Supporting Information for

Quantitative identification of unknown exposure pathways of phthalates based on measuring their metabolites in human urine

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Urinary phthalate analysis

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2 We measured five urinary phthalate metabolites using enzymatic deconjugation, solid-phase extraction,
3 and high-performance liquid-chromatography isotope-dilution tandem mass spectrometry
4 (isotope-dilution LC-MS/MS). The analytical methods used in the present study have been described in
5 detail previously (1). In addition to MBP and MEHP, which have been analyzed previously (1), three
6 phthalic acid monoesters, MMP, MEP, and MBzP, were newly targeted for the present analysis.

7 Standard substances, MMP (99.7%, Riedel-de Haën, Germany), MEP (98.4%, Hayashi Pure Chemical
8 Ind., Ltd., Osaka, Japan), and MBzP (> 98%, TCI Tokyo Kasei Kogyo co. Ltd., Tokyo, Japan), and their
9 corresponding isotopically-labeled internal-standard substances, MMP-$^{13}C_4$ (99%, Cambridge Isotope
10 Laboratories, Inc., Andover, MA, USA), MEP-d_4 (98.8%, Hayashi Pure Chemical Ind., Ltd., Osaka,
11 Japan), and MBzP-d_4 (99.9%, Hayashi Pure Chemical Ind., Ltd., Osaka, Japan), were newly purchased.

12 For the analysis of five analytes in urine samples, our past chromatographic conditions in the
13 LC-MS/MS procedure (1) were partially modified to ensure their chromatographic separation from
14 sample matrices (Table S1). A smaller-particle column (RS Pro C_{18}, 3μm, 2.1 mm i.d. × 100 mm, YMC,
15 Kyoto, Japan), lower flow rate, and gentle slope gradient were newly employed. A line-splitter was not
16 used in the present study. Incidentally, the separability of MBP from a structural isomer, mono-iso-butyl
17 phthalate (MiBP), was previously checked. Although their baseline separation was not always achieved,
18 the peak size of MiBP was adequately smaller than that of MBP. The reproducibility of the MBP
19 measurement value was relatively good (shown in Table S2). The MiBP reference substance was a
20 generous gift from Dr. Toshinari Suzuki (Tokyo Metropolitan Institute of Public Health, Tokyo, Japan).

21 The analytical conditions are summarized in Table S2. The analytical limits of detection (LODs) and
22 limits of quantification (LOQs) were calculated in three ways as follows. First, the instrumental LOD and
23 LOQ in LC-MS/MS were set at a signal-to-noise ratio of 3 and 6, respectively. Second, LOD and LOQ
24 were set as three and six times of the standard deviation of method-blank test values (n = 4), respectively.
25 Third, the standard deviations derived from the repeated measurements (n = 5) of the five lowest
26 standards were plotted against their concentrations. The intercept of their best-fit line was defined as the
27 standard deviation of the concentration approaches zero (SD_0). LOD was set as three times of SD_0. LOQ
28 was the concentration equal to 10%, which is calculated from the best-fit line of the relative standard
29 deviations of the same repeated measurements. Thus, the largest value among the three was finally
30 employed.

31 MEP, MBP, MBzP and MEHP were detected in all of the urine samples while MMP was detected in 35
32 of the samples. The MMP in one sample was less than the LOD. The MMP signal on LC-MS/MS was so
33 poor that the LOD of MMP was higher than that of the other four compounds. For the concentration in a
34 urine sample less than the LOD, a value equal to the LOD divided by the square root of 2 was substituted
35 (2, 3).

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Tables

3

4   Table S1. HPLC gradient method.

Time (min)	0.0	4.0	4.01	17	24	24.01	28	28.01	40
%A	90	90	85	75	35	0	0	90	90
%B	10	10	15	25	65	100	100	10	10

5   * Flow rate: 0.35 mL/min; mobile phase A: 1-mL acetic acid in 1-L ultra-pure water; mobile phase B:
6   1-mL acetic acid in 1-L acetonitrile.

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Table S2. Analytical conditions of phthalate monoesters.

Compound	Monitor ion (m/z) Precursor	Product	Cone [V]	Collision [eV]	RT* [min]	Mean recovery†	Reproducibility‡ [μg/L]	LOD [μg/L]	LOQ [μg/L]
MMP	178.9	76.9	50	23	7.70	–	3.7%	4.0	7.9
MMP-^{13}C$_4$	182.9	78.9	48	21	7.70	54%	–	–	–
MEP	192.9	76.9	48	23	11.6	–	27%	0.30	0.60
MEP-d$_4$	196.8	80.9	40	18	11.5	66%	–	–	–
MBP	220.8	76.9	50	23	22.4	–	0.74%	0.27	0.87
MBP-d$_4$	224.9	80.9	50	22	22.6	92%	–	–	–
MBzP	254.9	182.8	50	11	23.1	–	0.92%	0.15	0.30
MBzP-d$_4$	258.9	186.9	60	11	23.0	86%	–	–	–
MEHP	277.0	133.8	40	16	26.9	–	0.81%	0.27	0.54
MEHP-d$_4$	281.1	137.8	40	16	26.9	93%	–	–	–

* RT: retention time.

† Total surrogate recovery in 36 urine samples (tested using the previous HPLC gradient (*1*)).

‡ Reproducibility of measurement (n = 3, inter-day extraction, intra-day LC-MS/MS measurement).

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2 References

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