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Efficiency control of dietary pesticide intake reduction by human biomonitoring

Thomas Göen[a,*], Lukas Schmidt[a], Walter Lichtensteiger[b], Margret Schlumpf[b]

[a] Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany
[b] GREEN Tox, Zurich, Switzerland

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ABSTRACT

In spite of food safety controls for pesticide residues, a conventional diet still leads to a noticeable exposure of the general population to several pesticides. In a pilot study the response of exposure reduction by organic diet intervention on the urinary levels of pesticide metabolites was investigated. In the study two adult individuals were kept on a conventional diet for 11 days and morning urine voids were collected at the last four days of the period. Afterwards, the participants switched to exclusively organic food intake for 18 days and likewise morning urine samples were collected at the last four days of this period. In the urine samples six pyrethroid metabolites, six dialkylphosphates, four phenolic parameter for organophosphate pesticides and carbamates, 6-chloronicotinic acid (ClNA) as parameter for neonicotinoid insecticides, seven phenoxy herbicides, glyphosate and its metabolite AMPA were quantified using gas chromatographic mass spectrometric methods. Generally, the comparative analyses revealed greater shares as well as higher levels of the parameters in the samples taken during the common diet period compared to the organic diet period. Considerable decrease of the levels was found for almost all pyrethroid metabolites, dialkyphosphates and phenoxy herbicids, as well as for the phenolic metabolites 4-nitrophenol and 3,5,6-trichloropyridinol. In contrast, higher values were found for the organic diet period for ClNA and the metabolite of coumaphos in one of the volunteers. The present study confirms the results of former studies which indicated that an organic diet intervention results in considerable lower exposure to organophosphate pesticides and pyrethroids. It also verifies the former experience that monitoring of urinary parameters for non-persistent pesticides permits a reliable efficiency control of short-time effects by dietary interventions. Additionally to former studies, the results of the present study highlight the need of an extension of the parameter spectrum to all prominent pesticide groups.

1. Introduction

Since many decades pesticides are applied in agriculture to protect crops from fungal attacks, insects, and competing plants. In spite of food safety controls for pesticide residues, a conventional diet still leads to a noticeable exposure of the general population to several pesticides (Hamilton et al., 2004). The exposure situation may differ between the populations in different countries of the world, in principle but exists for both developed and developing countries. Particularly, residues in fruits and vegetables contribute to the daily pesticide intake via food (Hamilton et al., 2004; Zhang et al., 2008). Consequently, higher pesticide exposure was found in vegetarian communities (Berman et al., 2016). In contrast, lower pesticide exposure can be assumed in individuals who consume organic food due to refuse of synthetic pesticide application in organic agriculture (Johansson et al., 2014).

This hypothesis was supported by several studies on the relationship between dietary pesticide intake and the urinary concentrations of pesticides and their metabolites, respectively, in different populations (Kimata et al., 2009; McKelvey et al., 2013; Wielgomas, 2013; Morgan and Jones, 2013) as well as by studies on the effect of organic diet intervention (Macintosh et al., 2001; Lu et al., 2006; Oates et al., 2014; Bradman et al., 2015; Magnér et al., 2015). However, most of these studies focused on the exposure to organophosphate pesticides and pyrethroids only. Moreover, the determination of dialkylphosphates has been established for the human biomonitoring of organophosphate pesticides (Barr and

* Corresponding author at: Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine, Friedrich-Alexander-University Erlangen-Nuremberg, Schillerstrasse 25, D-91054, Erlangen, Germany.
E-mail address: Thomas.Goeen@fau.de (T. Göen).

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Needham, 2002; Berman et al., 2013), but does only depict an exposure to this pesticide unspecifically. Nevertheless, the human biomonitoring (HBM) offers much more parameters than the determination of dialkylphosphates and pyrethoid metabolites in urine (Barr and Needham, 2002; Göen, 2016).

In our pilot study the response of exposure reduction by dietary shift on the urinary levels of pesticide metabolites was investigated using a wide spectrum of HBM parameters for organophosphates, pyrethroids, carbamates, neonicotinoids, phenoxy herbicides and glyphosate.

2. Material and methods

2.1. Study design and parameters

The study was conducted in 2015 in Switzerland. Two adult individuals (1 male, 49 years old/1 female, 46 years old) living in Zurich (Switzerland) were kept on a conventional diet for 11 days and morning urine voids were collected at the last four days of the period. Afterwards, the participants switched to exclusively organic food intake for 18 days and likewise morning urine samples were collected at the last four days of this period. For the organic diet period the participants were demanded to buy and consume only food products and dishes, which were assigned as organic food or prepared based on the use of organic food. For the conventional diet period no special requirements for food supply was demanded. The dietary consumption was documented in dietary diaries for the whole study by the volunteers. Directly after sampling the urine samples were stored in a refrigerator at 4–8 °C not longer than 4 days. Then, urine samples were frozen at −20 °C and kept frozen until analysis. In the urine samples six pyrethroide metabolites, six dialkylphosphates as metabolites of organophosphate pesticides, four phenolic parameters for organophosphate pesticides and carbamates, 6-chloronicotinic acid as non-specific parameter for several neonicotinoid insecticides, seven phenoxy herbicides, glyphosate and its metabolite AMPA were quantified using gas chromatographic mass spectrometric methods (Table 1). Moreover, the creatinine content of the samples was determined to verify the supply of appropriately concentrated specimen. Both participants were informed about the aims of the study and gave their consent to their participation.

2.2. Analytical procedures

All analytical procedures were performed at the laboratory of the institute in Erlangen. The determination of TCPy, IPP, PNP and CHMC was carried out using a gas-chromatographic-tandem mass spectrometric (GC–MS/MS) method, which was described in detail elsewhere (Schmidt et al., 2013). In brief, 1 mL of the urine was used for the procedure. First, the conjugates of the phenolic structure to glucuronic acid and sulfate were cleaved enzymatically. Afterwards, the phenolic compounds were extracted from the urinary matrix by solid phase extraction and underwent a derivatization with *N-tert*-butyldimethylsilyl-*N*-methyltrifluoro-acetamide (MTBSTFA). Isotope-labeled equivalent structures were used as internal standards for each of the analytes. Calibration was carried out using standard solutions in pooled human urine. The limit of detection (LOD) and limit of quantification (LOQ) were determined by means of a seven equidistant point calibration near the proposed LOD, according to guideline DIN 32645 (Schmidt et al., 2013). The limit of detection (LOD) was 0.1 μg/L and the limit of quantification (LOQ) was 0.4 μg/L for TCPy, IPP, PNP and CHMC.

Urinary concentrations of the 6 dialkylphosphates were determined using a GC–MS/MS procedure (Barr et al., 2010; Berman et al., 2013). Isotope-labeled equivalent structures of the analytes were added to the urine, which was then freeze-dried. The lyophilized urine was extracted with diethyl ether and acetonitrile. Then the analytes were derivatized with pentafluorobenzyl bromide. After addition of water, liquid–liquid extraction was carried out twice with hexane to separate the derivatives from matrix components and excess of derivatization agent. Thereafter, GC–MS/MS analysis took place. Calibration was performed with standard solutions prepared in pooled urine. LOD and LOQ were estimated using a signal-to-noise ratio of 3:1 and 9:1, respectively. LOD ranged from 0.01 μg/L for DEDTP and 0.05 for DMDTP to 0.1 μg/L for DEP, DETP, DMP, and DMTP while the limit of quantification (LOQ) ranged from 0.03 μg/L for DEDTP and 0.15 for DMDTP to 0.3 μg/L for DEP, DETP, DMP, and DMTP.

The determination of pyrethroid metabolites were performed by a procedure described elsewhere (Schettgen et al., 2002). In brief, five milliliters of urine were pipetted into a 20-ml glass vial with teflon-lined screw top. Then, 25 μL of the working solution of the labelled internal standards ($^{13}C_6$-3-PBA and d_6-*trans*-Cl_2CA, 1 mg/L) was spiked. Hydrolysis of the conjugated carboxylic acids was performed by adding 1 mL of concentrated hydrochloric acid (37%) and heating for 1 h at 90 °C in a water-bath. After cooling to room temperature, the samples were further processed. The acidic urine samples were then extracted two times with 5 mL of *n*-hexane by short vortexing and subsequent mechanically shaking for 10 min. After centrifugation for 5 min at 1500*g*, the organic layers were taken up and combined in a 20-ml glass vial with teflon-lined screw top. For further cleanup, 2 mL of aqueous 0.1 N NaOH was added to the organic phase and the carboxylic metabolites were re-extracted into the aqueous phase by mechanically shaking for 10 min. After centrifugation for 5 min at 1500*g*, the organic phase was discarded. The remaining aqueous phase was again acidified by adding 100 μL of concentrated hydrochloric acid (37%) and once again extracted with 1.8 mL *n*-hexane. Following centrifugation at 1500*g* for 5 min, the upper layer was transferred to a micro-vial. Fifty microliters of toluene is added as a keeper, and the extract was evaporated under a gentle stream of nitrogen to a volume of approximately 50 μL (without heating). Then, 10 μL of *N-tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamid (MTBSTFA) were pipetted into the glass vial, and the solution was transferred to a microvial and sealed tightly. For derivatisation, the vial was heated at 80 °C for 60 min in an oven. One microliter volume of this sample was then analyzed by GC–MS/MS in electron ionization mode. LOD and LOQ were estimated using a signal-to-noise ratio of 3:1 and 9:1, respectively. LOD was 0.03 μg/L and LOQ was 0.1 μg/L for all six parameters.

The determination of chlorinated phenoxycarboxylic acids and 6-chloronicotinic acid were performed by a GC–MS procedure. In brief, two milliliters of urine were pipetted into a 8-ml glass vial with teflon-lined screw top. Then, 25 μL of the working solution of the labelled internal standards ($^{13}C_4$-2,4-D, $^{13}C_4$-2,4,5-T and $^{13}C_4$-MCPA, 1 mg/L, or $^{13}C_6$-ClNA, 1 mg/L) were added. Hydrolysis of the conjugated carboxylic acids was performed by adding 0.5 mL of concentrated hydrochloric acid (37%) and heating for 2 h at 80 °C in a water-bath. After cooling to room temperature, the samples were further processed. The acidic urine samples were then extracted with 4 mL of tert.-butylmethylether by mechanically shaking for 10 min. After centrifugation for 5 min at 2200*g*, the organic layers were taken up and combined in a 8-ml glass vial. The extract was evaporated under a gentle stream of nitrogen to dryness (without heating). Then, 250 μgL acetonitrile, 30 μL of hexafluoroisopropanol and 15 μL diisopropylcarbondiimide were pipetted into the glass vial, and the solution was shaken for 10 min. Thereafter, subsequently 1 mL 1 M $NaHCO_3$ solution and 500 μL iso-octane was added and the extraction was performed by 10 min shaking. After centrifugation (2200*g*) the organic layer was transferred in a 2 mL vial and the solution was reduced to 100 μL under a