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Biomonitoring of chlorophenols in human urine from several Asian countries, Greece and the United States☆

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ABSTRACT

Chlorophenols (CPs) are used in the production of pesticides and preservatives. Although human exposure to CPs has been known for years, current exposure levels to these chemicals in Asian countries are not known. In this study, we analyzed concentrations of eight CPs in 300 human urine samples collected from nine countries. Of these CPs, 2,5-dichlorophenol and 2,4-dichlorophenol were found at the highest median concentrations (median for all nine countries: 1.78 and 0.34 ng/mL, respectively). Pentachlorophenol was found in 59% of the samples analyzed at a median concentration of 0.07 ng/mL. Urine samples from Japan had the highest concentration of total CPs (median: 16.7 ng/mL) with 2,5-dichlorophenol accounting for 93.1% of the total concentration. The estimated daily intake (DI) for precursors of dichlorophenols varied widely, but several samples showed values higher than the acceptable DI recommended by the United States Environmental Protection Agency (EPA). These results suggest that CP exposure, especially to dichlorophenols, is prevalent in several countries, particularly in Asia, suggesting a pressing need for further assessment of the global sources and potential health effects of these chemicals.

1. Introduction

Chlorophenols (CPs), a group of halogenated chemicals encompassing several congeners, have been widely used as fungicides, insecticides, and wood preservatives for several decades (Favaro et al., 2008; Kauppinen et al., 1993; Kringstad and Lindström, 1984; Mueller et al., 1989). In the U.S., an estimated 10 063 kg of 2,4-dichlorophenol and 4295 kg of pentachlorophenol (PCP) were released into the environment in 2014 (U.S. EPA, 2017a). In addition, several CPs are known intermediates and metabolites of chlorophenoxy herbicides (Kilbane et al., 1982; Oh and Tuovinen, 1991).

Several studies have reported the occurrence of CPs in water, soil (Czaplicka, 2004; Gao et al., 2008; Muir and Eduljee, 1999; Persson et al., 2008), and in human specimens (Bartels et al., 1999; Frederiksen et al., 2014; Haines et al., 2016; Ye et al., 2014). Occupational exposure to PCP from its use in sawmills has been documented (Ruder and Yiin, 2011).

CPs are endocrine-disrupting chemicals (Terasaka et al., 2006), and studies have associated carcinogenicity (Garabedian et al., 1999; Hoovield et al., 1998), oxidative stress (Bukowska et al., 2007), apoptosis (Michałowicz and Sicińska, 2009), and teratogenicity (Zhao et al., 1995) to CPs. The International Agency for Research on Cancer (IARC) categorized CPs as Group 2B carcinogens (i.e., possible carcinogens). The U.S. Agency for Toxic Substances and Disease Registry listed CPs on the Priority List of Hazardous Substances. In recognition of these concerns, several countries have regulated commercial uses of CPs (Igbinosa et al., 2013; U.S. EPA, 1987).

Despite their toxic potential and ongoing use, biomonitoring surveys of human exposure to CPs are limited to North America and certain European countries (Becker et al., 2003; Casas et al., 2011; CDC, 2017; Health Canada, 2013). Little is known about human exposure to CPs in Asian countries. In this study, we determined urinary concentrations of eight CP congeners namely, 2,4- and 2,5-dichlorophenols (DCPs), 2,4,5- and 2,4,6-trichlorophenols (TCPs), 2,3,4,5-, 2,3,4,6- and 2,4,5,6-tetrachlorophenols (TeCPs), and PCP in 300 samples collected from China, India, Japan, Korea, Kuwait, Saudi Arabia, Vietnam, Greece and the U.S. to assess exposure

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levels, congener profiles, potential sources, and risks.

2. Materials and methods

2.1. Standards and reagents

Analytical standards of 2,4-dichlorophenol (24-DCP), 2,5-dichlorophenol (25-DCP), 2,3,4,5-tetrachlorophenol (2345-TeCP), 2,3,4,6-tetrachlorophenol (2346-TeCP), 2,3,5,6-tetrachlorophenol (2356-TeCP), and pentachlorophenol (PCP) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.). 2,4,5-trichlorophenol (245-TCP) and 2,4,6-trichlorophenol (246-TCP) were purchased from Cambridge Isotope Laboratories, Inc. (CIL; Andover, MA, U.S.). The isotopically labeled internal standards, d_3-2,4-DCP and $^{13}C_6$-PCP were purchased from Sigma-Aldrich. $^{13}C_6$-245-TCP and $^{13}C_6$-246-TCP were purchased from CIL. $^{13}C_6$-2456-TeCP was purchased from Toronto Research Chemicals Inc. (Downsview, ON, Canada). β-Glucuronidase (Type HP-2, 197 000 unit/mL, from *Helix pomatia*) was purchased from Sigma-Aldrich. All organic solvents used were of analytical grade.

2.2. Sample collection

Spot urine samples (n = 300) were collected from Greece, the U.S., and the seven Asian countries. Samples from Korea (n = 26) were collected in 2006–2007 and samples from China (n = 47), India (n = 41), Japan (n = 36), Kuwait (n = 40), Vietnam (n = 19) and the U.S. (n = 31) were collected during 2010–2012. Urine samples from Greece (n = 30) and Saudi Arabia (n = 30) were collected in 2012 and 2014, respectively. The age of donors from the nine countries ranged from 2 to 87. Details on sampling locations and samples have been reported earlier (Table S1, supporting information: Guo et al., 2011; Liao et al., 2012; Asimakopoulos et al., 2013). All samples were stored in polypropylene (PP) tubes and kept at −20 °C until analysis. The New York State Department of Health Institutional Review Board approved the study for the analysis of urine.

2.3. Sample extraction

CPs were extracted from urine by following the method described in Wang and Kannan (2013) with slight modification. Briefly, 0. 5 mL of urine sample was transferred into a 15-mL polypropylene (PP) tube and 300 μL of 1 M ammonium acetate buffer containing 100 unit/mL of ß-glucuronidase and 5 ng of internal standard mixture were added. After vortex mixing, samples were incubated overnight at 37 °C, and 3 mL of ethyl acetate was added, and shaken in an orbital shaker for 60 min. The organic and aqueous layers were separated by centrifugation at 3400 *g* for 5 min. The upper layer (organic extract) was transferred into a new 15-mL PP tube. The extraction was repeated twice with 3 mL aliquots of ethyl acetate. One milliliter of ultrapure water was then added to the combined extracts, which were shaken for 30 min, and centrifuged at 3400 *g* for 5 min. The upper layer was pipet transferred into a new 15-mL PP tube, which was evaporated to near dryness under a gentle stream of nitrogen. The extract was reconstituted with 0.5 mL of methanol, vortexed, and transferred into a liquid chromatograph (LC) vial for instrumental analysis.

2.4. Instrumental analysis

The extract was injected and analyzed by an ultra-high performance liquid chromatography (UPLC; Acquity I Class; Waters, Milford, MA, U.S.) coupled with an electrospray triple quadrupole tandem mass spectrometry (MS/MS; API 5500; AB SCIEX, Framingham, MA, U.S.). Separation and detection of DCPs were carried out by a Betasil C18 column (5 μm, 100 Å, 100 × 2.1 mm; Thermo Fisher Scientific Inc., Bellefonte, PA, U.S.) and APCI-negative ionization mode, respectively. Separation and detection of TCPs, TeCPs, and PCP were performed by a Kinetex F5 column (1.7 μm, 100 Å, 50 × 2.1 mm; Phenomenex, Torrance, CA, U.S.) and ESI-negative ionization mode, respectively. Both analyses were conducted using a multiple reaction monitoring (MRM) mode. Details of UPLC and MS/MS parameters are shown in Tables S2 and S3. The mass transition of target analytes, internal standard, and approximate retention times are shown in Table S4.

2.5. Quality assurance and quality control

A procedural blank, spiked blank, and matrix spiked sample were analyzed for each batch of 20 specimens. The results of quality control samples are shown in Table 1. Procedural blanks contained CPs at concentrations ranging from below the limit of quantification (LOQ) to 0.04 ng/mL. The average recoveries of CPs spiked in water and urine matrix ranged from 90.8 to 95.4% and from 84.7 to 99.3%, respectively. The LOQs of CPs ranged from 0.04 to 0.34 ng/mL (Table 1). The laboratory participated in various proficiency testing programs analyzing urine samples for select CPs, and the results were within ±20% of the reference values. The reported CP concentrations were not creatinine-adjusted. The urinary creatinine concentrations for the samples are available (see Guo et al., 2011) and the values were within the normal range. Furthermore, our aim was to provide baseline data on urinary CPs concentrations in populations in several countries studied here. Although creatinine adjustment is followed in the correction for urine dilution factors, such adjustment can also introduce other bias, because creatinine concentration not only depends on urine excretion volume but also on muscle mass of donors (Baxmann et al., 2008). Our results and conclusions are based on unadjusted concentrations of CPs in urine.

2.6. Statistical analysis

The concentrations of CPs below the LOQ were substituted with a value of LOQ/2 for the calculation of sum of all CPs analyzed (ΣCPs). DCPs and PCP had high detection rates (DR) and (LOQ/2) substitution for the LOQ values did not affect our conclusions. The analytical software ProUCL (5.0.00) was used to employ Kaplan-Meier (KM) method to calculate geometric mean (KM Mean) and standard deviation (KM SD). R software (version 3.2.1) was used to estimate the correlation between each CPs using Spearman's rank order correlation coefficient as a non-parametric statistical test. Significant differences between each CP and ΣCPs were examined by Steel-Dwass test as a non-parametric statistical test (Ihaka and Gentleman, 1996), with the *p* value set at 0.05.

3. Results and discussion

3.1. Concentrations of chlorophenols

Urinary concentrations of CPs in seven Asian countries, Greece, and the U.S. are shown in Table 2 and Table S5. Of the 300 samples analyzed from nine countries, 97.7% of the samples contained at least one CP. 24-DCP was the most frequently detected compound, with a DR of 92.7%, followed by 25-DCP (85.7%), PCP (58.7%), 246-TCP (27.0%), 245-TCP (25.7%), 2356-TeCP (7.3%) and 2346-TeCP (5.3%). 2345-TeCP was not detected in any sample. The overall trend in the DR of CP congeners was, DCP > PCP > TCP > TeCP. This pattern was noted for all countries except for Japan and Vietnam. In Japan, the DRs of both DCP congeners analyzed in this study were high (24-DCP: 91.7%, 25-DCP: 100%), but the DR of PCP was the

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