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ANALYTICAL TOXICOLOGY

Additional oxidized and alkyl chain breakdown metabolites of the plasticizer DINCH in urine after oral dosage to human volunteers

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Abstract Hexamoll® DINCH® (diisononyl-cyclohexane-1,2-dicarboxylate) is a new high molecular weight plasticizer and a non-aromatic phthalate substitute. In this follow-up study, we further investigated the extensive oxidative metabolism of Hexamoll® DINCH® after oral dosage of 50 mg to three male volunteers (0.552–0.606 mg/kg body weight). Urine samples were consecutively collected over 48 h post-dose. Chemical analysis was carried out by HPLC–MS/MS with labeled internal standards. New metabolites were tentatively identified and quantified via fragmentation analogies and new standard substances. In addition to the five urinary DINCH metabolites previously reported by us, we identified two groups of extensively oxidized metabolites characterized (a) by multiple side chain oxidation and breakdown and (b) by hydroxylation at the cyclohexane ring. The five newly identified carboxylated breakdown metabolites represented in sum 5.12 ± 0.49 % of the applied dose. MCHxCH (cyclohexane-1,2-dicarboxylic acid mono carboxyhexyl ester) was identified as a major metabolite (2.71 ± 0.34 %) and thus represents the second most important specific metabolite of DINCH after OH-MINCH (10.7 ± 2.1 %). Less than 1 % was excreted as ring-hydroxylated metabolites (four metabolites identified). Based upon a new reference standard, we can also update oxo-MINCH to 2.6 % of the applied dose. This follow-up study increases the total amount of the recovered dose from 39.2 to 45.7 % and describes a new major metabolite (MCHxCH) of DINCH that can be used as an additional valuable and specific biomarker to assess DINCH® exposure in future human biomonitoring studies.

Keywords DINCH · Phthalate substitute · Human metabolism · Urinary metabolites · Excretion fractions · Human biomonitoring

Introduction

Hexamoll® DINCH® (diisononyl-1,2-cyclohexanedicarboxylate) is a high molecular weight (HMW) plasticizer and phthalate substitute. HMW phthalates such as Di(2-ethylhexyl)phthalate (DEHP) and Diisononylphthalate (DINP) are under intensive scrutiny because of their reproductive and endocrine toxicity (Regulation (EC) No. 1907/2006 of the European Parliament and of the Council 2006; Directive 2005/84/EC of the European Parliament and of the Council 2005; Rothenbacher and Schwack 2009). In the USA and the European Union, certain HMW phthalates have been banned from using in toys and childcare products. Hexamoll® DINCH® has been introduced in the European market in 2002, mainly intended for sensitive applications such as toys, food contact materials, and medical devices (EFSA 2006; Biedermann-Brem et al. 2008; Bhat et al. 2014; David et al. 2015). The current toxicological

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DINCH = Hexamoll® and DINCH®.

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profile suggests that DINCH is neither a reproductive toxicant nor an endocrine disruptor (Furr et al. 2014). Thyroid hyperplasia (200 mg/kg body weight/day for males and 1000 mg/kg bw/day for both sexes) and signs of renal toxicity (300 mg/kg bw/day for male and 1000 mg/kg bw/day for both sexes) have only been observed at relatively high dosing levels (EFSA 2006). The TDI for DINCH has been set by EFSA to be 1 mg/kg bw/d (EFSA 2006). For DEHP, EFSA has derived a TDI of 50 µg/kg bw/day for DEHP (EFSA 2005), with the DEHP-specific restriction that only 50 % of this TDI may come from food.

Hexamoll® DINCH® is manufactured by catalytic hydrogenation of DINP2, CAS 28553-12-0 (Schütze et al. 2012). Based on this process, the planar aromatic ring of DINP2 is transformed by hydrogenation into a non-aromatic, non-planar cyclohexane ring existing predominantly in the most stable chair configuration, allowing cis- and trans-configuration. The typical commercial products consist of 90 % *cis*- and 10 % *trans*-isomers. The C9-alcohol moiety consists of approximately 10 % *n*-nonyl, 35–40 % methyloctyl, 40–45 % dimethylheptyl, and 5–10 % methylethylhexyl alcohol isomers. The basic human metabolism of DINCH was first described by Koch et al. (2013). In brief, metabolic degradation of DINCH starts with partial cleavage of the diester resulting in the simple monoester (MINCH). The alkyl chain of MINCH is then the target for various ω, ω-1, and ß-oxidations. Similar to phthalate plasticizers such as DEHP and DINP, the various oxidized monoesters are apt biomarkers of Hexamoll® DINCH® exposure (Anderson et al. 2011; Fromme et al. 2015; Human Biomonitoring Commission 2014; Koch et al. 2013; Silva et al. 2007, 2013; Schütze et al. 2014, 2015). Among these classical secondary metabolites, OH-MINCH is the most abundant (10.7 %), followed by cx-MINCH (2 %) and oxo-MINCH (2 %). MINCH and the above chain oxidized metabolites can be further broken down to the major metabolite CHDA (23.7 %), which, however, is not specific for DINCH anymore.

Previous human metabolism studies on phthalates have shown the importance of secondary, oxidized metabolites with an increasing alkyl chain length of the phthalate. The increasing C-chain length results in a higher lipophilicity of the HMW phthalates (Leng et al. 2014; Koch and Angerer 2007; Koch and Calafat 2009; Koch et al. 2004, 2005, 2012, 2013). While for DEHP simple oxidative modifications seem sufficient to facilitate renal excretion, additional oxidative modifications or breakdown seems to be necessary with increasing alkyl chain length. Silva et al. (2006) and Kato et al. (2007) already pointed out the possible importance of several multiple oxidized and/or chain breakdown metabolites of DINP and DIDP in the spectrum of urinary excreted metabolites. Recently, Silva et al. (2012) tentatively identified multiple oxidized metabolites in the urine of rats orally dosed with DINCH.

Recognizing the growing importance of oxidative metabolism for high molecular weight plasticizers such as HMW phthalates or DINCH, we reanalyzed the urine samples from our controlled DINCH human oral dosing study (Koch et al. 2013). We screened these samples for two groups of extensively oxidized metabolites characterized either by multiple side chain oxidation and consecutive side chain breakdown or by an (additional) hydroxylation at the cyclohexane ring (see, Fig. 1) and tentatively identified and quantified relevant metabolites based on their chromatographic behavior and mass spectrometric fragmentation pattern using either novel, custom synthesized analytical standards (MCHxCH; oxo-MINCH) or analytical standard substances with single oxidative modifications from our previous study as references.

Materials and methods

Study design

The full study design has already been published by Koch et al. (2013). Hexamoll® DINCH® was dosed to three male volunteers, aged between 26 and 38 years, weighing between 82 and 90 kg, and born and living in Germany. The volunteers had no known occupational exposure to DINCH or phthalates. 50 mg DINCH was added to 0.25 ml ethanol and ingested, mixed with coffee or tea, in a chocolate-coated waffle-cup for breakfast. The applied dose of 0.585 µg/kg bw is considerably lower than the TDI of 1 mg/kg bw for DINCH as derived by EFSA and included in Commission Regulation (EU) No. 10/2011. Urine samples were taken as follows: one sample before dosage (control sample) and consecutively after dosage for 48 h. We conducted this study in full accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and an ethical review of the medical faculty of the Ruhr-University Bochum (Reg. No.: 3866-10).

Chemicals

Hexamoll® DINCH® (cyclohexane-1,2-dicarboxylic acid, diisononylester CAS No. 166412-78-8 (US CAS No. 474919-59-0), purity ≥99.5 %) was provided by BASF SE (Germany). Specific monoester metabolites based upon the 4-methyloctyl side chain isomer were synthesized by Dr. Belov, Max-Planck Institute for Biophysical Chemistry, Germany: cyclohexane-1,2-dicarboxylate-mono-4-methyloctyl ester (MINCH), cyclohexane-1,2-dicarboxylate-mono-(7-carboxylate-4-methyl)heptyl ester (cx-MINCH), cyclohexane-1,2-dicarboxylate-mono-(7-hydroxy-4-methyl)octyl ester (OH-MINCH), cyclohexane-1,2-dicarboxylate-mono-(7-oxo-4-methyl)octyl ester

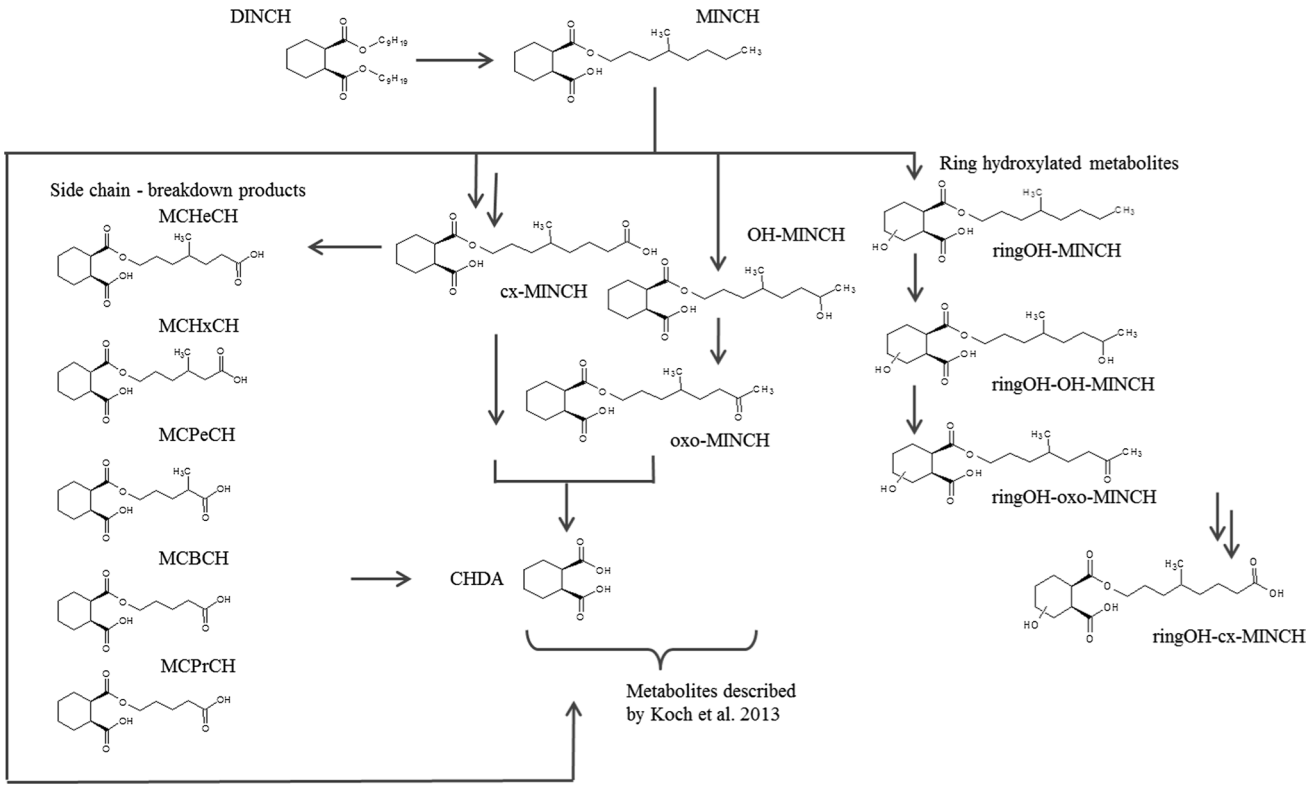

Fig. 1 Proposed DINCH metabolism including newly identified ring hydroxylation metabolites and side chain breakdown metabolites

(oxo-MINCH), and cyclohexane-1,2-dicarboxylate-mono-(5-carboxylate-4-methyl)pentyl ester (MCHxCH), and their side chain labeled analogous structures cyclohexane-1,2-dicarboxylate-mono-D2-4-methyloctyl ester (D2-MINCH), cyclohexane-1,2-dicarboxylate-mono-D2-(7-carboxylate-4-methyl)heptyl ester (D2-cx-MINCH), and cyclohexane-1,2-dicarboxylate-mono-D4-(7-hydroxy-4-methyl)octyl ester (D4-OH-MINCH). All synthesized compounds had a purity ≥95 %. The deuterated compounds used as internal standards had no detectable impurities of unlabeled and partially labeled compounds. Water and acetonitrile with a high purity (HPLC/MS grade), ammonium acetate p.a., acetic acid (glacial, extra pure), and ethanol p.a. were purchased from Merck, Darmstadt, Germany. K12 β-glucuronidase (from *E. coli*) was purchased from Roche Biomedical, Mannheim, Germany.

Analytical procedure

According to Schütze et al. (2012), urine samples were removed from −20 °C storage and thawed at room temperature prior to transferring 300 µl aliquots to 1.8 ml vials. 100 µl 1 M ammonium acetate buffer pH 6, 10 µl internal standard, and 6 µl β-glucuronidase (diluted 1:1 with 1 M ammonium acetate buffer pH 6.0) were added. After incubation at 37 °C in a water bath for 2 h, 10 µl acetic acid was added to adjust the pH value. 100 µl of aliquots was injected into an Agilent Technology LC1200 coupled with an AB Sciex QTrap 5500 tandem mass spectrometer. HPLC gradient and column assembly remain unchanged compared to Schütze et al. (2012). In short, a Capcell PAK 5u C18 MG-II column, for cleanup and enrichment in back flush mode, and an Atlantis dC18 (2.1 × 150 mm; 3 µm), for chromatographic separation, were used (for solvent gradient and source conditions, see Online Resource, Tables 1–3). The parameters for mass spectrometric detection and quantification were complemented to include oxo-MINCH and MCHxCH, now present as standard substances. The relative recovery for these two additional metabolites was between 75 and 110 %, the day-to-day precision >80 % in 1:10 diluted samples, presenting the smallest dilution used for this metabolism study.

Identification of the postulated, carboxylated side chain breakdown products was performed via specific fragmentation patterns. Similar to the phthalates, for MCHxCH and cx-MINCH, the carboxy group leads to stabilized negative charges on its resulting side chain fragment (Kato et al. 2007; Koch et al. 2007; Schütze et al. 2012). We chose these characteristic carboxylated alkyl chain fragments to identify and quantify all corresponding carboxy breakdown products (m/z 173-14*n). For the identification and quantification of metabolites hydroxylated at ring position

Table 1 HPLC-MS/MS parameters for urinary DINCH metabolites

DINCH metabolites	Abbreviation		Q1 mass (Da)	Q3 mass (Da)	RT (min)	Internal standard used for quantification
cyclohexanol-1,2-dicarboxylic acid mono carboxyoctyl ester	ringOH-OH-MINCH	Quan	330	151	15.1	D4-OH-MINCH
		Qual	330	169		
cyclohexanol-1,2-dicarboxylic acid mono oxononylester	ringOH-oxo-MINCH	Quan	328	151	15.4	D4-OH-MINCH
		Qual	328	169		
cyclohexanol-1,2-dicarboxylic acid mono hydroxynonylester	ringOH-MINCH	Quan	313	151	19.1	D2-MINCH
		Qual	313	169		
cyclohexanol-1,2-dicarboxylic acid mono carboxyoctylester	ringOH-cx-MINCH	Quan	343	173	14.2	D4-OH-MINCH
		Qual	343	151		
cyclohexanol-1,2-dicarboxylic acid mono carboxyoctyl ester	MCHeCH	Quan	314	159	16.3	D2-cx-MINCH
		Qual	314	153		
cyclohexane-1,2-dicarboxylic acid mono carboxyhexyl ester	MCHxCH	Quan	300	145	15.6	D2-cx-MINCH
		Qual	300	153		
cyclohexane-1,2-dicarboxylic acid mono carboxypentylester	MCPeCH	Quan	286	131	15.1	D2-cx-MINCH
		Qual	286	153		
cyclohexane-1,2-dicarboxylic acid mono carboxybutylester	MCBCH	Quan	272	117	14.6	D2-cx-MINCH
		Qual	272	153		
cyclohexane-1,2-dicarboxylic acid monocarboxypropylester	MCPrCH	Quan	257	103	14.1	D2-cx-MINCH
		Qual	257	153		

Quan Quantifier trace; *qual* qualifier trace

(ringOH-MINCH, ringOH-OH-MINCH, and ringOH-oxo-MINCH), we postulated specific hydroxylated ring fragments with m/z 151 (quantifier trace) and m/z 169 (qualifier trace). For ringOH-cx-MINCH, we postulated m/z151 (ring-hydroxylated fragment) and m/z 173 (carboxylated alkyl side chain fragment), see Table 1.

For the above-postulated metabolites, the tentative quantification was carried out with external calibration curves of OH-MINCH, oxo-MINCH, cx-MINCH, MINCH, and MCHxCH. Each carboxylated breakdown metabolite was quantified via MCHxCH, whereas for the ring-hydroxylated metabolites cx-MINCH was used for ringOH-cx-MINCH, OH-MINCH was used for ringOH-OH-MINCH, oxo-MINCH was used for ringOH-oxo-MINCH, and MINCH was used for ringOH-MINCH. Calibration curves of the structurally related DINCH metabolites were obtained using the peak area ratio of analyte to its isotope-labeled analog versus the concentration.

Results and discussion

We identified nine new urinary DINCH metabolites that could be separated into two groups (metabolite classes): ring-hydroxylated metabolites and side chain carboxylated breakdown metabolites. Figure 1 shows the postulated DINCH metabolism. Metabolites were identified by specific, postulated fragmentation patterns and their respective retention times in relation to the already known, simple side chain oxidized DINCH metabolites.

Chromatograms for each of the newly identified metabolite classes (based upon specific mass transitions) are shown in Fig. 2 (ring-hydroxylated metabolites) and Fig. 3 (side chain carboxylated breakdown metabolites). The left column of each figure shows chromatograms from a pre-dose urine sample, and the right column shows chromatograms from a urine sample taken 6.3 h after oral DINCH dosage. The chromatograms to the right clearly show the pronounced occurrence of the postulated DINCH metabolites resulting from the ingestion of DINCH. The chromatographic behavior of the ring-hydroxylated metabolites (Fig. 2b) follows the same pattern as the chromatographic behavior of non-ring hydroxylated but otherwise analogous classical DINCH metabolites: ringOH-cx-MINCH eluted first due to its highest hydrophilicity, while ringOH-MINCH eluted last due to its lowest hydrophilicity. Due to the isomeric nature of both the alkyl side chain and the different possible positions of oxidation, a multitude of individual and overlapping peaks can be observed. For the side chain carboxylated breakdown metabolites (Fig. 4b), retention times are getting shorter with shorter alkyl chain length, 15.6 min for MCHxCH and 14.1 min for MCPrCH. Cx-MINCH, the longest carboxylated side chain metabolite (represented by its deuterium-labeled analog in the chromatograms), has the longest retention time. The peak shapes were generally comparable to those known from the

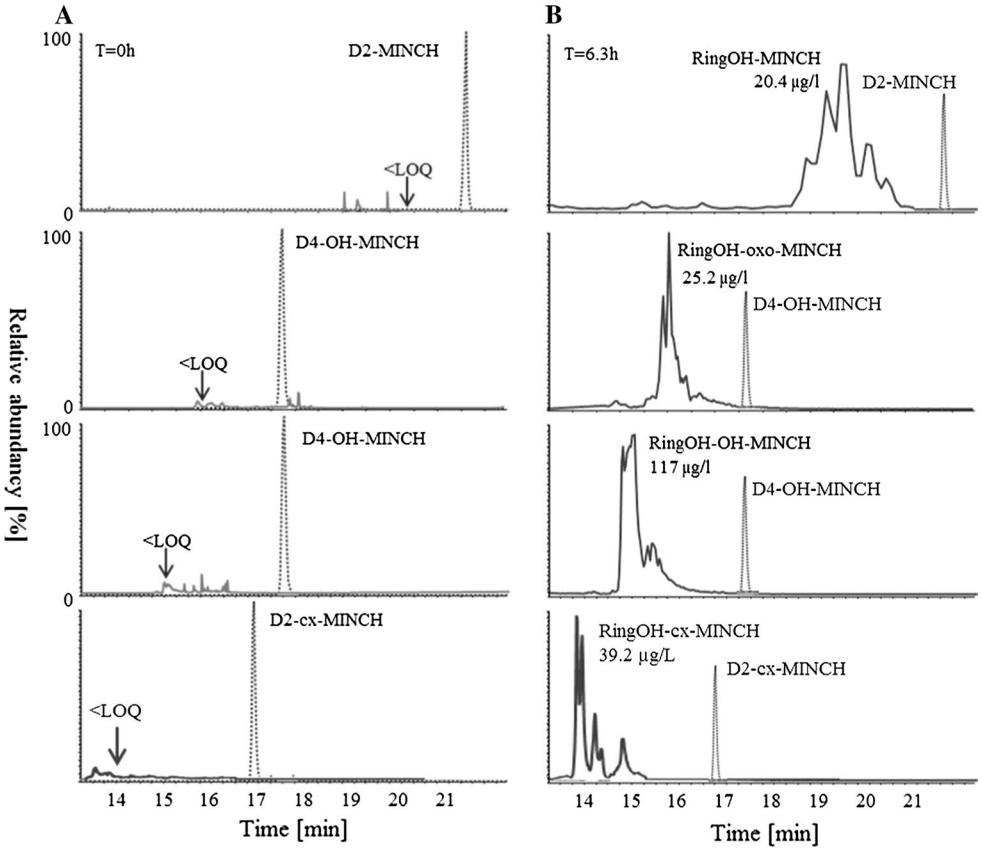

Fig. 2 Chromatograms of native ring-hydroxylated metabolites and concentrations: **a** the urine sample prior to dosage including the internal standard used for quantification, **b** oxidized metabolites emerging in a representative post-urine sample

classical DINCH metabolites as previously investigated by Koch et al. (2013). While the ringOH-metabolites generally show a broader elution profile due to the isomeric nature of the alkyl side chain and the various possibilities of polar modifications at both the alkyl side chain and the cyclo-aliphatic ring, the carboxylated metabolites elute rather condensed due to the more dominant influence of the (terminal) carboxy group on chromatography. Furthermore, with decreasing alkyl chain length, the isomeric composition of the alkyl side chain gets less and less complex and the peaks of the side chain carboxylated breakdown metabolites get sharper.

We obtained full-scan MS/MS spectra of the tentative metabolites at their corresponding retention times. In Fig. 4 (upper row), we compare the mass spectrum derived from the authentic cx-MINCH (a) and MCHxCH (b) standards. Both carboxylated metabolites share common daughter ions containing the cyclohexane moiety (m/z 171, m/z 153, and m/z 127). Furthermore, they share one common transition originating from the stabilized, carboxylated side chain (m/z 327–>173 for cx-MINCH and m/z 299–>145 for MCHxCH). All of these analogous transitions were also present in the other side chain carboxylated breakdown metabolites (data not shown). In Fig. 4 (lower row), we compare the mass spectrum of side chain OH-MINCH (c) recorded from the authentic analytical standard with the mass spectrum of ringOH-MINCH (d) recorded in the post-dose urine sample at the retention time 19.1 min after isolation of m/z 313 as the parent mass. For the ring-hydroxylated metabolites, namely ringOH-MINCH, ringOH-OH-MINCH, ringOH-cx-MINCH, and ringOH-oxo-MINCH, we identified m/z 169 and m/z 151 as specific ring fragments which probably originate from dehydration of the ring-hydroxylated cyclohexane-1,2-carboxylate moiety. These fragments are not present in the non-ring-hydroxylated metabolites.

For the purpose of tentative quantification, we integrated each m/z signal over the whole time range of elution. This approach has already been used to quantify our previously investigated DINCH metabolites (Koch et al. 2013) but also for metabolites of phthalates with isomeric alkyl side chain compositions such as DINP (Koch et al. 2007) or DIDP (Wittassek and Angerer 2008).

The urinary elimination kinetics of the newly identified DINCH metabolites are shown in Fig. 5. Figure 5a shows the elimination kinetics of the side chain carboxylated breakdown metabolites, and Fig. 5b shows the elimination kinetics of the ring-hydroxylated metabolites. In both figures, for orientation, we also included the classical and previously reported metabolites cx-MINCH, OH-MINCH, and oxo-MINCH. Similar to the previously reported DINCH metabolites, the new metabolites exhibit

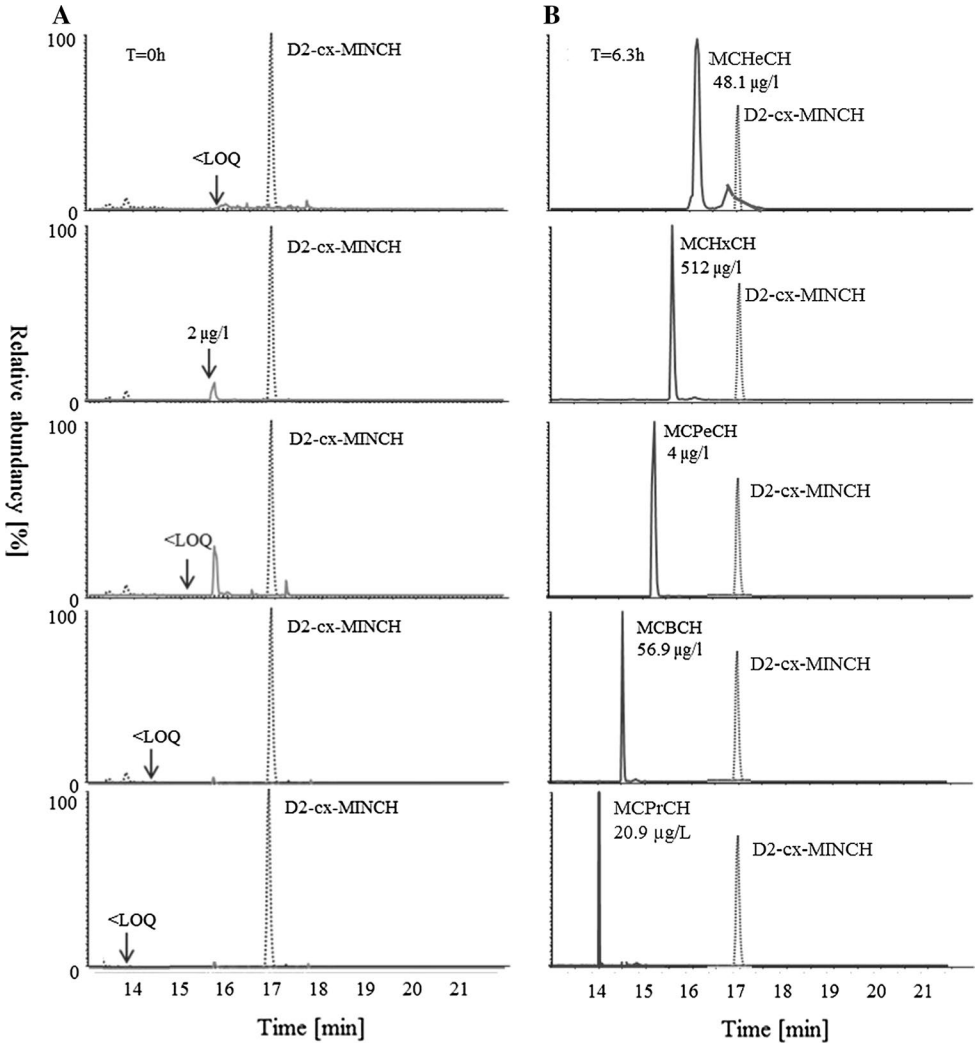

Fig. 3 Chromatograms of native carboxylated metabolites and concentrations: **a** the sample prior dosage including the internal standard used for quantification, **b** carboxylated metabolites emerging in a representative post-urine sample

their maximum of renal elimination 2–6 h post-dose. MCHxCH, the ß-oxidation product of cx-MINCH (characterized by a mass loss of m/z 28), is the predominant carboxy metabolite, even surpassing cx-MINCH itself (maximum concentration of 2720 µg/g creatinine versus 1750 µg/g creatinine). The next ß-oxidation product MCBCB (maximum concentration of 824 µg/g creatinine) is the third most prominent carboxylated metabolite. The other carboxylated metabolites (MCHeCH, MCPeCH, and MCPrCH) were of minor importance but still measurable in urine samples 48 h after the dose and also following similar elimination kinetics. The identification of MCHeCH as a metabolite of DINCH is interesting and was already reported by Silva et al. (2012). The classical ß-oxidation of the 9-carbon alkyl chain of DINCH would only result in 7-carbon (MCHxCH) and 5-carbon (MCBCB) alkyl chain metabolites. Thus, the occurrence of the carboxylated 8-carbon alkyl chain metabolite MCHeCH could either results from α-oxidation (Jansen and Wanders 2006) of the C9-alkyl chain of DINCH, or the—based on the production process unlikely—presence of C8- and/or C10-alkyl chains in DINCH. To test the alkyl chain lengths of DINCH, we directly infused a 1 mg/l (acetonitrile) solution of the Hexamoll® DINCH® used in our oral metabolism study into the mass spectrometer. We could not find any indication for C8 or C10 alkyl side chains which should have resulted in additional molecular masses compared to a purely C9-based DINCH (see, Online Resource, Fig. 1). Therefore, a metabolic mechanism that causes the loss of one carbon atom in the DINCH side chain is likely. Such a mechanism would be α-oxidation that, for example, occurs in the metabolism of 3-methyl branched (e.g., phytanol) and 2-hydroxy straight chain fatty acids (Casteels et al. 2007; Foulon et al. 2003; Wanders et al. 2011).

The ring-hydroxylated metabolites (Fig. 5b) were excreted at considerably lower concentrations (at least ten times lower) as compared to the non-ring-hydroxylated analogs. Among the ring-hydroxylated metabolites, ringOH-OH-MINCH is the most abundant metabolite, with 121 µg/g creatinine (mean over the three volunteers) at its

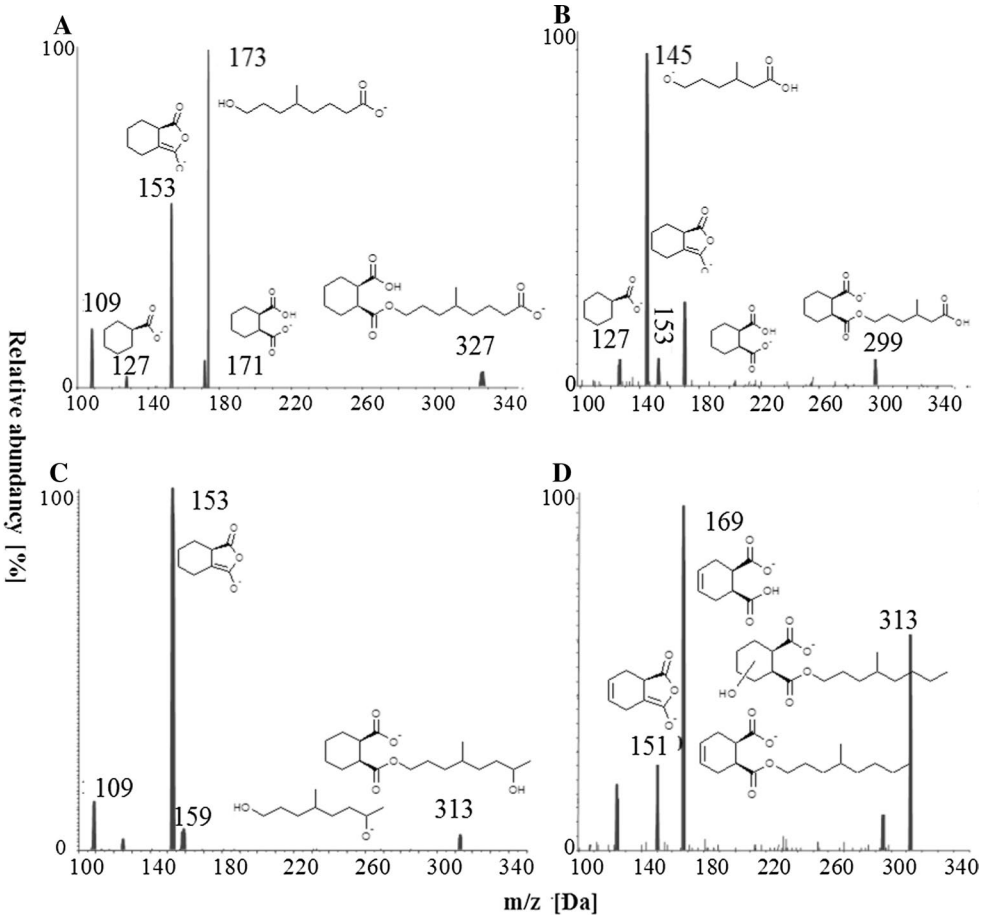

Fig. 4 Mass spectrometric fragmentation pattern of cx-MINCH (**a**). MCHxCH (**b**), OH-MINCH (**c**), and ringOH-MINCH (**d**)

maximum, followed by ringOH-cx-MINCH (100 µg/g creatinine), ringOH-oxo-MINCH (28.3 µg/g creatinine), and ringOH-MINCH (20.8 µg/g creatinine) (Fig. 5). Albeit at low concentrations and similar to the carboxylated metabolites, all of these metabolites were still measurable in urine samples 48 h after the dose.

5.12 % of the applied DINCH dose was excreted as the newly identified side chain carboxylated breakdown metabolites, with MCHxCH (2.71 ± 0.34 %) representing the dominant metabolite within this metabolite class (see, Table 2 with a summary of all previously and the newly established metabolite conversion factors). Thus, MCHxCH is the second most abundant specific DINCH metabolite in the whole DINCH metabolite spectrum after OH-MINCH (10.7 % ± 2.59). CHDA is the major urinary metabolite of DINCH; however, it cannot be used as a valid biomarker of DINCH exposure, because it is also a metabolite of other CHDA carrying chemicals. Regarding the ring-hydroxylated metabolites, ringOH-OH-MINCH was the major metabolite within this class of metabolites, representing 0.28 ± 0.34 % of the dose. All ring-hydroxylated metabolites taken together represent less than 0.5 % of the dose. For all metabolites investigated, more than 90 % of their individual share in metabolism is excreted within the first 24 h. This follow-up study increases the total amount of the DINCH dose recovered in urine as identified metabolites from 39.2 to 45.7 %.

Conclusion

In this study, we have proven the extensive oxidative human metabolism of DINCH prior to urinary excretion. We identified nine new urinary DINCH metabolites in humans: five side chain carboxylated breakdown metabolites and four ring-hydroxylated metabolites. While ring-hydroxylated metabolites represent only minor share in the metabolite spectrum, the five side chain carboxylated breakdown metabolites represent above 5 % of the oral DINCH dose excreted in urine. MCHxCH was identified the second most abundant specific metabolite of DINCH representing approximately 2.7 % of the dose. Still, more than 50 % of the oral DINCH dose remains unaccounted for. Sulfate conjugates might add to the share of the DINCH metabolites excreted via urine. We were not able to investigate sulfate conjugates in this study, because enzymes with lipase/arylsulfatase activity (e.g., HP-2 from H. pomatia) that would have cleaved sulfate conjugates would also have

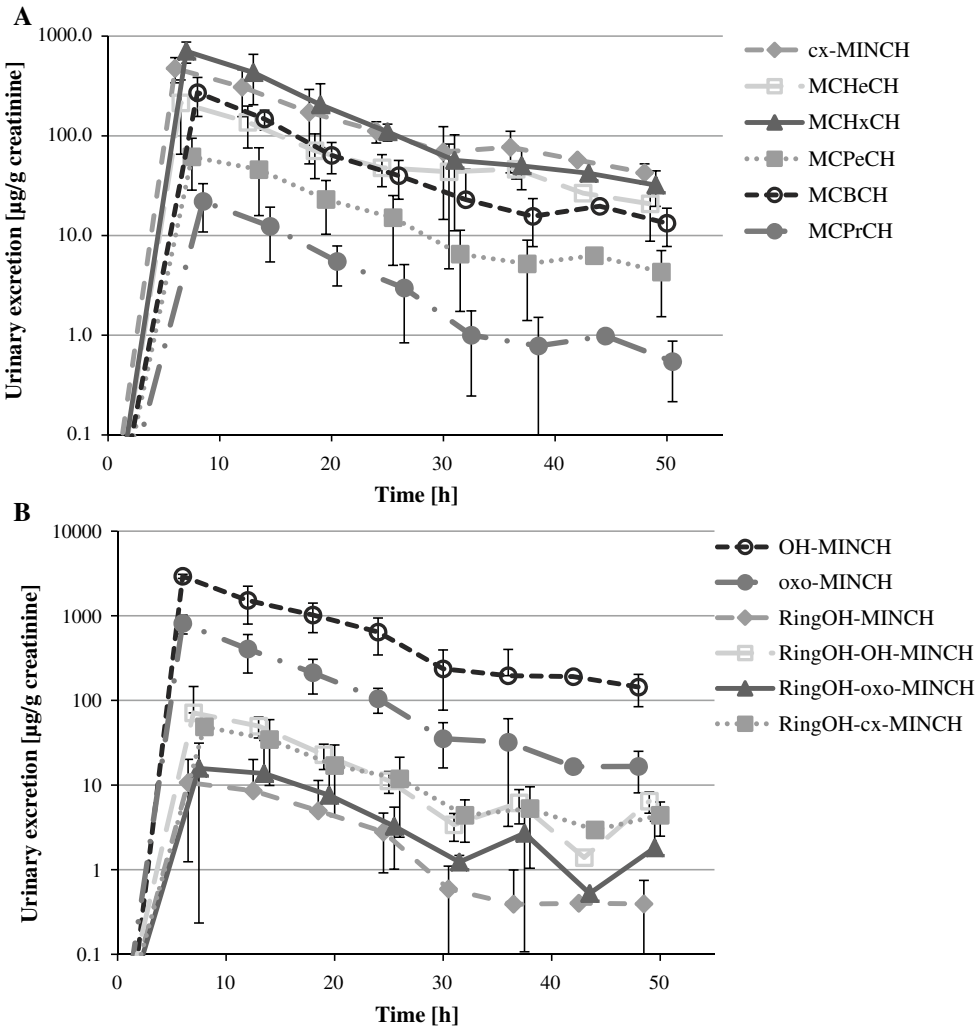

Fig. 5 Creatinine adjusted elimination kinetics of carboxylated (**a**, *top*) and ring-hydroxylated (**b**, *bottom*) metabolites in logarithmic scale from three volunteers

Table 2 Relative excretion of oxo-MINCH (based upon the reference standard oxo-MINCH), carboxylated side chain breakdown products, and ring-hydroxylated metabolites

	DINCH metabolites	Excretion 0–48 h (%)	
"Classical" metabolites (Koch et al. 2013)	CHDA	23.7 ± 3.36	
	MINCH	0.72 ± 0.48	
	OH-MINCH	10.7 ± 2.1	Sum: 15.6 ± 2.77
	oxo-MINCH[a]	2.60 ± 0.42	
	cx-MINCH	2.03 ± 0.3	
Carboxylated side chain breakdown metabolites	MCHeCH	0.96 ± 0.26	Sum: 5.12 ± 0.49
	MCHxCH	2.71 ± 0.34	
	MCPeCH	0.3 ± 0.15	
	MCBCH	1.07 ± 0.16	
	MCPrCH	0.09 ± 0.04	
Ring-hydroxylated metabolites	ringOH-MINCH	0.04 ± 0.04	Sum: 0.59 ± 0.53
	ringOH-OH-MINCH	0.28 ± 0.34	
	ringOH-oxo-MINCH	0.07 ± 0.09	
	ringOH-cx-MINCH	0.19 ± 0.08	
	Sum	45.7 ± 7.07	

[a] Update of the semi-quantitative value reported in Koch et al. (2013), based on an authentic standard in this study

cleaved the ester bonds of DINCH and its metabolites, an effect previously described for phthalates (Blount et al. 2000) and tere-phthalates (Lessmann et al. 2016). However, sulfate conjugation has been shown to be of subordinate relevance in human metabolism for the structurally analogous phthalates (Silva et al. 2003; Samandar et al. 2009). According to Bhat et al. (2014), in rats dosed with radiolabeled ^{14}C-DINCH (20 mg/kg), between 28 and 32 % of the DINCH was excreted via urine. Obviously, the dose equivalent excreted in humans via urine is higher, but most probably some part of the dose still seems to be excreted via feces.

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