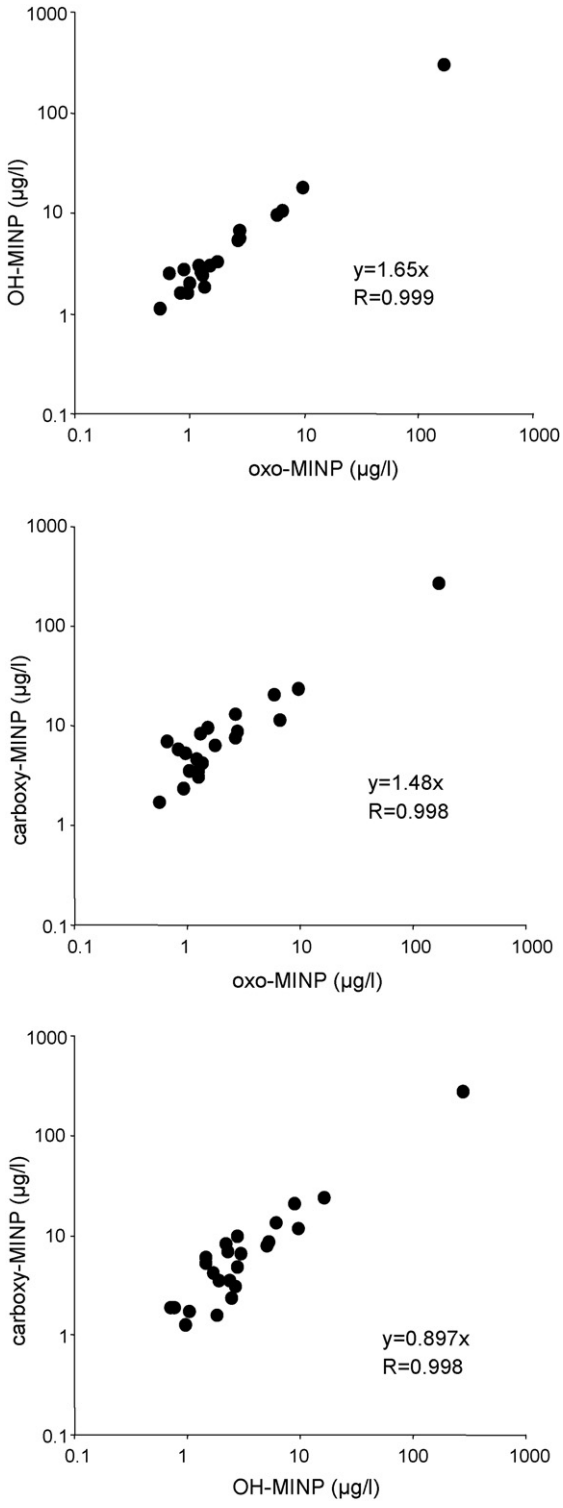

Fig. 6. Correlation analyses of urinary OH-MINP, oxo-MINP and carboxy-MINP. R represents the Pearson correlation coefficient. Levels below the LOD were excluded in the analysis.

3.4. Results of biological monitoring

The results of the biological monitoring of 25 persons occupationally not exposed to phthalates are summarized in Table 7. We for the first time are able to give reliable quantitative information on the renal DINP-metabolite excretion in the general population and thus can complement the exposure scenario already known for other important endocrine disrupting/modulating phthalates like DEHP, DnBP, DiBP (di-*iso*-butylphthalate) and BBzP (butylbenzylphthalate).

All except one urine samples contained OH-MINP and carboxy-MINP metabolites above the limit of quantification (96%), 21 out of the 25 samples contained oxo-MINP metabolites above the limit of quantification (80%). This pilot study therefore indicates the general population is ubiquitously exposed to DINP. This finding is in contrast to previous biomonitoring studies, which stated that only a small part of the population was exposed to DINP [16,18]. These studies were solely based on the determination of the simple monoester MINP. MINP, however, has recently been shown to be an inept parameter for urinary DINP biomonitoring because either none or only traces of MINP are excreted in urine even after high DINP exposures [9,19–21]. We found the oxidised DINP metabolites in almost all urine specimens from the general population over a wide concentration range, indicating to an omnipresent but intra-individually highly variable DINP exposure.

We checked the correlation for the excretion of the DINP-metabolites and found very close and highly significant ($p > 0.0001$) correlations between all of the three oxidised DINP metabolites with correlation coefficients according to Pearson of above 0.99 (see Fig. 6). Correlations of comparable quality have been previously described for the oxidised DEHP metabolites [13,36,37] and for tentatively identified DINP metabolites [20]. Major oxidised DINP metabolite in most of the 25 urine samples was carboxy-MINP. We found it in about two-times the concentration of OH-MINP and four-times the concentration of oxo-MINP. Oxidised DINP metabolites were therefore distributed similar to the DEHP metabolites with carboxy, hydroxy and oxo functional groups within this study (see Table 7) and previous studies [10,28,37–42].

4. Conclusions

Although various extensive risk assessments (ECB [3], NTP [4], EFSA [5]) have dealt with DINP both the chemical composition of the two different DINP types currently on the market and the internal exposure of the general population to these two types of DINP has remained obscure. Analysing the alcohol (INA) used for synthesis of DINP we identified and quantified the major alkyl side chain of both DINP types: the 4-methyloctyl-chain. We derived oxidised metabolites based on this specific side chain (7oxo-MMeOP, 7OH-MMeOP, 7carboxy-MMeHP) and thus quantified all DINP metabolites with hydroxy, oxo and carboxy functional groups which are excreted renally after DINP exposure. The methods limit of quantification is sufficiently low to quantify these metabolites in more than 80% of urine samples investigated from the general population. Labelled internal standards for each metabolite and the on-line sample preparation considerably contribute to the excellent quality criteria of the method. Previous biomonitoring studies solely based on the simple monoester MINP [16,18] have to be interpreted with utmost caution and it is questionable whether MINP can be regarded as a valid biomarker of DINP exposure at all [9,22]. Consequently, we did not implement the analysis of MINP in our method.

DINP metabolite levels determined in this study were approximately five-times lower than respective DEHP metabolite levels in the same population. However, a direct comparison between DEHP and DINP warrants utmost care. McKee et al. [19] have shown in rats, that a considerable portion (around 50%) of the orally dosed DINP is excreted via the feces while DEHP is known to be excreted almost exclusively via urine in humans [10,22–23]. Based on the analytical method presented here, we are currently investigating metabolism and elimination kinetics of DINP in humans, as we have previously done for DEHP [22,23].

By combining the analytical method described here for DINP metabolites with two similar analytical methods previously published for metabolites of other major phthalates [12,13], we are now able to determine eleven metabolites of five phthalates known for their endocrine disrupting potency: DINP, DEHP, DnBP, DiBP and BBzP. Exposure to all of these phthalates is omnipresent in the general population. Combining human metabolism data on DINP with human biomonitoring data will play an important role in future exposure (dose) and risk assessments for DINP and the other endocrine active phthalates.

Acknowledgements

We would like to thank the Deutsche Forschungsgmeinschaft (DFG) for their financial support (AN 107/16-4). We also would like to thank the major European DINP producers (represented by Dr. R. Otter of BASF, Dr. M. Penman of ExxonMobil and Dr. N. Scholz of OXENO) who generously provided us with their INA intermediates. Special thanks also to Dr. D. Rochat of UMR 1272, INRA, Centre de Versailles-Grignon for 4-methyloctanol-1 he gave to us as a generous gift.

References

[1] AgPU—Arbeitsgemeinschaft PVC und Umwelt e.V. Plasticizers market data, Am Hofgarten 1–2, 53113 Bonn, January 2006.
[2] D. Cadogan, The European Council for Plasticisers and Intermediates (ECPI), personal communication.
[3] European Union Risk Assessment Report, 1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich and di-“isononyl” phthalate (DINP), European commission Joint Research Centre, EUR 20784 EN, Office for Official Publications of the European Communities, 2003.
[4] R. Kavlock, K. Boekelheide, R. Chapin, M. Cunningham, E. Faustman, P. Foster, M. Golub, R. Henderson, I. Hinberg, R. Little, J. Seed, K. Shea, S. Tabacova, R. Tyl, P. Williams, T. Zacharewski, Toxicol. Reprod. 16 (2002) 679.
[5] European Food Safety Authority (EFSA), EFSA J. 244 (2005) 1.
[6] W. Kaufmann, K. Deckardt, R.H. McKee, J.H. Butala, R. Bahnemann, Regul. Toxicol. Pharmacol. 36 (2002) 175.
[7] Report to the U.S. Consumer Product Safety Commission by the Chronic Hazard Advisory Panel on Diisononyl Phthalate (DINP), U.S. Consumer Product Safety Commission, Directorate for Health Sciences, Bethesda, MD 20814, June 2001.

[8] L.E. Gray, J. Ostby, J. Furr, M. Price, D.N.R. Veeramachaneni, L. Parks, Toxicol. Sci. 58 (2000) 350.
[9] H.M. Koch, H. Drexler, J. Angerer. Reprod. Toxicol. 18 (2004) 759.
[10] H.M. Koch, R. Preuss, J. Angerer. Int. J. Androl. 29 (2006) 155.
[11] J. Angerer, H. Greim, in: J. Angerer, T. Weiss (Eds.), Biological Monitoring: Prospects in Occupational and Environmental Medicine, Wiley-VCH, Weinheim, Germany, 2002, p. 191.
[12] H.M. Koch, L.M. Gonzalez-Reche, J. Angerer, J. Chromatogr. B 784 (2003) 169.
[13] R. Preuss, H.M. Koch, J. Angerer, J. Chromatogr. B 816 (2005) 269.
[14] M.J. Silva, N.A. Malek, C.C. Hodge, J.A. Reidy, K. Kato, D.B. Barr, L.L. Needham, J.W. Brock, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 789 (2003) 393.
[15] K. Kato, M.J. Silva, L.L. Needham, A.M. Calafat, Anal. Chem. 77 (2005) 2985.
[16] M.J. Silva, D.B. Barr, J.A. Reidy, N.A. Malek, C.C. Hodge, S.P. Caudill, J.W. Brock, L.L. Needham, A.M. Calafat, Environ. Health Perspect. 112 (2004) 331.
[17] M.J. Silva, A.R. Slakman, J.A. Reidy, J.L. Preau Jr., A.R. Herbert, E. Samandar, L.L. Needham, A.M. Calafat, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 805 (2004) 161.
[18] Centers for Disease Control and Prevention, Third National Report on Human Exposure to Environmental Chemicals, Atlanta, GA, National Center for Environmental Health, Division of Laboratory Sciences, available: http://www.cdc.gov/exposurereport/3rd/pdf/thirdreport.pdf (accessed 22 March 2006).
[19] R.H. McKee, M. El Hawari, M. Stoltz, F. Pallas, A.W. Lington, J. Appl. Toxicol. 22 (2002) 293.
[20] M.J. Silva, J.A. Reidy, J.L. Preau Jr., L.L. Needham, A.M. Calafat, Environ. Health Perspect. 114 (2006) 1158.
[21] M.J. Silva, K. Kato, C. Wolf, E. Samandar, S.S. Silva, E.L. Gray, L.L. Needham, A.M. Calafat, Toxicology 223 (2006) 101.
[22] H.M. Koch, H.M. Bolt, J. Angerer, Arch. Toxicol. 78 (2004) 123.
[23] H.M. Koch, H.M. Bolt, R. Preuss, J. Angerer, Arch. Toxicol. 79 (2005) 367.
[24] H.M. Koch, H.M. Bolt, R. Preuss, R. Eckstein, V. Weisbach, J. Angerer, Arch. Toxicol. 79 (2005) 689.
[25] M.J. Silva, J.A. Reidy, J.L. Preau, E. Samandar, L.L. Needham, A.M. Calafat, Biomarkers 11 (2006) 1.
[26] M.J. Silva, E. Samandar, J.L. Preau, L.L. Needham, A.M. Calafat, Toxicology 219 (2006) 22.
[27] J. Hellwig, R. Jackh, Food Chem. Toxicol. 35 (1997) 489.
[28] R. Otter, BASF, personal communication, July 2003.
[29] European Council for Plasticizsers and Intermediates (ECPI), Information letter of D. Cadogan to the Ministère de l'Environnement and to the Institut National de Recherche et de Sécurité, on the differences between the various DINPs, Brussels, September 1997.
[30] D. Rochat, K. Mohammadpoor, C. Malosse, A. Avand-Faghih, M. Lettere, J. Beauhaire, J.P. Morin, A. Pezier, M. Renou, G.A. Abdollahi, J. Chem. Ecol. 30 (2004) 387.
[31] K. Larsen, Clin. Chim. Acta 41 (1972) 209.
[32] American Chemistry Council Phthalate Esters Panel, Comments on draft CERHR evaluation of di-isononyl phthalate (DINP), ACC, 2000.
[33] H.D. Gilsing, J. Angerer, D. Prescher, Monatsh. Chem. 133 (2002) 1147.
[34] H.D. Gilsing, J. Angerer, D. Prescher, Monatsh. Chem. 134 (2003) 1207.
[35] H.D. Gilsing, J. Angerer, D. Prescher, Monatsh. Chem. 136 (2005) 795.
[36] D.B. Barr, M.J. Silva, K. Kato, J.A. Reidy, N.A. Malek, D. Hurtz, M. Sadowski, L.L. Needham, A.M. Calafat, Environ. Health Perspect. 111 (2003) 1148.
[37] H.M. Koch, B. Rossbach, H. Drexler, J. Angerer, Environ. Res. 93 (2003) 177.
[38] H.M. Koch, H. Drexler, J. Angerer, Int. J. Hyg. Environ. Health 206 (2003) 77.
[39] H.M. Koch, H. Drexler, J. Angerer, Int. J. Hyg. Environ. Health 207 (2004) 75.
[40] K. Becker, M. Seiwert, J. Angerer, W. Heger, H.M. Koch, R. Nagorka, E. Roßkamp, C. Schlüter, B. Seifert, D. Ullrich, Int. J. Hyg. Environ. Health 207 (2004) 409.
[41] H.M. Koch, H. Drexler, J. Angerer, Int. J. Hyg. Environ. Health 207 (2004) 15.
[42] H.M. Koch, J. Angerer, H. Drexler, R. Eckstein, V. Weisbach, Int. J. Hyg. Environ. Health 208 (2005) 489.