Environmental exposure to organophosphorus and pyrethroid pesticides in South Australian preschool children: A cross sectional study

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ARTICLE INFO

Article history:
Received 20 February 2012
Accepted 21 July 2012
Available online 11 August 2012

Keywords:
Environmental exposure
Organophosphate
Pyrethroid
Neurotoxic insecticides
Children
South Australia

ABSTRACT

Organophosphorus (OP) and pyrethroid (PYR) compounds are the most widely used insecticides. OPs and PYRs are developmental neurotoxicants. Understanding the extent of exposure in the general population and especially in young children is important for the development of public health policy on regulation and use of these chemicals. Presented here are the results of the first investigation into the extent of environmental exposure to neurotoxic insecticides in preschool children in South Australia (SA).

Children were enrolled from different areas of SA and assigned into urban, periurban and rural groups according to their residential address. Residential proximity to agricultural activity, parental occupational contact to insecticides and use of insecticides within the household were investigated as potential indirect measures of exposure. We used liquid chromatography/tandem mass spectrometry to measure the following metabolites of OPs and PYRs in urine samples as direct indicators of exposure: dialkylphosphates, p-nitrophenol, 3-methyl-4-nitrophenol, 3,5,6-trichloro-2-pyridinol, cis- and trans-3-(2,2-dichlorovinyl)–2,2-dimethyl-cyclopropane-1-carboxylic acid, cis-3-(2,2-dibromovinyl)–2,2-dimethyl-cyclopropane-1-carboxylic acid, 2-methyl-3-phenylbenzoic acid and 3-phenoxycbenzoic acid. Results were analysed to assess factors affecting the risk and level of exposure. Results were also compared to the published data in similar age groups from US and German studies.

The results of this study demonstrate that there was widespread chronic exposure to OPs and and PYRs in SA children. OP metabolites were detected more commonly than PYR. Exposure to more than one chemical and contemporaneous exposure to chemicals from both OP and PYR groups was common in the study population. There were some differences in risks and levels of exposure between the study groups. Exposure to some restricted use of chemicals, for example, fenitrothion, was higher in periurban and rural children. There was no difference among the study groups in exposure to chlorpyrifos, used commonly in agriculture and in domestic settings and most frequently found OP pesticide in food in Australia. South Australian children appear to have higher levels of exposure compared their peers in US and Germany.

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1. Introduction

Insecticides are chemicals used to control insect pests that damage or destroy crops or transmit diseases among humans and animals. Organophosphorus (OP) and pyrethroid (PYR) compounds are the most widely used groups of insecticides in Australia and worldwide. OP and PYR insecticides are neurotoxins. Neurotoxicity is defined as any permanent or reversible adverse effect on the structure or function of the nervous system. The main mode of action of OPs in humans is inhibition of acetylcholinesterase, while the main mode of action of PYRs is modulation of voltage gated ion channels. Apart from main modes of action, both groups act on other biochemical and molecular targets within the nervous system. Extensive experimental data demonstrate that low-level exposure to some commonly used OPs and PYRs can negatively affect the development of nervous system in young laboratory animals via “non-classical” mechanisms that are independent of the main mode of action. International scientific literature shows that there is a concern

Abbreviations: SA, South Australia; OP, organophosphorus; PYR, pyrethroid; HPLC/MS–MS, high performance liquid chromatography tandem mass spectrometry; DAP, dialkylphosphates; DMP, dimethyl phosphate; DEP, diethyl phosphate; DETP, diethyl thio phosphate; DMTP, dimethyl thio phosphate; DEDTP, diethyl dithiophosphate; DMDTP, dimethyl dithiophosphate; TCPy, 3,5,6-trichloro-2-pyridinol; DCCA, cis- and trans-3-(2,2-dichlorovinyl)–2,2-dimethyl-cyclopropane-1-carboxylic acid; DRCA, cis 3-(2,2-Dibromovinyl)–2,2-dimethyl-cyclopropane-1-carboxylic acid; MPA, 2-methyl-3-phenylbenzoic acid; PBA, 3-phenoxynbenzoic acid; OR, odds ratio; CI, confidence interval; SE, standard error of the mean; ADI, acceptable daily intake; ATDS, Australian Total Diet Survey; CV, coefficient of variation; LOD, limit of detection.

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1 Please note, at the time of the study, Dr Babina was not an employee of SA Health and as such, her current affiliation with SA Health bears no relevance to the presented study.
and need for research into the effects of chronic low level (environmental) exposures to neurotoxic insecticides on the neurobehavioral and emotional development of young children as well as on children’s health in general (Bjørling-Poulsen et al., 2008). Understanding the extent of exposure in the general population is essential for evaluating whether chemicals may be linked to adverse health outcomes and for the development of scientifically sound public health policy on regulation and use of these chemicals.

South Australia has a well-developed agriculture sector, which includes wide acreage crops, livestock and horticulture. An estimated 54.1 million ha or 55% of South Australia’s land area is used for agricultural activity and there were 14,262 agricultural establishments in the 2002–2003 financial years (ABS, 2003). In addition, ‘lifestyle farming’ on small acreage and backyard gardening are very popular in Australia.

The Australian Pesticide and Veterinary Medicine Authority (APVMA) is Australia’s federal regulatory body whose roles are to review and allow (or disallow) chemicals onto the Australian market, to develop regulatory residue limits and to make recommendations on the use of registered chemicals, mostly by means of information collated on the chemical label. The APVMA maintains an online publicly accessible database of registered products, Public Chemical Registration Information System (PUBCRIS) [http://www.apvma.gov.au/pubcris]. The state health authorities are responsible for training and licensing restricted-use chemical users. However, Australian authorities neither have effective control over pesticide use beyond the point of sale nor do they collect information on where, when, how or how much product is being used.

Overall, thirty OP actives and twenty two synthetic PYR actives are included in over 300 and over 1000 of registered commercial formulations respectively (APVMA, 2012). Six OP actives and sixteen PYR actives are allowed for public use in Australia. Publicly accessible OP actives include: chlorpyrifos, diazinon, dichlorvos, fenthion, malathion and omethoate (APVMA, 2012). The OP and PYR formulations are available for domestic use by the general public as indoor surface sprays (fly, spider, cockroach), outdoor plant and barrier sprays, indoor and outdoor powder preparations, concentrate solutions, insect traps, flea bombs, mosquito coils, lice control lotions and shampoos as well as veterinary products. These formulations can be purchased over the counter in hardware stores, supermarkets and pharmacies in Australia. In addition, Australian homes are routinely treated with various insecticides including chlorpyrifos (an OP insecticide) and bifenthrin (a PYR insecticide) for termite control, both before and after construction.

This widespread availability of pesticides to the general public, large variety of registered products (both for public and restricted use) and extensive mixed agricultural land use pattern of South Australia suggests that there is a potential for significant widespread exposure in the general population. The extent of environmental exposure to OPs and PYRs in the Australian general population and its possible health effects so far have not been addressed in the scientific literature and in particular the extent of environmental exposure in children in Australia is unknown.

This study aimed to explore the extent of exposure to OP and PYRs pesticides in South Australian children and to investigate the factors that influence the risk of being exposed and the levels of exposure in the study population.

2. Study design and methods

2.1. Subjects

The study took place during 2003–2006. The subjects of this study were 340 healthy children aged 2.5 to 6 years old residing in metropolitan Adelaide (urban group), Adelaide Hills area (peri-urban group) and agricultural areas of South Australia including the Yorke Peninsula, mid-north region, Barossa Valley, Riverland, McLaren Vale and the Murray-Hills area (rural group) (Fig. 1). Study groups were chosen based on a priori descriptive knowledge of pesticide use patterns and the knowledge of land use and agricultural activity in each area. Differences in population density meant that larger areas needed to be covered to achieve the target sample size in rural areas. The description of pesticide use and agricultural activity patterns in each sample group’s area of residence are summarised in Table 1.

The study employed stratified random sampling approach. Children were recruited through local preschools (child care centres, kindergartens and reception year in primary schools). The contact details of the preschools within areas of interest were taken from the “Yellow Pages” phone directory. In peri-urban and rural areas all preschools listed in the phone directory were contacted, while urban preschools were chosen within a sample of 30 postcodes drawn in urban Adelaide. The urban postcode sample was drawn to represent the diversity of the socio-economic status (SES) of the urban Adelaide population based on SES profiles by Glover and Tennant (1999). At the time of initial contact, brief introduction to the project and sampling process were given to the preschool directors/principals. A proportion of preschools did not consent to participate in the study. The consented preschools were then asked to distribute the project materials among the parents of eligible children. The parents were given an opportunity to meet/contact the project officer to discuss any questions or concerns.

The sampling was conducted through early September to late November in 2003 and in 2004. Sampling at the same time of the year allowed accounting for possible seasonal variations in pesticide use.

Approvals were gained from human research ethics committees (including Flinders University, University of South Australia and the South Australian Department of Education).

2.2. Sample size estimation

An earlier study by Loewenherz et al. (1997) compared urinary DAP levels in children from pesticide applicator families and from reference families in a rural community in the USA. The difference in measured concentrations between the two groups reached up to 4 fold for one of the metabolites, DMTP. For the purpose of the sample size calculation for this study, the target difference of 50% between the groups was assumed with standard deviation of 1.0 (log-transformed concentrations). A similar approach has been used in other studies (Wilson et al., 2004). To detect the difference in exposure levels between the sample groups with at least 80% power (α = 0.05, 20% probability of Type II error, and 5% probability of Type I error) (Cohen, 1988), a sample size of at least 100 subjects in each sample group (urban, peri-urban and rural) would need to be achieved.

2.3. Indirect measures of exposure

Parents were asked to complete a General Questionnaire (GQ) that was designed to acquire information about pesticide use inside and outside each child’s residence, parental occupational contact with pesticides and parental smoking habits. The GQ provided the information that formed the basis of several indirect measures of exposure, the effects of which were tested in the statistical analysis. The GQ also provided information on the both mother’s and father’s occupation, which was used to estimate combined family income based on the ANU4 scales (Jones and McMillan, 2001). The subjects were assigned into “high income”, “medium income” and “low income” categories based on commonly used income classification approach: the 25th percentile of the population distribution in income comprises low income subjects, while 75th percentile consists of high income subjects (ABS, 2004). Proximity of every child’s residence to agricultural activity was measured using the web-based South
Australian Government’s Report on Generalized Land Use for 2003 (Atlas SA, 2004). This allowed the location of the residence to be classified as within 50 m, within 200 m, or beyond 200 m from agriculturally zoned areas. These distances were chosen mostly intuitively, since this study was the first of a kind in Australia and there were no preliminary data upon which the decisions of grouping by distance could be based.

2.4. Direct measures of exposure: Urinary metabolites

The extent of human exposure to chemicals may be characterised using biological monitoring—measurement of chemicals or their metabolites in body fluids or tissues, such as blood and urine. Biological monitoring of exposure to neurotoxic insecticides has been used extensively in occupational health in Australia and worldwide. Measurement of OP and PYR insecticides and/or their metabolites in urine samples is a widely accepted tool in occupational and environmental exposure assessment (Aprea et al., 1994; Heudorf et al., 2004; NCEH, 2009; Smith et al., 2002).

The parents of the study participants were asked to collect a single first morning void urine sample from their child. Urine samples were individually coded and stored frozen (−20 °C) until analysis.

The urine samples were analysed for the following metabolites of OP insecticides: dialkylphosphates (DAPs), \( p \)-nitrophenol, 3-methyl-4-nitrophenol, 3,5,6-trichloro-2-pyridinol (TCPy) and following metabolites of PYR insecticides: cis- and trans- 3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid (DCCA), cis- 3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid (DBCA), 2-methyl-3-phenylbenzoic acid (MPA) and 3-phenoxybenzoic acid (3-PBA). These metabolites have been used for the purposes of biological monitoring in a number of studies (Aprea et al., 2000; Barr et al., 2002; Esteban et al., 1996; Heudorf et al., 2004; Morgan et al., 2005; NCEH, 2009; Saieva et al., 2004; Smith et al., 2002). The metabolites were chosen based on their informative value and availability of the chemical standards. Table 2 summarises information on the metabolites measured in this study and their respective parent compounds.

While a number of gas chromatography coupled with mass spectrometry (GC/MS) methods have been developed and are thoroughly validated, recent developments in liquid chromatography coupled with mass spectrometry (LC/MS) allowed the development of a new

### Table 1

<table>
<thead>
<tr>
<th>Study group and location</th>
<th>Pesticide use patterns</th>
<th>Examples of chemicals used</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urban group</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban Adelaide</td>
<td>Indoor/outdoor pest (ant, spider, fly, cockroach) control by the household members</td>
<td>Indoor surface sprays, garden sprays, c (PYRs, OPs, carbamates, herbicides)</td>
</tr>
<tr>
<td></td>
<td>Pre- and post construction termite control by the pest control operators</td>
<td>Termite control (chlorpyrifos, bifenthrin, deltamethrin and others)</td>
</tr>
<tr>
<td></td>
<td>Head lice control</td>
<td>Shampoos and lotions (malathion, PYRs)</td>
</tr>
<tr>
<td></td>
<td>Veterinary products</td>
<td>Pet shampoos, collars (OPs and PYRs)</td>
</tr>
<tr>
<td><strong>Peri-urban group</strong></td>
<td>As in urban Adelaide, plus:</td>
<td>As in urban Adelaide</td>
</tr>
<tr>
<td>Adelaide Hills (Mount Lofty Watershed)</td>
<td>High density of agricultural activity within populated residential area</td>
<td>Anecdotal evidence of wide spread use and overuse of generally available and restricted use chemicals</td>
</tr>
<tr>
<td></td>
<td>“Lifestyle” and small scale farming (orchards, vineyards, sheep and dairy farms)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Large number of residents are licensed to use restricted use chemicals</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rugged terrain makes aerial spray common, especially for pastures</td>
<td></td>
</tr>
<tr>
<td><strong>Rural group</strong></td>
<td>As in urban Adelaide, plus:</td>
<td>As in urban Adelaide</td>
</tr>
<tr>
<td>Regional SA</td>
<td>Rural townships and farm residences surrounded by large agricultural establishments</td>
<td>Use of restricted chemicals</td>
</tr>
<tr>
<td></td>
<td>Wide acreage crops (barley, wheat), pastures, orchards, vineyards</td>
<td>Aerial spray on the pastures and fields</td>
</tr>
</tbody>
</table>

Fig. 1. Map of study groups and sample areas.
A Waters Alliance 2695 HPLC system (Waters, USA) interfaced with a Micromass Quattro tandem mass spectrometer (Micromass, UK) was used for the urine sample analysis in this study.

2.4.1. Method 1—DAPs

Chemical standards of dimethyl phosphate (DMP), diethyl phosphate (DEP), diethyli thiophosphate (DETP), dimethyl thiophosphate (DMTP), diethyl dithiophosphate (DEDTP) and dimethyl dithiophosphate (DMDTP) were kindly donated by WorkCover Laboratories, Sydney, Australia. HPLC grade acetonitrile and methanol were purchased from BDH Laboratory Supplies (UK). Pairing reagent, tetraethyl ammonium acetate (TEA) was purchased from SigmaAldrich (USA). Nitrogen and argon were purchased from BOC Gases (Adelaide, South Australia).

All chemicals were of an analytical grade.

Stock solutions of all six DAP standards were prepared in 100% acetonitrile at concentration 1 g/L. Mixed standard solution (MSS) was prepared in 50% methanol with resulting concentration of 10 mg/L for each of the compounds. MSS was then used to prepare calibration dilutions of DAPs in blank urine at the following levels: 5, 10, 25, 50, 75, 100, 125 μg/L. Blank urine was collected from unexposed individuals and was heat treated (60 °C for 30 min) in order to minimise amount of DAPs potentially present in blank urine. External standard method was used for compound detection and quantification. The calibration dilutions of DAPs prepared in blank urine at the levels: 0, 5, 10, 25, 50, 75, 100, 125 μg/L were run prior to each run in order to obtain calibration curves. Linear calibration curves (R² ranging between 0.97 and 0.99) were obtained for each run.

LC analysis employed Wakois II 5C18RS 5 μm × 50 × 2.0 mm analytical column coupled with Wakois C18RS 5 μm guard cartridge. Both were purchased from SGE (SGE International Pty Ltd).

Urine samples and standards were thawed at room temperature, spun at 2500 rpm for 10 min, then 1 mL of samples/standard was transferred into a vial, already containing 50 μL TEA. 10 μL was directly injected into LC system. Water/methanol mobile phase was used for gradient elution (0% methanol between 0 and 2 min, then increasing to 10% methanol by 7 min and to 100% methanol by 9 min, holding at 100% methanol until 10 min, then decreasing to 0% methanol by 12 min and holding at 0% methanol until 17 min to allow for column equilibration). Compounds retention times were between 1.5 and 4 min, making this method fast and convenient for analysis of a large number of samples.

The mass spectrometer was used in negative ionisation mode (ES-) in 8 channel multiple reaction monitoring (MRM) method. A series of infusion experiments were run for each compound in order to devise MS–MS parameters optimal for both specificity and selectivity. Two transitions were selected for each compound (with the exception of DEDTP). Nitrogen was used as a desolvation gas and argon was used as a collision gas. Desolvation gas flow rate was set at 800 L/h; collision cell pressure was maintained at 4.4–4.5 e⁻¹ mbar. Only four of six DAPs gave reliable retention, elution and signal. DMP was detected as an unretained compound, thus quantification of DMP was not possible. DEDTP coeluted with unidentified urine matrix interference and was excluded from the final method. The quantification of only four out of six DAPs still provided informative assessment of the general exposure to a wide range of OPs in the focus population. It would not though be sufficient if the assessment of exposure to particular OP compounds were the focus of the study.

To determine within-series variability of the method, a calibration standard (50 or 100 μg/L) was run after every 10 injections. Within-series coefficient of variation (CV) ranged from 4.3 to 10.4%. Between-day variability was determined by running duplicates of samples analysed the previous day. Between-day CV ranged between 6.2 and 12.7%. The overall mean recovery ranged between 89 and 103 (%±SD). The overall method limit of detection was 0.1 μg/L.

2.4.2. Method 2—PYRs and specific OP metabolites

HPLC grade acetonitrile and methanol were purchased from BDH Laboratory Supplies (UK). Glacial acetic acid was purchased from SigmaAldrich (USA). Chemical standards of cis- and trans-DCCA, DBCA, 3-PBA, 3-PBA-13C₆ where purchased from AccuStandard (USA). Chemical standard of MPA was synthesised at the Environmental Health Laboratories, Flinders University, South Australia (Smith et al., 2002). Chemical standards of TCPy and p-nitrophenol were purchased from SigmaAldrich (USA). Nitrogen and argon were purchased from BOC Gases (Adelaide, South Australia). All chemicals were of an analytical grade.

The analysis employed a Wakois II 5C18RS 5 μm × 50 × 2.0 mm analytical column coupled with Wakois C18RS 5 μm guard cartridge. Both were purchased from SGE (SGE International Pty Ltd).

Sample preparation procedure was conducted as described in Olsson et al. (2004). In brief, urine samples were thawed at room temperature, 2 mL of sample was used in the analysis. Available internal standard (3-PBA 16C₆) was added to achieve resulting concentration of 50 ppb. To release the glucuronide and/or sulphate conjugated compounds, β-glucuronidase type H-1 (SigmaAldrich, USA) with specific activity of ~500 U/mg was used. To each sample, an amount of enzyme giving ~800 U of activity was added in 0.2 M acetate buffer.

| Metabolite | Parent compound—use and availability in Australia | Diallylphosphates (DAPs): | Standard method was used for compound detection and quantification. The calibration dilutions of DAPs prepared in blank urine at the levels: 0, 5, 10, 25, 50, 75, 100, 125 μg/L were run prior to each run in order to obtain calibration curves. Linear calibration curves (R² ranging between 0.97 and 0.99) were obtained for each run. LC analysis employed Wakois II 5C18RS 5 μm × 50 × 2.0 mm analytical column coupled with Wakois C18RS 5 μm guard cartridge. Both were purchased from SGE (SGE International Pty Ltd). |
(3.1 mL of glacial acetic acid, 9.7 g of sodium acetate, 1 L of water). Samples were incubated at 37 °C overnight (Olsson et al., 2004).

Sample extraction was carried out next day using solid-phase extraction (SPE) procedure. SPE cartridges containing 50 mg of C18 media (United Chemical Technologies) were preconditioned using 1 mL methanol and then 1 mL of 1% acetic acid. Samples were filtered through cotton wool and then passed through the SPE cartridge. To reduce interferences, cartridges were washed with 5% methanol in 1% acetic acid (Olsson et al., 2004) and the compounds of interest were eluted with 2 mL 100% methanol. 1.5 mL of the eluent was transferred into LC vial and 10 μL of that was injected into the LC/MS–MS analytical system.

Stock solutions of analytical standards were prepared at concentration of 500 μg/L in methanol. Standard curve solutions were prepared in blank urine at concentrations: 5, 15, 30, 45, 60, 75, and 100 μg/L. The calibration solutions were subjected to the same sample preparation as the test samples. The blank urine only had 1.5 mL of 0.2 M acetic buffer added to it (without β-glucuronidase) before the overnight incubation. Addition of β-glucuronidase would release compounds potentially present in the blank urine and therefore introduce additional interference.

Calibration solutions were run at the beginning of each run and resulting calibration curve (linear; R² ranged between 0.93 and 0.98) was used to estimate the concentrations of the compounds of interest in urine samples from study participants.

Mobile phase composed of water and acetonitrile was used (20% acetonitrile between 0 and 0.5 min, then increasing to 100% acetonitrile by 5 min holding at 100% acetonitrile until 10 min, then decreasing to 20% acetonitrile by 12 min and holding at 20% acetonitrile until 19 min to allow for column equilibration). Samples and column were kept at room temperature. Injection volume was 10 μL. The flow rate was 0.2 mL/min.

Mass spectrometer was used in negative ionisation mode (ES-) in 13-channel (MRM) method. Nitrogen was used as a desolvation gas and argon was used as a collision gas. Desolvation gas flow rate was set at 800 L/h; collision cell pressure was maintained at 4 mBar. A flow rate was set at 4.5 e⁻⁴ mBar. A series of infusion experiments were run for each compound in order to devise MS–MS parameters optimal for both specificity and selectivity.

To determine within-series variability of the method, a calibration standard (15 or 30 μg/L) was run after every 10 injections. Within-series CV ranged from 4.3 to 9.8%. Between-day variability was determined by running duplicates of samples analysed the previous day. Between-day CV ranged between 6.4 and 12.1%. The overall mean recovery ranged between 98 and 113 (%±SD). The method limit of detection was 0.1 μg/L.

2.4.3. Creatinine correction

A spot sample urinary concentration of pesticide metabolite in the units of μg/L has uncertain meaning because variable fluid intakes may result in large variations in the excreted concentrations. Several methods are available for correction for urine dilution of spot samples including specific gravity, timed or daily urinary excretion, and creatinine correction. The creatinine correction approach involves calculating the ratio of analyte to creatinine and the resulting concentration is reported as microgram of analyte per gram of creatinine (μg/g creatinine). Creatinine correction has been used extensively in biomonitoring studies in both adult and children populations (Aprea et al., 2000; MacIntosh et al., 1999; NCEH, 2009). The urinary creatinine was measured by Jaffe kinetic reaction (Yatzidis, 1974) without deproteinisation on a Boehringer Mannheim Hitachi 917 automated analyser.

2.5. Statistical analysis

Statistical analysis was performed using SPSS statistical software package version 12.0.1 (SPSS Inc., Chicago, IL).

2.5.1. Data screening and diagnostics

Initially, data frequencies were screened for logical inconsistencies that may have been the result of erroneous handling of the data. Next, detection of outliers was conducted using the scatter, stem-and-leaf, and error bar plots (Gilbert, 1987). The outliers were investigated in the regression diagnostics and characterised by centred leverage (cutoff was set at 2 k/N, where k = sample groups number / 3), discrepancy (measured by externally studentised residuals, cutoff was set at 4.0) and influence (measured by Cook’s distance, cutoff was set at 1.0) (Cohen et al., 2003). Outliers were detected in this study comprised less than 5% of the study population and, based on the regression diagnostics, they were deemed conceptually and statistically tolerable (Cohen et al., 2003) and therefore remained unchanged within the dataset.

The interactions among predictor variables were monitored for each metabolite prior to the development of multivariate regression models in stepwise univariate regression analyses. The dependent variables (metabolite concentrations) were analysed against each independent variable (indirect exposure measures) and then new predictor variables were added to the model in succession while monitoring for large unexplainable changes in the value and/or direction of the coefficients which would suggest serious influence of collinearity (Cohen et al., 2003). There were no signs of unacceptable multicollinearity among independent variables in our dataset.

2.5.2. Data transformation

The creatinine corrected concentrations of urinary metabolites (measured in μg/g creatinine) were sorted into two types of variables to reflect different aspects of exposure: risk of exposure and level of exposure. Firstly, binary variables were created for each metabolite, allowing assignment of the study participants into either “exposed” or “non-exposed” groups: subjects whose urine samples contained metabolites at levels “below LOD” levels were assigned into “non-exposed” group, while subjects whose urine samples contained “above LOD” levels were assigned into “exposed” group. This approach allowed the analysis of the factors affecting the risk of being exposed within the whole study population. The shape of the raw concentrations of urinary metabolites (measured in μg/g creatinine) was departing significantly from normal distribution (skewness ranging from 2 to 10). The raw data was therefore logarithmically transformed (log-transformed) to ensure its normality. The log-transformed data was normally distributed (maximum skewness −0.6 for DETP and DMDTP). These log-transformed metabolite concentrations formed the second type of variables that allowed investigation of the factors affecting the levels of exposure among exposed study participants.

After initial descriptive analysis, a series of simple bivariate correlations were performed to explore the associative relationships among the variables in the dataset. The final step in the data analysis was either logistic (binary data) or linear (normally distributed continuous data) regression analysis.

2.5.3. Binary logistic regression analysis: Exploring factors predicting risk of exposure

To explore which of the indirect exposure measures are associated with the risk of exposure, binary logistic regression analysis was performed. The independent variables included “Area of residence” (rural, peri-urban, urban); “Type of residence” (a house in metropolitan Adelaide, a house in rural town, a farm, a lifestyle farm in peri-urban area); “Parental occupational exposure” and “Residing within 50 m from an agricultural activity” and “Residing within 200 m from an agricultural activity”. The other variables used in the analysis were “Child’s age” and “Family income”. The results are reported here as an odds ratio (OR) together with corresponding 95% confidence intervals (95%CI) and significance level (p) in Table 5.
2.5.4. Linear regression analysis: Exploring factors predicting levels of exposure

A series of stepwise linear regressions were run to explore the effects of indirect measures of exposure as independent (predictor) variables on the level of exposure among exposed subjects only estimated using log-transformed creatinine corrected urinary metabolite concentrations as dependent variables. The analysis was performed separately for each metabolite. The independent variables included “Area of residence” (rural, peri-urban, urban); “Type of residence” (a house in metropolitan Adelaide, a house in rural town, a farm, a lifestyle farm in peri-urban area); “Parental occupational exposure” and “Residing within 50 m from an agricultural activity” and “Residing within 200 m from an agricultural activity”. The other variables used in the analysis were “Child’s age”, “Child’s gender” and “Family income”. The results of this analysis, including model summary (standardised β and t statistics) and significance levels (p) are presented in Table 6.

3. Results

3.1. Study population: Descriptive analysis

The samples were collected from various areas of metropolitan Adelaide, Adelaide Hills and rural areas, aiming to include families from different socio-economic backgrounds ensuring that the final sample population reflected the socio-economic gradients existing in the general population in South Australia. A total of 340 children (115 urban, 111 peri-urban, 114 rural) participated in the study. Of those preschools that agreed to participate, the response (participation) rate among eligible families ranged between 1% and 40%, with an overall participation rate of 12.1%. Highest response rates were in rural areas and lowest response rates were in some less advantaged Southern suburbs of metropolitan Adelaide. The gender composition of the final sample population was comparable to the gender composition of the overall SA population of pre-schoolers (about 55% boys, estimated using 2000 SA Demographic [ABS, 2000]). Overall, the resulting sample of 340 children represented 1.41% of the total population of 3–6 year olds at the time of the study, satisfied estimated sample size requirement and was deemed representative of the general SA population aged 3 to 6 years. The demographic characteristics and indirect exposure parameters of the total sample and sample groups are summarised in Table 3.

3.2. Indirect exposure measures—descriptive analysis

Significantly more rural parents (52.6%, p < 0.001) reported occupational contact with pesticides than those from urban and peri-urban groups. Similarly, significantly more children were residing in close proximity to agricultural activity in peri-urban (40.5%) and rural (69.3%) groups compared to the urban group (0.9%, p < 0.001). Mean combined family income was significantly lower in peri-urban and rural groups compared to the urban group (p = 0.017 and < 0.001 respectively) (Table 3). The differences in income and age distribution were addressed in further analysis by assessing variables “Family income” and “Child’s age” for confounding effect.

Some variables initially intended to be assessed for use as indirect measures of exposure were excluded from the analysis. Many parents misreported the use of pesticides inside homes due to the misinterpretation of the term “pesticide”. For example, some parents reported use of domestic disinfectants, dishwashing detergents and even air fresheners in question 5 of the GQ (“Do you/your partner use pesticides inside the house?”). Therefore the reported use of pesticides could not be considered to be a true representation of the domestic use of pesticides in the community and variable “Reported use of pesticides” was excluded from further analysis. Similarly, most parents (over 80%) could not recall whether or not their house had been professionally treated for pest control, which led to the exclusion of the variable “House treated professionally” from further analysis.

3.3. Urinary metabolites data: Descriptive analysis

Tables 4 and 5 show the mean concentrations with standard deviations, range and major percentiles: 25th (P25), 50th (P50), 75th (P75) and 95th (P95) for each measured metabolite by sample group. Most commonly detected metabolites were TCPy (detected in between 92.2 and 98.2%), 3PB (detected in 80.9 to 84.2% of samples) and DAPs (detected in 53.0 to 82.6% of samples). Highest concentrations detected were for DMTP (periurban group, range < LOD–1615 μg/g creatinine and P95–697.9 μg/g creatinine), DEP (periurban group, range < LOD–1177 μg/g creatinine and P95–386.5 μg/g creatinine) and DETP (rural group, range < LOD–365.7 μg/g creatinine and P95–141.5 μg/g creatinine). The least frequently detected metabolite was DBCA (detected in between 10.8 and 14.9% of samples). There were differences in the levels of metabolites detected among the groups (Figs. 2–4), which were further explored in regression analyses.

3.4. Binary logistic regression analysis: Exploring factors predicting risk of exposure

There were no significant differences in the risk of exposure for most metabolites. Significantly higher risks of exposure to fenitrothion, as measured by urinary 3-methyl-4-nitrophenol, were detected for periurban (OR 2.228, 95%CI 1.214–4.09 and p = 0.001) and rural (OR 2.648, 95%CI 1.426–4.917 and p = 0.002) groups as compared to urban group. Similarly, farm children were more likely to be exposed to fenitrothion (OR 2.226, 95%CI 1.131–4.381 and p = 0.02) than children living in metropolitan Adelaide. At the same time, children living in rural towns were more likely than children living in urban Adelaide to be exposed to deltamethrin based on the detection of deltamethrin specific metabolite, DBCA (OR 2.097, 95%CI 1.046–4.203 and p = 0.037).

Risk of exposure to some OP insecticides (based on the detection of DEP and DMTP) was higher in children whose parents reported contacting pesticides at work (OR 2.839, 95%CI 1.153–6.976 and p = 0.023 for DEP and OR 2.403, 95%CI 1.328–4.346, p = 0.004 for DMTP). Risk of exposure to the methyl-containing group of OP

Table 3

<table>
<thead>
<tr>
<th>Study population: descriptive statistics by sample group.</th>
</tr>
</thead>
<tbody>
<tr>
<td>All samples</td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Number of subjects</td>
</tr>
<tr>
<td>Mean child’s age±SD</td>
</tr>
<tr>
<td>Gender % boys</td>
</tr>
<tr>
<td>Mean family income ($× 10^3)</td>
</tr>
<tr>
<td>% reported parental occupational contact with pesticides</td>
</tr>
<tr>
<td>% have some agricultural activity within 50 m of residence</td>
</tr>
</tbody>
</table>

* ANOVA, urban—peri urban contrast.
** ANOVA, urban—rural contrast.
Kruskal–Wallis test.
insecticides (based on the detection of DMTP in urine samples) was higher in peri-urban (OR 2.459, 95% CI 1.376–4.393, p = 0.002) and rural children (OR 3.484, 95% CI 1.885–6.437, p = 0.001).

3.5. Linear regression analysis: Exploring factors predicting levels of exposure

Compared to the urban group, the periurban group had higher levels of urinary p-nitrophenol (β = 0.214, p = 0.046), 3-methyl-4-nitrophenol (β = 0.242, p = 0.028), 3-PBA (β = 0.267, p = 0.006), DEP (β = 0.320, p = 0.001) and DMTP (β = 0.259, p = 0.018). At the same time, compared to urban group, rural group had higher levels of 3-methyl-4-ITP (β = 0.319, p = 0.02), 3-PBA (β = 0.479, p = 0.001), DEP (β = 0.390, p = 0.001) and DMTP (β = 0.277, p = 0.04). Residing within 50 m or less from an agricultural activity was significantly associated with higher levels of urinary DCCA (β = 0.381, p = 0.002). Parental occupational exposure was significantly associated with higher levels of urinary DEP (β = 0.141, p = 0.021) and DMTP (β = 0.209, p = 0.003). Residing on a farm was only marginally significantly associated with higher levels of urinary DETP (β = 0.223, p = 0.05).

4. Discussion

Since the human body metabolises OP and PYR insecticides rapidly, urinary metabolites only reflect recent exposure. However, the detection of short lived urinary metabolites in a significant proportion of urine samples from a large group of subjects in a cross-sectional study would be indicative of ongoing (chronic) exposure in the population. As such, the results of this study demonstrate that there is widespread chronic exposure to OPs and PYRs in South Australian children. Furthermore, exposure to more than one chemical and contemporaneous exposure to chemicals from both OP and PYR groups is common. We did not aim to conduct an in depth investigation of the exposure pathways in the study population. We did, however, aim to explore some of the potentially most significant pathways. The fact that we had to exclude the only variable that was directly reflective of the domestic use of pesticides due to common misinterpretation of the question ‘Do you use pesticides at home?’ is a noteworthy limitation in our study. This misinterpretation is still meaningful in that it demonstrates very low level of ‘chemical literacy’ among the general population in South Australia. Combined with the significant number of OP and PYR products being available to the public, this finding warrants further investigation. Interestingly, the question about occupational contact to pesticides did not cause as much confusion, perhaps due to the higher awareness of work-related hazards, which stems from occupational health and safety education.

The differences in frequency of detection (reflecting risk of exposure) and levels (reflecting levels of exposure) of urinary metabolites among the groups were complex, but seemed to be reflective of the land use patterns and chemical availability. There is no way for us to know what chemicals were used on what type of crops in which areas at the time of the study. Australian authorities do not have effective controls over chemical use beyond the point of sale and there is no systematic data collection on chemical use in Australia.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Group</th>
<th>N</th>
<th>% samples with detectable levels</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>P25</th>
<th>P50</th>
<th>P75</th>
<th>P95</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCCA (cis + trans−)</td>
<td>Urban</td>
<td>115</td>
<td>35.7</td>
<td>8.1 ± 2.0</td>
<td>&lt;LOD–137.7</td>
<td>1.5</td>
<td>2.9</td>
<td>4.8</td>
<td>43.2</td>
</tr>
<tr>
<td></td>
<td>Periurban</td>
<td>111</td>
<td>36.0</td>
<td>9.0 ± 2.3</td>
<td>&lt;LOD–134.6</td>
<td>1.8</td>
<td>4.6</td>
<td>7.8</td>
<td>30.6</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>114</td>
<td>49.1</td>
<td>5.6 ± 6.5</td>
<td>&lt;LOD–32.8</td>
<td>2.4</td>
<td>3.6</td>
<td>5.4</td>
<td>24.0</td>
</tr>
<tr>
<td>DBCA</td>
<td>Urban</td>
<td>115</td>
<td>13.9</td>
<td>3.0 ± 2.5</td>
<td>&lt;LOD–8.4</td>
<td>1.1</td>
<td>2.0</td>
<td>5.1</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>Periurban</td>
<td>111</td>
<td>10.8</td>
<td>4.7 ± 4.5</td>
<td>&lt;LOD–16.5</td>
<td>2.3</td>
<td>2.9</td>
<td>5.4</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>114</td>
<td>14.9</td>
<td>5.2 ± 4.9</td>
<td>&lt;LOD–17.9</td>
<td>1.9</td>
<td>3.7</td>
<td>7.2</td>
<td>−</td>
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<tr>
<td>MPA</td>
<td>Urban</td>
<td>115</td>
<td>25.2</td>
<td>1.9 ± 3.0</td>
<td>&lt;LOD–16.4</td>
<td>0.7</td>
<td>1.1</td>
<td>1.8</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
<td>Periurban</td>
<td>111</td>
<td>28.8</td>
<td>3.7 ± 7.0</td>
<td>&lt;LOD–34.7</td>
<td>1.1</td>
<td>1.6</td>
<td>2.6</td>
<td>27.6</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>114</td>
<td>25.4</td>
<td>1.9 ± 0.7</td>
<td>&lt;LOD–3.4</td>
<td>0.6</td>
<td>1.1</td>
<td>1.5</td>
<td>2.9</td>
</tr>
<tr>
<td>3-PBA</td>
<td>Urban</td>
<td>115</td>
<td>80.9</td>
<td>1.2 ± 3.2</td>
<td>&lt;LOD–30.0</td>
<td>0.2</td>
<td>0.5</td>
<td>1.1</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>Periurban</td>
<td>114</td>
<td>86.5</td>
<td>1.4 ± 2.6</td>
<td>&lt;LOD–18.6</td>
<td>0.3</td>
<td>0.8</td>
<td>1.3</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>114</td>
<td>84.2</td>
<td>1.6 ± 1.7</td>
<td>&lt;LOD–12.9</td>
<td>0.6</td>
<td>1.1</td>
<td>1.7</td>
<td>4.8</td>
</tr>
</tbody>
</table>

Table 4
Summary statistics: OP metabolites, mean concentrations (µg/g creatinine) by sample group.

Table 5
Summary statistics: PYR metabolites, mean concentrations (µg/g creatinine) by sample group.
The periurban group was comprised of children living in the Adelaide Hills region. This region is known for mixed pastoral and horticultural (wine growing and orchards) land use mixed with hobby farms, were farmers and many hobby farmers hold restricted agricultural use licences. While no comprehensive data has ever been collected, this area is anecdotally known for overuse of agricultural chemicals.

The rural group was comprised of children living on the farms and in small townships in several areas of country SA. These are areas of mixed pastoral, broad acre agricultural and intensive horticultural land uses, where a large variety of chemicals would be used.

4.1. Risk of exposure

Risk of exposure to a wide range of PYR insecticides (as measured by urinary levels of 3-PBA) did not differ among the study groups and was not affected by the predictor variables used in the analysis. Similarly, the risk of exposure to chlorpyrifos (as determined by urinary levels of TCPy) and bifenthrin (as determined by urinary levels of MPA) was not influenced by the type or area of residence, proximity to agricultural activity or parental occupational exposure. This can be explained by the fact that chlorpyrifos as well as bifenthrin and many PYRs are used extensively in agriculture and are freely available to the general public. This finding may also be confounded by low frequency of detection of urinary DBCA (less than 15% of population).

4.2. Level of exposure

Overall, the best predictors for higher levels of urinary metabolites were periurban and rural areas of residence and reported parental occupational exposure. This was true for non-specific metabolites of PYRs (3-PBA) and OPs (DEP, DETP and DMTP), potentially reflecting higher exposure to a wide range of PYRs and OPs compared to children living in urban Adelaide or children whose parents did not report occupational contact with pesticides. These findings support the previously reported observations of increased exposures in populations residing in rural agricultural areas, and in families of pesticide handlers (Castorina et al., 2010; Fenske et al., 2002). This did not however hold true for exposure to commonly available and widely used chemicals, such as chlorpyrifos and bifenthrin. This indicates that reliance on indirect exposure assessment without direct exposure measurement can lead to exposure misclassification. A similar observation but in respect of children’s exposure to herbicides has been highlighted in an earlier study by Arbuckle et al. (2004).

Of specific metabolites, periurban group had higher levels of p-nitrophenol and 3-methyl-4-nitrophenol, reflecting higher exposure to parathion and parathion-methyl and fenithrothion. Parathion was not registered for use in SA at the time of the sample collection while parathion-methyl and fenithrothion were registered for licensed use only. The rural group, similar to the risk of exposure, had higher levels of urinary 3-methyl-4-nitrophenol reflecting higher levels of exposure to fenithrothion compared to urban children. Significantly higher levels of urinary DCCA in children living within 50 m from an agricultural activity would be reflective of higher exposure to permethrin, cypermethrin and cyfluthrin, all of which are used widely in

Table 6
Risk of exposure: results of the binary logistic regression analysis comparing exposed and non-exposed subjects. Significant associations reported only.

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Predictor variables</th>
<th>OR</th>
<th>95%CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lower</td>
<td>Upper</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-Methyl-4-nitrophenol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Periurban group</td>
<td>2.228</td>
<td>1.214</td>
<td>4.090</td>
<td>0.010</td>
</tr>
<tr>
<td>Rural group</td>
<td>2.648</td>
<td>1.426</td>
<td>4.917</td>
<td>0.002</td>
</tr>
<tr>
<td>Residing on a farm</td>
<td>2.226</td>
<td>1.131</td>
<td>4.381</td>
<td>0.020</td>
</tr>
<tr>
<td>DBCA</td>
<td>2.097</td>
<td>1.046</td>
<td>4.203</td>
<td>0.037</td>
</tr>
<tr>
<td>DEP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parental occupational exposure</td>
<td>2.839</td>
<td>1.155</td>
<td>6.976</td>
<td>0.023</td>
</tr>
<tr>
<td>DMTDP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Periurban group</td>
<td>2.459</td>
<td>1.376</td>
<td>4.393</td>
<td>0.002</td>
</tr>
<tr>
<td>Rural group</td>
<td>3.484</td>
<td>1.885</td>
<td>6.437</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Parental occupational exposure</td>
<td>2.403</td>
<td>1.328</td>
<td>4.346</td>
<td>0.004</td>
</tr>
</tbody>
</table>

a Compared to urban group.

b Compared to subjects residing in the metropolitan Adelaide.
agriculture and are available for domestic use. No other factors were associated with urinary DCCA. Similar to the results of the analysis of the risk of exposure, there were no significant differences in the urinary levels of TCPy and MPA reflecting no significant differences in levels of exposure to chlorpyrifos and bifenthrin among the study groups. Unlike the risk of exposure to deltamethrin, the levels of urinary DBCA did not differ significantly by study group and were not associated with any other factor. This may be due to low frequency of detection of urinary DBCA in the study population.

4.3. Dietary exposure

Apart from chemical use in the area of residence or in the home, consumption of contaminated food may be a significant contributor to the overall exposure. OP and PYR residues have been measured in foods consumed by young children in many studies (Clayton et al., 2003; Fenske et al., 2002; MacIntosh et al., 2001). Curl et al. (2003a) and Lu et al. (2006) demonstrated lower levels of urinary metabolite excretion in children on organic diets. Lu et al. (2010) detected eleven OP and three PYR chemicals in their 24-h duplicate food sample study.

Dietary exposure to agricultural and veterinary chemicals has been recently explored in Australia in the 23rd Australian Total Diet Survey (ATDS) (FSANZ, 2011). Three PYR chemicals were reported in the 23rd ATDS: allethrin (in two food samples), bifenthrin (in one fruit sample) and permethrin (in one sample). Of the OPs, chlorpyrifos-methyl, chlorpyrifos and dimethoate were detected in several food samples, also detected were methamidfos and omethoate (each in one sample). While it appears that occurrence of pesticide residues on food is low in Australia, the 23rd ATDS also reported that chlorpyrifos residues in food contributed up to 20% of acceptable daily intake (ADI) in the 2–5 years old group (estimated at 90th percentile). This would account to approximately 0.6 μg/kg bw/day of chlorpyrifos in the general population of Australian children aged 2–5 years old. For comparison, Kawahara et al. (2007), estimated dietary intake of chlorpyrifos in children aged 3–6 years old residing in Tokyo at 0.007 μg/kg bw/day. Similarly, estimates for the US population based on the results of the US Food and Drug Administration’s Total Diet Study were around 0.03 μg/kg bw/day (estimated at 90th percentile) (Wright et al., 2002). Both comparisons seem to imply that, although below the regulatory levels of concern (below ADI), the Australian population of young children has higher levels of dietary exposure to chlorpyrifos, than their peers in Japan and the US. This may, in part, explain why there was little difference in levels of urinary TCPy was common among the study groups in our study.

Drinking water may be a contributor to the overall exposure. Contemporary water treatment methods can reliably remove pesticide residues from centralised drinking water supplies. Residents in periurban and rural SA, however, often rely heavily or solely on rainwater for their drinking water. We could not find any published reports of investigations into pesticide residues in domestic rainwater tanks in SA.

4.4. Preformed metabolites

The validity of using urinary metabolites as a golden standard of exposure assessment has been questioned and debated due to the fact that for some chemicals, environmental degradation of parent compounds may yield the same metabolites as human metabolism. TCPy has been shown to be common in children’s environment by Morgan et al. (2005) and has also been detected in the ambient air (Raina and Sun, 2008). DAPs have been detected in fruit juices (Lu et al., 2005). It is therefore possible that some proportion of urinary metabolites can come from ingesting preformed metabolites with food and environmental media rather than ingesting the parent compound (active insecticide). TCPy, DEP and DETP have been shown to be rapidly absorbed and eliminated with limited metabolism by rats when administered orally (Timchalk et al., 2007).

It is not clear what proportion of TCPy and DAPs excreted in urine would have come from actual parent compounds, their environmental oxon products or the preformed metabolites. This would potentially depend on route of exposure, environmental conditions and the individual’s metabolic make up. The preformed metabolites contribution needs to be taken into account in the future case–control and cohort studies, where good understanding of all factors affecting intra- and inter-individual variability is essential for investigating causative relationships between exposure and health effects. However, it is believed that a population-based cross sectional studies exploring associative relationships, such as the one presented here, would not dramatically suffer from potential interference from preformed metabolites.

4.5. International comparison

Urinary metabolite concentrations measured in this study do not have clinical guideline values to compare to in order to ascertain potential public health significance of the observed exposure levels. Also, being the first Australian study of its kind, we had no ‘background’ data for Australian population to compare to. The only option for conceptualising our findings was to compare the results of our study to the available published international data.

We compared our results with available published data on the concentrations of urinary DAPs in children under 6 years of age in Germany (Heudorf et al., 2004) and children aged 6 to 11 in the USA (NCEH, 2009). Figs. 5–7 demonstrate that SA children have higher exposures than both German and the US children. We used the international data in the formats they were available: German data were reported in mean μg/L (Heudorf et al., 2004), while US data were reported in μg/g creatinine by percentile group. While the comparison is of qualitative nature since it was not possible to conduct statistical comparison, the differences appear to be substantial. South Australian children appear to have substantially higher exposure levels to a wide range of OP and PYR pesticides than German and the US children.

The analytical methods employed by this study differed from methods used in Heudorf et al. (2004) and NCEH (2009) and were not cross validated against existing methods. Such validation was not possible at the time due mostly to resource limitations. Besides, Australia did not and still does not have another laboratory actively involved in biological monitoring of environmental exposures to pesticides in the general population, which could be used for cross-validation purposes. We have put every effort to adhere to good laboratory practices in our method development. While it is possible that some proportion of the differences seen between Australian, US and German populations may be due to potential methodological bias, the authors do not believe that such bias would be of significant magnitude. There is a need for thorough international cross-validation of the existing methods for future studies.

To further conceptualise this comparison, we looked at the number of registered OP and PYR-containing products in Australia, Germany, the US and the UK. Several international regulatory databases were examined in the preparation of this paper (APVMA, 2012; CERIS, 2012; HSE, 2012; National Ministry of Food, Agriculture and Consumer Protection, 2012). Table 7 summarises the numbers of OP and PYR (active constituents only) registered in Australia, UK and USA with numbers of actives available to the general public. It is clear from the numbers that Australia has substantially more OPs and PYRs registered for both restricted and domestic use by the general public than other comparable developed countries such as Germany, the USA and UK (Table 8). This difference in chemicals variety and in general availability to the general public in particular may be contributing to the differences seen in urinary metabolite concentrations.
**Fig. 5.** Concentration (by percentile groups, μg/g creatinine) of selected DAPs in urine samples from South Australian and US children (US samples collected 2003–2004, NCEH, 2009).

**Fig. 6.** Concentrations of selected specific metabolites (by percentile groups, μg/g creatinine) in urine samples from South Australian and US children (US samples collected 2001–2002, NCEH, 2009).
4.6. Further research

This study raises more questions than provides answers. Further research is needed to thoroughly investigate exposure pathways in Australian general population. Further studies should combine environmental monitoring, biological monitoring and testing of food for both pesticide residues and pre-formed metabolites. Repeated sampling spanning different seasons, thorough evaluation of domestic use of pesticides-containing products and cross-validation of analytical procedures are needed. Participation in an international research effort, such as AGRICOH (Leon et al., 2011) could be very beneficial for Australia.

5. Conclusions

Our findings demonstrate widespread chronic exposure to OP and PYR pesticides in young children in South Australia. There are differences in exposure risks and levels in different populations, but only for some chemicals, while the exposure seems to be ubiquitous for other chemicals. There appear to be higher levels of exposure in the study population as compared to similar populations in the US and Germany. Further research into pesticide exposure in the general population in Australia is warranted, as is public discussion on the current approaches to pesticide regulation and risk assessment and the widespread availability of neurotoxic pesticides to the general public.

Competing interests

Authors declare that they have no competing interests.

Acknowledgements

Authors express their appreciation to the participating families. The urine sample analyses were performed in Flinders Advanced Analytical Laboratory with invaluable help from Dr Daniel Jardine. Analytical standards for DAPs were kindly donated by the WorkCover Laboratories NSW. Kateryna Babina was supported by the FUSA Postgraduate Award (funded by the Flinders University). The study was funded by the Financial Markets for Children Foundation.

Table 7
Level of exposure: results of linear regression analysis of levels of exposure. Comparison among exposed subjects only (log-transformed creatinine corrected concentrations). Significant associations reported only.

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Predictor variables</th>
<th>β</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-Nitrophenol</td>
<td>Periurban group</td>
<td>0.214</td>
<td>2.007</td>
<td>0.046</td>
</tr>
<tr>
<td>3-Methyl-p-nitrophenol</td>
<td>Periurban group</td>
<td>0.242</td>
<td>2.218</td>
<td>0.028</td>
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<td></td>
<td>Rural group</td>
<td>0.319</td>
<td>2.344</td>
<td>0.020</td>
</tr>
<tr>
<td>DCCA</td>
<td>Periurban group</td>
<td>0.267</td>
<td>2.745</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>Rural group</td>
<td>0.479</td>
<td>4.051</td>
<td>0.000</td>
</tr>
<tr>
<td>3-PBA</td>
<td>Periurban group</td>
<td>0.214</td>
<td>2.007</td>
<td>0.046</td>
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<td>Rural group</td>
<td>0.319</td>
<td>2.344</td>
<td>0.020</td>
</tr>
<tr>
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<td>Periurban group</td>
<td>0.320</td>
<td>3.512</td>
<td>0.001</td>
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<td>Rural group</td>
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<td>Parental occupational exposure</td>
<td>0.141</td>
<td>2.315</td>
<td>0.021</td>
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<td>DETP</td>
<td>Residing on the farm</td>
<td>0.223</td>
<td>1.971</td>
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<td>Periurban group</td>
<td>0.259</td>
<td>2.375</td>
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<td></td>
<td>Rural group</td>
<td>0.277</td>
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<td></td>
<td>Parental occupational exposure</td>
<td>0.209</td>
<td>2.964</td>
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</tr>
</tbody>
</table>

* Compared to urban group.

Table 8
Summary of registered OP and PYR chemicals in Australia, USA, Germany and UK (May 2012).

<table>
<thead>
<tr>
<th>Registered chemicals</th>
<th>Australia</th>
<th>USA</th>
<th>Germany</th>
<th>UK</th>
</tr>
</thead>
<tbody>
<tr>
<td>OP Active constituents</td>
<td>30</td>
<td>10</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Active constituents available to the general public</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>PYR Active constituents</td>
<td>22</td>
<td>15</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Active constituents available to the general public</td>
<td>18</td>
<td>9</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>
References


FSANZ. Food Standards Australia New Zealand. The 23rd Australian Total Diet Survey. [accessed December 2011].


