Environment International 131 (2019) 104997

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

# Biomonitoring of common organophosphate metabolites in hair and urine of children from an agricultural community

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ARTICLE INFO

Handling Editor: Adrian Covaci

*Keywords:*
Pesticides
DAPs
Hair
Urine
Children
Biomonitoring

ABSTRACT

Levels of dialkylphosphate (DAP) metabolites were measured in hair and urine of children that lived close to intensively farmed areas of Almeria (Southeast Spain). The levels were used as proxies for exposure of these children to organophosphate pesticides (OPs). Determinants of exposure to DAPs were also examined. Urine and hair samples were collected from 222 children aged 3–11 years and information on lifestyle and dietary habits was collected from questionnaires administered to mothers. Urinary DAPs were analyzed by ultra-high performance liquid-chromatography coupled to triple-quadrupole tandem mass-spectrometry (UHPLC-QqQ-MS/MS) and hair DAPs by gas-chromatography coupled to mass spectrometry (GC–MS). Detection rates ranged from 21.8% for diethylphosphate (DEP) and diethylthiophosphate (DETP) to 35.9% for dimethylphosphate (DMP) in urine; and from 42.3% for DETP to 92.8% for DMP in hair. Diethyldithiophosphate (DEDTP) was detected in 0.5% of urine samples (one child), and in 26.6% of children's hair samples. A lack of correlation was observed for individual DAP metabolites and ΣDAPs between urine and hair samples, except for DEDTP. Urinary DAP levels of our child population were lower than those reported for children from other countries, including NHANES 1999–2000 data. The main determinants of hair DAP levels were age, sex, vegetable intake, parental exposure to pesticides at work, time spent playing indoors, monthly income and father's education level. Conversely, none of the predictors studied was significantly associated with urinary DAPs except age. Overall, hair has advantages over urine as it is easier to collect, handle and store, and allows for assessment of cumulative exposure to OPs, thus providing a greater insight for human biomonitoring.

## 1. Introduction

Organophosphate (OP) pesticides are commonly used insecticides for crop protection in agriculture, for pest control in public health programs and for domestic use inside homes. According to the nationwide survey of the Spanish pesticide market, based on European Regulation 1185/2009, the amount of OP insecticides used in Spain from 2011 to 2015 ranged from 2162 to 2572 tons. This accounts for about 33% of the total amount of insecticides, acaricides and nematocides sold during the same period, and approximately 3.3% of the total pesticides (on a tons weight basis) sold as plant protection products.[1] In the study area (Almería province, SE Spain), the amount of insecticides, acaricides and nematocides used in the year 2010 accounted for 62.6% of the total pesticide consumption. This figure contrasts with the total

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[1] Ministerio de Agricultura, pesca y alimentación. Estadística anual de consumo de productos fitosanitarios en la agricultura. https://www.mapa.gob.es/es/estadistica/temas/estadisticas-agrarias/agricultura/estadisticas-medios-produccion/fitosanitarios.aspx.

https://doi.org/10.1016/j.envint.2019.104997
Received 5 March 2019; Received in revised form 6 July 2019; Accepted 6 July 2019
Available online 27 July 2019

insecticides, acaricides and nematocides used in Andalusia (South Spain) during the same year (36.9%). Unfortunately disaggregated data by pesticide class are not available (Andalusian Statistics Institute).[2] This higher use reflects the type of crops grown in the large area of intensive agriculture in Almería. The type of agricultural crops and the production estimates in the study area for 2010 (the year of the study) are characterised as follows. They consisted chiefly of: tomato (858,621 tons produced/9939 ha of cultivated land), peppers (437,403 tons/7475 ha), cucumber (378,317 tons/4610 ha), watermelon (331,809 tons/5516 ha), zucchini (274,158 tons/5210 ha), lettuce (158,502 tons/6854 ha), melons (141,964 tons/4039 ha), and aubergines (139,676 tons/1824 ha).[3]

Once ingested, OPs do not accumulate appreciably in humans and over 75% is rapidly metabolized to at least one of six possible dialkylphosphates (DAPs) and then excreted in the urine within 6 to 24 h after exposure (Barr and Angerer, 2006). DAP metabolites are, therefore, group-specific and suitable for biological monitoring of exposure to OPs as a class, with longer biological half-lives than the parent insecticides. DAPs are not toxicologically active because they do not inhibit acetylcholinesterase (AChE) and are not considered toxic (CDC, 2016), but have been widely used as biomarkers of OPs exposure in epidemiological studies (Millenson et al., 2017).

Although OPs were the most frequently applied pesticides in agricultural use over the last three decades of the twentieth century, only 11 are currently approved for use in agriculture in the European Union (EU): chlorpyrifos, chlorpyrifos-methyl, dimethoate, ethoprophos, fenamiphos, fosthiazate, malathion, phosmet, pirimiphos-methyl and the fungicides fosetyl and tolclofos-methyl. All these OPs are metabolized to DAPs except ethoprophos, fenamiphos, fosthiazate and fosetyl.

A wide range of the population, including children, is exposed to OPs from different sources and by different routes. Children living in agricultural areas may be exposed to pesticides through inhalation of pesticide drift and/or volatilization from applications made in nearby crop fields, by parental take-home exposures and by residential use (Curl et al., 2002; Fenske et al., 2002). Occupational exposure of parents, especially if they are agricultural workers, has been reported to predict higher exposure of their children (Bouvier et al., 2005). Regarding non-occupational exposures, diet probably represents the major source of pesticide exposure in children because residues of OPs are detectable in many treated food commodities, particularly fruits and vegetables (Bradman et al., 2011; EFSA, 2018).

According to biomonitoring studies, children are widely exposed to pesticides, including OPs, pyrethroid, and organochlorine insecticides. Hence, identification of determinants of pesticide exposure, including sources and pathways of exposure, may offer opportunities to reduce children's exposure to these chemicals. Importantly, exposure factors vary over time as a function of changes in the diet, behaviour, and family practices occurring as children mature (Bradman et al., 2011).

A large number of epidemiological studies have reported urinary DAP concentrations as biomarkers for OP pesticide exposure; however, only a few studies have addressed DAPs levels in hair, which is an unconventional matrix that holds promise for assessing cumulative exposure to non-persistent pesticides (Kavvalakis and Tsatsakis, 2012; Kavvalakis et al., 2014). While the measurement of OPs or their urinary metabolites typically reflects recent exposure because of their short biological half-lives, the determination of DAPs in other biological matrices, such as hair, represents an indication of cumulative exposure in the past months through different routes of entry (Bouchard et al., 2006).

Hair testing has proven to be a useful toxicological procedure to assess past exposures to chemicals (drugs, metals and several organic pollutants) (Hernández et al., 2019). This is possible because of their long-term storage in this biological matrix, where chemicals remain stable for a long time. As for pesticides, hair analysis has been used to assess exposure to organochlorines, OPs, pyrethroids, neonicotinoids and carbamates (Kavvalakis et al., 2014). The measurement of DAP metabolites in hair segmental analysis of OP-poisoned patients has also been used to assess past acute exposure (Tsatsakis et al., 2012).

This study aims to assess exposure to OP pesticides by measuring their non-specific DAP metabolites in urine and hair samples from children living near to intensive agricultural areas where fruits and vegetables are grown in plastic-covered greenhouses. We also examined the relationship between urinary and hair DAP concentrations and assessed potential determinants of exposure, including sex, age, dietary habits, child's behaviour, parental work status and some socioeconomic features (household income and parental education). We focused on OPs because these are commonly used insecticides in the study area and many studies are available for comparison purposes. However, to the best of our knowledge only one study has reported simultaneous measurements of DAPs levels in hair and urine from 120 adults occupationally exposed to OPs (Kokkinaki et al., 2014) and another study analyzed DAPs in hair and blood from 29 children and 49 parents (Michalakis et al., 2014). Three additional studies assessed DAP levels in adult hair but not in urine (Knipe et al., 2016; Margariti and Tsatsakis, 2009; Tsatsakis et al., 2010), with sample sizes ranging from 6 to 50 individuals. Finally, we examined the correlation between DAP levels in urine and hair to ascertain whether these two matrices can be equivalent for biomonitoring purposes in children with environmental or dietary exposure to OP pesticides.

## 2. Material and methods

### 2.1. Study population

Study participants were recruited from state schools in El Poniente (Almería, South-Eastern Spain), an area of intensive farming activities where horticultural products are grown within over 20,000 ha of plastic greenhouses. Accordingly, large amounts of pesticides, including OPs, are used to improve fresh produce yield (see Introduction for more details). Six schools were randomly selected from a total of 48 public schools. Complete information was obtained from 591 children who met the inclusion criteria and returned an informed consent signed by their parents or guardians. From them, sufficient urine and hair were available for 222 children (68 boys and 154 girls, mean age 7.5 ± 2.3 years), who made up the final study population. Authorizations from the Andalusian Council of Health and Council of Education were obtained to gain access to these schools. The study was approved by the Ethics Committee of Hospital Virgen de las Nieves (Granada, Spain).

### 2.2. Collection of biological samples

Child urine and head hair samples were collected in October 2010. Instructions for sample collection and a clean polypropylene container were given to children and parents to collect an overnight urine sample. This was brought to school on the next day before the classes began and then children were weighed and their height measured for body mass index (BMI) calculation. Urine samples were kept in cool-boxes at 2–3 °C and immediately brought to Laboratorio Analitico Bioclinico (LAB) in Almería where they were aliquoted and stored frozen at −40 °C until analyzed within twelve months. An overnight sample of urine has been considered a proper surrogate for 24 hour urine in a validation study (Scher et al., 2007) and has been confirmed recently by Sinha and Banda (2018). A lock of hair (approximately 100 mg in

[2] Instituto de Estadística y Cartografía de Andalucía. https://www.juntadeandalucia.es/institutodeestadisticaycartografia/anuario/anuario14/cap06/anuario14-6_6_02.xls.

[3] https://www.juntadeandalucia.es/organismos/agriculturaganaderiapescaydesarrollosostenible/consejeria/sobre-consejeria/estadisticas/paginas/agrarias-superficies-producciones.html.

weight) was cut from the back of the children's head, as close to the scalp as possible, in the school on the same day as the urine collection had taken place. Hair samples were stored in paper envelopes in a dry and dark place at room temperature (~20–25 °C) until analysis. Hair samples were cut to obtain the 1 cm segment closest to the scalp, which corresponds to the last month exposure period. Hair samples were shipped to the Laboratory of Toxicology of the University of Crete, School of Medicine, for analysis.

### 2.3. Interviews – collection of information

At the time of sample collection mothers completed two structured questionnaires administered by trained personnel. The first was a general questionnaire to gather information on parental occupation (children's father or mother working in agriculture or otherwise exposed to pesticides, expressed as a binary variable: yes/no), socio-economic factors (household total net income (< 500 €, 500–1000€, 1000–2000€, > 2000€), education level of child's father and mother (none, primary, secondary, University degree)). The time children spent playing outdoors was recorded as total number of hours and then categorized as a binary variable (< 1 h, ≥1 h per day). A validated semi-quantitative food frequency questionnaire (Vioque et al., 2013) was also administered to gain information on children's consumption of fresh food products, specially fruits and vegetables. The number of servings per week of these food commodities was then categorized into tertiles.

### 2.4. Reagents

Dimethylphosphate (DMP, 98%, CAS # 813-78-5) and diethylphosphate (DEP, 98.9%, CAS # 311-45-5) were obtained from Acros Organics (Geel, Belgium, USA) and Chem Service (West Chester, New York, USA), respectively. *O,O*-diethylthiophosphate potassium salt (DETP, 98%, CAS # 5871-17-0) and diethyldithiophosphate salt (DEDTP, 95%, CAS # 298-06-6) were from Sigma–Aldrich (3050 Spruce Street, St. Louis, USA) while the dibutylphosphate (used as internal standard) (DBP, 97%, CAS # 107-66-4) was purchased from Fluka (Steinheim, Germany). HPLC-grade methanol was supplied by Sigma (Madrid, Spain), analytical grade dichloromethane and ethyl acetate were obtained from Panreac (Barcelona, Spain) and analytical grade formic acid was purchased from Fluka (Seelze, Germany).

### 2.5. Urine analysis

Four non-specific OPs pesticide metabolites (dialkylphosphates, DAPs) were measured in children's urine samples: DMP, DEP, DETP and DEDTP. Analyses were conducted in Laboratorio Analitico Bioclinico (LAB, Almería), an analytical chemistry laboratory authorized (A.359/l) and accredited (AC.463/III) by the Regional Ministry of Health of Andalusia. DAPs were extracted from urine samples using a procedure previously validated for the simultaneous extraction of polar and non-polar pesticides in urine samples by Cazorla-Reyes et al. (2011). Briefly, 5 mL urine samples were extracted using C-18 Sep-Pak cartridges (500 mg) previously conditioned with 4 mL of dichloromethane followed by 4 mL of ultrapure MilliQ water. Cartridges were dried for 2 h under vacuum and the retained analytes were then eluted with 5 mL of dichloromethane. The extracts were evaporated to dryness with a vacuum rotary evaporator at 40 °C and the residue dissolved in 2 mL of ethyl acetate solution. One mL was taken and evaporated under a gentle nitrogen stream and the concentrated extract was re-dissolved with 1 mL of a mixture 1:1 (v/v) of methanol and an aqueous solution of formic acid (0.01%, v/v) prior to ultra-high performance liquid chromatography (UHPLC) coupled to triple-quadrupole (QqQ) tandem mass spectrometry (MS/MS) analysis. Separations were achieved using an Acquity UPLC™ BEH $C_{18}$ column (100 mm × 2.1 mm, 1.7 µm particle size). The solvent gradient elution consisted of methanol and an aqueous solution of formic acid (0.01%, v/v) (Cazorla-Reyes et al., 2011). To provide overall assessments of precision, accuracy, and reliability of the method, quality control (QC) samples were analyzed along with collected samples. QC samples were prepared as blank samples and inserted blindly among the study samples. The limit of detection (LOD) was 1 µg/L for DMP and DEP and 0.5 µg/L for DETP and DEDTP. The intra- and inter-day variability measures yielded a result below 20% for all DAP metabolites studied.

Creatinine concentration in children's urine was determined using Jaffe's Method in a Hitachi 917 automatic chemistry analyzer in Hospital El Poniente (Almería).

### 2.6. Hair analysis

The same DAPs metabolites were analyzed as in urine. The analytical procedure used for the extraction and analysis of DAPs in hair samples has been previously published and validated (Tsatsakis et al., 2010; Kokkinaki et al., 2014). Briefly, hair samples were decontaminated by washing twice with water and once methanol, then 50 mg of dried hair were transferred to a test tube and 2 mL of methanol was added before incubating in an ultrasonic bath for 4 h at room temperature. Then, liquid–solid extraction was performed with another 2 mL of methanol for 30 min with mechanical shaking. The mixture was centrifuged at 4000 rpm for 5 min and the supernatant was transferred through an econofilter (0.2 µm pore size) to a test-tube containing 15 mg of $K_2CO_3$ and 50 mg $Na_2S_2O_5$. The solvent was evaporated to dryness under a gentle stream of nitrogen at room temperature. Fifteen mg of $K_2CO_3$ were added to the residue, reconstituted in 1 mL of acetonitrile and a 0.1 mL solution of pentafluorobenzylbromide (PFBBr) in acetonitrile (1,3, v/v) as derivatization agent and incubated at 80 °C in a water bath for 30 min with intermittent swirling. The solution was then brought to room temperature and evaporated to dryness under nitrogen at room temperature. Finally, 100 µL of toluene were added and the mixture was centrifuged at 4000 rpm for 5 min in order to separate $K_2CO_3$. Two microliters of the solution were injected into the GC–MS system and analyzed according the conditions described below.

Electron ionization mass spectrometric analysis was performed on a GC–MS system (QP-2010, Shimadzu) equipped with a BPX5 (30 m × 0.25 mm × 0.25 µm) capillary column and pure helium as carrier gas at a flow of 1 mL/min. The column temperature was initially held at 60 °C for 1 min, raised to 180 °C (20 °C/min), stable for 1 min, raised to 250 °C (4 °C/min) and was finally raised to 300 °C (at 25 °C/min), where it was held for 2 min. The injector, interface and ion source temperatures were set as 270 °C, 300 °C and 230 °C, respectively. Quantitative analysis was achieved in selected ion monitoring (SIM) mode with a scan time of 0.2 s, using two qualifier ions for the confirmation of each compound and one target ion for quantitation (in bold): *m/z* 110, **306** for DMP; **258**, 334 for DEP; **350**, 274 for DETP; **366**, 185 for DEDTP and **335** for DBP (dibutyl phosphate, used as internal standard). The LOD was 6 pg/g for DMP, 5 pg/g for DEP and 3 pg/g for DETP and DEDTP. Inter- and intra-day variability measures yielded a result of < 16% for all DAP metabolites studied. This protocol has been developed and validated in a previous study (Tsatsakis et al., 2010).

### 2.7. Statistical analysis

We calculated detection frequencies (percentage of samples above the LOD) along with descriptive statistics and percentiles for each DAP metabolite in both urine and hair samples. Concentrations of DMP, DEP, DETP and DEDTP were expressed as geometric mean, median and 25, 75 and 95 percentiles for urine and hair samples because of skewed distributions. Although many urine samples had concentrations below detection limits for some metabolites, none of the children studied had all DAPs below their LOD. Samples with concentrations below the respective detection limit were assigned an imputed value of one-half the

LOD for statistical analyses. As dimethyl metabolites are only generated by dimethyl OP pesticides and diethyl metabolites only by diethyl OP pesticides, we calculated the molar sum of diethyl- and dimethyl- metabolite concentrations to create a combined DAP measure (ΣDAPs) as described elsewhere (Arcury et al., 2006). This sum, named ΣDAPs, was expressed as nanomoles per gram of urine creatinine or picomoles per mg hair and represents the total DAP metabolite concentrations and therefore an overall indication of OP exposure. Statistical analysis of the samples was then carried out.

The non-parametric Mann–Whitney *U* test and Kruskal-Wallis tests were used to assess bivariate associations between DAP metabolite levels and potential determinants of exposure selected a priori, including sex, age (stratified into two categories according to the children's scholar status: 3–5 years was the range for preschool-age children and 6–11 years for school-age children), fruit and vegetables intake (as a continuous variable, number of servings/week), occupation of household members, household total net income and parental education level. The influence of these variables was evaluated for single DAPs and for ΣDAPs metabolites expressed as nanomoles per gram urinary creatinine or picomoles per mg hair. Bivariate correlations between DAP metabolite levels in urine and hair were assessed using Spearman's rank-order correlation test.

The SPSS Statistic package (version 21.0) was used for statistical analysis of data and a level of significance was set to 0.05.

## 3. Results

Basic demographic data of the 222 children that participated in this study are presented in Table 1. Sixty eight children were male (30.6%) and 154 females (69.4%), with a mean age of 7.48 ± 2.30 and mean BMI 18.75 ± 3.74 $kg/m^2$. No significant differences were observed between boys and girls when compared by age, BMI, total fruit or vegetable intake, and time playing outdoors. Also, no difference was observed when comparing exposure of father and mother to pesticides in occupational or residential settings (Table 1). When boys and girls were stratified by age-range (3–5 and 6–11 years), no statistically significant differences were observed when compared by BMI, total fruit or vegetable intake, time playing outdoors or parents working in agriculture or otherwise exposed to pesticides (data not shown).

Table 2 shows the descriptive statistics (geometric mean, 95% confidence interval, percentiles 25, 50, 75 and 95, and maximum value) for each individual DAP metabolite and ΣDAPs concentrations in urine and hair samples. Spearman's rho correlation coefficient between DAP levels in these two biological samples is given. Percentages of samples below the LOD are also shown. Each of the DAP metabolites was detected in < 50% of the urine samples. In contrast, a higher percentage of positive detections were found in hair samples, ranging from 92.8% for DMP to 26.6% for DEDTP. While 45.9% of children had detectable concentrations of at least one DAP metabolite in urine, 98.6% of children showed detectable levels of at least one DAP in hair. The diethyl metabolites were dominated by DEP (21.8% detection frequency in urine and 87.4% in hair). DMP levels were higher than any diethyl metabolite, and even higher than the sum of all diethyl metabolites in either urine or hair samples.

In relation to the determinants of exposure studied, none except age were significantly associated with urinary DAP metabolites, as children at age 3–5 years had slightly higher DEDTP values than children aged 6–11 years. Likewise, the correlation analysis showed an inverse relationship between age and urinary DEP (rho = −0.152, $p < 0.05$) and DEDTP (rho = −0.341, $p < 0.01$). Conversely, a few significant associations were found for hair. In particular, age was inversely correlated with DETP (rho = −0.181, $p < 0.01$) and DEDTP (rho = −0.261, $p < 0.01$). Younger children (3–5 years) had significantly higher DEP, DETP and DEDTP levels than children in the 6–11 year old age group. Girls had significantly higher DAP levels than boys with the exception of DMP, where hair concentration was significantly lower (Tables 3–5). Regarding BMI, no significant association was found with DAP levels in either urine or hair with the exception of urinary DEDTP (rho = −0.205, $p < 0.01$).

Greater hair DMP levels were found in children playing outdoors for < 1 h (Table 3): hair DEP concentration was greater in the top tertile of overall vegetable intake and with lower father's education (Table 3): hair DETP levels were higher when either parent was working in agriculture or otherwise exposed to pesticides, and with

**Table 1**
Demographic characteristics of the total population of children stratified by sex.

| | Total Mean ± SD or N | Boys Mean ± SD or N (%) | Girls Mean ± SD or N (%) | p |
|---|---|---|---|---|
| Age (years) | 7.48 ± 2.30 | 7.54 ± 2.23 | 7.45 ± 2.33 | 0.789[a] |
| Sex | 222 (100%) | 68 (30.6%) | 154 (69.4%) | – |
| BMI ($kg/m^2$) | 18.75 ± 3.74 | 18.46 ± 3.32 | 18.88 ± 3.91 | 0.437[a] |
| Total intake of fruits | 24.80 ± 14.35 | 25.12 ± 14.98 | 24.67 ± 14.12 | 0.885[b] |
| Total intake of vegetables | 20.59 ± 13.36 | 20.40 ± 14.79 | 20.68 ± 12.70 | 0.552[b] |
| Time playing outdoors (≥1 h) | 85 | 29 (34.1%) | 56 (65.9%) | 0.379[c] |
| Father working in agriculture/or exposed to pesticides | 93 | 28 (30.1%) | 65 (69.9%) | 0.693[c] |
| Mother working in agriculture/or exposed to pesticides | 57 | 14 (24.6%) | 43 (75.4%) | 0.303[c] |
| Household total net incomes | 204 | 63 (30.9%) | 141 (69.1%) | 0.947[c] |
| < 500 € | 12 | 3 (25%) | 9 (75%) | |
| 500–1000 € | 34 | 10 (29.4%) | 24 (70.6%) | |
| 1001–2000 € | 92 | 30 (32–6%) | 62 (67.4%) | |
| > 2000 € | 66 | 20 (30.3%) | 46 (69.7%) | |
| Father's education | | | | 0.462[c] |
| None | 34 | 8 (23.5%) | 26 (76.5%) | |
| Primary school | 92 | 25 (27.2%) | 67 (72.8%) | |
| Secondary school | 47 | 18 (38.3%) | 29 (61.7%) | |
| University | 27 | 8 (29.6%) | 19 (70.4%) | |
| Mother's education | | | | 0.173[c] |
| None | 16 | 4 (25.0%) | 12 (75.0%) | |
| Primary school | 102 | 24 (23.5%) | 78 (76.5%) | |
| Secondary school | 55 | 19 (34.5%) | 36 (66.5%) | |
| University | 42 | 17 (40.5%) | 25 (59.5%) | |

[a] Student *t*-test.
[b] Mann-Whitney test.
[c] Chi square.

**Table 2**
Descriptive statistics of concentration of DAP metabolites in urine (data are adjusted for creatinine) and hair.

| | Urine (μg/g creatinine) | | | | | | | | | Hair (pg/mg) | | | | | | | | | Rho[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | % < LOD | GM | 95% CI | P25 | P50 | P75 | P95 | Max | n | % < LOD | GM | 95% CI | P25 | P50 | P75 | P95 | Max | |
| *DMP* | | | | | | | | | | | | | | | | | | | |
| Both sexes | | | | | | | | | | | | | | | | | | | |
| Total | 222 | 64.1% | 0.31 | (0.23–0.43) | ND | ND | 3.57 | 23.51 | 66.01 | 222 | 7.2% | 149.7 | (123.5–181.5) | 94.3 | 166.6 | 335.6 | 1271.2 | 8634.5 | 0.040 |
| 3–5 years | 52 | 71.2% | 0.28 | (0.15–0.52) | ND | ND | 3.80 | 20.50 | 60.21 | 52 | 13.5% | 101.7 | (61.2–168.9) | 24.3 | 126.2 | 338.2 | 1271.2 | 5937.5 | −0.005 |
| 6–11 years | 170 | 61.9% | 0.32 | (0.22–0.47) | ND | ND | 3.56 | 25.12 | 66.01 | 170 | 5.3% | 168.5 | (138.3–205.3) | 113.0 | 173.5 | 335.6 | 1325.8 | 8634.5 | 0.053 |
| Boys | | | | | | | | | | | | | | | | | | | |
| Total | 68 | 64.7% | 0.27 | (0.16–0.47) | ND | ND | 2.12 | 17.93 | 60.21 | 68 | 2.9% | 203.8 | (151.8–273.5) | 126.2 | 217.1 | 361.3 | 892.6 | 8634.5 | 0.217* |
| 3–5 years | 14 | 78.6% | 0.21 | (0.05–0.84) | ND | ND | ND | 60.21 | 60.21 | 14 | 7.1% | 204.2 | (84.6–492.8) | 125.9 | 249.9 | 370.6 | 5937.5 | 5937.5 | 0.090 |
| 6–11 years | 54 | 61.1% | 0.29 | (0.16–0.54) | ND | ND | 2.28 | 17.93 | 32.12 | 54 | 1.9% | 203.6 | (149.2–278) | 128.0 | 202.6 | 354.3 | 892.6 | 8634.5 | 0.225 |
| Girls | | | | | | | | | | | | | | | | | | | |
| Total | 154 | 63.8% | 0.33 | (0.22–0.49) | ND | ND | 4.50 | 25.12 | 66.01 | 154 | 9.1% | 130.7 | (102.4–166.8) | 85.6 | 147.4 | 310.7 | 1325.8 | 3820.7 | −0.015 |
| 3–5 years | 38 | 68.4% | 0.31 | (0.15–0.63) | ND | ND | 4.27 | 20.50 | 30.39 | 38 | 15.8% | 78.7 | (42.5–145.6) | 20.7 | 117.4 | 305.8 | 1271.2 | 3188.3 | −0.019 |
| 6–11 years | 116 | 62.3% | 0.34 | (0.21–0.54) | ND | ND | 4.93 | 27.57 | 66.01 | 116 | 6.9% | 154.3 | (119.9–198.6) | 109.8 | 157.3 | 315.6 | 1591.6 | 3820.7 | −0.008 |
| *DEP* | | | | | | | | | | | | | | | | | | | |
| Both sexes | | | | | | | | | | | | | | | | | | | |
| Total | 222 | 78.2% | 0.13 | (0.11–0.17) | ND | ND | ND | 4.75 | 14.70 | 222 | 12.6% | 79.1 | (64.2–97.6) | 36.9 | 85.4 | 231.6 | 852.7 | 3846.0 | 0.104 |
| 3–5 years | 52 | 82.7% | 0.14 | (0.09–0.21) | ND | ND | ND | 5.07 | 11.33 | 52 | 13.5% | 109.1 | (65.4–182) | 35.0 | 137.9 | 403.6 | 1488.4 | 3846.0 | 0.052 |
| 6–11 years | 170 | 76.8% | 0.13 | (0.10–0.17) | ND | ND | ND | 4.48 | 14.70 | 170 | 12.4% | 71.7 | (57.2–89.9) | 36.9 | 76.6 | 190.9 | 644.0 | 2032.9 | 0.099 |
| Boys | | | | | | | | | | | | | | | | | | | |
| Total | 68 | 80.9% | 0.11 | (0.08–0.16) | ND | ND | ND | 3.27 | 11.33 | 68 | 19.1% | 46.0 | (30.9–68.6) | 28.2 | 48.6 | 80.0 | 1612.8 | 3846.0 | 0.149 |
| 3–5 years | 14 | 78.6% | 0.16 | (0.05–0.48) | ND | ND | ND | 11.33 | 11.33 | 14 | 14.3% | 91.1 | (27.7–299.6) | 33.0 | 70.9 | 363.0 | 3846.0 | 3846.0 | 0.174 |
| 6–11 years | 54 | 81.5% | 0.10 | (0.07–0.15) | ND | ND | ND | 2.59 | 3.37 | 54 | 20.4% | 38.5 | (25.6–58) | 28.1 | 46.7 | 68.9 | 409.6 | 2032.9 | 0.106 |
| Girls | | | | | | | | | | | | | | | | | | | |
| Total | 154 | 77.0% | 0.14 | (0.11–0.19) | ND | ND | ND | 5.20 | 14.70 | 154 | 9.7% | 100.5 | (79.2–127.6) | 44.9 | 122.3 | 266.5 | 852.7 | 1972.0 | 0.049 |
| 3–5 years | 38 | 84.2% | 0.13 | (0.08–0.21) | ND | ND | ND | 5.07 | 7.73 | 38 | 13.2% | 116.5 | (65–208.8) | 37.0 | 149.5 | 424.8 | 1462.6 | 1488.4 | 0.007 |
| 6–11 years | 116 | 74.6% | 0.15 | (0.11–0.21) | ND | ND | 0.40 | 5.84 | 14.70 | 116 | 8.6% | 95.7 | (74–123.9) | 48.8 | 116.2 | 233.3 | 696.7 | 1972.0 | 0.056 |
| *DETP* | | | | | | | | | | | | | | | | | | | |
| Both sexes | | | | | | | | | | | | | | | | | | | |
| Total | 222 | 78.2% | 0.11 | (0.09–0.13) | ND | ND | ND | 2.59 | 5.73 | 222 | 57.7% | 12.3 | (9.3–16.4) | ND | ND | 111.1 | 375.8 | 8675.3 | 0.054 |
| 3–5 years | 52 | 90.4% | 0.09 | (0.07–0.12) | ND | ND | ND | 1.12 | 5.35 | 52 | 38.5% | 23.6 | (13.4–41.6) | ND | ND | 148.3 | 270.6 | 395.5 | 0.144 |
| 6–11 years | 170 | 74.4% | 0.12 | (0.09–0.14) | ND | ND | 0.21 | 2.93 | 5.73 | 170 | 63.5% | 10.1 | (7.3–14) | ND | ND | 101.5 | 385.1 | 8675.3 | 0.032 |
| Boys | | | | | | | | | | | | | | | | | | | |
| Total | 68 | 80.9% | 0.10 | (0.08–0.14) | ND | ND | ND | 1.86 | 3.30 | 68 | 73.5% | 7.0 | (4.3–11.4) | ND | ND | 80.6 | 385.1 | 881.2 | 0.134 |
| 3–5 years | 14 | 92.9% | 0.09 | (0.05–0.15) | ND | ND | ND | 1.12 | 1.12 | 14 | 50.0% | 21.0 | (5.3–84.2) | ND | ND | 199.5 | 395.5 | 395.5 | 0.523* |
| 6–11 years | 54 | 77.8% | 0.11 | (0.07–0.16) | ND | ND | ND | 2.25 | 3.30 | 54 | 79.6% | 5.2 | (3.2–8.6) | ND | ND | | 385.1 | 881.2 | 0.017 |
| Girls | | | | | | | | | | | | | | | | | | | |
| Total | 154 | 77.0% | 0.11 | (0.09–0.14) | ND | ND | ND | 3.04 | 5.73 | 154 | 50.6% | 15.9 | (11.3–22.3) | ND | ND | 130.0 | 324.5 | 8675.3 | 0.001 |
| 3–5 years | 38 | 89.5% | 0.10 | (0.07–0.13) | ND | ND | ND | 4.24 | 5.35 | 38 | 34.2% | 24.6 | (13.1–46.3) | ND | 65.2 | 142.4 | 250.3 | 270.6 | −0.116 |
| 6–11 years | 116 | 72.8% | 0.12 | (0.09–0.16) | ND | ND | 0.24 | 3.04 | 5.73 | 116 | 56.0% | 13.7 | (9.1–20.6) | ND | ND | 116.1 | 419.0 | 8675.3 | 0.023 |
| *DEDTP* | | | | | | | | | | | | | | | | | | | |
| Both sexes | | | | | | | | | | | | | | | | | | | |
| Total | 222 | 99.5% | 0.06 | (0.05–0.06) | ND | ND | ND | ND | 1.32 | 222 | 73.4% | 6.4 | (4.9–8.2) | ND | ND | 33.5 | 242.4 | 15,074.7 | 0.142** |
| 3–5 years | 52 | 100.0% | 0.07 | (0.06–0.08) | ND | ND | ND | ND | 0.29 | 52 | 48.1% | 16.7 | (9.1–30.6) | ND | 5.9 | 133.0 | 366.1 | 562.2 | 0.048 |
| 6–11 years | 170 | 99.4% | 0.06 | (0.05–0.06) | ND | ND | ND | ND | 1.32 | 170 | 81.2% | 4.7 | (3.6–6.2) | ND | ND | ND | 165.7 | 15,074.7 | 0.062 |
| Boys | | | | | | | | | | | | | | | | | | | |
| Total | 68 | 100.0% | 0.06 | (0.05–0.07) | ND | ND | ND | ND | 1.32 | 68 | 91.2% | 3.3 | (2.3–4.8) | ND | ND | ND | 195.4 | 15,074.7 | 0.225* |
| 3–5 years | 14 | 100.0% | 0.07 | (0.05–0.11) | ND | ND | ND | ND | 0.29 | 14 | 71.4% | 7.6 | (2.2–26.3) | ND | ND | 34.5 | 449.4 | 449.4 | 0.050 |
| 6–11 years | 54 | 100.0% | 0.06 | (0.05–0.07) | ND | ND | ND | ND | 1.32 | 54 | 96.3% | 2.7 | (1.9–3.8) | ND | ND | ND | ND | 15,074.7 | 0.197 |
| Girls | | | | | | | | | | | | | | | | | | | |
| Total | 154 | 99.3% | 0.06 | (0.05–0.07) | ND | ND | ND | ND | 1.25 | 154 | 65.6% | 8.5 | (6.2–11.7) | ND | ND | 92.6 | 270.1 | 5724.9 | 0.096 |
| 3–5 years | 38 | 100.0% | 0.07 | (0.06–0.08) | ND | ND | ND | ND | 0.19 | 38 | 39.5% | 22.4 | (11.1–45.1) | ND | 44.1 | 140.5 | 366.1 | 562.2 | 0.034 |

(*continued on next page*)

**Table 2** (*continued*)

| | Urine (μg/g creatinine) | | | | | | | | | Hair (pg/mg) | | | | | | | | Rho[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | % < LOD | GM | 95% CI | P25 | P50 | P75 | P95 | Max | n | % < LOD | GM | 95% CI | P25 | P50 | P75 | P95 | Max | |
| 6–11 years | 116 | 99.1% | 0.06 | (0.05–0.06) | ND | ND | ND | ND | 1.25 | 116 | 74.1% | 6.2 | (4.4–8.7) | ND | ND | 28.4 | 194.5 | 5724.9 | 0.015 |
| *ΣDAPs*[b] | | | | | | | | | | | | | | | | | | | |
| Both sexes | | | | | | | | | | | | | | | | | | | |
| Total | 222 | 54.1% | 7.56 | (5.86–9.74) | 1.36 | 3.10 | 42.76 | 227.55 | 548.43 | 222 | 1.4% | 3.14 | (2.70–3.61) | 1.78 | 2.83 | 4.86 | 26.70 | 96.74 | 0.027 |
| 3–5 years | 52 | 67.3% | 5.99 | (3.55–10.08) | 1.42 | 2.24 | 44.68 | 213.38 | 482.88 | 52 | 0.0% | 3.71 | (2.82–4.82) | 1.78 | 3.17 | 6.40 | 34.66 | 66.30 | 0.118 |
| 6–11 years | 170 | 50.0% | 8.12 | (6.06–10.89) | 1.35 | 6.78 | 42.76 | 233.43 | 548.43 | 170 | 1.8% | 3.00 | (2.62–3.51) | 1.78 | 2.71 | 4.50 | 26.67 | 96.74 | −0.002 |
| Boys | | | | | | | | | | | | | | | | | | | |
| Total | 68 | 54.4% | 6.73 | (4.36–10.39) | 1.24 | 3.67 | 31.62 | 195.00 | 482.88 | 68 | 0.0% | 2.88 | (2.32–3.73) | 1.64 | 2.53 | 4.27 | 56.54 | 96.74 | 0.167 |
| 3–5 years | 14 | 64.3% | 6.17 | (1.81–20.86) | 1.13 | 2.24 | 61.66 | 279.55 | 482.88 | 14 | 0.0% | 4.00 | (2.23–9.00) | 1.97 | 2.52 | 5.78 | 60.05 | 66.30 | 0.393 |
| 6–11 years | 54 | 51.9% | 6.89 | (4.43–10.78) | 1.32 | 5.95 | 31.19 | 166.92 | 255.39 | 54 | 0.0% | 2.64 | (1.99–3.50) | 1.57 | 2.60 | 3.57 | 50.69 | 96.74 | 0.080 |
| Girls | | | | | | | | | | | | | | | | | | | |
| Total | 154 | 53.9% | 7.96 | (5.81–10.91) | 1.41 | 3.04 | 54.82 | 233.60 | 548.43 | 154 | 1.9% | 3.26 | (2.80–3.81) | 1.88 | 3.12 | 5.11 | 23.41 | 56.70 | −0.043 |
| 3–5 years | 38 | 68.4% | 5.92 | (3.36–10.36) | 1.56 | 2.22 | 38.59 | 200.06 | 242.26 | 38 | 0.0% | 3.62 | (2.78–4.86) | 1.74 | 3.50 | 6.67 | 23.02 | 36.25 | 0.008 |
| 6–11 years | 116 | 49.1% | 8.78 | (6.13–12.66) | 1.36 | 7.28 | 63.31 | 257.91 | 548.43 | 116 | 2.6% | 3.16 | (2.62–3.81) | 1.93 | 3.03 | 4.81 | 26.66 | 56.70 | −0.053 |

ND: not detectable.

[a] Spearman's rho correlation coefficient; (*) $p < 0.10$; (**) $p < 0.05$.

[b] Sum DAPs are expressed on a molar basis.

greater monthly income (Table 4); and nearly-significant greater DEDTP and ΣDAP concentrations were observed when fathers were agricultural workers or were otherwise exposed to pesticides, and with greater monthly income (Tables 4 and 5, respectively). ΣDAP also showed a significant association with greater monthly income (Table 5).

The correlation between participants' hair and urine DAP metabolites concentration is shown in Supplementary Table 1. No significant correlation was observed except for DEDTP, whose levels were positively correlated between these two biological matrices. On the other hand, all DAP metabolites were associated with each other in urine and the same holds true for hair, with the exception of DMP which failed to show a significant correlation with DEP and DETP (Supplementary Table 1). In the subpopulation of boys, hair and urine DMP and DEDTP showed a nearly-significant and positive correlation (Table 2). Regarding DETP, a nearly-significant correlation was also found, but only for boys 3–5 year-old. However, no significant correlation was observed for ΣDAPs between urine and hair (Table 2).

## 4. Discussion

This study concurrently measured DAP levels in urine and hair from children living in agricultural communities from Southeast Spain along with their main determinants of exposure. To our knowledge, only one prior study examined the concentration of DAP metabolites in hair and urine of adults (Kokkinaki et al., 2014) and another one in hair and blood from children with hypospadias and their parents (Michalakis et al., 2014), although with a limited sample size. Hair DAP levels in our children population showed roughly similar median concentrations to those found in the general Greek population (Tsatsakis et al., 2010) and concentrations in-between two separate rural Greek populations (Margariti and Tsatsakis, 2009; Kokkinaki et al., 2014). The lowest results for DAPs in hair that have been reported are for pregnant women from the ELFE French nationwide birth cohort (Béranger et al., 2018). No comparison with other child populations could be made because there is an apparent lack of studies addressing DAP levels in children's hair. To date only 5 studies have examined DAP levels in human hair (all of them conducted in adult populations). These are shown in Supplementary Table 2 along with the results from the present study. Although the analytical methods used for hair testing were similar (were carried out in the same laboratory except in the case of Béranger et al. (2018), exposure patterns to OP pesticides may not be comparable.

The following order of positive detections was observed in hair samples from our study: DMP (92.8%) > DEP (87.4%) > DETP (42.3%) > DEDTP (26.6%), which is roughly comparable to results from 311 pregnant women from the ELFE French birth cohort (Béranger et al., 2018), although the latter study detected DMP in a lower percentage of samples and in lower concentrations than our study. In contrast, an adult population (n = 50) occupationally exposed to pesticides from Sri Lanka (Knipe et al., 2016) had detectable DETP levels in 90% of hair samples, followed by DEP and DEDTP in 82% and DMP only in 42% of samples. In another study (Tsatsakis et al., 2010), the percentage of positive hair samples in 27 individuals from the general Greek population was 96.3% for DEP, 70.4% for DEDTP, 66.7% for DETP and 63.0% for DMP. While these three studies on adult populations had a lower percentage of positive detections for DMP than for DEP, in our child population DMP was more frequently detected than any diethyl metabolite. Overall, the high percentage of positive detects for all DAP metabolites in hair points to the great potential of hair testing for biomonitoring long-term OP exposure given the short half-lives of these pesticides. This conclusion is supported by experimental studies where rabbits exposed to the OP insecticides chlorpyrifos and diazinon incorporated diethylphosphate metabolites into the hair in a dose- and duration-dependent manner (Maravgakis et al., 2012).

While 98.6% of our child population had detectable levels of any

**Table 3**
Geometric means and 95% confidence intervals for urinary and hair concentrations of DMP and DEP with respect to sex, fresh food consumption, BMI, any parent working in agriculture or otherwise exposed to pesticides, time spent playing outdoors, household total net income and parents' education level.

| | Urine | | | | Hair | | | | Urine | | | | Hair | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DMP (μg/g creatinine) | | | | DMP (μg/g creatinine) | | | | DEP (μg/g creatinine) | | | | DMP (μg/g creatinine) | | | |
| | n | GM | 95% CI | p[a] | n | GM | 95% CI | p[a] | n | GM | 95% CI | p[a] | n | GM | 95% CI | p[a] |
| Age | | | | 0.510 | | | | 0.107 | | | | 0.131 | | | | 0.074 |
| 3–5 years | 52 | 0.28 | (0.07–0.49) | | 52 | 101.70 | (35.07–168.33) | | 52 | 0.14 | (0.04–0.24) | | 52 | 109.01 | (54.52–163.49) | |
| 6–11 years | 168 | 0.32 | (0.21–0.43) | | 170 | 168.52 | (111.55–225.49) | | 168 | 0.13 | (0.08–0.18) | | 170 | 71.71 | (52.40–91.03) | |
| Sex | | | | 0.543 | | | | 0.045 | | | | 0.198 | | | | < 0.001 |
| Boy | 68 | 0.27 | (0.09–0.45) | | 68 | 203.8 | (76.78–330.81) | | 68 | 0.11 | (0.04–0.18) | | 68 | 46.0 | (14.05–77.94) | |
| Girl | 152 | 0.33 | (0.21–0.45) | | 154 | 130.7 | (94.20–167.20) | | 152 | 0.14 | (0.09–0.19) | | 154 | 100.5 | (77.59–123.40) | |
| Body mass index (BMI) | | | | 0.990 | | | | 0.940 | | | | 0.774 | | | | 0.637 |
| Normal weight | 121 | 0.33 | (0.21–0.5) | | 121 | 144.9 | (108.99–192.64) | | 121 | 0.14 | (0.10–0.19) | | 121 | 84.6 | (63.70–112.40) | |
| Overweight | 62 | 0.36 | (0.2–0.67) | | 62 | 172.9 | (125.44–238.33) | | 62 | 0.13 | (0.09–0.2) | | 62 | 61.5 | (41.12–91.92) | |
| Obese | 39 | 0.21 | (0.1–0.45) | | 39 | 131.8 | (85.48–203.21) | | 39 | 0.12 | (0.07–0.2) | | 39 | 95.9 | (57.05–161.09) | |
| Total intake of fruits | | | | 0.177 | | | | 0.865 | | | | 0.878 | | | | 0.901 |
| 1st tertile | 71 | 0.34 | (0.19–0.59) | | 71 | 131.0 | (92.76–185.12) | | 71 | 0.12 | (0.08–0.17) | | 71 | 81.1 | (57.22–114.97) | |
| 2nd tertile | 65 | 0.29 | (0.16–0.52) | | 65 | 205.6 | (144.63–292.4) | | 65 | 0.11 | (0.08–0.17) | | 65 | 66.8 | (42.72–104.32) | |
| 3rd tertile | 67 | 0.26 | (0.14–0.46) | | 67 | 123.5 | (83.65–182.21) | | 67 | 0.16 | (0.10–0.26) | | 67 | 98.9 | (67.79–144.18) | |
| Total intake of vegetables | | | | 0.777 | | | | 0.174 | | | | 0.588 | | | | 0.017 |
| 1st tertile | 72 | 0.26 | (0.15–0.45) | | 72 | 117.9 | (79.22–175.51) | | 72 | 0.11 | (0.08–0.16) | | 72 | 85.1 | (56.11–129) | |
| 2nd tertile | 71 | 0.38 | (0.21–0.66) | | 71 | 156.6 | (115.87–211.64) | | 71 | 0.12 | (0.08–0.18) | | 71 | 70.8 | (51.79–96.68) | |
| 3rd tertile | 66 | 0.31 | (0.17–0.57) | | 66 | 176.7 | (122.84–254.13) | | 66 | 0.17 | (0.11–0.28) | | 66 | 94.8 | (63.59–141.46) | |
| Mother working in agriculture (or exposed to pesticides) | | | | 0.368 | | | | 0.906 | | | | 0.283 | | | | 0.283 |
| No | 158 | 0.30 | (0.21–0.43) | | 158 | 135.1 | (106.91–170.6) | | 158 | 0.13 | (0.10–0.17) | | 158 | 79.8 | (63.22–100.67) | |
| Yes | 57 | 0.40 | (0.2–0.79) | | 57 | 197.2 | (135.51–286.83) | | 57 | 0.16 | (0.10–0.27) | | 57 | 83.9 | (51.65–136.34) | |
| Father working in agriculture (or exposed to pesticides) | | | | 0.605 | | | | 0.789 | | | | 0.631 | | | | 0.712 |
| No | 108 | 0.30 | (0.19–0.47) | | 108 | 144.9 | (110.82–189.49) | | 108 | 0.14 | (0.10–0.19) | | 108 | 72.5 | (54.31–96.72) | |
| Yes | 93 | 0.35 | (0.22–0.57) | | 93 | 163.7 | (118.16–226.67) | | 93 | 0.14 | (0.10–0.20) | | 93 | 91.2 | (64.42–129.03) | |
| Time (h) spent playing | | | | 0.552 | | | | 0.056 | | | | 0.408 | | | | 0.945 |
| < 1 h | 131 | 0.35 | (0.23–0.54) | | 131 | 162.0 | (125.63–208.85) | | 131 | 0.14 | (0.10–0.18) | | 131 | 87.1 | (66.42–114.21) | |
| ≥1 h | 85 | 0.25 | (0.16–0.41) | | 85 | 127.3 | (93.48–173.27) | | 85 | 0.14 | (0.10–0.19) | | 85 | 69.1 | (49.00–97.36) | |
| Household total net income | | | | 0.859 | | | | 0.322 | | | | 0.813 | | | | 0.591 |
| < 500 € | 11 | 0.63 | (0.08–1.34) | | 12 | 96.3 | (55.76–136.94) | | 11 | 0.15 | (0.01–0.31) | | 12 | 41.1 | (15.29–66.91) | |
| 500–1000 € | 34 | 0.26 | (0.01–0.51) | | 34 | 138.8 | (80.31–197.27) | | 34 | 0.10 | (0.02–0.22) | | 34 | 96.7 | (40.64–152.71) | |
| 1001–2000 € | 92 | 0.35 | (0.21–0.49) | | 92 | 138.9 | (77.11–200.6) | | 92 | 0.15 | (0.08–0.22) | | 92 | 78.4 | (43.13–113.59) | |
| ≥2000 € | 66 | 0.27 | (0.12–0.42) | | 66 | 185.0 | (80.10–290.0) | | 66 | 0.12 | (0.04–0.20) | | 66 | 94.0 | (54.40–133.60) | |
| Father's education level | | | | 0.117 | | | | 0.947 | | | | 0.562 | | | | 0.016 |
| None | 33 | 0.21 | (0.05–0.37) | | 34 | 137.4 | (48.80–225.9) | | 33 | 0.13 | (0.02–0.24) | | 34 | 172.9 | (97.18–248.66) | |
| Primary | 92 | 0.28 | (0.15–0.41) | | 92 | 152.3 | (86.00–218.6) | | 92 | 0.12 | (0.07–0.17) | | 92 | 67.7 | (39.15–96.28) | |
| Secondary | 47 | 0.71 | (0.32–1.10) | | 47 | 164.0 | (107.40–220.7) | | 47 | 0.16 | (0.04–0.28) | | 47 | 76.4 | (42.80–109.90) | |
| University | 27 | 0.23 | (0.05–0.41) | | 27 | 156.1 | (41.60–323.9) | | 27 | 0.17 | (0.06–0.28) | | 27 | 63.0 | (30.93–129.89) | |
| Mother's education level | | | | 0.925 | | | | 0.902 | | | | 0.704 | | | | 0.093 |
| None | 15 | 0.30 | (0.08–0.68) | | 16 | 184.4 | (40.70–373.4) | | 15 | 0.07 | (0.02–0.15) | | 16 | 171.9 | (47.27–296.49) | |
| Primary | 102 | 0.30 | (0.16–0.44) | | 102 | 145.2 | (74.40–216.0) | | 102 | 0.14 | (0.08–0.20) | | 102 | 73.45 | (51.34–95.56) | |
| Secondary | 55 | 0.34 | (0.16–0.52) | | 55 | 149.0 | (71.63–226.3) | | 55 | 0.16 | (0.05–0.27) | | 55 | 70.52 | (32.29–108.75) | |
| University | 42 | 0.37 | (0.10–0.64) | | 42 | 145.5 | (43.60–247.4) | | 42 | 0.15 | (0.03–0.27) | | 42 | 91.2 | (28.16–155.72) | |

[a] Mann-Whitney or Kruskal-Wallis test.

DAP metabolite in hair, this figure was far lower for urine (45.9%, see Supplementary Table 3). This finding suggests that levels of DAP metabolites are exposure- and time-dependent, and different for urine (recent exposure) and hair (cumulative exposure). In contrast, over 90% of the children assessed in California and Japan had positive detections for at least one DAP metabolite in urine (Supplementary Table 3). In our study, the order of positive detects in urine samples was: DMP (35.9%) > DEP (21.8%) > DETP (21.8%) > DEDTP (0.5%), with DMP concentrations being higher than those of diethyl metabolites. These figures parallel those found for hair and indicate higher exposure to dimethyl OP pesticides, which are the majority of OPs authorized for agricultural use in the EU.

Urinary DAP levels of our child population were compared with other studies carried out on children from different geographical areas from 2001 onwards (Supplementary Table 3). Overall, our children's levels showed lower values than other studies, regardless of whether these were conducted in urban areas or agricultural communities. Only newborns from a French cohort (Dereumeaux et al., 2017), children enrolled in the Chamacos birth cohort (Bradman et al., 2011), and children 6–11 years from the National Health and Nutrition

**Table 4**
Geometric means and 95% confidence intervals for urinary and hair concentrations of DETP and DEDTP with respect to sex, fresh food consumption, BMI, any parent working in agriculture or otherwise exposed to pesticides, time spent playing outdoors, household total net income and parents' education level.

| | Urine | | | | Hair | | | | Urine | | | | Hair | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DMP (μg/g creatinine) | | | | DMP (μg/g creatinine) | | | | DEP (μg/g creatinine) | | | | DMP (μg/g creatinine) | | | |
| | n | GM | 95% CI | p[a] | n | GM | 95% CI | p[a] | n | GM | 95% CI | p[a] | n | GM | 95% CI | p[a] |
| Age | | | | 0.839 | | | | 0.011 | | | | < 0.001 | | | | < 0.001 |
| 3–5 years | 52 | 0.09 | (0.01–0.17) | | 52 | 23.60 | (16.06–31.14) | | 52 | 0.07 | (0.06–0.09) | | 52 | 16.72 | (9.95–23.49) | |
| 6–11 years | 168 | 0.12 | (0.08–0.16) | | 170 | 10.01 | (1.44–18.58) | | 168 | 0.06 | (0.05–0.08) | | 170 | 4.37 | (0.96–9.89) | |
| Sex | | | | 0.322 | | | | 0.012 | | | | 0.378 | | | | < 0.001 |
| Boy | 68 | 0.10 | (0.05–0.15) | | 68 | 7.0 | (3.22–10.77) | | 68 | 0.06 | (0.03–0.09) | | 68 | 3.3 | (2.78–9.34) | |
| Girl | 152 | 0.11 | (0.07–0.15) | | 154 | 15.8 | (2.45–29.15) | | 152 | 0.06 | (0.04–0.08) | | 154 | 8.5 | (1.38–15.62) | |
| Body mass index (BMI) | | | | 0.876 | | | | 0.598 | | | | 0.972 | | | | 0.970 |
| Normal weight | 121 | 0.10 | (0.08–0.13) | | 121 | 0.10 | (0.08–0.13) | | 121 | 0.06 | (0.06–0.07) | | 121 | 6.2 | (4.43–8.80) | |
| Overweight | 62 | 0.13 | (0.09–0.19) | | 62 | 0.13 | (0.09–0.19) | | 62 | 0.06 | (0.05–0.07) | | 62 | 6.2 | (3.72–10.16) | |
| Obese | 39 | 0.11 | (0.07–0.16) | | 39 | 0.11 | (0.07–0.16) | | 39 | 0.05 | (0.04–0.06) | | 39 | 7.1 | (3.74–13.51) | |
| Total intake of fruits | | | | 0.622 | | | | 0.947 | | | | 0.231 | | | | 0.574 |
| 1st tertile | 71 | 0.10 | (0.08–0.13) | | 71 | 13.0 | (7.78–21.87) | | 71 | 0.06 | (0.05–0.07) | | 71 | 7.0 | (4.31–11.3) | |
| 2nd tertile | 65 | 0.10 | (0.08–0.14) | | 65 | 10.0 | (5.75–17.22) | | 65 | 0.06 | (0.05–0.06) | | 65 | 5.5 | (3.51–8.49) | |
| 3rd tertile | 67 | 0.13 | (0.09–0.19) | | 67 | 16.8 | (10.08–28.12) | | 67 | 0.06 | (0.05–0.07) | | 67 | 7.1 | (4.33–11.55) | |
| Total intake of vegetable | | | | 0.675 | | | | 0.945 | | | | 0.578 | | | | 0.973 |
| 1st tertile | 72 | 0.12 | (0.09–0.17) | | 72 | 16.8 | (9.74–28.81) | | 72 | 0.06 | (0.05–0.07) | | 72 | 10.0 | (5.96–16.69) | |
| 2nd tertile | 71 | 0.11 | (0.08–0.15) | | 71 | 9.3 | (5.87–14.67) | | 71 | 0.06 | (0.05–0.07) | | 71 | 5.3 | (3.53–7.83) | |
| 3rd tertile | 66 | 0.11 | (0.08–0.15) | | 66 | 15.0 | (8.75–25.71) | | 66 | 0.06 | (0.05–0.07) | | 66 | 5.9 | (3.65–9.61) | |
| Mother working in agriculture (or exposed to pesticides) | | | | 0.357 | | | | 0.023 | | | | 0.253 | | | | 0.275 |
| No | 158 | 0.11 | (0.09–0.14) | | 158 | 11.6 | (8.28–16.26) | | 158 | 0.06 | (0.05–0.07) | | 158 | 6.9 | (5.01–9.47) | |
| Yes | 57 | 0.12 | (0.08–0.17) | | 57 | 14.2 | (8.09–24.83) | | 57 | 0.06 | (0.05–0.07) | | 57 | 5.8 | (3.69–9.24) | |
| Father working in agriculture (or exposed to pesticides) | | | | 0.271 | | | | 0.048 | | | | 0.929 | | | | 0.089 |
| No | 108 | 0.11 | (0.09–0.14) | | 108 | 10.9 | (7.39–16.12) | | 108 | 0.06 | (0.05–0.07) | | 108 | 6.2 | (4.31–9.03) | |
| Yes | 93 | 0.12 | (0.09–0.15) | | 93 | 12.2 | (7.84–19.01) | | 93 | 0.06 | (0.05–0.07) | | 93 | 7.1 | (4.69–10.77) | |
| Time (h) spent playing | | | | 0.692 | | | | 0.797 | | | | 0.265 | | | | 0.290 |
| < 1 h | 131 | 0.11 | (0.09–0.14) | | 131 | 14.1 | (9.62–20.78) | | 131 | 0.06 | (0.05–0.06) | | 131 | 7.0 | (4.92–10.05) | |
| ≥1 h | 85 | 0.12 | (0.09–0.15) | | 85 | 10.2 | (6.61–15.66) | | 85 | 0.06 | (0.06–0.08) | | 85 | 5.9 | (4.02–8.59) | |
| Household total net income | | | | 0.605 | | | | 0.047 | | | | 0.659 | | | | 0.905 |
| < 500 € | 11 | 0.10 | (0.01–0.21) | | 12 | 3.8 | (1.06–8.74) | | 11 | 0.05 | (0.04–0.06) | | 12 | 4.5 | (2.43–11.41) | |
| 500–1000 € | 34 | 0.08 | (0.07–0.17) | | 34 | 21.4 | (12.16–30.60) | | 34 | 0.06 | (0.04–0.08) | | 34 | 6.3 | (2.57–10.05) | |
| 1001–2000 € | 92 | 0.11 | (0.06–0.16) | | 92 | 9.8 | (6.25–13.25) | | 92 | 0.06 | (0.05–0.07) | | 92 | 6.8 | (2.65–14.33) | |
| ≥2000 € | 66 | 0.12 | (0.06–0.18) | | 66 | 15.8 | (3.30–34.80) | | 66 | 0.06 | (0.03–0.09) | | 66 | 7.0 | (4.85–18.77) | |
| Father's education level | | | | 0.331 | | | | 0.139 | | | | 0.256 | | | | 0.532 |
| None | 33 | 0.10 | (0.02–0.18) | | 34 | 17.9 | (7.63–28.15) | | 33 | 0.06 | (0.04–0.09) | | 34 | 9.6 | (8.05–27.15) | |
| Primary | 92 | 0.10 | (0.05–0.15) | | 92 | 8.3 | (5.31–11.35) | | 92 | 0.05 | (0.04–0.06) | | 92 | 5.8 | (3.08–8.54) | |
| Secondary | 47 | 0.15 | (0.06–0.24) | | 47 | 16.6 | (10.10–23.04) | | 47 | 0.08 | (0.03–0.13) | | 47 | 5.8 | (3.69–15.31) | |
| University | 27 | 0.12 | (0.01–0.23) | | 27 | 11.1 | (3.89–18.33) | | 27 | 0.06 | (0.04–0.07) | | 27 | 8.6 | (2.41–14.81) | |
| Mother's education level | | | | 0.925 | | | | 0.902 | | | | 0.563 | | | | 0.218 |
| None | 15 | 0.08 | (0.02–0.18) | | 16 | 16.8 | (4.99–28.54) | | 15 | 0.06 | (0.04–0.07) | | 16 | 6.2 | (1.52–13.96) | |
| Primary | 102 | 0.11 | (0.06–0.16) | | 102 | 10.2 | (6.92–13.50) | | 102 | 0.06 | (0.04–0.08) | | 102 | 7.0 | (0.81–14.87) | |
| Secondary | 55 | 0.15 | (0.07–0.23) | | 55 | 11.4 | (5.15–28.03) | | 55 | 0.06 | (0.03–0.09) | | 55 | 4.2 | (1.61–6.76) | |
| University | 42 | 0.10 | (0.02–0.18) | | 42 | 18.4 | (8.79–27.91) | | 42 | 0.06 | (0.04–0.07) | | 42 | 10.4 | (7.62–28.48) | |

[a] Mann-Whitney or Kruskal-Wallis test.

Examination Survey (NHANES) beyond the year 2001 (Barr et al., 2011) had lower levels than our child population. However, our results are roughly similar to those reported by another Spanish study (Roca et al., 2014). Remarkably, all the studies shown in Supplementary Table 3 have urinary concentrations of DMP, DEP and DETP below the Reference values set by the Human Biomonitoring Commission of the German Federal Environmental Agency for children 3–14 years, i.e. DMP 75 μg/L (0.595 mmol/L), DEP 30 μg/L (0.195 mmol/L) and DETP 10 μg/L (0.059 mmol/L) (Schulz et al., 2011). Reference values have not been defined for DEDTP, as for the majority of the biomonitoring studies DEDTP levels are below the LOD of 1 μg/L. Based on DAP urinary data, exposure to OP pesticides have declined over time mainly due to regulatory restrictions or cancellation of OP pesticides (Barr et al., 2011).

From the 11 OPs approved for use in the EU as plant protection products in 2018 (EU Pesticides Database, http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.selection&language=EN), six can be broken down to dimethyl metabolites and only one (chlorpyrifos) to diethylmetabolites (DEP and DETP, but not DEDTP). The remaining four OPs (ethoprophos, fenamiphos, fosthiazate and fosetyl) do not yield any DAP metabolite. On the other hand, DEDTP metabolites are less directly associated with exposure than DMP and DEP because they break down rapidly to DETP and then to DEP (Coye et al., 1986). These reasons may explain the low proportion of positive results for DEDTP found in urine and hair samples from our study.

The fact that all urinary DAP metabolites were correlated with each other suggests common pathways for both exposure and excretion. Since the concurrent use of diethyl OP pesticides in applications with dimethyl pesticides is unlikely, the correlation between dimethyl and diethyl metabolites suggest dietary exposures from vegetable and fruit intake where both groups of OP pesticides are regularly used (Barr

**Table 5**
Geometric means for urinary and hair concentrations of ΣDAPs with respect to sex, fresh food consumption, BMI, any parent working in agriculture or otherwise exposed to pesticides, time spent playing outdoors, household total net income and parents' education level.

| | Urine Σ DAPs (nmol/g creatinine) | | | | Hair Σ DAPs (pmol/mg) | | | |
|---|---|---|---|---|---|---|---|---|
| | n | GM | 95% CI | p[a] | n | GM | 95% CI | p[a] |
| Age | | | | 0.615 | | | | 0.182 |
| 3–5 years | 52 | 5.99 | (2.24–9.74) | | 52 | 3.72 | (2.01–5.43) | |
| 6–11 years | 168 | 8.12 | (5.85–10.50) | | 170 | 2.99 | (2.07–3.91) | |
| Sex | | | | 0.495 | | | | 0.066 |
| Boys | 68 | 6.73 | (2.96–10.45) | | 68 | 2.88 | (1.08–4.68) | |
| Girls | 152 | 7.98 | (5.56–10.39) | | 154 | 3.27 | (2.47–4.07) | |
| Body mass index (BMI) | | | | 0.878 | | | | 0.536 |
| Normal weight | 118 | 7.42 | (5.30–10.40) | | 120 | 3.34 | (2.75–4.05) | |
| Overweight | 62 | 8.25 | (5.16–13.15) | | 62 | 2.81 | (2.22–3.56) | |
| Obese | 39 | 7.18 | (4.01–12.85) | | 39 | 2.93 | (2.14–4.02) | |
| Total intake of fruits | | | | 0.152 | | | | 0.957 |
| 1st tertile | 64 | 10.35 | (6.64–15.91) | | 65 | 0.89 | (0.71–1.11) | |
| 2nd tertile | 73 | 7.15 | (4.49–11.24) | | 73 | 0.81 | (0.61–1.08) | |
| 3rd tertile | 64 | 5.54 | (3.48–8.82) | | 65 | 0.86 | (0.67–1.11) | |
| Total intake of vegetables | | | | 0.467 | | | | 0.669 |
| 1st tertile | 62 | 10.82 | (7.14–16.45) | | 62 | 3.53 | (2.68–4.65) | |
| 2nd tertile | 68 | 6.18 | (3.97–9.63) | | 68 | 3.13 | (2.64–3.71) | |
| 3rd tertile | 76 | 7.00 | (4.26–11.44) | | 78 | 2.90 | (2.18–3.85) | |
| Mother working in agriculture (or exposed to pesticides) | | | | 0.427 | | | | 0.142 |
| No | 155 | 7.12 | (5.36–9.47) | | 157 | 3.10 | (2.63–3.65) | |
| Yes | 57 | 9.20 | (5.36–15.65) | | 57 | 3.32 | (2.56–4.30) | |
| Father working in agriculture (or exposed to pesticides) | | | | 0.970 | | | | 0.079 |
| No | 107 | 7.54 | (5.27–10.61) | | 107 | 2.85 | (2.35–3.46) | |
| Yes | 91 | 7.42 | (5.07–10.88) | | 93 | 3.47 | (2.81–4.28) | |
| Time (h) spent playing outdoors | | | | 0.716 | | | | 0.340 |
| < 1 h | 128 | 7.79 | (5.57–10.83) | | 130 | 3.19 | (2.65–3.83) | |
| ≥1 h | 85 | 7.21 | (4.96–10.44) | | 85 | 3.07 | (2.53–3.73) | |
| Household total net income | | | | 0.477 | | | | 0.025 |
| < 500 € | 11 | 13.58 | (1.00–28.16) | | 12 | 1.89 | (1.42–2.36) | |
| 500–1000 € | 34 | 5.34 | (0.70–9.98) | | 34 | 2.91 | (1.40–4.42) | |
| 1001–2000 € | 92 | 8.42 | (5.57–11.27) | | 92 | 3.00 | (1.94–4.06) | |
| ≥2000 € | 66 | 7.37 | (3.85–10.89) | | 66 | 4.11 | (2.13–6.09) | |
| Father's education level | | | | 0.070 | | | | 0.172 |
| None | 33 | 5.73 | (2.15–9.31) | | 34 | 4.17 | (1.29–7.05) | |
| Primary | 92 | 6.64 | (3.97–9.31) | | 92 | 2.84 | (1.77–3.92) | |
| Secondary | 47 | 15.98 | (8.55–23.41) | | 47 | 3.19 | (1.98–4.40) | |
| University degree | 27 | 6.49 | (2.13–10.86) | | 27 | 3.45 | (0.81–6.08) | |
| Mother's education level | | | | 0.544 | | | | 0.109 |
| None | 15 | 5.44 | (2.98–11.86) | | 16 | 4.50 | (0.94–8.06) | |
| Primary school | 102 | 6.95 | (4.11–9.79) | | 102 | 2.94 | (1.97–3.91) | |
| Secondary school | 55 | 9.47 | (5.29–13.66) | | 55 | 2.90 | (1.40–4.40) | |
| University degree | 42 | 9.25 | (3.76–14.74) | | 42 | 3.70 | (1.23–6.17) | |

[a] Mann-Whitney or Kruskal-Wallis test.

et al., 2011). However, the lack of correlation found between DAP levels in urine and hair samples likely indicates the large daily variation in OP pesticide residues present in food items consumed by children, along with the relatively short biological half-lives of these pesticides (Lu et al., 2006).

We also investigated the potential exposure determinants of DAP metabolites in children's urine and hair. While none of the determinants studied were associated with urinary DAP levels (with the exception of age), some significant associations were found for hair. Overall, age was inversely correlated with diethyl metabolites of OP pesticides in hair samples, which might be due to different food intake or exposure time in children aged 3–5 years as compared to children 6–11 years. However, when these two age categories were compared by vegetable and food intake or by time spent playing outdoors, no significant differences were observed (data not shown). Only household income was significantly associated with the two age-ranges studied, as children 6–11 years were overrepresented in those families with monthly household income over 1000 € ($p = 0.001$). Other factors not addressed in this study (e.g., the frequency and duration of hand-to-mouth activity, proximity to floors, carpets, lawns, soil, and other daily activities) might also contribute to the higher DAP levels observed in younger children. As differences were observed only for diethyl metabolites, and chlorpyrifos is the unique OP authorized for agricultural use in the EU rendering these metabolites, exposure to pesticide residues through the occupational take-home pathway might also play a role (Hyland and Laribi, 2017). Children 6–11 years have been reported to show higher urinary DAP concentrations than adolescents and adults (Barr et al., 2004), suggesting a greater risk of exposure to OP pesticides in children than other population subgroups. However, there exist controversial results as Heudorf et al. (2004) found no correlation between urinary DAP levels and children's age. On the other hand, we found that sex (i.e., girls) and occupational exposure of parents to pesticides were associated with increased hair DAP levels (Tables 3–5). Regarding urinary DAPs, while high levels have been reported for females and children (Health Canada, 2013; Sinha and Banda, 2018), other studies found no significant differences in relation to children's age and sex, family income, housing situation, and season (Lu et al., 2001).

Regarding diet, children with the highest vegetable intake had significantly greater hair DEP levels (Table 3). Intake of fruit and vegetables has been consistently and positively associated with urinary DAP metabolites in children at all ages, suggesting that diet is an important

source of pesticide exposure to children (Bradman et al., 2011; Lu et al., 2005, 2006). Hair DEP levels encountered in our study population may be explained because chlorpyrifos is the only OP pesticide with diethyl moiety authorized for use in agriculture in the EU and one of the pesticides more often detected in the food samples analyzed in the EU Coordinated Programme on Pesticide Residues in Food for the year 2011 (EFSA, 2014).

Parental occupational exposure to pesticides, especially for agricultural workers, has been considered as a predictive factor for higher exposure of their children (Bouvier et al., 2005). Children residing in agricultural areas may also be exposed to pesticides as a result of drifting from the application areas to household environments where children live. Moreover, parents who are farmers can transfer OPs to their children by take-home exposure (Rohitrattana et al., 2014). For these reasons, children living in agricultural areas tend to have a higher probability of pesticide exposures than those who do not. In our study, the increased hair levels of DMP in children that spent < 1 h playing outdoor (Table 3) is consistent with other studies and suggests that time spent indoors may contribute to higher urinary DAP concentrations (Barr et al., 2011). After indoor pesticide applications, pesticide residues within rooms may increase young children's exposure because they play on the floor and put things in their mouths (oral non-dietary and cutaneous exposure). Pesticides may also be present in house dust (e.g., due to the use of pesticides in the house or the garden), on dirt brought into the house on shoes or by pets, or on cut flowers and ornamental plants (Guodong et al., 2012). However, non-dietary intake of DEP in dust has been estimated to be ≤5% of the dose calculated from DEP levels in urine, suggesting that ingestion of dust is not a significant source of DAPs in urine (Quirós-Alcalá et al., 2012). Conversely, other studies found no clear association between urinary DAP metabolite levels and child behaviour patterns or residential proximity to crop fields (Koch et al., 2002).

The associations found between high household income and greater hair DETP and ΣDAPs levels can be considered as proxies for differences in food consumption patterns between socioeconomic groups (Spaan et al., 2015). Nevertheless, low-income children may experience higher exposures to pesticides because of poor housing quality and residential pesticide use to control pest infestations (Bradman et al., 2015).

Exposure determinants of the two classes of DAPs (dimethyl and diethyl metabolites) do not necessarily have to be the same because of differences in usage patterns of OPs, physical-chemical properties, metabolism of dimethyl versus diethyl OP pesticides, field degradation and environmental fate (Bradman et al., 2011). Further research is needed to understand better what factors contribute to hair and urinary DAPs concentrations in children.

This study has several limitations. One of them is the lack of specificity of DAP metabolites with respect to the parent OPs from which they are derived, and their toxicological potency. Accordingly, in a setting where diverse OP pesticides are used, measurement of non-specific DAP metabolites does not provide information on exposure to the specific parent OP compound. Another limitation is the collection of only a single urine sample, which may not reflect the average exposure. A single urinary measurement has limited ability to determine the variation of biological concentrations over time. It has been argued that, under a chronic OP pesticide exposure scenario, urinary elimination of DAPs may reach a steady state that reflects the average exposure (Kapka-Skrzypczak et al., 2011). However, such consideration may not be sufficient for assessing dietary exposure to OP pesticides which is dynamic in nature. Depending upon the timing of pesticide residue intake with certain meals, overnight urine samples may (or may not) represent true exposures. Only exposures that occurred during the previous few hours or days can be captured. Significantly, hair testing provides an advantage over urine testing because DAP metabolites can accumulate in hair over time. In principle, and as demonstrated here, this allows more reliable and more sensitive measurement of OP exposure.

Additionally, while urinary DAPs are widely used in epidemiological studies to assess exposure to OP pesticides, DAPs are both urinary metabolites and breakdown products of OPs. Since abiotic hydrolysis, photolysis, and plant metabolism can convert OP residues to DAP metabolites on or in the fruits and vegetables, environmental or dietary exposure to these toxicologically irrelevant metabolites can overestimate OP pesticide exposure in occupational and environmental studies. Indeed, over 70% of urinary DAP metabolite concentrations may be attributable to exposure to the preformed metabolites in the environment (Barr et al., 2011). Hence, exposure to preformed DAPs may affect the reliability of these metabolites as biomarkers of exposure to OP pesticides in non-acute settings (Weerasekera et al., 2009). Notwithstanding that, DAP biomonitoring data may still be useful as an indicator for the maximum potential of OP pesticide exposure over time.

Finally, the food frequency questionnaire used allowed mothers to report servings of fruits and vegetables consumed by their children each day, but it was not calibrated to specific portion sizes. While the use of reported servings in the analyses may have introduced uncontrolled variability, this type of non-differential exposure misclassification would tend to bias results toward the null hypothesis (Bradman et al., 2011).

The risk of potential selection bias was addressed by exploring differences in children's age and sex, household total net income, parental education, and urinary concentrations of DAP metabolites between children from the original study population (n = 591) and the final population included in this research (n = 222 children) for which sufficient amounts of urine and hair were available. No significant differences were observed for age and urinary levels of DAP metabolites, with the exception of DMTP as children who provided hair samples had significantly higher urinary DMTP levels compared to children who did not. This might lead to overestimation of the magnitude of the associations observed for this metabolite. On the other hand, a significantly greater percentage of girls than boys from the original population provided hair samples. As girls usually have longer hair, they were likely more prone to give hair samples than boys. Nevertheless, no significant differences in urinary DAP levels were observed by sex in the original sample. Furthermore, potential differences by sex regarding other variables potentially associated with urinary DAP levels were explored in the original sample to rule out a modification of effect related to sex. However, we did not find significant differences by sex in the effect of household net income on urinary DAPs, as both girls and boys from families reporting higher monthly income tended to show higher urine DAP levels. Likewise, no significant differences were observed in the effect of fathers' and mothers' education on urine DAPs between boys and girls, as higher fathers' and mother's education level was associated with increased urinary concentrations of these metabolites in both sexes. Although at the age range studied boys and girls usually do not play outside similarly, no differences were observed in our study regarding time they spent outdoors. Accordingly, although girls outnumbered boys this did not affect the magnitude of the associations found in our study.

In conclusion, we found that children living in an area of intensive agriculture are probably exposed to OP pesticides from multiple pathways. Hair DAP levels arise in part from the intake of food containing OP-generating DAPs, from agricultural or residential pesticide use, and indirectly from some dimensions of socioeconomic status (e.g., household income and parental education level). Conversely, urinary DAPs failed to be associated with the determinants of exposure studied. Overall, hair testing of DAPs (and even of parent OP pesticides) may provide greater insights for human biomonitoring purposes than measuring urinary DAP metabolites as hair is easier to collect, handling, store and ship than urine or blood. Besides, the wider window of exposure and the higher detection rates of the compounds of interest make hair an alternative promising matrix to urine or blood.

## Declaration of Competing Interest

Authors declare that they have no conflict of interest.

## Acknowledgements

This study was partially funded by the Ministry of Economy, Innovation, Science and Employment, Government of Andalucia (reference number P08-CTS-04313, FEDER funds). The authors thank the school's staff and the children and families, without whom this study would not have been possible. The authors also thank Prof. Colin Ockleford for his review of the manuscript and his helpful suggestions.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.104997.

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