Chemosphere 118 (2015) 219–228

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

The potential use of diisononyl phthalate metabolites hair as biomarkers to assess long-term exposure demonstrated by a rat model

Jen-Yi Hsu [a,b], Hsin-Hui Ho [a], Pao-Chi Liao [a,*]

[a] Department of Environmental and Occupational Health, College of Medicine, National Cheng Kung University, 138 Sheng-Li Road, Tainan 704, Taiwan
[b] Analytical Center, Chia Nan University of Pharmacy and Science, 60 Sec. 1, Erren Road, Rende Dist., Tainan 717, Taiwan

HIGHLIGHTS

- DINP metabolites were generated by *in vitro* liver enzyme incubation for mass and retention time matching.
- Demonstrated that the eight DINP metabolites reported previously were detected in hair samples.
- Profiles of the eight DINP metabolites were different in urine and hair samples.
- Dose–response relationship was observed for using DINP metabolites in hair as exposure biomarkers.
- Dose–response saturation was less pronounced in hair than in urine samples.

ARTICLE INFO

Article history:
Received 25 June 2014
Received in revised form 29 August 2014
Accepted 7 September 2014
Available online 29 September 2014

Handling Editor: Myrto Petreas

Keywords:
Diisononyl phthalate
Metabolites
Long-term exposure
Hair
Biomarker

ABSTRACT

Diisononyl phthalate (DINP) is a widely used industrial plasticizer. People come into contact with this chemical by using plastic products made with it. Human health can be adversely affected by long-term DINP exposure. However, because the body rapidly excretes DINP metabolites, the use of single-point urine analysis to assess long-term exposure may produce inconsistent results in epidemiologic studies. Hair analysis has a useful place in biomonitoring, particularly in estimating long-term or historical exposure for some chemicals. Several studies have reported using hair analysis to assess the concentrations of heavy metals, drugs and organic pollutants in humans. As a biomarker, DINP metabolites were measured in rat hair in animal experiments to evaluated long-term exposure to DINP. In addition, we evaluated the correlation between the levels of DINP metabolites in hair and in urine. The levels of DINP metabolites in rat hair were significantly higher in the exposure group, relative to the control group ($p < 0.05$). DINP metabolites had a positive correlation with increasing administered dose. Significant positive correlations for MINP, MOINP and MHINP were found between hair and urine ($r = 0.86$, $r = 0.79$ and $r = 0.74$, respectively, $p < 0.05$). Several metabolites in urine showed earlier saturation than in hair. In this report, we detected eight metabolites in hair and demonstrate that hair analysis has potential applications in the assessment of long-term exposure to DINP.

1. Introduction

Phthalates are a group of synthetic industrial chemicals that are widely used in plastics, building materials, and personal care products. More than 18 billion pounds of phthalates are used each year. They are produced worldwide and are found in children's toys, food packaging, and medical devices (Crinnion, 2010; Colacino et al., 2011; Saravanabhavan and Murray, 2012). Phthalates are endocrine disruptors and affect reproductive outcomes, the development of the male reproductive tract (Waterman et al., 1999; Gray et al., 2000; Kavlock et al., 2002), and sexual differentiation in male rats (Gray et al., 2000; Tyl et al., 2004; Foster, 2006). Epidemiological studies have also revealed that exposure to several commonly used phthalates can alter sex steroid hormone levels in human subjects (Main et al., 2006; Pan et al., 2006; Lin et al., 2011). Phthalates are easily released from plastic products because they are not chemically bonded to it. Due to the high consumption of phthalates, humans are exposed to phthalates from a wide range of consumer products (Wormuth et al., 2006; Wittassek et al., 2011). Phthalates are considered ubiquitous compounds to which humans are frequently exposed. Phthalates are divided into types, short chain and long chain, based on the number of carbon atoms in their alcohol chain. These types have very different applications, toxicological properties, and classifications. The long-chain phthalates, mainly DINP, are increasingly

* Corresponding author. Tel.: +886 6 2353535x5566; fax: +886 6 2752484.
E-mail address: liaopc@mail.ncku.edu.tw (P.-C. Liao).

http://dx.doi.org/10.1016/j.chemosphere.2014.09.025
0045-6535/

used to replace di(2-ethylhexyl) phthalate (DEHP) as the major plasticizer in PVC products since the use of DEHP has been legally prohibited (TURI, 2006; Abb et al., 2009). DINP and diisodecyl phthalate (DIDP) account for approximately 60% of the total plasticizer market in Europe (Wittassek et al., 2011). The illegal use of DEHP and DINP as clouding agents in food and beverages was recently reported in Taiwan in May 2011 (Juan et al., 2011). Therefore, the evaluation of phthalate exposure in humans is a critical issue (Lin et al., 2010).

When animals are exposed to DINP, DINP can be metabolized in two steps: phase I (e.g., hydrolysis and oxidation) and then phase II (conjugation) (Frederiksen et al., 2007). Three kinds of major DINP metabolites have been extracted from urine: mono-oxoisononyl phthalate (MOINP), mono-carboxyisooctyl phthalate (MCIOP), and mono-hydroxyisononyl phthalate (MHINP) (Silva et al., 2006). A previous study in our laboratory used an alternative approach in which the signals derived from stable isotope-labeled precursors with varied labeling ratios were analyzed to detect likely DINP metabolite. With this method, seven metabolite signals were identified as potential markers for DINP exposure (Hsu et al., 2011). DINP metabolites have been previously used as exposure biomarkers (Preau et al., 2010). Epidemiology studies have measured DINP metabolites in biofluids to prove ongoing exposure to DINP in general population and in special subpopulations, such as pregnant women and children (Lin et al., 2011). Human biomonitoring uses different matrices, such as blood, urine, and saliva, to measure DINP exposure (Niino et al., 2001; Frederiksen et al., 2010; Lin et al., 2011). Urine is the most common biofluid used for biological monitoring. The urinary concentrations of DINP metabolites can be used to assess the exposure of a person at a single point in time. However, previous studies have shown that personal exposure to phthalates varies based on lifestyle. Thus, the concentration of DINP metabolites in urine also varies with different lifestyles (Koch et al., 2013). Due to the short elimination half-life of DINP, measuring exposure over weeks or months may require multiple measurements (Preau et al., 2010). DINP exposure assessments in epidemiologic studies are not consistent because of different sampling time points (Saravanabhavan and Murray, 2012). The general population is continually exposed to DINP through food packaging and personal products (Koch et al., 2013).

DINP metabolites are rapidly excreted from the body, and a single-point urine test only report personal exposure within a few days. Several studies have discussed the importance of sampling time on epidemiological studies (Preau et al., 2010; Frederiksen et al., 2012). Human health can be affected by long-term exposure to DINP. Using a single-point urine analysis to assess personal long-term exposure will produce inconsistent results in epidemiologic studies. We thus need to identify a long-term exposure biomarker to assess the correlation between DINP exposure and health. Measuring DINP metabolites to assess the effects of long-term exposure can be challenging. In contrast to other biological samples such as urine, hair analysis could potentially determine the duration of chemical exposure, ranging from several months to years. In addition, hair analysis provides additional advantages, such as low cost and ease to store and transport (Li et al., 2012).

The purpose of the present study was to assess the measurement of DINP metabolites in rat hair as biomarkers for long-term exposure to DINP. Furthermore, the correlation between the levels of DINP metabolites in hair and urine were evaluated.

2. Materials and methods

2.1. Chemicals and reagents

Glucose 6-phosphate, glucose 6-phosphate dehydrogenase, β-nicotinamide adenine dinucleotide phosphate (NADP), β-glucuronidase, sulphatase, disodium hydrogen phosphate (≧99.5%), magnesium chloride (for molecular biology), and trifluoroacetic acid (TFA, ≧99.0%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Methanol (HPLC grade), formic acid (FA, ≧99.9%), and acetic acid (≧99.9%) were purchased from Merck (Darmstadt, Germany). Hydrated sodium dihydrogen phosphate (>98%) and hydrochloric acid (36.5–38.0%) were purchased from J.T. Baker (Phillipsburg, NJ, USA). Sodium dodecyl sulphate (SDS) was purchased from SERVA (Heidelberg, Germany). Deionized water was acquired using a Millipore system (Milford, MA, USA). Native mono-iso-nonylphthalate (D_0-MINP) and deuterium-labeled (on the benzene ring) mono-iso-nonylphthalate (D_4-MINP) were synthesized according to the procedure reported by Koch and Angerer (Koch and Angerer, 2007). Commercial DINP, CAS No. 28553-12-0, was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.2. Animal experiments

For the single-dose treatment study, twelve 7-week-old male Wistar rats weighing approximately 250 g were chosen and housed in metabolism cages individually for 30 d. Rats were randomly divided into two groups. The exposure group ($N = 6$) was orally administered with commercial DINP (300 mg kg^{-1}) by gavage. The control group ($N = 6$) was administered only corn oil by gavage. For each rat, hair samples were collected one month before and after continuous exposure to DINP. Urine samples were collected, and the collection time was 24 h after DINP exposure. After collection, the hair samples were placed at room temperature, and the urine samples were frozen at −20 °C until analysis. The hair samples were pretreated with acidic extraction (TFA/methanol) overnight at 45 °C. The DINP metabolites in urine were extracted by off-line C_{18} solid-phase extraction (SPE) after enzymatic (β-glucuronidase and sulphatase) hydrolysis at 37 °C for 90 min.

For the dose-dependent study, fifteen 7-week-old male Wistar rats weighing approximately 250 g were chosen and housed in individual metabolism cages for 30 d. The rats were randomly divided into five groups. Each group was orally administered by gavage individually with commercial DINP (0, 150, 300, 600, or 1200 mg kg^{-1}). For each rat, samples were collected and stored as described previously. All of the samples were prepared following the previous procedures.

2.3. Rat hair pretreatment

The first step for hair pretreatment is wash. The purpose is to remove external contamination. Several common washing solvents have been reported, including water, methanol and acetone. The most common wash procedure is using 0.1% SDS and water to wash the hair alternately 3–5 times each (Takayama et al., 2003; Miyaguchi et al., 2007). To remove external contamination of hair, the wash procedure is necessary. The wash steps followed previous studies. Here, we chose the most common solvents and procedures, using 0.1% SDS and water to wash hair alternately 3–5 times with each solvent (Takayama et al., 2003; Miyaguchi et al., 2007). All of the hair samples were alternately washed with 0.1% SDS and water three times for 10 min in a sonicator for decontamination.

Hydrolysis methods of hair samples are divided into three categories: acidic digestion, alkaline digestion, and enzymatic digestion. Acidic digestion does not destroy the hair structure but is a slow process. Alkaline and enzymatic digestions require less time than acidic digestion, but the resulting solution contains many low-molecular-weight compounds that may interfere with the analysis. An addition clean-up procedure is therefore necessary