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Exposure of Portuguese children to the novel non-phthalate plasticizer di-(iso-nonyl)-cyclohexane-1,2-dicarboxylate (DINCH)

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ABSTRACT

Di-(iso-nonyl)-cyclohexane-1,2-dicarboxylate (DINCH) is used as substitute for high molecular weight phthalate plasticizers such as di-(2-ethylhexyl) phthalate (DEHP) and di-(iso-nonyl) phthalate (DINP). Due to a rapid substitution process we have to assume omnipresent and increasing DINCH exposures. The aim of this study was to evaluate DINCH exposure in 112 children (4–18 years old) from Portugal, divided in two groups: 1) normal-/underweight following the usual diet; and 2) obese/overweight but under strict nutritional guidance. First morning urine samples were collected during the years 2014 and 2015. Oxidized DINCH metabolites (OH-MINCH, oxo-MINCH, cx-MINCH) were analyzed after enzymatic hydrolysis via on-line HPLC-MS/MS with isotope dilution quantification. We detected DINCH metabolites in all analyzed samples. Urinary median (95th percentile) concentrations were 2.14 μg/L (15.91) for OH-MINCH, followed by 1.10 μg/L (7.54) for oxo-MINCH and 1.08 μg/L (7.33) for cx-MINCH. We observed no significant differences between the two child-groups; only after creatinine adjustment, we found higher metabolite concentrations in the younger compared to the older children. Median (95th percentile) daily DINCH intakes were in the range of 0.37 to 0.76 (2.52 to 5.61) μg/kg body weight/day depending on calculation model and subpopulation. Body weight related daily intakes were somewhat higher in Group 1 compared to Group 2, irrespective of the calculation model. However, in terms of absolute amounts (μg/day), DINCH intakes were higher in Group 2 compared to Group 1. In regard to age, we calculated higher intakes for the younger children compared to older children, but only with the creatinine-based model. This new data for southern European, Portuguese children adds information to the scarce knowledge on DINCH, confirming omnipresent exposure and suggesting higher exposures in children than adults. Significant sources and routes of exposure have yet to be unveiled. For now, all calculated daily intakes are far below established health benchmark levels (TDI, RfD). However, rapidly increasing exposures have to be expected over the next years.

1. Introduction

Di-iso-nonyl-cyclohexane-1,2-dicarboxilate (DINCH) is used as substitute for some High Molecular Weight (HMW) phthalate plasticizers like di (2-ethylhexyl) phthalate (DEHP) and diisononyl phthalate (DINP). These two HMW phthalates have been going through intensive scrutiny worldwide, due to their endocrine disrupting and reprotoxic activity (CPSC, 2008; EU, 2005; EU, 2006). Since 1999 (EU, 1999) (Directive 1999/815/EC) the European Union (EU) has banned DEHP in toys and childcare articles in concentrations above 0.1%, and DINP, di-isodecyl phthalate (DIDP) and di-n-octyl phthalate (DnOP), in toys and childcare articles which are intended for mouthing at concentrations above 0.1% (entry 51/52 of Annex XVII of the Regulation EC No. 1907/2006) (EU, 2006). Since 2001, DEHP is classified as a reproductive toxicant in the EU (Directive 2001/59/EU) (EU, 2001); since February 2015, DEHP (listed in Annex XIV of the Regulation EC No. 1907/2006) (EU, 2006) must not be placed on the EU market any more (REACH sunset date). Due to these changes in the regulatory landscape and increasing evidence for adverse health effects on humans the demand for safe non-phthalate, non-aromatic substitutes increased.

In 2002, the plasticizer DINCH was introduced into the market, intended for the use in sensitive products such as toys, food contact materials and medical devices (Bhat et al., 2014; Biedermann-Brem et al., 2008; David et al., 2015; EFSA, 2006). DINCH is produced by catalytic hydrogenation of the aromatic ring of DINP, and commercialized as

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Hexamoll® DINCH® (Koch et al., 2013a). In 2014, 200,000 tons were produced according to data from BASF SE (BASF, 2015) with a rapidly increasing production volume over the past decade. It is expected that production volumes will continue to grow in the future.

The currently available data suggest that DINCH, contrary to phthalates such as DEHP and DINP, is neither an endocrine disruptor nor a reproductive toxicant (EFSA, 2006; Furr et al., 2014). According to the data summarized by European Food Safety Authority (EFSA) (EFSA, 2006), in a sub chronic oral toxicity study in rats only at high level doses (1000 mg/kg bw/day for both genders and 300 mg/kg bw/day for males) DINCH could cause renal toxicity. The same study showed an increased incidence of thyroid hyperplasia in males at all tested doses and at high level dose for females. However, these effects were contributed to substantial differences between rats and humans. The NOAEL for thyroid hyperplasia was considered inapplicable to establish a tolerable daily intake (TDI). A TDI of 1 mg/kg bw/day with a factor of uncertainty of 100 was established by EFSA (EFSA, 2006) based on the NOAEL for renal effects. Recently, Bhat et al. (2014) derived an oral reference dose (RfD) of 0.7 mg/kg/day for thyroid hypertrophy/hyperplasia seen in F1 rats from a two-generation study.

Similar to other external plasticizers, DINCH is physically dissolved in the polymer and not chemically bound to it. In a Swiss market survey in 2005, DINCH was found to migrate from gaskets of metal closures into oily food (Fankhauser-Noti et al., 2006). DINCH seems to have emission rates similar to DINP from plastic material (Holmgren et al., 2012). EFSA assigned a specific migration limit of 1 mg/kg food (EFSA, 2006).

Once DINCH has entered the human body it is rapidly broken down to its simple monoester by ester cleavage. The alkyl side chain of this monoester is further oxidized to the cyclohexane-1,2-dicarboxylic acid-mono(hydroxyl-iso-nonyl) ester (OH-MINCH), the cyclohexane-1,2-dicarboxylic acid-mono(carboxy-iso-octyl) ester (cx-MINCH) and the cyclohexane-1,2-dicarboxylic acid-mono(oxo-iso-nonyl) ester (oxo-MINCH). These oxidized metabolites are the major DINCH metabolites excreted in urine and currently used for human-biomonitoring (HBM) purposes (Koch et al., 2013a; Schütze et al., 2015; Silva et al., 2012; Völkel et al., 2016).

The results from HBM population studies currently available (CDC, 2015; Fromme et al., 2016; Giovanoulis et al., 2016; Gomez Ramos et al., 2016; Schütze et al., 2014; Schütze et al., 2012; Silva et al., 2013) suggest that DINCH exposure is already widespread across the globe. Additionally, a steep increase in DINCH exposure has been reported (Schütze et al., 2014; Silva et al., 2013) since its introduction in the market, with children presenting higher urinary metabolite levels and daily intakes (DI) than adults (Fromme et al., 2016; Schütze et al., 2014; Silva et al., 2013).

The aim of this study was to investigate DINCH exposure in Portugal, as another country in the EU, in a group of 112 children. Moreover, according to other studies children seem to be a population of special concern regarding plasticizer exposure, as their exposure levels are higher when compared to adults (Cutanda et al., 2015; Den Hond et al., 2015; Kasper-Sonnenberg et al., 2014), which makes the results of this study of special interest. Finally, as our study population was composed by two groups, one with healthy normal-/underweight and another with obese/overweight children without other known associated diseases (with nutritional counselling) another aim was to assess possible differences in DINCH exposures among these two groups.

2. Material and methods

2.1. Subjects and urine samples

The present study is part of an ongoing study to assess possible differences between obese/overweight and normal-/underweight children regarding the exposure to several environmental compounds. The initial aim of this project was the determination of exposure to several suspected or confirmed endocrine disruptors and/or obesogens. Later, considering the regulation and the increasing and relevant substitution by novel compounds, DINCH was added to list. Children were recruited from the pediatric appointment at Hospital de S. João, and several local schools, in the years of 2014 and 2015. Children came from two Portuguese districts, Oporto and Aveiro, belonging respectively to the North and Central region of the country. In all, one hundred and twelve children (55 boys, 57 girls) participated in this study with an age range of 4 to 18 years (median 10 years).

The children were divided in two groups according to the body mass index (BMI). In the Portuguese public health system, the Direcção-Geral de Saúde adopted the World Health Organization (WHO) growth charters since 2012 (WHO, 2007). Group 1 included healthy children (without associated diseases) which were normal-/underweight. Group 2 included children diagnosed for obesity/overweight without other known associated diseases. The obese/overweight group was recruited from a pediatric nutritional appointment, thus counselled for healthy and balanced nutrition and was set on a prescribed diet (at least for three months), based on fresh food and less packaged and processed food items. The children in Group 1 continued with the usual diet.

A summary of anthropometric data for the two groups of children is given in Table 1.

The majority of the children was overweight/obese (62%; $n = 69$). While the discriminators body weight and BMI differed significantly ($p < 0.05$) between the 2 groups, age, gender, height and urinary creatinine were evenly distributed ($p > 0.05$) (Table 1).

During the course of the study, we collected a first morning urine sample from each participating child. All the specimens were kept cool during transportation and then stored at −20 °C until analyses.

The study was approved by the ethics committee of the Centro Hospitalar S. João/FMUP (Medicine Faculty of Oporto University ref. 163.13) and all the parents provided written consent.

2.2. Analysis of DINCH metabolites in urine

Oxidized DINCH metabolites were analyzed after enzymatic hydrolysis via on-line HPLC-MS/MS with isotope dilution quantification (Schütze et al., 2012). Briefly, to 300 μL urine 100 μL of 1 M ammonium acetate buffer (pH 6.0), 10 μL of internal standard solution and 6 μL of β-glucuronidase (from *E. coli* strain K-12, without arylsulfatase activity) were added. Then, the samples were gently mixed and placed in a water bath at 37 °C for 2 h. After adding 10 μL of acetic acid, the samples were stored at −18 °C overnight to precipitate proteins. The samples were then thawed at room temperature and centrifuged at 1900 × *g* for 10 min, and 25 μL supernatant were injected into an Agilent Technology LC1200 system coupled with an AB Sciex QTrap 5500 tandem mass spectrometer. We used a Capcell PAK 5u C18 MG-II column for clean-up and enrichment and, after back flush, an Atlantis dC18 (2.1 × 150 mm; 3 μm) for chromatographic separation. Based on the 1/x weighted calibration curves of the 4-methyl octyl derived standard substances the sum of all C9 alkyl chain isomers of MINCH and the alkyl chain isomers with oxidative functional groups were quantified. Mean accuracies for

Table 1
General characteristics of the studied population.

Population characteristics	Group 1 Normal-/underweight ($n = 43$)[a] usual diet	Group 2 Overweight/obese ($n = 69$)[a] special nutritional guidance
Age (years) mean	10.81	10.20
Gender (%)	44% Female 56% Male	55% Female 45% Male
Weight (kg) mean	36.50	52.48
Height (cm) mean	139.94	142.26
BMI (kg/m^2) mean	17.53	25.25
Creatinine (g/L) mean	1.08	1.08

[a] The underweight/normal weight and obese/overweight groups were defined according to the WHO charters (WHO, 2007).