

Urinary Metabolites of Organophosphate and Pyrethroid Pesticides in Children from the PHIME Cohort (Trieste): A European Pediatric Environmental Exposure Study

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Abstract

Urinary metabolites of organophosphate (OP) and pyrethroid (PYR) pesticides from seven years old children of a birth cohort study (n=199; PHIME cohort of Trieste, Italy) have been measured. Six OP and two PYR metabolites have been investigated, 2-Diethylamino-6-methylpyrimidin-4-ol (DEAMPY, pirimiphos metabolite) was the one found at higher concentrations, median 3.4 ng/mL specific gravity adjusted (SG adjusted), followed by 4-nitrophenol (PNP, median 1.4 ng/mL SG adjusted) and 3,5,6-trichloro-2-pyridinol (TCPY, median 0.36 ng/mL SG adjusted), parathion and chlorpyrifos metabolites, respectively. TCPY concentrations were low in comparison to other distributions of OP metabolites in children from other studies. Accordingly, the PHIME cohort showed a distinct OP metabolite distribution with high concentrations of pirimiphos and parathion. Another specific characteristic of this cohort was the high concentration of 3-phenoxybenzoic acid (3-BPA, median 0.36 ng/mL SG adjusted), a general metabolite of PYR pesticides.

Evaluation of anthropometric and socio-demographic characteristics of children and families only showed a positive association between family educational level and urinary concentrations of DEAMPY metabolite ($p < 0.05$), which could reflect distinct dietary habits depending on the educational level. Estimated daily intakes were evaluated, all studied metabolites were found within safe levels.

Keywords: Organophosphate pesticides, Pyrethroids, Human Biomonitoring, Children, Estimated Daily Intakes.

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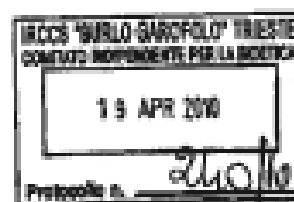
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1. Introduction

Organophosphate (OP) and pyrethroid (PYR) pesticides are commonly used in agriculture and for domestic and gardening use. They have been designed to eliminate insects but they can also affect non-targeted species, including humans, causing them adverse health effects (Barr, 2008). The PYR insecticides were developed as a synthetic version of the naturally occurring pesticide pyrethrin. They are currently replacing OP and carbamate insecticides in domestic and agricultural use as they are safer for the mammalian species (Narahashi, 2007).

Nevertheless, public concern on pesticide use is increasing, not only for the negative impacts on wildlife and environment but also for the potential health effects on humans. OP and PYR pesticide exposure has been related to respiratory, digestive, reproductive and neurological problems, among others (Ye et al., 2017; Arcury et al., 2016; Llop et al., 2017).

Lately, these chemicals have been found in different matrices, including dietary products, water, outdoor and indoor air and house dust (Banerjee et al., 2012; Sousa et al., 2018; Coscollà et al., 2017; Gibbs et al., 2017; Mercier et al., 2011; Tang et al., 2018). In addition, recent studies have found them even in freshwater and edible fish (Arisekar et al., 2019, Barbieri et al., 2019, Pico et al., 2019), being in some cases related with pollution accidents, like the death fish episode by pyrethroid exposure in Northern Italy (Bille et al., 2017).

Exposure to OPs and PYR pesticides is thought to occur via ingestion, direct skin contact in domestic uses and inhalation by proximity to spraying areas. Diet has been identified as the primary source of exposure in the general population (Becker et al., 2006; McKone et al., 2007). Several studies have recently investigated the influence of various foods and food groups (Fortes et al., 2013; Lewis et al., 2015; Jardim et al., 2018).

When OPs and PYR enter human body, they are metabolized and then excreted in the urine, either in free form or bound to glucuronic acid or sulfates (Barr, 2008). A two steps metabolic pathway transforms OPs into dialkyl phosphate (DAP) and hydroxylated organic moieties that are specific of each pesticide (Chambers and Russell, 1995). The metabolic reactions of pyrethroids proceed in two steps, involving the generation of an acid and an alcohol moiety per compound which are excreted (Mikata et al., 2011).

Children are especially susceptible to environmental toxicants such as OP and PYR pesticides due to their developmental immaturity. Their organs are more vulnerable (particularly the brain and nervous system), they have lower capacity to absorb and eliminate chemicals and they are exposed at higher levels, increasing their risk, compared with adults (National Research Council, 1993, Landrigan et al., 2004, Katsikantami et al., 2019, Landrigan et al., 2019). The discovery of OPs and PYR pesticides in amniotic fluid and meconium (Berton et

al., 2014, Bradman et al., 2003) indicated that fetuses are already exposed to these chemicals. After birth, babies and toddlers can be also exposed via breastfeeding, repeated hand-to-mouth ingestion, diet and ambient or airborne exposure (Zartarian et al., 2000; Weldon et al., 2011; Yusa et al., 2017). Early life exposure may be particularly detrimental given the rapid and formative brain development occurring during these periods (Eskenazi et al., 2014). In addition, children younger than seven years of age do not have adult levels of enzymes to detoxify OP pesticides (Huen et al., 2009).

There is an increasing evidence of the relationship between OP pesticide and PYR exposure and health effects. Adult exposure has been associated with various adverse health outcomes, including cancer (Engel et al., 2017), impacts on the reproductive and endocrine systems (Kamijima et al., 2004; Ram, 2017; Lerro et al., 2018) or diabetes (Starling et al., 2014; Park, et al. 2018). Prenatal and postnatal exposure has also been linked with children health effects. The former has been associated with neurodevelopmental problems (González-Alzaga et al., 2014), low birth weight (Rauch et al., 2012), increased child blood pressure (Harari et al., 2010), shorter time of gestation (Eskenazi, 2004), respiratory outcomes (Reardon et al., 2009, Raanan et al., 2016), obesity and diabetes (Bost-Legrand et al., 2016; Slotkin 2011). The latter was found to affect negatively neurodevelopmental outcomes, such as working memory, attention or motor speed (Ruckart et al., 2004, Rohlman et al., 2005, Oulhote et al., 2013, Cartier et al., 2016). It is worth noting that adverse OP effects, which include cognitive impairment and attention deficit, occur at levels well below those causing inhibition of blood cell AChE. These levels are still considered as toxicological end point references in risk assessment to establish adverse daily intakes (ADIs) in humans (for an updated review see Hertz-Picciotto et al., 2018).

These results evidence the need for a more detailed assessment of the effects to these compounds during all human age periods but particularly in the early life stages. This information may also be useful for designing adequate remediation actions towards the minimization of the exposure to these pesticides, particularly in the first years of age.

Unfortunately, the information available on the occurrence of these pesticides in children is scarce. The present study is aimed to contribute to fill this gap by analysis of urine from 7 years old children in Trieste (Italy). Pesticides are one of the most frequently detected classes of pollutants in Mediterranean countries such as Italy (Meffe and de Bustamante, 2014) due to their widespread use, particularly in areas of extensive agriculture. According to the Statistical Office of the European Union, Italy is one of the countries with the highest use of pesticides in Europe (Eurostat, 2016).

2. Methods

2.1. Population and study design

The present work is focused on 7 year-old children, who belong to the Italian prospective mother-child cohort, Northern Adriatic Cohort II (NAC II), of the “Public health impact of long-term, low-level mixed element exposure in susceptible population strata” (PHIME) project.

The aim of PHIME was to assess the association between mercury exposure from food consumption during pregnancy and development of the children nervous system at 18 months. A detailed description of the study protocol has been published previously (Valent et al., 2013). In brief, 900 pregnant women permanent residents in the study area for at least 2 years, having 18 years of age or more, and not having been absent from the study area for more than 6 weeks during pregnancy were recruited between 2007 and 2009 at the Institute for Maternal and Child Health IRCCS Burlo Garofolo, Trieste, Italy (Figure 1).

The follow-up of the Italian children born within the NAC II cohort of PHIME proceeded until children’s 7 years, with the purpose of assessing the effect of low level mercury exposure through foods and in particular through fish consumption on the developing nervous system at this age. Participant subjects were the 767 children born within the NAC II cohort of PHIME between April 2007 and April 2009 and reaching the 7 years old between 2014 and 2016. Children whose parents gave consent to be involved in the NAC II cohort of PHIME and in its continuation were included in the study. The 7 years old follow-up took place at the Institute for Maternal and Child Health IRCCS Burlo Garofolo in Trieste, Italy.

The present work involved a subset of 199 children followed up between August 2014 and December 2015, within the “Crome Life-Cross Mediterranean Environment and Health Network” project.

The research protocol was approved by the ethics Committee of the University of Udine and of the Institute for Maternal and Child Health IRCCS Burlo Garofolo in Trieste (Italy).

2.2 Sample collection and questionnaires

Children urine at 7 years were collected in Kartell™ bottles by mothers at home and then brought in to research personnel. Successively, urine samples were transferred, by research personnel, into Falcon polypropylene tubes and stored in freezer (initially at -20°C and then at -80°C).

Socio-demographic information was obtained through questionnaires administered to parents during the pregnancy period (Valent et al., 2013) and when children were 7 years old. The variables used in this study were: children's gender and BMI and parents' educational level and if they smoke or not at home, at child age of 7 years.

Family dietary habits were collected after delivery by administration of a questionnaire to the mothers which concerned consumption of 138 food items and was adapted from a previously validated food frequency questionnaire (Franceschi et al., 1993; Franceschi et al., 1995; Decarli et al., 1996). Supplementary questionnaires were administered when children were eighteen months and seven years old, respectively. These questionnaires assessed changes in anthropometric measures and developmental milestones of the child, breastfeeding history, child fish intake, diseases and daycare attendance. Regarding fish intake, servings per week were transformed into amounts considering one serving as 150 g and half serving as 80 g. Four different kinds of fish were taken into account: fresh fish, shellfish, clams and canned fish.

2.3 Sample preparation and instrumental analysis

Sample preparation and analysis follows the method described in Garí et al. (2018). Briefly, centrifuged and filtered urine samples (1 mL) were introduced into 10 mL centrifuge tubes for hydrolysis with β -glucuronidase. A mixture of the available isotopically labelled internal standards (25 μ L) was also added. The hydrolysed samples were cleaned up by solid-phase extraction (SPE). The SPE cartridges (Oasis HLB 3 cm³, 60 mg) were preconditioned with a mixture of MeOH:Acetone (25:75 v/v) followed by H₂O containing 1% acetic acid. The OPs and PYR metabolites were eluted with 1.5 mL of MeOH:Acetone (25:75 v/v). The collected extracts were reduced under N₂ to near dryness and transferred to chromatographic vials with 120 μ L of MeOH:H₂O (25:75 v/v).

Identification and quantification of six specific OP metabolites and the 2 PYR biomarkers showed in Table 1, was carried out by isotope dilution solid phase extraction UPLC-MS/MS using an Ultra-Performance Liquid Chromatography (UPLC Acquity H-Class, Waters, Milford, MA, USA) coupled to a Triple Quadrupole Mass Spectrometer (XEVO-TQ-S, Waters, Milford, MA, USA) equipped with an electrospray ionization (ESI) interface. The chromatographic separation was performed on a Betasil C18 column (100 mm x 2.1 mm, 3 μ m particle size). The injection volume was 10 μ L, at a flow rate of 0.3 mL/min. The column temperature was kept at 30 °C during the analysis. A gradient elution with a mobile phase of acetonitrile and a mixture of H₂O with 1% acetic acid and 5% methanol was used. Target compounds were positively identified by their

retention times and the ratio of the two MRM transitions, which had to fall within $\pm 20\%$ of the average ratio obtained from standard solutions.

Synthetic urine (Surine, Preserve Free, Sigma-Aldrich) was used for blanks, quality control (QC) materials and calibration curves. Accuracies were assessed at two levels (QCLow, 1 $\mu\text{l/L}$ and QCHigh 10 $\mu\text{l/L}$). Calibration curves were prepared by adding 25 μl of standard solutions at concentrations between 2.5 and 800 ppb into 1 mL of synthetic urine. The quantification was performed using the isotopically-labelled internal standards (Garí et al., 2018). This methodology was additionally checked out by participation in rounds of the German External Quality Assessment Scheme since 2016 (G-EQUAS), which includes the OP metabolites PNP and TCPY and the 2 PYR pesticide metabolites 3-PBA and 4-F-3-PBA, providing results within the range of 20% of the consensus values. To our knowledge there is no proficiency testing program for the other analytes or a program including all the analytes from the present study.

Specific gravity (SG) was measured in all urine samples. This parameter ranged between 1.001 and 1.020 g/mL (mean = 1.0077 g/mL) and was used to normalize the measured individual metabolite concentrations to the general dilution pattern of the whole cohort. The SG corrected concentrations were obtained by applying the formula: $[\text{OPs/PYR}]_{\text{SG}}^i = [\text{OPs/PYR}]^i * (\bar{x}_{\text{SG}} - 1) / (\text{SG}^i - 1)$ where \bar{x}_{SG} is the average specific gravity of the cohort, $[\text{OPs/PYR}]^i$ is the measured concentration of the i individual, and SG^i is the specific gravity of the urine of i (Boeniger, M. F. et al. 1993).

2.4 Calculation of OP and PYR daily intakes

Biomonitoring studies present concentrations of biomarkers in biological matrices; however, pesticide regulation committees (European Commission) indicate the maximum acceptable doses as acceptable daily intakes (ADI) and reference dose (RfD) expressed as $\mu\text{g/kgbw/day}$. Thus, OPs and PYR daily intakes were estimated for the analysed pesticides based on the molar levels of the urinary metabolites, using the following toxicokinetic model by Katsikantami et al., 2019: $EDI \left(\frac{\mu\text{g}}{\text{kg bw } d} \right) = \frac{C_U \left(\frac{\mu\text{mol}}{\text{L}} \right) * V_U (\text{L}) * MW_P \left(\frac{\text{g}}{\text{mol}} \right)}{F_{UE} * BW (\text{kg})}$, where EDI is Estimated Daily Intake, C_U the molar concentration of metabolite, V_U the total urinary volume excreted within 24 h, MW_P the molecular weight of the parent compound, F_{UE} the urinary excretion factor of the parent compound and BW the body weight.

Data used for the calculation are shown in Table 2, 24-h urine volumes were estimated as 0.82 L (Cequier et al., 2017). Molecular weight for pirethroids with 3-PBA as main metabolite was estimated as the mean of the most common pirethroids (permetrin, deltametrin, fenvalerate and cypermethrin). Excretion factor data were available for all metabolites except

DEAMPY and only applicable to adults, hence two approximations were done, one using the found F_{UE} and the other assuming a minimum of 5 and a maximum of 100% of excretion.

2.5 Data analysis

Data analysis and graphics were performed using the statistical software R (R Development Core Team, 2018). The *plyr* and *ggplot* R packages were used. Statistics were focused on the metabolites found above the limit of detection (LOD) in more than 20% of the samples: DEAMPY, PNP, TCPY, 3-PBA and 4-F-3-PBA. One-half of the LOD was assigned to non-detected values.

Geometric mean (GM) and its 95% confidence interval (CI), median and interquartile ranges were used for descriptive analysis. Mann Whitney U test was used to evaluate concentration differences between the socio-demographic groups.

Multivariate models were performed to evaluate the relations of sociodemographic factors and child's fish consumption with pesticide concentration, compound concentrations were transformed into the natural logarithms because of its skewed distribution.

3. Results

3.1. Socio-demographic characteristics

Table 3 summarizes the descriptive characteristics of the 199 families included in this study. Thirty-eight percent of children were boys and 62% girls, their average weight and height were 25.7 kg and 123.9 cm, respectively. Concerning BMI, most of the children had normal weight (69%), while 19 and 12% were overweight or obese, none of the children were classified as underweight (WHO, 2007).

The highest educational level of one of the parents was chosen for family classification. Due to the uneven distribution of the five categories (Table 3), the participant families were grouped into two categories, with and without university studies ($n = 92$, 46% and $n = 106$, 54%, respectively). Concerning home smoking, the families were included in the "smoking group" if one of the parents was smoker (63%). Non-smoking families were 37%.

3.2. Urinary concentrations of OPs and PYR metabolites

Descriptive statistics for the measured urinary concentrations of the analysed metabolites are summarized in Table 4. Detection frequencies (DFs; >LOD) of each compound ranged from below LOD to 97% of detection, being PNP (DF=97%) and DEAMPY (DF=96%) the compounds found most frequently above the LOD, followed by TCPY (detected in 80%), and the PYR pesticide metabolites, 3-PBA and 4F-3-PBA, with DF of 81% and 24%, respectively.

The sum of the three most abundant OPs metabolites, DEAMPY, PNP, TCPY, ranged between 0.018 and 73 ng/mL SG adjusted with a median of 8.0 ng/mL SG adjusted. The median of the sum of PYR metabolites, 3-PBA and 4-F-3-PBA, was more than ten times lower compared with the sum of OPs, 0.71 ng/mL SG adjusted, ranging between below the limit of detection and 69 ng/mL SG adjusted.

The most abundant OP pesticide was DEAMPY, the metabolite of pirimiphos (median 4.5 ng/mL SG adjusted), followed by PNP and TCPY, the metabolites of parathion and chlorpyrifos, respectively, with medians of 1.5 and 0.41 ng/mL SG adjusted. Regarding pyrethroids, 3-PBA metabolite was the one found at highest concentration with a SG adjusted median of 0.57 ng/mL against 0.015 ng/mL of 4-F-3-PBA. The higher concentration of 3-PBA is expected since it reflects contributions from several pyrethroids whereas 4-F-3-PBA is specific of cyfluthrin.

Median values, interquartile range and results obtained after using the Mann-Whitney U-test for two independent samples are shown in Table 5. There are no significant differences with respect to children gender and BMI or if parents smoke at home or not (Figure S1. Supplemental material). However, children with at least one parent with higher educational level (university degree) had higher concentrations of DEAMPY metabolite (Table 5).

Multivariate linear regression analysis adjusted by children's BMI, gender and family education was performed to explore the influence of fish consumption on the OP and PYR concentrations (Table S1. Supplemental material). Fish intake was categorized between children who had 1 or more servings per week and those having less than 1. Fish consumption was not significant for the urinary OP and PYR concentrations.

3.3 Estimated daily intakes (EDI)

Median EDIs with interquartile range (Q_1 - Q_3) for children are presented in Figure 2 in three different cases of urinary excretion factor (F_{UE}), ADIs are marked in red for each compound. The hypothetical worse-case for F_{UE} has been placed at 5% of excretion, the only metabolite crossing this threshold is PNP, Parathion metabolite. Using the values for F_{UE} found in previous studies (Morgan et al., 1977, Wollen et al., 1992, Leng et al., 1997, Bouchard et al., 2003, Payne-

Sturges et al., 2009, Sams and Jones, 2012 and Ratelle et al., 2015), as mentioned before they are usually done with a very small population and just for adults, all the EDIs are below the ADIs, within safe levels, for all the studied pesticides.

4. Discussion

The present study combines the measurement of OPs (six metabolites) and PYR (two metabolites) to obtain a more comprehensive description of children exposure to pesticides.

4.1. Organophosphate pesticides

DEAMPY (metabolite of pirimiphos) is the most abundant OP in the present cohort, 3.0 ng/mL (Table 4), and a distinct feature when compared with the OP concentrations found in children of other studies (Table 6). The median of this metabolite was below LOD in Seattle and in Valencia (Lu et al., 2008; Roca et al., 2014) and below 1 ng/mL in North Carolina and Thailand (Arcury et al., 2007; Panuwet et al., 2009). Northern Italy has high levels of cereals production (Eurostat, 2016) and pirimiphos is an insecticide and acaricide used for the protection of stored grain but in our study it is not possible to define the source of exposure. However, a previous study analysing the occurrence and distribution of pesticides in the province Bologna (Northern Italy) found pirimiphos between the most detected compounds (Ghini et al., 2004).

TCPY (metabolite of chlorpyrifos), the dominant OP in the other cohorts, was also present in Trieste but at a median concentration, 0.36 ng/mL, much lower than the other studies, 2.5-3.7 ng/mL (Table 6). The Ministry of Health Report on Plant Protection Products in Food (Ministerio della Salute, 2018), based on data collected in Italy between 2015 and 2016, indicates among the pesticide residuals more frequently found in the Friuli Venezia-Giulia Region, a significant presence of pirimiphos in cereals, as well as of chlorpyrifos and several pyrethroids in fruits.

Concerning other OP metabolites, PNP in the Trieste cohort, 1.2 ng/mL, was in the range of the concentrations observed in other studies, 0.93-2.9 ng/mL (Roca et al., 2014; Panuwet et al., 2009; Arcury et al., 2007). Despite the European banning of parathion since 2004, this is one of the OP found in higher concentrations in children from the compared cohorts. Given the reactivity of this compound and the low persistence in environmental samples, its rather high concentration must reflect recent inputs in the studied area. This compound has been found in North Italy as one of the most abundant pesticide (Ghini et al., 2004).

In the case of IMPY (diazinon) and CMHC (coumaphos), the samples examined in the present cohort have median values below the limit of detection which are similar with those observed in previous studies with children (Arcury et al., 2007; Lu et al., 2008; Panuwet et al., 2009). These results are consistent with the ban of these pesticides by the European Union. However, in other studies, e.g. the Valencian cohort (Roca et al., 2004), the IMPY metabolite was dominant, 5.2 ng/mL.

MDA (malathion) is also present at low median concentrations, < LOD, which is a distinct feature comparing from studies in USA or Thailand (Barr et al., 2005; Arcury et al., 2007; Lu et al., 2008, Panuwet et al., 2009). The low contents in the Trieste cohort are in contrast with the occurrence of this OP in some Italian rivers (Mountuori et al., 2015).

A statistically significant difference between parents' educational level and children DEAMPY metabolite concentrations has been observed in the present cohort (Table 5), involving higher levels in the group of higher education. Previous studies on general population of women have found similar significant differences with OP pesticide exposure. Thus, women with lower/middle studies were those showing lower concentrations of TCPY in New York (Berkowitz et al., 2003). Similar patterns were observed in women from Cincinnati (Yolton et al., 2013) and Canada (Sokoloff et al., 2016), where women with higher education presented higher urinary concentrations of OP metabolites, dialkylphosphates in these cases. This positive association with education could reflect distinct dietary habits related with cultural differences and higher consumption of products treated with OPs.

4.2 Pyrethroids

Comparing with previous studies, children from Trieste show the highest concentrations of the 3-BPA metabolite, 0.56 ng/mL (Becker et al., 2006; Arcury et al., 2007; Panuwet et al., 2009; Roca et al., 2014). In contrast, the median concentration of 4-F-3-BPA in the studied cohort, <LOD, is similar to those found in previous studies, <LOD-0.14 ng/mL. Some pyrethroids have authorized use in Italy for beekeeping activities (Perugini et al., 2018). They have been also found in wastewater (Rousis et al., 2016).

Although PYR have been found in fish (Corcellas et al., 2015; Muir et al., 1994) no statistically significant associations have been found between PYR or OP concentrations and weekly fish consumption. These results are in agreement with previous studies that did not observe any association with PYR exposure and fish consumption in Rome (Fortes et al., 2013), and in disagreement with a study in a Norwegian cohort (Cequier et al. 2017) that found a positive association with one non-specific OP metabolite. In any case, the EDI calculations from

the present cohort shows that children's exposure to OPs pesticides and PYR were within safe levels.

5. Conclusions

DEAMPY was the metabolite found at higher concentration in the urine of Italian children belonging to the NAC-II -PHIME cohort (median 4.5 ng/mL SG adjusted). This metabolite of pirimiphos was followed by PNP and TCPY, the metabolites of parathion and chlorpyrifos, respectively. In contrast, the concentrations of TCPY were low when compared to other distributions of OP metabolites in children. These compositional differences make the Trieste cohort very distinct in terms of OP pesticide composition from other cohorts of children described in previous studies, showing high influence of pirimiphos. Another specific feature of this cohort is the high abundance of 3-BPA, a general marker of PYR pesticides.

A positive association between higher paternal educational levels and higher child urinary concentrations of OPs and PYR metabolites was observed. This difference could reflect distinct dietary habits, with regard to fish consumption, depending on paternal and maternal education. Dietary assessment through a three-day diet diary is ongoing in this cohort, and this will eventually allow one to establish significant association between OP and PYR metabolites and intake of specific food items, e.g. fruits, vegetables, dairy products, further to fish. The concentrations of these OP and PYR pesticides are not related to fish consumption, and estimated daily intakes of all studied metabolites were found within safe levels. However, further investigation is needed to determine the urinary excretion factor in children to being able to evaluate EDIs.

Declaration of interests

The paper reflects only the authors' views, and the European Union is not liable for any use that may be made of the information. The authors declare they have no competing interests.

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Table 1. Metabolite's acronyms and principal uses of the pesticides analysed.

Acronym	Analyte	Pesticide	Principal uses	Status¹ (legislation)
DEAMPY	2-diethylamino-6-methylpyrimidin-4-ol	Pirimiphos		Approved ²
IMPY	2-isopropyl-6-methyl-4-pyrimidol	Diazinon	All crops, specially fruits and citrus plantations and agricultural facilities	Not approved (2007/393)
MDA	Malathion dicarboxylic acid	Malathion		Approved
PNP	4-nitrophenol	Parathion		Not approved (01/520/EC)
TCPY	3,5,6-trichloro-2-pyridinol	Chlorpyrifos		Approved
CMHC	3-chloro-4-methyl-7-hydroxycoumarin	Coumaphos	Farm and domestic animals to control mite	Not approved ³
3-PBA	3-phenoxybenzoic acid	Common pyrethroids	Parks and gardens, forestry plantations, agricultural	Approved ⁴
4-F-3-PBA	4-fluoro-3-phenoxybenzoic acid	Cyfluthrin	crops, pets and lice	Not approved (460/2014)

¹European Comission; ²Pirimiphos-ethyl is not approved (2002/2076); ³Never notified and authorised in EU; ⁴Approved pyrethroids: Cypermethrin, Deltamethrin, Esfenvalerate or Etofenprox.

Table 2. Data used for the calculation of children's EDI

	MW_M (g/mol)	MW_P (g/mol)	F_{UE}	Study
DEAMPY	181.23	305.33	-	
IMPY	152.19	304.35	0.6	<i>Payne-Sturges, 2009</i>
PNP	139.11	291.26	0.36	<i>Morgan, 1977</i>
TCPY	198.43	350.57	0.7	<i>Payne-Sturges, 2009</i>
3-PBA	214.22	462.92	0.24	<i>Various authors^a</i>
4F-3-PBA	232.21	434.30	0.4	<i>Leng, 1997</i>

MW_m, Metabolite molecular weight; MW_p, Parent compound molecular weight; F_{UE}, Urinary excretion factor; ^aWollen et al., 1992, Sams and Jones, 2012, Ratelle et al., 2015

Table 3. Sociodemographics of study participants

	Children
All participants	199 (100)
Gender (n=198)	
Male	76 (38)
Female	122 (62)
Weight (n=178)	25.7±4.7
Boys (n=66)	25.6±3.7
Girls (n=112)	25.8±5.2
Height (n=178)	123.9±5.1
Boys (n=66)	124.0±4.2
Girls (n=112)	124.0±5.6
BMI (n=178)	
Normal	122 (69)
Overweight	34 (19)
Obese	22 (12)
Family education (n=198)	
Elementary	1 (1)
Middle	16 (8)
High school	89 (45)
University	92 (46)
Smoking at home (n=194)	
Yes	71 (37)
No	123 (63)

Table 4. Specific gravity (SG) adjusted and non-adjusted urinary levels (ng/mL) of OPs and PYR metabolites. Full names of the metabolites in Table 1.

Metabolite	LOD ¹ (ng/mL)	LOQ ² (ng/mL)	DF ³ (%)	SG adjusted results (n=198)			Non-adjusted results (n=199)		
				GM (CI) ³	Median	Range	GM (CI) ³	Median	Range
DEAMPY	0.017	0.025	96	3.4 (2.6-4.5)	4.5	<LOD -71	2.7 (2.1-3.5)	3.0	<LOD -110
IMPY	0.014	0.021	6	<LOD	<LOD	<LOD -0.50	<LOD	<LOD	<LOD -0.98
MDA	0.069	0.10	0	<LOD	<LOD	-	<LOD	<LOD	-
PNP	0.017	0.025	97	1.4 (1.1-1.7)	1.5	<LOD -37	1.1 (0.92-1.3)	1.2	<LOD -14
TCPY	0.020	0.039	80	0.29 (0.22-0.38)	0.41	<LOD -30	0.23 (0.18-0.30)	0.36	<LOD -6.0
CMHC	0.026	0.030	1	<LOD	<LOD	<LOD -0.96	<LOD	<LOD	<LOD -0.25
3-PBA	0.018	0.027	81	0.36 (0.28-0.48)	0.57	<LOD -69	0.29 (0.22-0.38)	0.56	<LOD -36
4F-3-PBA	0.019	0.028	24	0.027 (0.022-0.034)	0.015	<LOD -3.0	0.022 (0.018-0.027)	<LOD	<LOD -1.3
ΣOP ⁴	-	-	91	7.4 (6.1-9.1)	8.0	0.018-73	5.9 (5.0-7.0)	6.6	0.026-120
ΣPYR ⁵	-	-	53	0.55 (0.44-0.69)	0.71	<LOD -69	0.43 (0.35-0.54)	0.59	0.019-36

¹LOD: Limit of detection; ²LOQ: Limit of quantification; ³DF: Detection frequencies (>LD); GM (CI): Geometric mean and 95% confidential interval;

⁴ΣOP = [DEAMPY] + [PNP] + [TCPY]; ⁵ΣPYR = [3-PBA] + [4-F-3-PBA]

Table 5. Median urinary concentration differences (ng/mL SG adjusted) of principal metabolites detected by characteristics of study population.

Characteristics	n (%)	DEAMPY		IMPY		PNP		TCPY		3-PBA		4F-3-PBA	
		P50	IQR ¹	P50	IQR	P50	IQR	P50	IQR	P50	IQR	P50	IQR
Gender													
Boys	76 (38)	4.7	0.92-20	0.0089	0.0045-0.012	1.5	0.85-3.7	0.30	0.069-0.87	0.62	0.20-1.4	0.015	0.0073-0.077
Girls	122 (62)	4.5	1.3-10	0.011	0.0054-0.013	1.4	0.57-3.4	0.47	0.075-1.6	0.47	0.070-1.4	0.015	0.0085-0.077
BMI													
Normal	122 (69)	4.6	1.3-13	0.011	0.0054-0.013	1.4	0.56-3.8	0.4	0.044-1.2	0.65	0.069-1.6	0.015	0.0077-0.077
Overweight/Obese	54 (31)	3.2	0.82-11	0.0077	0.0041-0.011	1.7	0.75-3.2	0.47	0.16-1.1	0.48	0.16-0.95	0.015	0.0059-0.077
Family education													
Below university	106 (54)	2.8*	0.96-8.5	0.0089	0.0045-0.013	1.3	0.52-3.2	0.43	0.059-1.4	0.49	0.069-1.0	0.015	0.0077-0.13
University degree	91 (46)	6.1*	1.5-16	0.0089	0.0054-0.013	1.8	0.75-3.7	0.39	0.083-1.3	0.7	0.16-1.8	0.015	0.0077-0.038
Smoking at home													
Yes	71 (37)	3.4	0.82-11	0.0089	0.0045-0.013	1.6	0.59-4.2	0.37	0.054-1.1	0.41	0.17-1.2	0.015	0.0064-0.038
No	123 (63)	5.7	1.2-13	0.0089	0.0054-0.013	1.4	0.59-3.4	0.45	0.082-1.4	0.65	0.077-1.6	0.015	0.0096-0.12

¹IQR: Interquartile range (P25-P75); * *p-value* for Mann-Whitney U test <0.05

Table 6. Comparison of the median urine concentrations in children from Trieste with those in children from other cohort studies (ng/mL). Full names of the metabolites in Table 1

	N	Year	DEAMPY	IMPY	MDA	PNP	TCPY	CMHC	3-PBA	4-F-3-PBA	Study
Italy (Trieste)	199	2014-2016	3.0 [2.7] ²	<LOD ¹ <LOD	<LOD <LOD	1.2 [1.1]	0.36 [0.23]	<LOD <LOD	0.56 [0.29]	<LOD [0.022]	<i>Present study</i>
US (NHANES)	481	1999-2000			0.49		2.7 [2.88]				<i>Barr, D. B. et al., 2005</i>
US (North Carolina)	60	2004	0.14	0.49 [0.56]	0.21	1.6 [1.0]	2.5 [1.9]	0.14	0.07		<i>Arcury, T. A. et al., 2007</i>
US (Seattle)	23	2003-2004	<LOD	<LOD	1.6		3.7	<LOD			<i>Lu, C. et al., 2008</i>
Thailand	207	-	0.14 [0.16]	<LOD	0.21 [0.27]	2.9 [2.7]	2.6 [2.4]		0.07 [0.20]	0.14 [0.15]	<i>Panuwet, P. et al., 2009</i>
Spain (Valencia)³	125	2010	<LOD [0.47]	5.2 [3.3]		0.93 [0.96]	3.4 [3.36]		<LOD	<LOD	<i>Roca, M. et al., 2014</i>
Germany	396	2001-2002							0.29 [0.31]	<LOD	<i>Becker, K. et al., 2006</i>

¹<LOD data below limit of detection; ²Geometric mean into []; ³Data in ng/g creatinine

FIGURE CAPTIONS

Figure 1. Sampling location. Trieste, Italy.

Figure 2. Median estimated daily intakes with interquartile range (Q_3-Q_1) for children. In red, acceptable daily intakes (ADI).