14 Metabolomics Profiling of Di-(2-propylheptyl) Phthalate (DPHP) Biotransformation Products as Exposure Markers: Analytical Strategy and Application

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Contents

Abstract

Phthalates are widely used in the manufacture of plastics and personal care products, and humans are easily exposed to phthalates through the usage of these products. Phthalates and di-(2-propylheptyl) phthalate (DPHP), the alternative plasticizer to replace traditional phthalates, are toxicants and their half-life of the original chemical forms in humans is less than 24 h. Therefore, the biological monitoring of phthalates and DPHP are commonly performed by measuring the

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corresponding metabolites instead of the original chemical form. This chapter presents the strategy of combining high-resolution mass spectrometry-based metabolomics method and dose-response study in laboratory animals to discover DPHP biotransformation products as exposure markers. This strategy included screening biomarkers, verification of dose-response relationships in laboratory animals, and application in human subjects. First, the multivariate data analysis method (orthogonal partial least squares-discriminant analysis OPLS-DA and mass defect filter, MDF) and isotope tracing method (signal mining algorithm with isotope tracing, SMAIT) were used to screen and find meaningful signals in mass spectrometry (MS) dataset generated from urine samples collected from DPHP-administered rats or in vitro-incubation sample of DPHP. Next, the meaningful MS signals were verified as exposure marker candidates by assessing dose-response relationships in an animal feeding study. Finally, the exposure marker candidates are applied in human subjects and identified the chemical structures. A biotransformation product of DPHP, mono-(2-propyl-7-dihydroxy-heptyl) phthalate, was suggested as a DPHP exposure marker for general human exposure assessments.

Keywords

Phthalate · DPHP · Metabolite · Biotransformation · Exposure marker · Biomarker · OPLS-DA · SMAIT · MDF

Abbreviations

BBP	Butyl-benzyl phthalate
cx-MEPP	Mono-(2-ethyl-5-carboxypentyl) phthalate
cx-MPBP	Mono-(2-propyl-4-carboxybutyl) phthalate
cx-MPHxP	Mono-(2-propyl-6-carboxyhexyl) phthalate
DBP	Di-n-butyl phthalate
DEHP	Di-(2-ethylhexyl) phthalate
DIA	Data-independent acquisition
DINP	Di-iso-nonyl phthalate
Di-OH-MPHP	Mono-(2-propyl-7-dihydroxyheptyl)-phthalate
DPHP	Di-(2-propylheptyl) phthalate
FT-ICR	Fourier transform ion cyclotron resonance
GC	Gas chromatography
IP	Isotopic pair
IPRR	IP response ratio
LC-HRMS	Liquid chromatography-high resolution mass spectrometry
MDF	Mass defect filter
MPHP	Mono-(2-propylheptyl)-phthalate
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MW	Molecular weight

OH-MPHP	Mono-(2-propyl-6-hydroxyheptyl) phthalate
OPLS-DA	Orthogonal partial least squares-discriminant analysis
oxo-MPHP	Mono-(2- propyl-6-oxoheptyl) phthalate
PVC	Polyvinyl chloride
Q-TOF	Quadrupole-TOF
RTW	Retention time window
S/N ratio	Signal-to-noise ratios
SDS	Sodium dodecyl sulfate
SMAIT	Signal mining algorithm with isotope tracing
TOF	Time of flight
ΔMZ	Mass shift
ΔRT	Retention time difference

Introduction

Phthalates are widely used in industry, plastics, construction materials, and personal care products, so humans are frequently exposed to phthalate compounds. More than 18 billion pounds of phthalates are used every year. Phthalates are used to be plasticizers in the manufacture of polyvinyl chloride (PVC) products and solvents or fixatives in fragrances (Bagchi et al. 2018; Weinstein et al. 2017). Phthalates are lipophilic and can be absorbed via inhalation, ingestion, and direct skin contact. Phthalates can rapidly be metabolized into their respective monoesters body to increase water solubility through phase I biotransformation in the human (Latini 2005; Frederiksen et al. 2007; Marklund et al. 2010; Fierens et al. 2012). In addition, the monoesters could conjugate the chemical to a polar glucuronide, result in increasing the aqueous solubility and promoting excretion (Zhang et al. 2009; Marklund et al. 2010). In recent years, exposure to phthalates has attracted a large number of concerns because of their adverse effects on human health, especially on the reproductive system (Lin et al. 2010). For example, phthalates are detected in human breast milk and can cause incomplete virilization in newborn boys (Main et al. 2006). The free testosterone of serum is decreased in the workers that were exposed to high doses of di-n-butyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP) in China (Pan et al. 2006). In addition, DEHP, DBP, di-iso-nonyl phthalate (DINP), and butyl-benzyl phthalate (BBP) are scrutinized due to the endocrine-disrupting effects (Hsu et al. 2019). Therefore, the use of traditional phthalates is prohibited.

Di-(2-propylheptyl) phthalate (DPHP) is used as an alternative plasticizer to manufacture PVC. DPHP could be easily released from plastic products, because it is not chemically bound in the polymer (EC JRC 2003; Johnson et al. 2010). Thus, the risk of human exposure to DPHP may be high. It was reported that the detection rate of DPHP in Germany has risen from 3.3% in 2009 to 21.7% in 2012, and the global consumption of DPHP is continuously increasing over time (Schütze et al. 2015). Previous studies reported that DPHP does not affect fetal testicular testosterone production (Furr et al. 2014), but experimental evidence demonstrates that

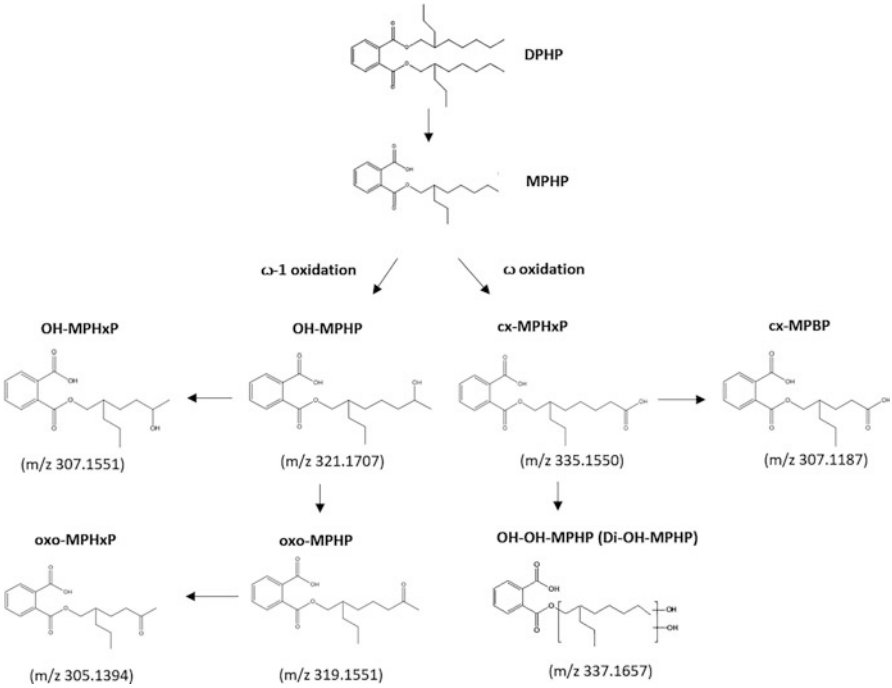


Fig. 1 Proposed biotransformation products of DPHP. (The data comes from the literature reported by Hsu et al. (2019) and Shih et al. (2019b))

DPHP is a subchronic toxicant (CPSC 2011). Some adverse effects were detected in rats exposed to DPHP, including decreasing body weight, increasing liver weight, altered thyroid and pituitary functions, and histopathology changes in adrenal, liver, and soft tissue histopathology (BASF 2009; CPSC 2010). Four biotransformation products have been postulated as DPHP biotransformation products in humans, including mono-(2-propylheptyl)-phthalate (MPHP), mono-(2-propyl-6-oxoheptyl) phthalate (oxo-MPHP), mono-(2-propyl-6-hydroxyheptyl) phthalate (OH-MPHP), and mono-(2-propyl-6-carboxyhexyl) phthalate (cx-MPHxP) (Fig. 1) (Wittassek and Angerer 2008; Gries et al. 2012). A human volunteer study showed that urinary elimination half-lives of oxo-MPHP, OH-MPHP, and cx-MPHP are less than 8 h, and within 48 h after oral application, only 24% of the applied doses were eliminated in the urine as the three DPHP biotransformation products (Leng et al. 2014; Klein et al. 2018). Hence, an effective method for discovering exposure markers (original forms or biotransformation products) with a longer half-life and a higher amount in humans is essential.

Mass spectrometry (MS) plays a critical role in metabolomics research, especially in endogenous metabolites and the biotransformation products of xenobiotics detection and identification in complex matrices (Cooks et al. 2015; Hsu et al. 2017). Endogenous metabolites in biological samples are often present at low levels and

have an excessive matrix background (Hsu et al. 2017). Despite the complexity of endogenous metabolites/biotransformation products and the matrix environment, the availability of high-resolution mass spectrometry (HRMS) with improved mass accuracy has dramatically improved the applicability and productivity of metabolite identification processes. However, handling complex MS datasets to provide positive metabolite identification is still a challenge. Therefore, it is necessary to develop an efficient strategy for metabolite signal filtering (Forsberg et al. 2018; Lu et al. 2017). Multivariate analysis methods are one of the approaches to finding meaning peaks in metabolomics datasets in which the peaks contributing the most to variations or separation are identified for further targeted analysis, such as principal component analysis (PCA), partial least squares projection to latent structures (PLS), and orthogonal partial least squares discriminant analysis (OPLS-DA). This chapter presents the strategy of combining high-resolution mass spectrometry-based metabolomics method and dose-response study in laboratory animals to discover DPHP biotransformation products as exposure markers.

DPHP Exposure Marker Discovery

Workflow of DPHP Exposure Marker Discovery

Figure 2 outlines the general workflow for MS-base exposure marker discovery, includes sample preparation and MS data acquisition, MS data processing, and chemical structure identification. In addition, the biospecimen collected from the DPHP-administered laboratory animal model and in vitro-enzyme incubation sample of DPHP can be used as the target samples. Here report the strategy of combining high-resolution mass spectrometry-based metabolomics method and dose-response study in laboratory animals to discover DPHP biotransformation products as exposure markers. This strategy included screening biomarker candidates from MS data, exposure marker candidates verification of dose-response relationships in laboratory animals, and application in human subjects. After the peak table generated from the biological samples by HRMS, the multivariate data analysis method (OPLS-DA, MDF, etc.) and isotope tracing method (signal mining algorithm with isotope tracing, SMAIT) were used to screen and find meaningful peaks in the MS dataset. Next, the meaningful MS peaks were verified as exposure marker candidates by assessing dose-response relationships in an animal feeding study. Finally, the exposure marker candidates are applied in human subjects and identified the chemical structures.

Sample Preparation and LC-MS Analysis

Various specimens, such as urine, blood, hair, etc., have been exploited for exploring biological exposure. Blood samples usually have to deproteinize by precipitating with methanol, acetone, acetonitrile, or hydrochloric acid. Urine samples, which

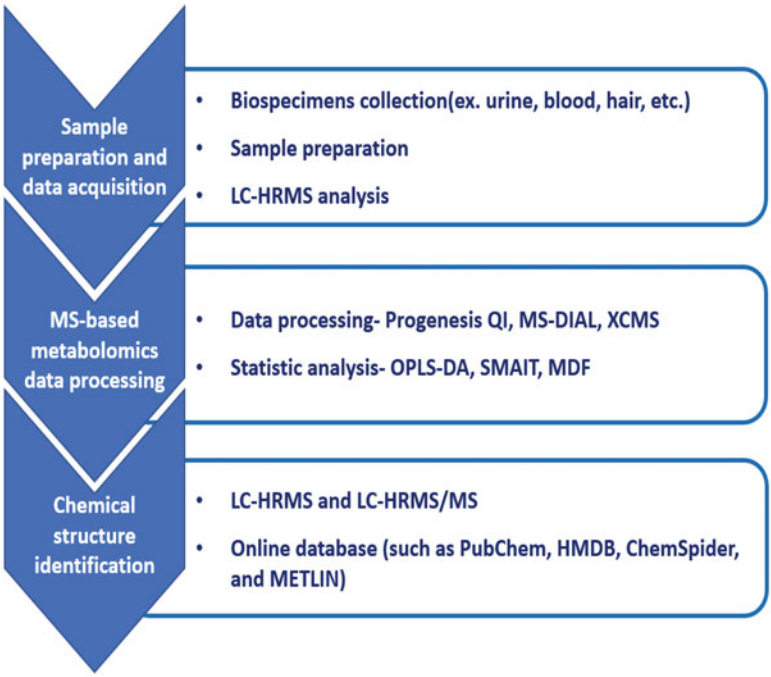


Fig. 2 Workflow for the discovery of exposure markers. Overall, three steps for exposure marker discovery, including sample preparation and data acquisition, metabolomics data processing, and chemical structure identification

commonly add an antibacterial agent like sodium azide to prevent microbial contamination after collection, are hydrolyzed with β-glucuronidase and sulfatase to remove the conjugates of phase II metabolites (Marklund et al. 2010). To reduce interference and preconcentration of the analytes, biospecimens are prepared by common procedures, such as solid-phase extraction, liquid-liquid extraction, and QuEChERS. Hair samples are washed with sodium dodecyl sulfate (SDS), followed by deionized water three times in the ultrasonic bath, and then air-dried (Shih et al. 2019a). The hair samples are cut into 1–2 mm snippets and homogenized. Then, a hair sample (about 50–100 mg) is used to extract the analytes by a methanol and trifluoroacetic acid mixture with sonicated. The extracted sample is concentrated to dryness by nitrogen stream evaporation or speed vac and then dissolved in formic acid (Shih et al. 2019a, b).

LC and gas chromatography (GC), both highly efficient for separation and often hyphenated with HRMS, identify and quantify components of a highly complicated mixture, such as endogenous metabolite detection and identification. HRMS, including the time of flight (TOF), Orbitrap, magnetic sector, and Fourier transform ion cyclotron resonance (FT-ICR), have highly increased the accuracy and selectivity of mass spectrometry, whereas LC is generally coupled with quadrupole-TOF (Q-TOF) and Orbitrap to be used in liquid form samples. The advantages of HRMS are fast

scan rate, high mass accuracy, excellent resolution, superior sensitivity, and multiple-stage mass spectrometry scanning (MS/MS). In addition, HRMS can provide high resolution (>10,000 at full-width at half-maximum) of ion measurements and accurate mass (<5 ppm deviation) capabilities that can determine the elemental composition of metabolite ions and their fragments.

MS Data Processing Approaches

The MS data processing can include generating a peak table, statistical analysis, and structure identification. For peak table generation, a great majority of the software can be used, such as XCMS (Smith et al. 2006; Tautenhahn et al. 2012), MS-DIAL (Tsugawa et al. 2015), Progenesis QI, etc. In addition, several strategies or statistical analysis methods have been used to screen and filter the meaning peaks from LC-HRMS data. In this chapter, three approaches, orthogonal partial least squares-discriminant analysis (OPLS-DA), the signal mining algorithm with isotope tracing (SMAIT), and mass defect filter (MDF), are used to identify the candidates of DPHP biotransformation products.

Peak table generation. Because of accurate mass measurement and comprehensive signal recording, HRMS is widely used in many laboratories, untargeted analysis becomes practical. The peak table generation is the first step in the typical untargeted analysis process. MS data processing requires processing more than 10,000 mass spectral signals in a complex HRMS dataset for untargeted analysis, so data processing automation is essential. The chromatographic peaks in the raw data are automatically integrated and aligned between samples. A great majority of the software, such as XCMS (Smith et al. 2006; Tautenhahn et al. 2012), MS-DIAL (Tsugawa et al. 2015), Progenesis QI, etc., can further convolute to group peaks with different charge states or ion adduct types generated by the same compound into a "feature." Among them, MS-DIAL and XCMS are open sources, as well as Progenesis QI is commercial software. In addition, MS-DIAL implements a new deconvolution algorithm for data-independent acquisition (DIA) datasets in LC-MS/MS, whereas XCMS Online can process data format from various instrument vendors.

Feature filter. Various methods have been developed to objectively search/filter LC-HRMS data to facilitate target peak detection. This chapter introduces the three methods of OPLS-DA, SMAIT, and MDF. OPLS-DA is one of the multivariate analysis methods. It utilizes multivariate data to discriminate between two groups and is based on the development of the PLS method (Worley and Powers 2013). It is a distinguished sample classification to help identify possible markers. The easy interpretability of OPLS-DA modeling with a dimensionality reduction and data fusion step are the advantages of OPLS-DA (Boccard and Rutledge 2013). Figure 3 shown an example of result figures of OPLS-DA. The OPLS-DA score plot shows the variation between the peak profiles of the two different groups with different treatments. Each dot indicates a score value for a result from a sample. The S-plot shows the direction of the hyperplane relative to the original X variable, which can

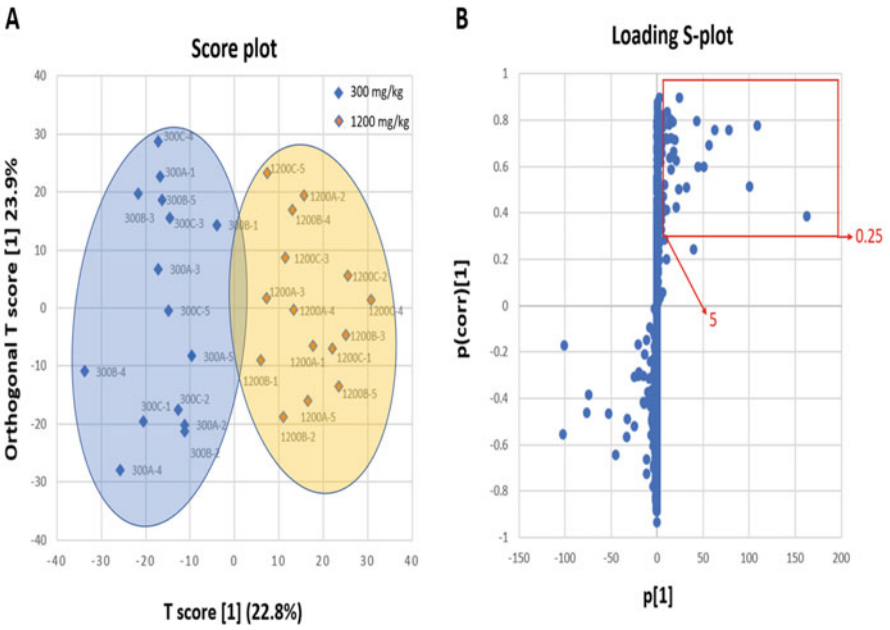


Fig. 3 Score plot and S-plot of OPLS-DA multivariate data analysis. (**a**) The OPLS-DA score plot shows the variation between the metabolite signal profiles of the two groups of DPHP exposure doses. Each point indicates a score value for a result from an animal. (**b**) The OPLS-DA loading S-plot shows the relative contributions of the signal variables to the clustering of rats dosed with 300 and 1200 mg/kg DPHP. Each circle indicates the loading value of a signal. (From the literature of Hsu et al. (2019))

well summarize the influence of the variable on the pattern (Hsu et al. 2019). S-plot shows that the covariance (P[1]) and correlation (P(corr)[1]) are the covariance and correlation of T score [1] and signal abundance, respectively. Thus, S-plot can filter the interesting signals to identify the candidates for DPHP exposure markers. The OPLS-DA analysis is free for use on MetaboAnalyst 5.0 website.

SMAIT is an algorithm based on the isotope tracing concept and used to filter target biotransformation products of xenobiotics (Lin et al. 2010). It contains three steps, including isotopic pair (IP) finding, IP response ratio (IPRR), and IPRR correlation analysis (Fig. 4) (Hsu et al. 2017). IP finding is used to find the signals with mass shifts in samples with varying isotope-labeled concentration ratios by performing the signal processing on LC-MS signal peaks extracted by peak extraction computational tools. Sample mixtures with varying isotope-labeled concentration ratios (naïve: isotope-labeled = 3:7, 4:6, 5:5, 6:4, and 7:3, respectively) are incubated with the liver enzyme to generate metabolites and then analyzed by LC-HRMS. The mass shift (ΔMZ) within the user-defined RT difference (ΔRT) is found between the native and isotope-labeled peak doublets in the hierarchical peak list of the isotope pair. If the alternation of the RTs and mass between the two peaks is less/equal than a given ΔRT and a given ΔMZ, these peaks are judged to be IP. In the

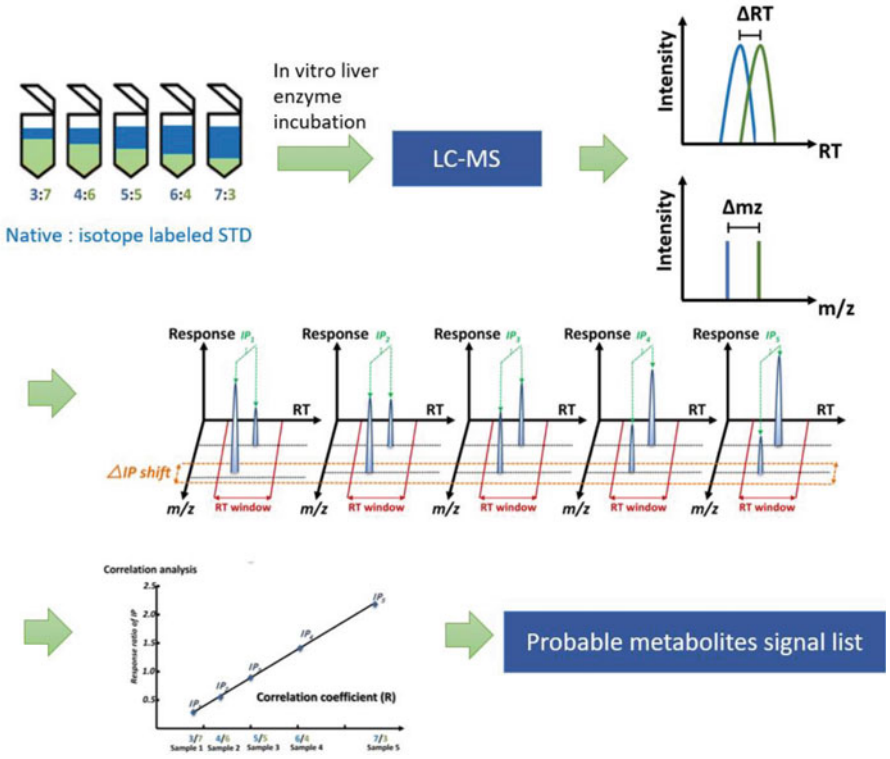


Fig. 4 The analytical approach of the SMAIT. There are three steps in the SMAIT approach, including isotopic pair (IP) finding, IP response ratio (IPRR), and IPRR correlation analysis. (From the literature of Hsu et al. (2017))

IPRR step, the IPs are located in five samples with different ratios (native: isotope-labeled) that give the response ratios correlated with the expected concentration ratios. Because the RT shifts may cause the loss of IP for a specific concentration ratio and raise the difficulty of excavating the metabolite signal using the SMAIT strategy, the user-defined sliding RT window (RTW) is similar to the RT tolerance, is used to replace the peak alignment process. The peak values of each sample in RTW are grouped to calculate Pearson's correlation coefficients. The RTW scan depends on the peaks in five UPLC-HRMS datasets with the different ratios and finds the IP combinations and their correlation coefficients. In the step of IPRR correlation analysis, this analysis locates the IP that gives a response ratio related to the expected concentration ratio. It is supposed that the high correlation coefficients of the IPs have a higher probability of containing probable metabolite signals.

MDF technique was first introduced and utilized for the identification peak of drug biotransformation products in 2003. It is developed to detect xenobiotics' endogenous metabolites and biotransformation products in complex matrixes using a high mass accuracy instrument, HRMS (Zhang et al. 2003; Zhu et al. 2006). The target compound

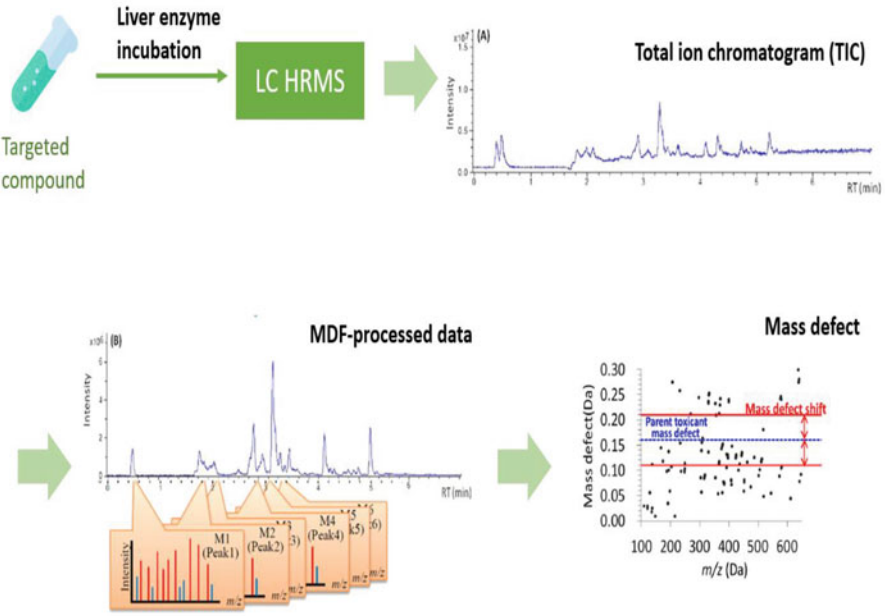


Fig. 5 The workflow of MDF. There are three steps to identify the biotransformation products, including in vitro incubation with liver enzyme, LC-HRMS analysis, and MDF-processed data. (From the literature of Hsu et al. (2017))

is incubated with the liver enzyme to obtain the biotransformation product mixture and then measurement by LC-HRMS, MS data processed by MDF software according to the defined mass defect change from the target compound's mass. Figure 5 is a schematic diagram for the MDF concept process. Each parent compound has a mass defect associated with the metabolites of phase I and phase II. Because many parent compounds structures of natural metabolites typically remain unchanged, the mass defect change of metabolites associated with their parent compounds situates within a relatively narrow range (usually within ± 0.050 Da). Depending on the parent compound's molecular weight (MW), the MW range of these metabolites can be estimated to filter off the ions that go beyond the expected MW range. The signals of mass defect change within the boundaries are retained by MDF, whereas the signals out of the mass defect shift are excluded. MDF-processed chromatogram automatically produces after processing, and the possible metabolites of target compounds with signal-to-noise ratios (S/N ratio) greater than 3 in the chromatogram processed can be obtained. This data processing approach of MDF permits users to concentrate on the analysis of potential chemical metabolite candidates.

Dose-Response Verification

A suitable exposure marker can increase the abundance correspondingly as the concentration or time of exposure increases. To verify the biotransformation

products candidates of DPHP identified by the workflow (Fig. 2), the different doses of DPHP are administered to experimental animals further to perform the dose-response validation of exposure marker candidates. The biotransformation products candidates of DPHP that can show a dose-response relationship under different DPHP exposure doses were validated as exposure marker candidates of DPHP.

Chemical Structure Identification of DPHP Exposure Markers

The chemical structure of DPHP exposure marker candidates is identified based on their accurate masses and product ion spectra in negative mode obtained by LC-HRMS and MS/MS. In addition, the chemical structures of the exposure marker candidate peaks were identified by online databases (such as PubChem, HMDB, ChemSpider, and METLIN) (Marco-Ramell et al. 2018; Housley et al. 2018), and the strategy integrated knowledge-based metabolic predictions of biotransformation routes established in-house. The possible fragments of the inferred structures of DPHP biomarker candidates can be predicted using Mass Frontier software (HighChem, Thermo Fisher Scientific, USA), which delivers small-molecule structural clarification for metabolism and metabolomics research, and then be additionally verified by the MS/MS spectra of the DPHP exposure marker candidates.

Applications of DPHP Exposure Markers in Biospecimens

The DPHP exposure marker candidates were further applied in 24H-urine samples collected from human subjects aged 17–79 and hair samples collected from long-term DPHP administrated rats. The urine sample can represent the internal exposure dose for recent DPHP exposure and the hair sample for long-term DPHP exposure.

DPHP Exposure Markers in the Human Urine Sample

A previous study reported by Hsu et al. (2019) has demonstrated the overall strategy for DPHP exposure marker discovery that integrates the HRMS-based metabolomics approach under the OPLS-DA method with the dose-response verification method in laboratory animals and finally applied the exposure marker candidates in human subjects. The overall strategy includes screening biomarkers by multivariate data analysis, verifying dose-response relationships (laboratory animals), and applying the filtered exposure marker candidates in human subjects is shown in Fig. 6 (Hsu et al. 2019). In stage I, Hsu and colleagues performed two groups of Wistar rats treated with two levels of DPHP (300 and 1200 mg/kg body weight (bw) in corn oil; $n = 15$ for each group) by oral administration to identify the DPHP exposure marker candidates (Fig. 6). Subsequent 24-h rat urine samples were collected and analyzed using LC-HRMS in full-scan mode followed by LC-MS/MS. After making the raw data of LC-HRMS into a peak list table by Progenesis QI and examining a multivariate statistical analysis by importing into OPLS-DA (the selection criteria P(corr)

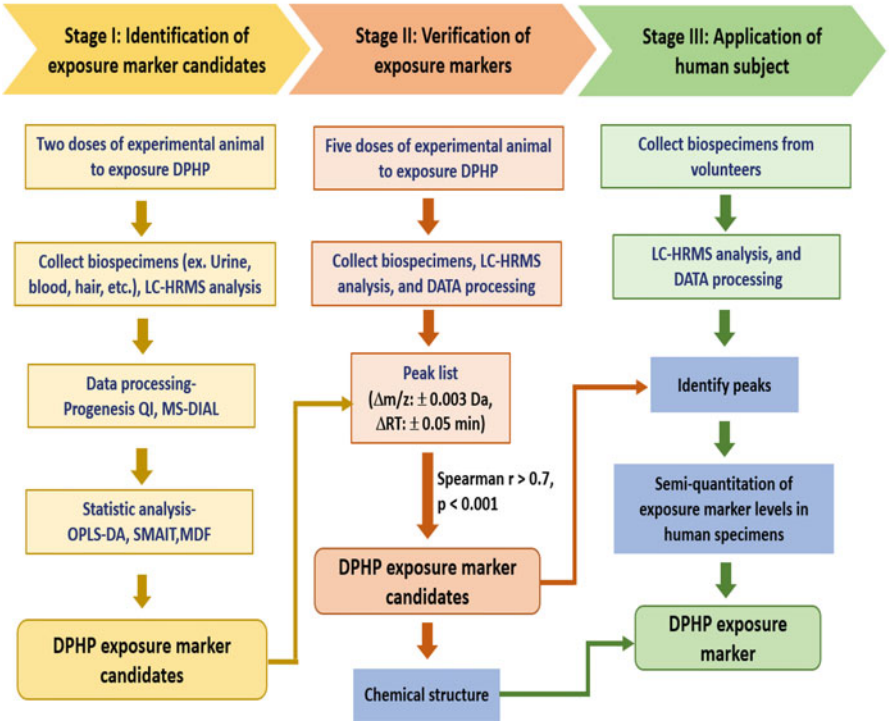


Fig. 6 Scheme of DPHP exposure marker discovery. There are three stages of DPHP exposure marker discovery, including identification of DPHP exposure marker candidates (stage I), verification of DPHP exposure marker candidates (stage II), and application in human subjects (stage III). (From the literature of Hsu et al. (2019))

[1] > 0.25 and P[1] > 5), 39 DPHP exposure marker candidates were identified (Fig. 3). Next, five groups ($n = 6$) of Wistar rats were treated with different doses of DPHP (0, 150, 300, 600, and 1200 mg/kg bw in corn oil) by oral administration to verify DPHP exposure marker candidates in stage II (Fig. 6). Thirty-six DPHP exposure marker candidates were further determined by verifying dose-response relationships for 39 DPHP exposure marker candidates. In stage III, the 36 DPHP exposure marker candidates were then investigated in 116 urine specimens from human subjects aged 17–79 years old by LC-HRMS. Twelve of the 36 DPHP exposure marker candidate signals were detected in over 30% of the human urine specimens. According to the chemical information, these 12 DPHP exposure marker signals can be divided into 7 chemicals and their corresponding isomers with the same m/z but different retention times, and 5 possible chemical structures were identified.

Three of these five possible chemical structures were oxidized the fatty acids (hydroxycapric acid [Peak 1 and the isomer P2 and P3], sebacic acid [P4 and the isomer P5], and hydroxysebacic acid [P10]) that were the typical urinary acids

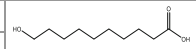
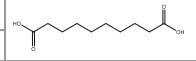
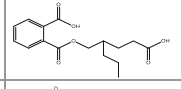
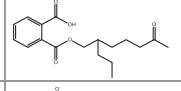
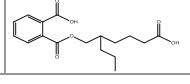
in the human body and not the specific biotransformation products of DPHP (Table 1). The other two possible chemical structures containing an aromatic dicarboxylic acid, the basic structure of phthalate, could be the specific biotransformation products of DPHP. Among them, mono-(2-propyl-4-carboxybutyl) phthalate (cx-MPBP, the P15 in Table 1) was detected in 99% of the human urine samples reported in the previous reports, but the m/z value of cx-MPBP (exact mass 307.1187) is the same with mono-(2-ethyl-5-carboxypentyl) phthalate (cx-MEPP, m/z 307.1187) that is a DEHP biotransformation product (Koch et al. 2005). The same mass of cx-MEPP and cx-MPBP cannot be distinguished through the mass spectrometer, but they can be resolved by chromatography or monitoring difference mass transitions (precursor ion/product ion). This characteristic renders cx-MPBP to be a nonspecific exposure marker for DPHP. In addition, cx-MEPP can be an appropriate DPHP exposure marker due to the extensive distributed cx-MEPP levels in the human research subjects if the isomers (cx-MEPP and cx-MPBP) can be discriminated by chromatographic methods and measured in a single LC-MS/MS run. Mono-(2-propyl-7-dihydroxyheptyl)-phthalate (di-OH-MPHP, P25 and the isomer P26 in Table 1) contains a complete side chain derived from DPHP and shows the high detection rates (96%, 111/116 and 74%, 86/116, respectively) and the maximum signal abundance among the 36 exposure marker candidate signals (Table 1). Thus, di-OH-MPHP is thought over to be a DPHP specific exposure marker.

DPHP Exposure Markers in Hair Samples

Hair has been increasingly used as a suitable matrix to assess the exposure of short- and long-term exposure reflection because chemicals found in serum can migrate into hair (Wennig 2000; Covaci et al. 2002; Alves et al. 2014). Shih and colleagues used the urine and hair samples from experimental animals to clarify whether the exposure markers in urine can be applied in hair samples as the long-term exposure marker (Shih et al. 2019a). The urine and hair sampled were collected from Wistar rats treated with five different doses of DPHP (0, 150, 300, 600, and 1200 mg/kg bw in corn oil) by oral administration once every 24 h for 7 days, in which the urine samples were collected on day 1, 7, 14, and 28 after the first exposure of DPHP, as well as the hair samples, were collected on day 28. The raw MS data were converted to peak lists using Progenesis QI software and identify metabolite candidates by OPLS-DA (the selection criteria P(corr) [1] > 0.5 and P[1] > 0.1 for urine samples, whereas P(corr) [1] > 0.5 and P[1] > 0.01 for hair samples).

The number of signals shows a dose-dependent response in the urine samples on days 1, 7, 14, and 28, and the hair samples on day 28 were 37, 43, 31, 7, and 29 candidates, respectively. After 7 continuous days of exposure, the number of DPHP metabolites present in urine is higher than the amount after 1 day of exposure. However, more tentative DPHP metabolites can be identified in urine samples on day 7 (43 candidates) than in hair samples on day 28 (29 candidates), and only 10 DPHP metabolites in the urine samples were also found in the hair samples,

Table 1 Characteristics of validated DPHP exposure markers in urine samples from humans and rats. (From the literature of Hsu et al. (2019), Shih et al. (2018, 2019a, b)

Peak no.	m/z for biotransformation product signals	RT (min)	Markers verified by rat	Literature	Chemicals information	
					Structure	Name
P1	187.1346	3.57	+			Hydroxycapric acid
P2	187.1346	3.67	+			
P3	187.1346	3.75	+			
P4	201.1138	2.29	+			Sebacic acid
P5	201.1138	2.59	+			
P6	203.1294	2.84	+			
P7	203.1295	2.41	+			
P8	203.1295	2.95	+			
P9	215.1296	3.22	+			
P10	217.1088	1.93	+			Hydroxysebacic acid
P11	228.1613	3.45	+			
P12	253.1124	2.38	+			
P13	292.1234	2.45	–			
P14	300.1827	3.34	+			
P15	307.1196	2.98	+			Mono-(2-propyl-4-carboxybutyl) phthalate (cx-MPBP)
P16	319.1563	3.53	+			Mono-(2-propyl-6-oxoheptyl phthalate
P17	321.1352	3.31	+			Mono-(2-propyl-5-carboxypenyl) phthalate

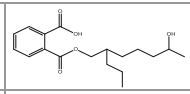
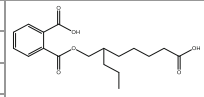
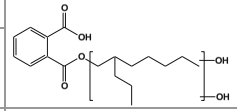
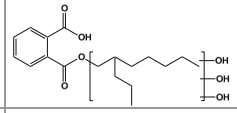
P18	321.1719	3.84	+			Mono-(2-propyl-6-hydroxyheptyl) phthalate
P19	323.1146	2.13	+			
P20	335.1509	3.57	+			Mono-(2-propyl-6-carboxyhexyl) phthalate (cx-MPHxP)
P21	335.1509	3.68	+			
P22	335.1511	3.93	+			
P23	335.1511	3.06	+			
P24	337.1308	2.78	+			
P25	337.1664	2.52	+			Mono-(2-propyl-dihydroxyheptyl) phthalate (Di-OH-MPHP)
P26	337.1664	2.89	+			
P27	351.1454	2.43	+			
P28	351.1456	2.95	+			
P29	351.1456	3.09	+			
P30	353.1613	2.14	+			Mono-(2-propyl-trihydroxyheptyl) phthalate (Tri-OH-MPHP)
P31	361.1509	2.52	–			
P32	363.1459	2.69	+			
P33	365.1615	3.21	+			
P34	367.1405	2.08	+			
P35	367.1405	2.15	+			
P36	499.2198	2.27	+			
P37	511.1833	2.18	–			
P38	513.1989	2.27	+			
P39	513.1989	2.63	+			

Table information are from the literature of Hsu et al. (2019), Shih et al. (2018, 2019a, b)

proving that the DPHP metabolites in urine and hair are different. To clarify why the metabolites were different between urine and the 28th-day hair samples, the chemical structures of the identified metabolites were determined by UPLC-MS/MS. The structures of tentative metabolites in the 7th-day urine and 28th-day hair samples were identified. These metabolites seem to be structurally related to DPHP, including three known DPHP metabolites (oxo-MPHP, OH-MPHP, and cx-MPHxP), one previously reported DPHP structure-related metabolite that has been suggested as a suitable DPHP exposure marker (P25, di-OH-MPHP) searched/filtered by MDF and SMAIT (Shih et al. 2018), and three groups of novel DPHP structure-related metabolites (m/z = 321.1358, 337.1670, and 337.1671, respectively) (Shih et al. 2019a). These DPHP-related signals showed a dose-dependent response in urine samples but no dose-dependent response in hair samples. Among the urine exposure markers, only the cx-MPHxP signal can be detected in the first visit samples. These results indicate that the hair samples can be long-term exposure samples and use different exposure markers than urine samples.

Mini-dictionary of Terms

- ***Biotransformation products*** are metabolized from an exogenous compound to increase the water solubility and excretion efficiency.
- ***HRMS*** is a high-resolution mass spectrometer that can provide accurate mass measurement and comprehensive recording of the signal.
- ***Metabolomics*** is a scientific research of chemical processes involving metabolites, small molecule substrates, intermediates, and products of cell metabolism and providing a direct functional readout of the physiological state in organisms.
- ***Dose-response*** is that an increasing level of exposure is related to either an increasing or a decreasing risk of the outcome.
- ***MS/MS*** is a particular m/z value of interest selected from the mass spectrum and collided into fragment ions to identify the chemical structure.
- ***Multiple reaction monitoring (MRM)*** is a precise and sensitive mass spectrometry technique to selectively quantify compounds within complex mixtures.

Key Facts of DPHP Exposure Markers

DPHP is widely used in personal care products and plastic products in recent years. Due to the subchronic toxicity of DPHD, it is necessary to biomonitor and estimates the DPHP exposure in the human body. DPHP has short half-lives in the human body and exhibits rapid ADME (absorption, distribution, metabolism, and excretion) processes within a day. Biomonitoring of DPHP exposure is commonly performed by measuring its respective metabolites instead of their original chemical forms. Discriminant the DPHP metabolic features and identification of DPHP exposure markers are essential to discover the exposure markers.

Summary Points

- This chapter shows an HRMS-based metabolomics profiling to screen the DPHP exposure markers.
- The DPHP exposure markers are identified and verified in experimental animals and then be applied to human subjects.
- Three metabolomics data-screening approaches, OPLS-DA, SMAIT, and MDF, were introduced in this chapter to discover the exposure marker candidates.
- The biotransformation product of DPHP (oxo-MPHP, OH-MPHP, cx-MPHxP, and di-OH-MPHP) can be the exposure marker for general human exposure assessments in urine samples.

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