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Variability of urinary excretion of pyrethroid metabolites in seven persons over seven consecutive days—Implications for observational studies

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HIGHLIGHTS

- Urinary 3PBA concentrations show fairly stable levels in seven adults over one week.
- Spot or multiple urine spot samples should be considered as the most reliable for exposure assessment.
- Creatinine adjustment was shown to improve overall 3PBA measurement reliability in spot samples.

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ABSTRACT

Concentration of urinary metabolites is frequently used for biomonitoring of exposure to synthetic pyrethroids, the class of non-persistent insecticides. These chemicals are currently widely used in agriculture, households and public health all over the world. Most of them are easily metabolized in mammals and in the form of metabolites excreted in urine. The concentration in urine is thus susceptible to significant variations, even within a short period of time. In this study, temporal changes in urinary metabolites concentrations in seven subjects (four females and three males aged: 24–71) were monitored over seven consecutive days. All urine voids (281 in total) were collected and analyzed for *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylic acid (*cis*-Cl_2CA and *trans*-Cl_2CA), *cis*-3-(2,2-dibromo-vinyl)-2,2-dimethylcyclo-propanecarboxylic acid (Br_2CA) and 3-phenoxybenzoic acid (3PBA) using a validated gas chromatography ion-trap mass spectrometry method. Only 3PBA was detectable in more than 60% of the collected samples enabling a reliable statistical analysis. Statistical analysis was performed to evaluate temporal variability in urinary excretion of 3PBA over the studied period. Both volume and creatinine (Cre) adjusted concentrations were evaluated with the latter one being the most reliable. Among all samples, first morning voids (FMV) were the least reproducible (inter-class correlation coefficient – ICC, 0.551 and 0.350 for volume and creatinine adjusted concentrations, respectively). Spot and reconstructed 24-h samples were more reproducible in this study. ICC values for ng/mL concentrations were 0.599 and 0.681 (in spot and 24-h samples) and 0.846 and 0.796 for μg/g creatinine concentrations.

Results of this study suggest fairly constant short-term exposure to pyrethroids metabolized to 3PBA among the urban population in Poland. Creatinine adjustment should be performed in epidemiological studies and spot or multiple spot samples should be preferentially collected for the highest reliability of the measurement.

1. Introduction

Insecticides play a very important role in the protection of human health and significantly improve agricultural productivity. This is a group of pesticides of particular interest in toxicology because they exhibit the same toxicity mechanism in target organisms as in humans. One of the most widely used classes of insecticides are actually synthetic pyrethroids (PYRs) which partly replaced more toxic organophosphates and carbamates. PYRs as active ingredients are components of many commercial formulations employed in agriculture, gardening, households and are of special importance in public health to prevent spreading of the vector transmitted infections like malaria, tropical fevers, etc. This group of insecticides is the only one recommended by WHO for impregnation of anti-mosquito bednets (WHO, 2009). Numerous biomonitoring surveys demonstrated wide exposure among the populations of several countries: Germany (Heudorf and Angerer, 2001), Japan (Kimata et al., 2009), Canada (Fortin et al.,

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2008a) and USA (Barr et al., 2010). Recently, quantifiable concentrations of 3PBA in the urine of a convenience sample of the Polish population have been also reported (Wielgomas et al., 2013). Estimation of systemic exposure is usually performed using urinary metabolite concentration measurement, because once ingested the human body, PYRs undergo effective biotransformation and are quite rapidly excreted in urine partly in conjugated forms as glucuronides and sulphates (Eadsforth et al., 1988; Sams and Jones, 2012; Eadsforth and Baldwin, 1983). This approach based on the urinary metabolite excretion combines all routes and possible sources of exposure. In the general population, non occupationally exposed to pesticides, dietary exposure seems to be the most significant source of pyrethroids, however episodic exposure can occurs also from the other sources (periodic or systematic indoor pesticide application, dust, clothes and wool carpet impregnation). Besides the diet, also pet care products and other household products containing PYRs may play a significant source of exposure (Keenan et al., 2009). The dose recovered in urine is a result of combined exposure to both parent compounds (native pyrethroids) and their degradation products/metabolites that could be ingested with food, air, dust or water (Morgan, 2012). It is still unidentified to what extent exposure to degradation products plays a role in systemic exposure, measured as urinary excretion. Based on the animal and human experiments, toxicokinetics for most pyrethroids is well known. It is ascertained that the half-lives for most PYRs are short. In a few hours, the majority of the adsorbed dose is eliminated through the kidneys, thus, urine analysis can estimate very recent exposure (Eadsforth and Baldwin, 1983; Eadsforth et al., 1988). Since the excretion is efficient and rapid, single measurement of urinary concentration would be inappropriate and not reliable to evaluate averaged individual exposure. Biomonitoring studies are designed frequently to measure relationships or predict health outcomes and exposure to hazardous factors. Many variables can affect temporal variability of urinary excretion of specific biomarkers: gender, time of the year, diet, episodic pesticide application, indoor/outdoor activity, etc. (Meeker et al., 2005; Lambert et al., 2005). It is of the highest importance to precisely design a sampling strategy in large scale observational studies. Depending on the type of collected urine samples (spot sample, first morning void or 24 h collections), a study outcome could be affected due to the differences in concentrations in those samples. Thus, studying short- and long-term reliability of sampling is very important. If a single spot sample is to be collected per day, first morning voids (FMV) are often preferred on the basis of the assumption that the FMV provides an integrated sample over a relatively long part of the day and is therefore more likely to be representative. However, this assumption has not been validated, and no clear standards exist for the correction for urine volume, contaminant excretion, dilution, or time between exposure and void (Hwang et al., 1997).

Due to the short half-lives and mixed exposure sources, PYRs are suspected to show high variability over time that could aggravate interpretation of the biomonitoring results, over- or underestimating of real exposure based on a single measurement approach. Since levels of biomarkers of exposure in urine reflect temporal variations in both exposure and physiological processes, sampling design may significantly affect analytical results (Hinwood et al., 2002).

Until now, short-term variability was studied for urinary concentrations of polycyclic aromatic hydrocarbon metabolites (Li et al., 2010), bisphenol A (Ye et al., 2011), phthalate metabolites (Peck et al., 2010; Preau et al., 2010), and organophosphate pesticide metabolites (Kissel et al., 2005). Usually the concentrations exhibited moderate to high variability described by an interclass correlation coefficient (ICC).

In this work, we attempted to evaluate intra- and inter-day personal variations in urinary 3-phenoxybenzoic acid over seven consecutive days in seven people. The study was designed to provide detailed information on exposure variability by observing urinary biomarker concentrations. In regard to the epidemiological studies, this knowledge can be used for designing exposure assessment strategies and for adjusting for measurement errors in pyrethroid exposure.

2. Materials and methods

2.1. Chemicals

The following chemicals were obtained from Sigma–Aldrich (Germany): 1,1,1,3,3,3-hexafluoroisopropanol (HFIP), diisopropylocarbodiimide (DIIC), 3-phenoxybenzoic acid (3PBA) and 2-phenoxybenzoic acid (2PBA) which was used as an internal standard. Other metabolites: *cis*-3-(2,2-dibromo-vinyl)-2,2-dimethylcyclo-propanecarboxylic acid (Br_2CA), *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylic acid (*cis*-Cl_2CA and *trans*-Cl_2CA) were from Dr. Ehrenstorfer GmbH (Germany). All other reagents were of analytical grade. Standard stock solutions of 1 mg/mL were prepared in acetonitrile and stored at −20 °C, protected from light. Working standard solutions were prepared in acetonitrile and were stored at +4 °C.

2.2. Subjects and sample collection

In this study (June–October 2011), we recruited four women and three men aged 24–71, residents of Gdańsk (Poland), who agreed to collect urine samples according to a study protocol. All procedures were approved by the Ethics Committee of the Medical University of Gdańsk, Poland (NKEBN/301/2011). Prior to enrolment in the study, each subject signed the informed consent form. The study participants were asked to collect all urine voids in precleaned, calibrated glass beakers (to measure total void volume) and save about 30 mL from each void in the polypropylene storage tubes. No dietary restrictions were forced, so the volunteers were allowed to follow their own, regular dietary habits. All seven participants declared no occupational exposure to pesticides or recent application at place of residence. Two male participants (P2 and P3) were active cigarette smokers. Study participants characteristics are shown in Table 1.

Collected urine samples were stored at the participant location in a portable freezer (−18/−20 °C) until the end of the collection period and were then transported to the laboratory and stored further at −20 °C until analysis.

All subjects collected in total 281 spot samples including 49 first morning voids. None of the subjects declared to miss any sample.

2.3. Chemical analysis

The concentrations of 3PBA were measured by gas chromatography ion-trap mass spectrometry (GC–MS) using electro-ionization operated in selected ion storage detection mode. The extraction step was adopted from a previously established method (Schettgen et al., 2002). Briefly, 3 mL of thawed urine were transferred into 10 mL screw-top glass tube and 25 μL of IS solution (2PBA, 1 μg/mL of acetonitrile) along with 0.6 mL concentrated hydrochloric acid were added. Samples were incubated at 95 °C in a laboratory oven for 90 min. After bringing samples to room temperature, 4 mL of hexane were added and tubes were shaken for 15 min. Following centrifugation, the hexane layer was transferred to the next screw-top glass tube, and extraction with the same volume of hexane was repeated. Extracts were combined and then reextracted with 0.5 mL of 0.1 M NaOH. Hexane was discarded, while 0.1 mL of concentrated HCl and 2 mL of hexane were added to the remaining aqueous phase. Samples were shaken again, centrifuged and the resulting supernatant was evaporated to dryness under the stream of nitrogen at 45 °C. The residue was treated with 10 μL of HFIP, 15 μL of DIIC and 250 μL of hexane. Samples were mixed for 10 min at room temperature and then 1 mL of 5% K_2CO_3 was added. After vigorous shaking, tubes were centrifuged and hexane layer was transferred to an autosampler vial. Two microliters of the final extract were analyzed by GC–MS.

Analyses were performed using a Varian GC-450 gas chromatograph equipped with 220-MS ion-trap mass spectrometer working in the selected ion storage (SIS) mode. Derivatives were separated on VF-5 ms (Varian, Palo Alto, USA) low bleed column (30 m × 0.25 mm I.D., 0.25 μm film thickness) using the following oven program: 60 °C – 1 min, 60–150 °C (8 °C/min), 150–280 °C (30 °C/min), 280 °C – 11 min. Selected ion (364 *m*/*z*) was monitored for quantitation of 2PBA and 3PBA. Two microliters of the final extract were injected (injector temperature 280 °C) in splitless mode with 25 psi pulse pressure lasting 1.0 min. Chromatograph was equipped with 1079 programmable temperature vaporizing injector and SGE focusliner glass inlet.

Urinary creatinine was measured with spectrophotometric Jaffe method.

2.4. Internal and external quality control

Quality of the assay was systematically monitored throughout the study. It was accomplished by the inclusion in each analytical batch a series of quality control samples. Physiological human urine spiked with analytes at three levels (0.5 ng/mL, 1 ng/mL and 5 ng/mL) was prepared in our lab, frozen and used when needed. In addition, ClinCal® Urine Calibrator (Cat. No. 9969), lyophilized, for Toxic Organic