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RESEARCH ARTICLE

Cross-sectional biomonitoring study of pesticide exposures in Queensland, Australia, using pooled urine samples

AL Heffernan[1,2] · K English[3,4] · LML Toms[5] · AM Calafat[6] · L Valentin-Blasini[6] · P Hobson[7] · S Broomhall[8] · RS Ware[4,9] · P Jagals[4,9] · PD Sly[4] · JF Mueller[1]

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Abstract A range of pesticides are available in Australia for use in agricultural and domestic settings to control pests, including organophosphate and pyrethroid insecticides, herbicides, and insect repellents, such as N,N-diethyl-meta-toluamide (DEET). The aim of this study was to provide a cost-effective preliminary assessment of background exposure to a range of pesticides among a convenience sample of Australian residents. De-identified urine specimens stratified by age and sex were obtained from a community-based pathology laboratory and pooled (n = 24 pools of 100 specimens). Concentrations of urinary pesticide biomarkers were quantified using solid-phase extraction coupled with isotope dilution high-performance liquid chromatography–tandem mass spectrometry. Geometric mean biomarker

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✉ AL Heffernan
amy.heffernan@uqconnect.edu.au

K English
k.english@uq.edu.au

LML Toms
leisamaree.toms@qut.edu.au

AM Calafat
aic7@cdc.gov

L Valentin-Blasini
lvb5@cdc.gov

P Hobson
Peter_Hobson@snp.com.au

S Broomhall
Sara.Broomhall@environment.gov.au

RS Ware
r.ware@uq.edu.au

P Jagals
p.jagals@uq.edu.au

PD Sly
p.sly@uq.edu.au

JF Mueller
j.mueller@uq.edu.au

[1] National Research Centre for Environmental Toxicology, The University of Queensland, 39 Kessels Rd, Coopers Plains, QLD 4108, Australia

[2] The Florey Institute of Neuroscience and Mental Health, Parkville, VIC, Australia

[3] School of Medicine, The University of Queensland, Brisbane, QLD, Australia

[4] Children's Health and Environment Program, Child Health Research Centre, The University of Queensland, Brisbane, QLD, Australia

[5] School of Public Health and Social Work and Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, QLD, Australia

[6] Centers for Disease Control and Prevention, Atlanta, GA, USA

[7] Sullivan Nicolaides Pathology Taringa, Taringa, QLD, Australia

[8] Australian Government Department of the Environment, Canberra, ACT, Australia

[9] School of Public Health, The University of Queensland, Brisbane, QLD, Australia

concentrations ranged from <0.1 to 36.8 ng/mL for organophosphate insecticides, <0.1 to 5.5 ng/mL for pyrethroid insecticides, and <0.1 to 8.51 ng/mL for all other biomarkers with the exception of the DEET metabolite 3-diethylcarbamoyl benzoic acid (4.23 to 850 ng/mL). We observed no association between age and concentration for most biomarkers measured but noted a "U-shaped" trend for five organophosphate metabolites, with the highest concentrations observed in the youngest and oldest age strata, perhaps related to age-specific differences in behavior or physiology. The fact that concentrations of specific and non-specific metabolites of the organophosphate insecticide chlorpyrifos were higher than reported in USA and Canada may relate to differences in registered applications among countries. Additional biomonitoring programs of the general population and focusing on vulnerable populations would improve the exposure assessment and the monitoring of temporal exposure trends as usage patterns of pesticide products in Australia change over time.

Keywords Biomonitoring · Urine · Pesticides · Organophosphate · Pyrethroid · Children

Introduction

Pesticides are used in agricultural and domestic settings to control pests. Pesticides include but are not limited to insecticides, such as pyrethroids and organophosphates, herbicides, and insect repellents. In Australia, limited information exits about use and availability of insect repellents, but a range of pyrethroid and organophosphate pesticides are commonly available (Table 1). Pyrethroids, the most prevalent insecticide class on the Australian market, are found in an array of domestic (e.g., aerosols, medical treatments, veterinary products) and agricultural pest control products (APVMA 2015). Diazinon, maldison/malathion, and chlorpyrifos, the most commonly available organophosphates for domestic applications, are found in spray products, veterinary products, headlice treatments, and garden treatments (APVMA 2015). Chlorpyrifos and dimethoate are the most commonly found organophosphate residues in foods sold in Australia (FSANZ 2011).

Pyrethroids and organophosphates are neurotoxins, and chronic early life exposure has been associated with a range of adverse health effects in humans, including cognitive deficits (Bouchard et al. 2011; Rauh et al. 2012; Koureas et al. 2012; Shelton et al. 2014) and increased incidence of childhood cancers (Roberts and Karr 2012; Turner et al. 2010). Routes of pesticide exposure include dermal, inhalation, and dietary/non-dietary ingestion, with food residues being an important source that varies markedly by region (Becker et al. 2006; Riederer et al. 2008; Morgan 2012; Trunnelle et al. 2014). Additional exposure pathways and sources specific to young children, such as mouthing and lower breathing zone compared to adults (Tulve et al. 2002; WHO 2011), may place young children at greater risk of both acute and chronic pesticide exposures; in one survey of insecticide-related calls to an Australian poison control center, children under the age of 4 years accounted for half of all calls (English et al. 2015).

Table 1 Pyrethroid and organophosphate insecticides commonly available for domestic and agricultural use in Australia identified via Public Chemical Registration Information System Search

Pyrethroids	Organophosphates
Allethrin	Azinphos-methyl
Bioallethrin	Chlorfenvinphos
Bifenthrin	Chlorpyrifos
Bioresmethrin	Diazinon
Cyfluthrin	Dichlorvos
Cyhalothrin	Dimethoate
Cypermethrin	Ethion
Deltamethrin	Fenamiphos
Esbiothrin	Fenitrothion
Esfenvalerate	Maldison/malathion
Flumethrin	Methidathion
Fluvalinate	Mevinphos
Imiprothin	Omethoate
Permethrin	Phorate
Phenothrin	Prothiofos
Pyrethrin	Profenofos
Pyrethrum	Propetamphos
Transfluthrin	Temephos
Tetramethrin	Terbufos
	Trichlorfon

Source: APVMA (2015)

The number of pesticides, particularly insecticides and herbicides, registered for domestic and agricultural use in Australia exceeds those in other countries and regions, including the USA and the European Union (Babina et al. 2012). Annual insecticide and herbicide sales in Australia exceeded 1.8 billion Australian dollars in 2012–2013 financial year (APVMA 2014). However, as no integrated pesticide usage reporting system exists in Australia, quantitative data are scarce (Radcliffe 2002).

Biomonitoring is a tool increasingly used for exposure assessment (NRC 2006), and urinary metabolites are commonly used biomarkers of exposure for non-persistent chemicals. Biomonitoring data of pesticides exist for populations in Europe (Becker et al. 2006; Schettgen et al. 2002; Becker et al. 2006; Roca et al. 2014), North America (CDC 2015; Fortin et al. 2008), and China (Guodong et al. 2012), but there are no large-scale data currently available for the general Australian population. Here, we present a preliminary age and sex-stratified characterization of exposure to select organophosphate insecticides, pyrethroid insecticides, the insect repellent N,N-diethyl-meta-toluamide (DEET), and the

herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in a convenience sample of Australian residents using a simple and cost-effective pooled urine-sampling approach.

Materials and methods

Study population and sample collection

For this cross-sectional study, de-identified specimens were obtained from a community-based pathology laboratory (Sullivan Nicolaides Pathology, Taringa) from surplus-archived urine that had been collected and analyzed as part of routine testing throughout the state of Queensland, Australia, with the majority of samples collected from sub-tropical South East Queensland. Urine specimens were collected from November 2012 to November 2013 in sterile polyethylene urine specimen containers, refrigerated at 4 °C for up to 3 days, and then frozen. As this was a pre-existing, convenience population, no specific sampling protocols were employed. This work was approved by the University of Queensland ethics committee (approval number 2013000397). The involvement of the Centers for Disease Control and Prevention (CDC) laboratory was determined not to constitute engagement in human subject research.

Pooling protocol

Descriptive information about each specimen was limited to date of sample collection, sex, and date of birth of the individual. Before pooling, samples were stratified by age and sex into the following strata: 0–4, 5–14, 15–29, 30–44, 45–59, and >60 years. The mean age of each pool was calculated from the average age of the individuals making up that pool. A total of 2400 individual specimens were combined into 24 pools, with 100 individual specimens contributing to each pool; there were 2 pools for each of the 12 age-sex strata. Specimens were pooled based on volume, where each individual in the pool contributed the same volume; thus, the concentration measured in each pool is equivalent to the arithmetic mean of the concentration in each individual sample contributing to the pool (Caudill 2010). During pooling, individual urine specimens were thawed, homogenized, and aliquoted, after which the pooled sample was well mixed, divided into smaller aliquots, and frozen until analysis. A synthetic urine sample was included as a procedural blank (Calafat and Needham 2009). No measures of creatinine or specific gravity were available for individual samples.

Chemical analysis

Pooled urine samples were shipped on ice to the CDC (Atlanta, USA) for chemical analysis and analyzed for several pesticide biomarkers (Table 2), specifically six non-specific organophosphate metabolites, dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), dimethyl dithiophosphate (DMDTP), diethyl phosphate (DEP), diethyl thiophosphate (DETP), and diethyl dithiophosphate (DEDTP); four specific organophosphate metabolites, 3,5,6-trichloro-2-pyridinol (TCPY), malathion dicarboxylic acid (MDA), 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPY), and paranitrophenol (PNP); four pyrethroid metabolites, 3-phenoxybenzoic acid (3-PBA), 4-fluoro-3-phenoxybenzoic acid (4-F-3-PBA), cis-3-(2,2-dibromovinyl)-2,2-dimethyl cyclopropane carboxylic acid (DBCA), and trans-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylic acid (trans-DCCA); two phenoxy acid herbicides, 2,4-D and 2,4,5-T; and DEET and its metabolites 3-diethylcarbamoyl benzoic acid (DCBA) and N,N-diethyl-3-(hydroxymethyl) benzamide (DHMB). For analysis, we used 96-well plate-based or online solid-phase extraction and high-performance liquid chromatography–isotope dilution–tandem mass spectrometry approaches as described before (Kuklenyik et al. 2013; Davis et al. 2013; Odetokun et al. 2010). Accuracy and precision for each analytical run were monitored through the use of calibration standards, reagent blanks, and quality control materials of high and low concentrations. The limits of detection (LODs) ranged from 0.08 to 0.50 ng/mL and are listed in Table 3.

Statistical analysis

The influence of age (in years) on chemical concentration was assessed via curvilinear regression, as follows:

$$\text{Concentration} = A + \beta_1 \times \text{Age} + \beta_2 \times \left(\text{Age}–\text{Mean age}\right)^2$$

Concentrations below the LOD were replaced with the LOD divided by the square root of 2 (Hornung and Reed 1990). All regression analyses were conducted using Stata statistical software v12.1 (StataCorp, College Station, TX, USA). Criteria for significance were set as $p < 0.05$.

Results

Organophosphate insecticide metabolites were detected in >96 % of pooled samples with the exception of DMDTP (75 %), DETP (83 %), and DEDTP, which was not detected in any sample. Overall, the concentrations of these organophosphate metabolites were relatively low (geometric mean [GM] 13.6, 10.6, 0.41, 6.18, and 1.25 ng/mL for DMP, DMTP, DMDTP, DEP, and DETP, respectively) and ranged

Table 2 Compound abbreviations, parent chemicals, and metabolites by chemical class

Abbreviation	Full name	Parent chemical	Chemical class
DMP	dimethyl phosphate	Organophosphate insecticides	Organophosphate
DMTP	Dimethyl thiophosphate	Organophosphate insecticides	Organophosphate
DMDTP	Dimethyl dithiophosphate	Organophosphate insecticides	Organophosphate
DEP	Diethyl phosphate	Organophosphate insecticides	Organophosphate
DETP	Diethyl thiophosphate	Organophosphate insecticides	Organophosphate
DEDTP	Diethyl dithiophosphate	Organophosphate insecticides	Organophosphate
TCPY	3,5,6-Trichloro-2-pyridinol	Chlorpyrifos, chlorpyrifos-methyl	Organophosphate
MDA	Malathion dicarboxylic acid	Malathion	Organophosphate
IMPY	2-Isopropyl-4-methyl-6-hydroxypyrimidine	Diazinon	Organophosphate
PNP	Paranitrophenol	Parathion, methyl parathion	Organophosphate
4-F-3-PBA	4-Fluoro-3-phenoxybenzoic acid	Cyfluthrin	Pyrethroid
DBCA	cis-3-(2,2-dibromovinyl)-2,2-dimethyl cyclopropane carboxylic acid	Deltamethrin	Pyrethroid
3-PBA	3-Phenoxybenzoic acid	Cyhalothrin, cypermethrin, deltamethrin, fenpropathrin, permethrin, tralomethrin	Pyrethroid
trans-DCCA	trans-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylic acid	Permethrin, cypermethrin, cyfluthrin	Pyrethroid
2,4-D	2,4-Dichlorophenoxyacetic acid	2,4-Dichlorophenoxyacetic acid (and its esters)	Phenoxy acid herbicide
2,4,5-T	2,4,5-Trichloro phenoxyacetic acid	2,4,5-Trichloro phenoxyacetic acid	Phenoxy acid herbicide
DEET	N,N-diethyl-meta-toluamide	N/A	DEET
DCBA	3-(diethylcarbamoyl) benzoic acid	N,N-diethyl-meta-toluamide	DEET
DHMB	N,N-diethyl-3-(hydroxymethyl) benzamide	N,N-diethyl-meta-toluamide	DEET

from <0.1 to 19.2 ng/mL for DAPs and from <0.1 to 3.09 ng/mL for the specific metabolites (GM 1.00, 0.38, and 1.76 ng/mL for MDA, IMPY, and PNP, respectively). The highest concentrations were for TCPY with GM 23.0 ng/mL and range 2.0–36.8 ng/mL (Table 3).

Pyrethroid metabolites DBCA, 3-PBA, and trans-DCCA were detected at GM 1.25, 1.21, and 1.89 ng/mL, respectively, with a maximum concentration of 5.51 ng/mL for trans-DCCA in females, 5–15 years (Table 3). 4-F-3-PBA was undetectable in all samples. Similarly, phenoxy acid herbicide metabolites 2,4-D and 2,4,5-T were low (maximum concentration 7.83 ng/mL and undetectable in all pools, respectively). The detection frequency of parent compound DEET was low (17 %), but metabolites DCBA and DHMB were detected in all pooled samples, with GM concentrations of 65.9 and 0.83 ng/mL, respectively (Table 4). All analytes were non-detectable in the synthetic urine sample with the exception of DCBA (0.55 ng/mL), a concentration quite close to the LOD for this analyte (0.48 ng/mL). Because the lowest DCBA concentration detected in the pools was nearly one order of magnitude greater (4.23 ng/mL; Table 3), we applied no blank correction to any DCBA results.

The association of age with urinary concentrations was examined using curvilinear modeling (see Supplementary Material 1 for regression coefficients). There was a significant association with age only for the organophosphate metabolites DMDTP, DETP, DMTP, TCPY, and PNP (Fig. 1). The percentage of variability explained by the model was 56, 42, 27, 28, and 22 %, respectively (Supplementary Material 1). The association of age with concentration was generally U-shaped, with highest concentrations at increasing extremes of age (i.e., youngest and oldest age strata), as well as a slight additional increase in concentration for the younger age groups only.

Discussion

For the first time, we report age- and sex-stratified urinary metabolites of organophosphate and pyrethroid insecticides, an insect repellent, and select phenoxy acid

Table 3 Summary of pool characteristics and chemical concentration (ng/mL) for organophosphate and pyrethroid insecticide metabolites, phenoxy acid herbicides, and DEET and its metabolites

Pool number	Age strata (years)	Average age (years)	Sex	Organophosphate insecticides										Pyrethroid insecticides				Phenoxy acid herbicides		DEET		
				Non-specific metabolites						Specific metabolites												
				DMP	DMTP	DMDTP	DEP	DETP	DEDTP	TCPY	MDA	IMPY	PNP	4-F-3-PBA	DBCA	3-PBA	trans-DCCA	2,4-D	2,4,5-T	DEET	DCBA	DHMB
1	0–4	2.93	M	14.5	21.1	1.64	5.54	2.24	<LOD	36.8	1.71	<LOD	2.14	<LOD	1.14	2.02	2.50	0.46	<LOD	3.43	33.2	0.32
2		2.74	M	11.6	11.6	1.53	5.54	2.62	<LOD	24.8	1.31	0.27	1.63	<LOD	1.14	0.95	1.54	0.38	<LOD	<LOD	25.1	0.31
3		3.33	F	12.6	14.2	1.35	5.75	3.49	<LOD	23.7	0.96	0.31	2.61	<LOD	1.17	1.97	3.24	0.33	<LOD	0.45	217	1.64
4		3.24	F	9.03	8.20	0.58	6.57	2.34	<LOD	12.0	0.70	0.26	1.44	<LOD	0.79	1.10	1.61	0.31	<LOD	<LOD	16.8	0.18
5	5–14	8.83	M	15.7	12.4	0.83	6.35	2.59	<LOD	28.8	0.96	0.43	1.68	<LOD	1.70	1.46	2.44	0.70	<LOD	<LOD	368	2.13
6		9.21	M	18.2	12.5	0.96	7.45	4.20	<LOD	23.2	0.73	0.22	1.59	<LOD	1.94	0.76	1.40	0.42	<LOD	<LOD	61.6	0.60
7		8.74	F	14.0	12.4	0.79	5.41	1.06	<LOD	28.5	0.80	0.38	1.55	<LOD	1.23	4.01	5.51	0.38	<LOD	<LOD	769	4.52
8		9.54	F	14.3	11.1	1.00	6.66	2.89	<LOD	25.1	0.78	0.36	1.68	<LOD	0.93	1.79	3.07	0.45	<LOD	<LOD	62.6	0.63
9	15–29	24.3	M	19.2	9.77	0.71	5.01	1.03	<LOD	23.5	0.93	0.45	1.09	<LOD	1.29	0.88	1.15	0.41	<LOD	<LOD	20.6	0.42
10		24.0	M	12.8	7.87	<LOD	4.80	0.99	<LOD	19.7	0.87	0.37	1.04	<LOD	0.68	0.86	1.62	0.38	<LOD	<LOD	53.1	0.70
11		24.0	F	19.2	12.9	0.80	10.4	2.36	<LOD	29.2	1.55	0.41	1.91	<LOD	1.87	2.09	2.81	0.55	<LOD	<LOD	21.3	0.45
12		23.4	F	15.2	11.8	0.64	6.25	2.22	<LOD	23.3	0.80	0.35	1.4	<LOD	1.17	1.02	2.04	0.32	<LOD	<LOD	11.0	0.29
13	30–44	37.8	M	15.4	13.3	<LOD	5.79	1.45	<LOD	19.0	0.77	1.29	1.61	<LOD	1.09	1.04	1.52	0.45	<LOD	<LOD	149	1.42
14		37.3	M	9.60	10.0	<LOD	3.74	1.13	<LOD	19.0	0.84	0.49	2.94	<LOD	0.75	2.04	2.48	0.35	<LOD	<LOD	26.1	0.29
15		36.7	F	12.3	7.64	0.41	5.51	<LOD	<LOD	18.1	0.80	0.30	1.28	<LOD	1.26	0.75	1.34	0.40	<LOD	<LOD	10.1	0.24
16		36.8	F	16.4	5.55	<LOD	6.95	<LOD	<LOD	16.6	0.81	0.37	1.26	<LOD	1.63	0.96	1.52	0.41	<LOD	<LOD	51.8	0.52
17	45–59	52.9	M	13.9	9.60	0.69	5.63	1.69	<LOD	21.3	2.97	<LOD	1.47	<LOD	0.71	0.82	1.31	0.60	<LOD	<LOD	197	2.03
18		53.2	M	15.0	9.54	<LOD	6.24	1.24	<LOD	24.8	1.79	0.36	2.19	<LOD	1.51	1.69	2.36	7.83	<LOD	<LOD	850	4.75
19		53.3	F	6.75	3.89	0.25	12.4	<LOD	<LOD	20.0	1.28	0.45	3.09	<LOD	1.68	0.84	1.55	0.48	<LOD	0.19	304	3.69
20		53.0	F	12.3	9.76	0.91	3.88	1.54	<LOD	14.7	0.92	0.26	1.49	<LOD	0.97	0.51	1.24	0.33	<LOD	<LOD	363	8.25
21	>60	73.7	M	16.0	17.8	0.71	5.91	1.37	<LOD	31.5	0.78	0.36	2.34	<LOD	1.81	2.37	3.78	0.69	<LOD	<LOD	101	1.39
22		71.9	M	17.4	14.9	<LOD	10.0	2.87	<LOD	32.3	1.15	0.69	1.47	<LOD	1.34	1.46	1.42	0.63	<LOD	<LOD	11.5	0.24
23		75.1	F	9.99	6.28	0.29	4.89	<LOD	<LOD	24.8	0.73	0.38	2.42	<LOD	2.33	0.91	1.23	0.29	<LOD	<LOD	4.23	0.17
24		76.1	F	14.3	16.1	0.69	7.57	1.17	<LOD	28.3	0.80	0.34	3.07	<LOD	1.63	0.58	1.12	0.46	<LOD	0.55	288	3.49
	GM	21.4		13.6	10.6	0.41	6.18	1.25	NC	23.0	1.00	0.33	1.76	NC	1.25	1.21	1.89	0.48	NC	NC	65.9	0.83
	LOD			0.50	0.10	0.10	0.10	0.25	0.50	0.10	0.50	0.10	0.10	0.10	0.50	0.10	0.60	0.15	0.10	0.08	0.48	0.09

Each pool represents 100 individuals

GM geometric mean, *LOD* limit of detection, *NC* not calculated as detection frequency was <60 %

Table 4 Summary of selected biomonitoring studies of specific and non-specific organophosphate pesticide, pyrethroid insecticide, and phenoxy acid herbicide metabolites in non-exposed populations since the year 2000. Concentrations are reported in ng/mL

Country	Year of sample collection	Population age (years) (*n*)	Measure of central tendency	Organophosphate pesticides										Pyrethroid insecticides				References
				Non-specific metabolites						Specific metabolites								
				DMP	DMTP	DMDTP	DEP	DETP	DEDTP	TCPY	MDA	IMPY	PNP	4-F-3-PBA	DBCA	3-PBA	trans-DCCA	
Australia	2012–2013	0–>60 (24)	GM	13.6	10.6	0.74	6.18	1.84	NC	23.0	1.00	0.38	1.76	NC	1.25	1.21	1.89	This study
Australia[a]	2003–2006	3–6 (115)[b]	AM	–	20.2	12.4	7.4	8.3	–	21.5	–	–	5.1	–	3.0	1.2	8.1[c]	Babina et al. (2012)
Australia	NR	NR (48)	AM	9	10	1	3	2	1	–	–	–	–	–	–	–	–	Oglobline et al. (2001)
Canada[d]	2007–2009	6–79 (~2600)[e]	GM	3.02	2.07	–	2.38	–	–	–	–	–	–	–	–	–	–	Haines and Murray (2012)
				2.91	1.99	–	2.22	–	–	–	–	–	–	–	–	–	–	
		6–79 (~2600)[f]																
Canada	2005	6–12 (120)	Median	–	–	–	–	–	–	–	–	–	–	<0.005	<0.006	0.20	0.24	Fortin et al. (2008)
		18–64 (120)		–	–	–	–	–	–	–	–	–	–	<0.005	<0.006	0.19	0.24	
China	2008	2 (301)	GM	2.52	1.56	–	1.78	3.18	NC	–	–	–	–	–	–	–	–	Guodong et al. (2012)
France	NR	24–62 (39)	AM	–	–	–	–	–	–	–	–	–	–	NC	–	0.77	0.50	Le Grand et al. (2012)
Germany	NR	0–65 (1177)	Median	–	–	–	–	–	–	–	–	–	–	<0.2	<0.1	–	0.4	Schettgen et al. (2002)
Germany	2001–2002	0–17 (363)	GM	13.5	8.23	<1.0	3.31	1.12	<1.0	–	–	–	–	<0.1	<0.1	0.31	0.20	Becker et al. (2006)
Israel	2010–2012	27–54 (120)	GM	14.4	9.5	0.4	2.1	0.5	0.03	–	–	–	–	–	–	–	–	Abdeen et al. (2015)
		21–35 (148)[g]	GM	3.7	8.6	0.4	2.9	0.9	0.02	–	–	–	–	–	–	–	–	
			GM	10.3	12.7	0.5	2.7	0.6	0.09	–	–	–	–	–	–	–	–	
		26–36 (72)[g]																
Israel	2011	20–74 (247)	GM	13.5	8.0	0.4	1.9	0.6	0.03	–	–	–	–	–	–	–	–	Berman et al. (2013)
Puerto Rico	2010–2012	18–40 (54)[g]	GM	–	–	–	–	–	–	–	–	–	–	<0.1	<0.5	0.2	<0.6	Lewis et al. (2014)
Puerto Rico	2010–2012	18–40 (54)[g]	GM	1.4	0.8	0.2	0.9	0.5	<0.1	0.4	<0.2	<0.1	0.5	–	–	–	–	Lewis et al. (2015)
Poland	2010–2011	5–77 (132)	AM	–	–	–	–	–	–	–	–	–	–	–	NC	0.39	NC	Wielgomas et al. (2013)
			GM	–	–	–	–	–	–	–	–	–	–	–	NC	0.26	NC	
Poland[h]	2012	<18 (128)	GM	–	–	–	–	–	–	–	–	–	–	–	NR	0.25	NR	Weilgomas and Piskunowicz (2013)
		>18 (134)		–	–	–	–	–	–	–	–	–	–	–	NR	0.20	NR	
		All (261)		–	–	–	–	–	–	–	–	–	–	–	NC	0.22	NC	
Spain[a]	2010	6–11 (125)	AM	8.60	5.40	0.95	4.00	1.70	<0.4	5.65	–	9.84	1.37	<0.2	0.9	4.76	2.16	Roca et al. (2014)
			GM	NC	NC	NC	1.77	NC	NC	3.36	–	3.31	0.96	NC	NC	NC	NC	
USA	2000–2001	1.7–5.5 (128)	AM	–	–	–	–	–	–	7.3	–	–	–	–	–	–	–	Morgan et al. (2005)
			GM	–	–	–	–	–	–	5.2	–	–	–	–	–	–	–	
USA	2001		AM	–	–	–	–	–	–	–	–	–	–	–	–	0.90	–	Morgan et al. (2007)

Table 4 (continued)

Country	Year of sample collection	Population age (years) (*n*)	Measure of central tendency	Organophosphate pesticides										Pyrethroid insecticides				References
				Non-specific metabolites						Specific metabolites								
				DMP	DMTP	DMDTP	DEP	DETP	DEDTP	TCPY	MDA	IMPY	PNP	4-F-3-PBA	DBCA	3-PBA	trans-DCCA	
		1.7–5.6 (127)	GM	–	–	–	–	–	–	–	–	–	–	–	–	0.39	–	
USA	2001	4–6 (203)	AM	9.6	17.4	2.8	6.0	1.6	0.2	–	–	–	–	–	–	5.0	3.6	Naeher et al. (2010)
USA	2003–2004	3–11 (23)	AM	–	–	–	–	–	–	–	–	–	–	0.02	0.004	1.22	1.24	Lu et al. (2006)
			GM	–	–	–	–	–	–	–	–	–	–	0.08	0.05	0.58	0.54	
USA	2007–2008	6–>60	GM	NC	2.28	NC	NC	NC	NC	1.29	NC	NC	0.67	NC	NC	0.40	NC	CDC (2015)
	2009–2010	(~2740)	GM	–	–	–	–	–	–	0.779	NC	NC	0.45	NC	NC	0.42	NC	
		6–>60 (~2740)																
USA	2012	>18 (55)	AM	–	–	–	–	–	–	1.84	1.18	0.75	1.40	0.84	<0.4	1.52	3.41	Davis et al. (2013)

AM arithmetic mean; *GM* geometric mean; *NC* not calculated, the proportion of results beneath the limit of quantification was too high to produce a valid result; *NR* not reported

[a] Concentrations reported as μg/g creatinine

[b] Urban population

[c] Sum of cis and trans isomers

[d] Twelve-hour nighttime collection

[e] Males

[f] Females

[g] Pregnant women

[h] Urban population

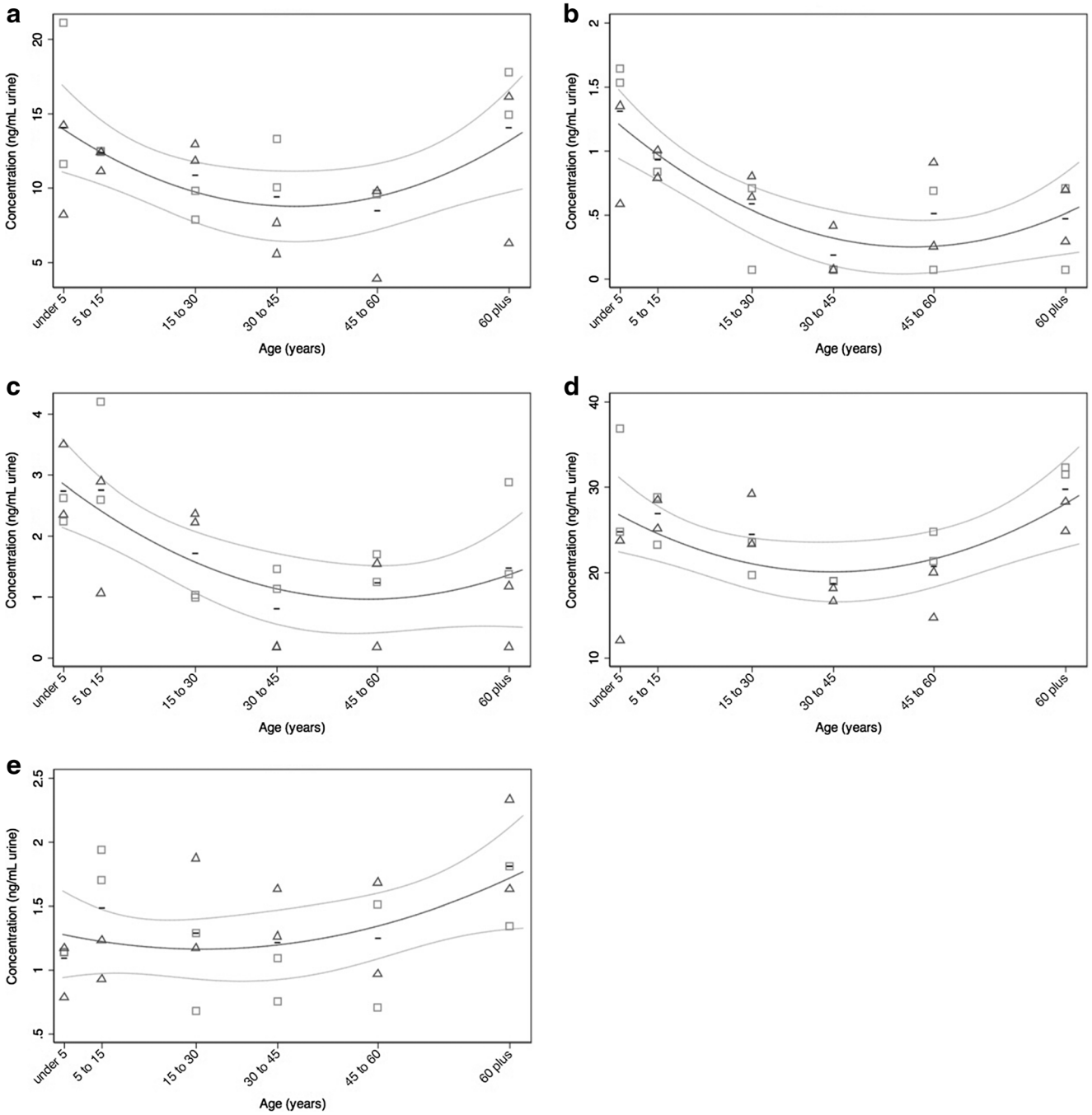

Fig. 1 Urinary total concentration (ng/mL) versus age (years) for pesticide metabolites with significant age-concentration relationships, DMTP (**a**), DMDTP (**b**), DETP (**c**), TCPY (**d**), and DCBA (**e**). The triangles denote the female pools, and the squares denote the male pools. The horizontal line indicates the mean concentration of four pools in each age stratum. The curvilinear regression line (*solid line*) with 95th confidence intervals (*dotted lines*) are presented

herbicides in a convenience sample of the general Australian population using samples pooled by age and sex. Pyrethroid and organophosphate insecticides are widely available in Australia (Table 1), and this fact was reflected by the high detection frequency (>90 %) of all metabolites measured, with the exception of DEDTP and 4-F-3-PBA. The low detection frequency of DEDTP and 4-F-3-PBA (we did not detect these compounds in any samples) is similar to studies in other countries (CDC 2015; Roca et al. 2014; Becker et al. 2006; Guodong et al. 2012). The herbicide 2,4-D and metabolites of the insect repellent DEET, DHMB, and DCBA were also detected in all pooled samples and at relatively high concentrations.

Age has previously been demonstrated to be associated with insecticide concentrations, with higher

concentrations typically found in young children (CDC 2015). However, for most of the metabolites in this study, we observed no association between concentration and age (Supplementary Material 1). Age was only significantly associated with concentrations of five organophosphate metabolites, namely, DMDTP, DMTP, DETP, TCPY, and PNP, and the association was curvilinear (Fig. 1), with higher concentrations in the younger and older (>60) age strata. With the exception of DMDTP (56 %) and DETP (42 %), the variability in metabolite concentration explained by age was low (<30 %) and the magnitude of the effect size of age on measured concentration was relatively small (Supplementary Material 1), where the greatest difference in concentration between age strata was less than one order of magnitude. This suggests that factors other than age and sex, such as specific behavioral and lifestyle factors, for example, domestic use patterns of pesticide products, also influence urinary concentrations.

The concentration differences in the youngest (<5 years) and oldest age strata (>60 years) may be related to absolute differences in external exposure compared to other age groups and/or age-related differences in behavior and physiology. Young children and older persons may have greater exposure to insecticides because of a relatively greater period of time spent in the indoor environment, where concentrations of these compounds are typically higher than outdoors (Rudel and Perovich 2009). Infants occupy different microenvironments than adults and often for prolonged periods of time. Because the floor is a critical zone for very young children (<2 years of age), consumer products that result in widespread floor contamination or are only applied to the floor, such as "trigger-spray" insecticides or aerosols, may be particularly relevant for children's exposures. Young children experience proportionally greater chemical exposures than adults due to physiological differences, including a relatively greater surface area to volume ratio (for dermal exposure), and increased respiratory and metabolic rates (Makri et al. 2004; WHO 2011). Early life exposures are of particular concern because of the disproportionate future years of life, providing a longer time frame to manifest a disease that has a long latency period (Scheuplein et al. 2002; WHO 2011).

Older adults may also have relatively higher urinary concentrations of certain pesticide biomarkers because of differences in lifestyle factors and physiology compared with younger age groups. Glomerular filtration rate decreases with age and is associated with decreased clearance of metabolites from the blood (ABS 2013). Additionally, the age distribution of the Australian population varies geographically, with older adults (>65 years) making up a larger proportion of the population in inner regional areas than in major cities (Baxter et al. 2011). A previous study demonstrated that urinary concentrations of several insecticide metabolites were higher for participants residing in inner regional areas of South Australia compared to the major city, and this was attributed to agricultural practices (Babina et al. 2012). In the current study, no data was available regarding the geographic distribution of individuals contributing to the pooled samples or regarding the potential for occupational pesticide exposures. It is, therefore, possible that geographic differences across the age pools may also have contributed to the observed association of greater concentrations of some pesticide metabolites in urine pools from older adults. Stratification of pooled samples via sex, age, and geographic distribution would be necessary to determine the individual effect of age and geographic distribution on concentrations. Additionally, the potential contribution of occupationally exposed individuals is likely to be a very small proportion of the 2500 individual specimens, as the pathology collection center used to source the specimens is located in South East Queensland and primarily serves residential Brisbane.

Concentrations of non-specific organophosphate metabolites DMTP, DEP, and DETP measured in Australian pooled urine (GM 10.6, 6.18, and 1.84 ng/mL, respectively) were generally higher than concentrations reported in general populations in Canada (~2.03, 2.30, and not measured, respectively; Haines and Murray 2012) and the USA (2.28, <LOD, and <LOD, respectively; CDC 2015). DMTP, DEP, and DETP are non-specific organophosphate biomarkers derived from the metabolism of a wide array of commonly available organophosphates in Australia, such as chlorpyrifos (128 products), diazinon (45 products), and maldison/malathion (25 products; APVMA 2015). Urinary concentrations of TCPY, a specific metabolite of chlorpyrifos, reported in this study (GM 22.6 ng/mL [0–4 years] and 26.3 ng/mL [5–14 years]) were similar to those reported in children in South Australia (3–6 years, n = 115, arithmetic mean 21.5 μg/g creatinine; Babina et al. 2012) but substantially higher than urinary concentrations reported in children in Spain (6–11 years, n = 125, GM 3.36 ng/mL; Roca et al. 2014) and USA (6–11 years, n = 386, GM 1.12 ng/mL; CDC 2015) (Table 4). Chlorpyrifos is still available for limited domestic applications in Australia and widely available for agricultural use. By contrast, in the USA, in 2000, chlorpyrifos was restricted from homeowner applications as well as some agricultural applications (US EPA 2000), and since then, urinary concentrations of TCPY in the general USA population have been in decline (CDC 2015). PNP, a metabolite of parathion and parathion methyl, was detected in all pools in our study (GM 1.76 ng/mL, range 1.04–3.09 ng/mL). Interestingly, PNP concentrations in children 0–4 years (GM 1.90 ng/

mL) were lower than those reported by Babina et al. (2012) during the period of 2003–2006, which may reflect declining exposures as use of parathion and parathion methyl was phased out in Australia in 2011, prior to the commencement of this study (Agriculture Victoria 2015).

The concentrations of pyrethroid metabolites DBCA, 3-PBA, and trans-DCCA (GM 1.25, 1.21, and 1.89 ng/mL, respectively) in this study were similar to concentrations reported in Spain (0.9, 4.76, and 2.16 ng/mL, respectively; Roca et al. 2014) and some adult populations in the USA (<0.4, 1.52, and 3.41 ng/mL, respectively; Davis et al. 2013) but higher than those reported in Canada (Fortin et al. 2008), France (Le Grand et al. 2012), Germany (Becker et al. 2006; Schettgen et al. 2002), Poland (Wielgomas and Piskunowicz 2013; Wielgomas et al. 2013), and USA National Health and Nutrition Examination Survey (NHANES) (CDC 2015) (all <0.1, <0.8, and <0.5 ng/mL, respectively; Table 4). With the exception of DBCA, which is a metabolite of deltamethrin, 3-PBA and trans-DCCA are non-specific metabolites for the commonly available pyrethroids cypermethrin, deltamethrin, and permethrin. Studies in the USA have shown that pyrethroid metabolite concentrations vary by geographical region and by pest control practices in the home, with dietary habits having less of an impact than for organophosphates (Lu et al. 2006). Higher pyrethroid metabolite concentrations in this study may therefore reflect more frequent or intense use of these household insecticides in South East Queensland.

Concentrations of primary DEET metabolite DCBA, ranging from 4.23 to 850 ng/mL (GM 65.9 ng/mL), are similar to the range reported in 2007–2008 NHANES (<0.93 to 5760 ng/mL; CDC 2016a) but not as high as in the 2009–2010 survey cycle (<0.48 to 30,400 ng/mL; CDC 2016b). These relatively high urinary concentrations (compared with other pesticide metabolites; Tables 3 and 4) may be related to low cost and high availability of DEET-containing products (Costanzo et al. 2007), particularly in Northern Queensland where DEET is used to protect against mosquito-borne diseases (Larson et al. 2000). Queensland has a hot, sub-tropical climate with a high-pest burden, which may explain relatively greater use of household insecticides. For this reason, the sampled population is unlikely to be representative of the general Australian population for all pesticide metabolites.

We have made a number of assumptions that must be considered when interpreting the results of this study: (1) pathology specimens do not introduce significant bias into the study population, (2) pooled samples provide an accurate measurement of mean concentration, and (3) spot samples provide a reasonable estimate of internal exposure over a given time frame. The study population consisted of convenience samples collected during the course of routine pathology testing. No creatinine or specific gravity data were available for the samples used in this study. However, for the interpretation of pooled measurements as representative measures of average concentration, variation in individual sample hydration status is expected to be averaged out and not introduce significant bias to the estimated average concentrations and excretion rates. For some compounds, there was a considerable difference in measured concentration for replicate pools within an age strata. For example, measured concentrations of TCPY in 0–4-year-old pools ranged from 12 to 36.8 ng/mL, while DCBA concentrations ranged from 61.6 to 769 ng/mL in 5–14-year-old pools (Table 3). As each pool contains a large number of individual samples (n = 100), the concentration of any one individual is unlikely to influence the pool mean, and thus, this variability is likely a reflection of actual variation in exposure within the given demographic stratum. One limitation of a pooled sampling approach is that it cannot provide any information on variance within a population. Ad hoc methods can be applied to estimate upper bound reference values, which may be important in a health risk context (Aylward et al. 2014).

The use of pooled specimens is advantageous as it saves significantly on analytical costs, reduces the time and resources required for recruitment, and may avoid ethical difficulties associated with reporting individual results (reviewed in Heffernan et al. 2014), and pooled pathology specimens have been successfully used previously to measure other short half-life chemicals in the Australian population (Gomez-Ramos et al. 2016; Thai et al. 2016; Heffernan et al. 2015; Van den Ede et al. 2015).

Here, we present results of the first large-scale biomonitoring study of pesticide metabolites in a convenience sample of the general Australian population. We demonstrate broad exposure to organophosphate and pyrethroid insecticides as well as to one insect repellent in Australia consistent with the availability of commercial pesticide-containing products in Australia for domestic and agricultural applications.

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Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

Disclaimer The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the CDC or the views of the Australian Department of the Environment.

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