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Biomonitoring of pyrethroid exposure among rural and urban populations in northern Poland

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HIGHLIGHTS

- Higher exposure to pyrethroids is noticed in rural populations and children.
- Diet is the main source of exposure to pyrethroids in urban areas.
- The use of pet-care products increases exposure.

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ABSTRACT

The aim of this study was to determine for the first time in Poland, levels of exposure to synthetic pyrethroids in preschool and school age children and their parents living in urban and rural areas. For this purpose concentrations of pyrethroid metabolites: 3-phenoxybenzoic acid (3-PBA), 2,2-dibromovinyl-2,2-dimethylcyclopropanecarboxylic acid (Br_2CA), *cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (*cis*-Cl_2CA) and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (*trans*-Cl_2CA) were determined in 374 urine samples using a validated GC–MS method.

All measured metabolites were detected more frequently and in higher concentrations in rural areas. 3-PBA was detected in 77.4% and 93.8% of samples from urban and rural areas, respectively. Its geometric mean (GM) concentration in rural population was higher than in urban (0.364 vs. 0.223 ng mL^{-1} and 0.272 vs. 0.155 μg g^{-1} creatinine, $p < 0.0001$). Among remaining metabolites, only *cis*- and *trans*-Cl_2CA were detected in more than 50.0% of samples in rural population. Average concentrations of 3-PBA in children were higher than in adults, both in urban and rural areas. Moderate to very strong positive correlations were noticed between concentrations of pairs of pyrethroid metabolites in urine samples.

Significant, moderate correlations between the concentrations of particular metabolites in parents and their children were observed in the urban population (range: $r = 0.2911–0.3919$ for non-adjusted and 0.3827–0.4693 for creatinine adjusted concentrations), while in rural areas there were no such relationship.

Application of pesticide formulations on pets in the past 6 months was associated with increased 3-PBA urinary concentration.

Further studies on pesticide exposure among children in rural areas are needed to identify and possibly reduce or eliminate the sources of exposure.

1. Introduction

One of the most valuable tools in assessing the extent of exposure to various chemicals is biological monitoring, which allows the estimation of the absorbed dose by measuring biomarker concentration in biological material (Angerer et al., 2007). The concentration of biomarker in biological material integrates all possible routes of exposure. In general, in the case of rapidly metabolized chemicals, urine is the material of choice, which is readily available in large quantities and in which the concentrations of metabolites are usually higher than in the blood. Another advantage of the urine is relatively uncomplicated sampling even from children of preschool age.

Synthetic pyrethroids are currently among the most commonly used insecticides in the agriculture, households and public health. There is a quantitative increase in consumption in relation to the organophosphorus insecticides which are to be replaced by less toxic alternatives. Pyrethroids are characterized by a relatively low acute toxicity to humans: they are quickly metabolized and

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excreted as metabolites in the urine (Eadsforth and Baldwin, 1983; Eadsforth et al., 1988; Leng et al., 1997). Concentration of metabolites in urine is used as a quantitative indicator of the exposure to synthetic pyrethroids and their degradation products. Urinary metabolites were used for several years in the assessment of exposure to synthetic pyrethroids in occupationally exposed persons, as well as in the general population, for which the main source of pyrethroids is the diet and the use of these insecticides in dwellings (Lu et al., 2006; Morgan et al., 2007; Kimata et al., 2009; Barr et al. 2010; Naeher et al., 2010; Egeghy et al., 2011; Schulz et al., 2011; Wielgomas et al., 2013).

Observational studies, although very expensive, provide extremely valuable information that allow to identify highly exposed populations, identify sources of exposure and their elimination or the use of appropriate preventive measures (Angerer et al., 2007; Aylward et al., 2012). In some countries nation-wide cross-sectional studies on exposure to chemicals are performed at regular time intervals (USA – The National Health and Nutrition Examination Survey (NHANES), Germany – The German Environmental Survey (GerES)), but the number of these countries is still limited (Barr et al., 2010; Schulz et al., 2011). It is however worth to note that important results are also obtained from smaller and unique populations of Australia (Babina et al., 2012), China (Wu et al., 2013), Italy (Fortes et al., 2013), Japan (Ueyama et al., 2009), Poland (Wielgomas et al., 2013) and UK (Bevan et al., 2013).

One of the weak points of biomonitoring is still the inability to predict the health consequences based on biomarker concentrations, due to missing or incomplete data on dose response relationships (Koureas et al., 2012).

In some cases, the presence of preformed metabolites in diet and surrounding environment leads to the overestimation of exposure – this is well documented for labile compounds such as organophosphorus insecticides (Zhang et al., 2008) and also for synthetic pyrethroid: fenpropathrin (Chen et al., 2012).

A pilot observational study was conducted recently in our laboratory to study the exposure to synthetic pyrethroids in urban population with a low contribution of children in the studied cohort (Wielgomas et al., 2013). The purpose of the current study was to assess for the first time in Poland exposure to pyrethroids in family members (parents and their children under 18) in rural and urban locations in northern Poland.

2. Experimental

2.1. Study design

All the procedures used in this study were approved by the Ethics Committee of the Medical University of Gdańsk, Poland.

In the period of May–June 2012 we visited pre-schools and primary schools in four different locations in northern Poland to meet the parents of attending children. During those meetings the aims of the study were presented to the parents and additional information was distributed at the same time in the form of brochures together with an informed consent form. In this study we included those families who returned their signed consent form within 7 d, following the first visit. Parents were allowed to include in the study all their children despite of age (up to 18 years).

Then the parents who returned signed consent forms were given detailed instructions on urine sampling and how to fill the survey forms. Urine was collected only on Mondays, so that the concentrations of metabolites in urine reflect exposure to pyrethroids only over the weekend. For convenience, the first morning voids were collected by investigators and transported to the laboratory and were stored at −20 °C until analysis. The samples and questionnaires were blindly coded at the time of reception.

The questionnaire contained a general household information (type of residential building, storage and use of pesticide products indoor, keeping pets, application of pesticides on pets in the last 6 months, presence of woolen products) and detailed information about each of the family members involved in the study.

A total of 374 urine samples were collected form 184 children and 190 adults.

In this project, participants were recruited in: Gdańsk and Łęgowo in the Pomeranian province and Lubawa and Tuszewo in the region of Warmia and Mazury. Gdańsk is the eighth largest city in Poland, with a population of over 450,000 inhabitants. Łęgowo is a suburban village located about 15 km from the center of Gdańsk with a population of about 3000 inhabitants. Lubawa is a town located about 160 km south-east of Gdańsk with a population of about 10,000 inhabitants. Tuszewo is a small village 4 km away from Lubawa with a population of about 650 inhabitants, where residential buildings are located in the direct vicinity of the fields and orchards of high-intensity production.

All participants of the study were given insight into their own results with reference to the results of the entire study population.

2.2. Chemical analysis

2.2.1. Materials and reagents

The following chemicals: 1,1,1,3,3,3-hexafluoroisopropanol (HFIP), diisopropylocarbodiimide (DIIC), 3-phenoxybenzoic acid (3-PBA) and 2-phenoxybenzoic acid (2-PBA) which was used as an internal standard (IS) were obtained from Sigma–Aldrich (Germany). Remaining metabolites: 2,2-dibromovinyl-2,2-dimethylcyclopropanecarboxylic acid (Br_2CA), *cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (*cis*-Cl_2CA) and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (*trans*-Cl_2CA) were obtained from Dr. Ehrenstorfer Laboratories, Germany. All other reagents were of analytical grade. Standard stock solutions of 1 mg mL^{-1} were prepared in acetonitrile and were stored at −20 °C, protected from light. Working standard solutions were prepared in acetonitrile and were stored at +4 °C. Stock and working standard solutions were replaced every 6 and 3 months respectively.

2.2.2. Chemical analysis

The concentrations of *cis*-, *trans*-Cl_2CA, Br_2CA and 3-PBA were simultaneously measured by gas chromatography–mass spectrometry (GC–MS) using electron-impact ionization operated in selected ion storage detection mode (SIS). The extraction step was adopted from the previously established method (Schettgen et al., 2002b) described below. Urinary creatinine was measured with spectrophotometric Jaffe method.

2.2.3. Pyrethroid metabolites in urine – sample preparation

Briefly, 3 mL of thawed urine were transferred into a 10 mL screw-top glass tube and 25 μL of IS solution (2-PBA, 1 μg mL^{-1} of acetonitrile) along with 0.6 mL concentrated hydrochloric acid were added. Hydrolysis was performed at 95 °C in the oven for 90 min. Four mL of hexane were added to the cooled samples and the tubes were shaken for 15 min. When separated by centrifugation, the hexane layer was collected to the next screw-top glass tube, and extraction was repeated. Hexane 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 again shaken, 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,