Environment International 163 (2022) 107206

Contents lists available at ScienceDirect

Environment International

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

Full length article

Pesticide exposure in New Zealand school-aged children: Urinary concentrations of biomarkers and assessment of determinants

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ARTICLE INFO

Handling Editor: Adrian Covaci

Keywords:
Organophosphates
Pyrethroids
Human biomonitoring
Children
Risk factors

ABSTRACT

This study aimed to assess pesticide exposure and its determinants in children aged 5–14 years. Urine samples (n = 953) were collected from 501 participating children living in urban areas (participant n = 300), rural areas but not on a farm (n = 76), and living on a farm (n = 125). The majority provided two samples, one in the high and one in the low spraying season. Information on diet, lifestyle, and demographic factors was collected by questionnaire. Urine was analysed for 20 pesticide biomarkers by GC–MS/MS and LC-MS/MS. Nine analytes were detected in > 80% of samples, including six organophosphate insecticide metabolites (DMP, DMTP, DEP, DETP, TCPy, PNP), two pyrethroid insecticide metabolites (3-PBA, trans-DCCA), and one herbicide (2,4-D). The highest concentration was measured for TCPy (median 13 μg/g creatinine), a metabolite of chlorpyrifos and triclopyr, followed by DMP (11 μg/g) and DMTP (3.7 μg/g). Urine metabolite levels were generally similar or low compared to those reported for other countries, while relatively high for TCPy and pyrethroid metabolites. Living on a farm was associated with higher TCPy levels during the high spray season. Living in rural areas, dog ownership and in-home pest control were associated with higher levels of pyrethroid metabolites. Urinary concentrations of several pesticide metabolites were higher during the low spraying season, possibly due to consumption of imported fruits and vegetables. Organic fruit consumption was not associated with lower urine concentrations, but consumption of organic food other than fruit or vegetables was associated with lower concentrations of TCPy in the high spray season. In conclusion, compared to other countries such as the U.S., New Zealand children had relatively high exposures to chlorpyrifos/triclopyr and pyrethroids. Factors associated with exposure included age, season, area of residence, diet, in-home pest control, and pets.

1. Introduction

Pesticide exposure is associated with a wide range of health effects in both children and adults. For example, it is reported that exposure to organophosphorus pesticides is associated with adverse reproductive and neurologic/neurobehavioral effects, and thyroid dysfunction (Koureas et al., 2012; Sánchez-Santed et al., 2016; Lacasaña et al., 2010). Exposure may occur via inhalation, dietary ingestion, dust/soil ingestion and dermal contact (Becker et al., 2006; Morgan, 2012; Riederer et al., 2008; Trunnelle et al., 2014). In very young children, mouthing is another important exposure route, and their breathing zone is lower to the ground compared to adults (Tulve et al., 2002; WHO, 2011). Children are also more susceptible to the effects of pesticides due to differences in dietary patterns, dose of pesticides in relation to weight,

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https://doi.org/10.1016/j.envint.2022.107206
Received 15 November 2021; Received in revised form 21 March 2022; Accepted 22 March 2022
Available online 5 April 2022

and for particular compounds, different pharmacokinetics (Neri et al., 2006; Osaka et al., 2016; Weiss et al., 2004).

New Zealand has an estimated pesticide-use of 7.89 kg/ha in 2017, 3 times higher than the world average (2.63 kg/ha) (Worldatlas website, Accessed Feb 2020). Currently, approximately 3500 pesticide products are registered in New Zealand (New Zealand Food Safety Website, accessed March 2020), with the breakdown as herbicides (60%), fungicides and bactericides (25%), plant growth regulators (8.6%), and insecticides (6.4%) (Manktelow et al., 2005). Organophosphorus (OP; mostly used among insecticides), pyrethroids (PYRs; 6th among insecticides), dithiocarbamates (1st among fungicide and bactericides) and phenoxy herbicides (1st among herbicides) are among the most widely used ingredients. Several chemicals in these groups have been identified, or are suspected, to lead to adverse health effects. For example, studies conducted by the US-EPA have identified environmental and health risks posed by the use of organophosphate pesticide chlorpyrifos (US-EPA, 2017) and this chemical has been banned in the EU, as well as several other countries and regions (European Union, 2007). Also, chlorpyrifos is currently considered to be added to global treaties such as the Stockholm Convention for regulation (Stockholm Convention Website, 2022). However, despite the high pesticide-use and potential health risks, no large exposure surveys have previously been conducted in New Zealand.

Urine is the most common matrix to assess recent pesticide exposure as currently used pesticides generally have short half-lives (<24 h) and metabolites/biomarkers are mostly polar, and they are therefore rapidly excreted with urine (Angerer et al., 2007; Barr et al., 2002; Roca et al., 2014). The importance of the role of urinary analysis to assess pesticide exposure will likely continue to increase in the future as the traditional persistent pesticides have been mostly banned and replaced by more polar and less persistent ingredients. For example, the exposure assessment for chlorpyrifos is likely more effective through urinary analysis of its human metabolite TCPy, compared with blood/serum analysis due to chlorpyrifos's relatively short human half-life (~24 h) (Nolan et al., 1984).

Urinary analysis also has limitations, including that the biomarkers are mostly measured in a single sample despite often large intra- and inter-individual variations in urinary biomarker concentrations (Aylward et al., 2014; Aylward et al., 2012; Lu et al., 2008; Hyland et al., 2021). Collecting a relatively large number of samples in a randomly selected population as done in The US National Health and Nutrition Examination Surveys (NHANES) may help address these limitations by averaging the variability associated with differences in meal ingestion, bladder emptying times, and hydration status between individuals.

In the current study, urine samples from 501 New Zealand children aged 5–14 years, collected during high and low pesticide spray seasons, were analysed for 20 biomarkers covering five classes of pesticides commonly used in New Zealand. The aim was to evaluate the exposure of school-aged children to pesticides, examine associations with demographic characteristics and lifestyle factors, and assess relevant exposure risks.

2. Materials and methods

2.1. Study population and design

This study took place between 2015 and 2017 as part of a larger project studying pesticide exposure and neuropsychological effects in children and involved the recruitment of urban, rural and farm children (aged 5–14 years) in the lower half of the North Island (the greater Wellington area, the Manawatu, the Wairarapa, and the Hawkes Bay) and the upper half of the South Island of New Zealand (Fig. 1). The targeted regions allowed this study to recruit sufficient participants in each group whilst minimising the distance between participants and the study centre (thus minimising travel time and cost). Rural regions were further selected to ensure a reasonable representation of different

Fig. 1. Map of sampling areas and geographical distribution of participants, with each dot on the map indicating the location of participants.

agricultural activities, hence the inclusion of some participants from regions that are further away from the research centre. A total of 784 participants were contacted through urban and rural schools including those known to have a relatively high number of children enrolled from farm areas. In total, parents of 603 participants (participation rate = 77%) completed a questionnaire, and urine samples were successfully collected from 501 participants, representing 418 families. Urine sampling was conducted during the "high pesticide spraying season" (November to March) and "low pesticide spraying season" (April to October). In total, 953 urine samples were collected from 501 participants, with 452 participants contributing 2 samples (one each from the low and high season) and 49 participants contributing only a single urine sample. Among the 501 participants, 300 lived in urban areas (urban), 125 lived on a farm (farm), and 76 lived in rural areas but not on a farm (rural).

Approval of human research ethics were gained from The Central Health and Disability Ethics Committee, Ministry of Health, New Zealand (Ethics reference: 13/CEN/134).

2.2. Urine sample collection and questionnaire

The first morning void urine sample was provided in a 60 mL polypropylene sterile container. Samples were stored at −20 °C, and an 18 mL aliquot of each sample was sent on dry ice to the analytical laboratory (Queensland Alliance for Environmental Health Science, The University of Queensland, Australia).

Parents were asked to complete a questionnaire about demographic characteristics such as the child's age, sex, ethnicity, body weight, and household gross income, as well as lifestyle factors, pesticide use at home, fruit and vegetable consumption, washing fruit/vegetable prior to consumption, organic food consumption, and whether pets are present in the home.

2.3. Urine analyses

2.3.1. Target compounds

This study measured 20 biomarkers from five classes of pesticides including organophosphate insecticides, pyrethroid insecticides, several herbicides, an insect repellent, and dithiocarbamate fungicides (Table 1).

2.3.2. Materials

Solvents, reagents and chemical standards were purchased from multiple suppliers. Details are provided in Section S1 in the Supplemental Materials (SM).

2.3.3. Urine sample preparation

The analytical methods, except for ETU and PTU, have been published in detail elsewhere (Li et al., 2019). Briefly, the six dialkylphosphate (DAP) metabolites of OPs were prepared using a lyophilisation-derivatisation method and analysed by gas chromatography coupled to a triple quadrupole mass spectrometer (GC–MS/MS). For all other compounds, samples were extracted via a solid phase extraction (SPE) method after deconjugated by β-glucuronidase (HP-2) and an incubation process, prior to analysis using a liquid chromatography coupled with a tandem mass spectrometer (LC-MS/MS).

The method for analysing ETU and PTU was modified from Montesano et al. (2007). Each sample (2 mL) was thawed at room temperature and spiked with 50 μL of internal standard (ethylene-D_4 thiourea; 100 ng/mL in methanol (MeOH)) for quantification purposes. The samples were then sealed and frozen at −80 °C for at least 4 h before placing into a freeze dryer overnight. The lyophilised samples were reconstituted into 2 mL of dichloromethane and mixed on a shaker at 200 rpm for 10 min. The extract was filtered using a 0.22 μm Captiva ND^{lipids} filter and evaporated under a gentle nitrogen stream to near dryness before being reconstituted into 100 μL of 5% acetonitrile in water. Sample analysis was conducted using a LC-MS/MS as described above.

2.3.4. Creatinine and specific gravity analysis

20 μL of each sample was aliquoted and diluted into 2 mL of MilliQ water. A 20 μL aliquot of diluted urine was taken and diluted again into 930 μL of 5% acetonitrile (ACN) in water. The samples were then spiked with 50 μL of internal standard (D_3-creatinine, 1000 ng/mL in MeOH) prior to being filtered through a 0.2 μm PTFE filter. Creatinine was analysed by LC-MS/MS as published elsewhere (Thai et al., 2014).

Identification of the above analytical responses was confirmed using a combination of signal to noise ratio, relative retention time to specific internal standard and response ratio for the two transitions monitored. Analyte concentrations were quantified from their relative response to a specific internal standard against the slope of a seven-point calibration curve, with the target compounds ranging from 0.05 to 50 ng/mL in urine. For creatinine, it was a four-point calibration curve ranging from 100 to 1000 ng/mL in urine. Details are provided in Table S1 in the SM.

In addition, specific gravity (SG) of these samples was also measured, using a digital refractometer (UG-α; ATAGO CO., LTD., Japan) which was calibrated by MilliQ water before each use.

2.4. Quality assurance and control (QA/QC)

Aliquots of synthetic urine (prepared based on the NHANES procedure (USCDC, 2013)) were used as blanks (n = 38) and analysed with the actual samples in every batch, thus allowing regular monitoring of

Table 1
Chemicals selected for analysis.

Metabolite	Abbreviation	Specific/non-specific	Parent compounds	Chemical class
Dimethyl phosphate	DMP	Non-specific	Various	Organophosphate insecticides
Dimethyl thiophosphate	DMTP	Non-specific	Various	Organophosphate insecticides
Dimethyl dithiophosphate	DMDTP	Non-specific	Various	Organophosphate insecticides
Diethyl phosphate	DEP	Non-specific	Various	Organophosphate insecticides
Diethyl thiophosphate	DETP	Non-specific	Various	Organophosphate insecticides
Diethyl dithiophosphate	DEDTP	Non-specific	Various	Organophosphate insecticides
3,5,6-Trichloro-2-pyridinol	TCPy	Specific	Chlorpyrifos, chlorpyrifos-methyl, triclopyr	Organophosphate insecticides
Malathion dicarboxylic acid	MDA	Specific	Malathion	Organophosphate insecticides
Para-nitrophenol	PNP	Specific	Parathion, methyl-parathion	Organophosphate insecticides
3-Phenoxybenzoic acid	3-PBA	Non-specific	Cyhalothrin, cypermethrin, deltamethrin, fenpropathrin, permethrin, tralomethrin	Pyrethroid insecticides
4-Fluoro-3-phenoxybenzoic acid	4F-3-PBA	Specific	Cyfluthrin	Pyrethroid insecticides
Cis-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic	*Cis*-DBCA	Specific	Deltamethrin	Pyrethroid insecticides
Trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid	*Trans*-DCCA	Specific	Cyfluthrin, permethrin, cypermethrin	Pyrethroid insecticides
Metolachlor mercapturate	MET	Specific	Metolachlor	Chloroacetanilide herbicides
Atrazine mercapturate	ATZ	Specific	Atrazine	Chlorotriazine herbicides
2,4-Dichlorophenoxyacetic acid	2,4-D	NA[#]	NA	Phenoxyacetic herbicides
2,4,5-Trichlorophenoxyacetic acid	2,4,5-T	NA	NA	Phenoxyacetic herbicides
N,N-diethyl-*m*-toluamide	DEET	NA	NA	Insect repellent
Ethylene thiourea	ETU	Specific	Ethylene bisdithiocarbamates	Dithiocarbamate fungicides
Propylene thiourea	PTU	Specific	Propineb	Dithiocarbamate fungicides

[#] NA: The chemical is parent compound itself.

potential contamination from the laboratory environment and materials.

Low and high-concentration QC samples (QCL, QCH) were made up by fortifying the synthetic urine with target compounds resulting in a concentration of 2 and 20 ng/mL respectively for each compound. Analytical accuracy was assessed by comparing the resulting concentrations of QCL and QCH samples against the expected concentrations stated above. Analytical precision was examined by the relative standard deviation (RSD) of the replicated QC samples (n = 6 for both QCL and QCH respectively). Briefly, the accuracy ranged from 0.47 to 1.5 depending on chemicals and mostly ranged within 0.80 to 1.2; the RSD for precision was ≤ 30% for the majority.

The limit of detection (LOD) for each analyte was calculated as the average concentration of the blanks plus three times the standard deviation. If a compound was not detected in the blanks, 3.3 × IDL (instrument detection limit, defined as the level of target compound when a signal to noise ratio of 3 is observed for the corresponding peak) was used to calculate the LOD. The LOD for each compound ranged from 0.0050 to 0.70 ng/mL in urine, which was generally lower or comparable with other studies including NHANES. Detailed QC results can be found in Table S2 in the SM.

To further validate the analytical methods, randomly selected archives of urine samples (n = 5) that were analysed by USCDC for a previous study (Heffernan et al., 2016) were re-analysed. Compared with the reported values in Heffernan et al. 2016, RSD for the target compounds was ≤ 27% and for most (~90%) samples it was ≤ 20%, reflecting good agreement with the analysis results by USCDC. Quality of data obtained in this study were also assessed in an inter-laboratory data comparison project – German External Quality Assessment Scheme (G-EQUAS), which includes chemicals such as DMP, DMTP, DMDTP, DEP, DETP, DEDTP, TCPy, PNP, 3-PBA, 4F-3-PBA, and *trans*-DCCA. All results were within the tolerance range provided by G-EQUAS, suggesting a comparable analytical capability of the QAEHS lab with other labs internationally. Detailed data are not shown here but an example of TCPy data is provided in Table S3 in the SM.

2.5. Statistical analysis

Statistical analyses were performed with Graphpad Prism 8 (San Diego, CA), SPSS Statistics (Version 25, IBM, Armonk, NY, USA) and SAS for Windows version 9.4 (SAS Institute, Cary, NC, USA). Concentrations of metabolites/biomarkers were presented in ng/mL and adjusted for creatinine and presented as μg/g creatinine. Data analyses were conducted on both crude and creatinine-adjusted concentrations but results for only the latter analyses are shown and discussed unless a difference in trends/results was observed. Such differences are discussed in Sections 3 and 4 for specific observations, and only the correlation observed using both crude and adjusted concentrations are summarised in the graphical abstract. Statistical analyses were conducted for analytes with a detection frequency > 80% in urine samples (Rennard et al., 2006) and half of the LOD was used as the value for non-detectable compounds.

D'Agostino & Pearson normality test (alpha = 0.05) was used to test normal distribution and no chemicals passed the normal distribution test. The raw data was therefore logarithmically transformed (log-transformed) to better approximate a normal distribution before further data analysis. Paired t-tests were used to assess differences in biomarker concentrations (log-transformed) between seasons (high vs low). One-way ANOVA (Kruskal-Wallis test) was used to compare the concentrations in participants from different areas (farm, urban and rural). Multiple linear regression was conducted to assess associations of the concentration for each analyte with demographic characteristics and lifestyle factors. Regression assumptions and influence of potential outliers were verified by residual plots. As urinary concentrations were log-transformed prior to regression analyses, regression coefficients represent relative differences, or exposure ratios (ERs), with an ER of e.g. 1.5 indicating that the exposure for a particular group is 1.5 times higher compared with the reference group. Overall, a total of 20 factors were included in the regression model. "Age" and "number of types of pesticides used in home (up to five)" were used as continuous variables and the other 18 factors were categorical variables (either nominal or ordinal). In specific, the weight status was computed using centiles and z-scores for height/weight-for-age according to WHO criteria – for age 5–19 years (WHO, 2007). Deprivation quintile was determined based on New Zealand Index of Deprivation (NZDep) 2013, which is an area-based index of socioeconomic deprivation in New Zealand (Atkinson et al., 2014). It measures the level of deprivation for people in each small area and is based on nine Census variables. For the purpose of this study these were presented as quintiles with the lowest quintile representing the least deprived participants. Further details about included variables can be found in Table S4 in the SM. Spearman correlation was used to assess correlations between concentrations of biomarkers from the same and different classes.

3. Results

3.1. Population characteristics

The demographic characteristics are summarised in Table 2. Overall, 49.3% of participants were male, and the age range was 5–14 years, with the majority (69%) being aged 6–9 years. The majority were of New Zealand European descent (87.4%), followed by Māori (9.4%), the indigenous population of New Zealand. Most children were recruited from the Wairarapa and Hawkes Bay (38.1%) and the greater Wellington area (33.7%; Fig. 1). Participants from the greater Wellington area were mostly urban (89%), while for participants from the other three areas combined only 45% was classified as urban. Except for region, which was related to the farm-urban–rural definition as noted above, there were no significant differences in demographic characteristics between the urban, farm and rural groups.

3.2. Brief description of questionnaire data

Over 97% of the participants' parents reported using pesticides in the house at least once, and this was similar for urban, rural and farm children (Table S4). Flea treatment was identified as the highest specific use category (67.4%), with pesticides "to kill other insects" being used by 82.4%. There was no significant difference in fruit or vegetable consumption between urban, rural and farm children. For the majority of participants, at least two portions (one portion = one child's handful) of fruit and vegetables were consumed per day and they were washed "sometimes" (32.2% for fruit and 20.7% for vegetables) or "almost always" (43.3% and 70.3%) before eaten. Over 60% of the participants responded that they never or only rarely consumed organically grown fruit or vegetables. Over 80% of the participants had pets in the home, with the highest proportion of pet ownership (96%) reported for participants living on a farm. Treatment for fleas, ticks or mites for at least "a few times a year" was reported by almost half of the pet owners (Table S4).

3.3. Concentration of metabolites/biomarkers measured in urine

Descriptive statistics of urine metabolite/biomarker concentrations expressed as μg/g creatinine are presented in Table 3. Biomarkers with a detection frequency < 10% were MDA, 4F-3PBA, DBCA, 245-T, MET, ATZ, DEET, ETU and PTU, and are not included in this table or further discussed (Summary statistics for the full data is provided in Table S5, and results expressed in ng/mL are provided in Table S6). Nine analytes were detected in > 80% of samples, including six organophosphate insecticide metabolites (DMP, DMTP, DEP, DETP, TCPy, PNP), two pyrethroid insecticide metabolites (3-PBA, *trans*-DCCA), and one herbicide (2,4-D) (Table 3). Among the six DAPs, the compounds with lower detection frequency were DMDTP (66.6%) and DEDTP (15.8%). There

Table 2
Demographic characteristics of the studied population.

	Total		Urban		Rural		Farm		Chi-Sq
	501 (n)	%	300 (n)	%	76 (n)	%	125 (n)	%	p-value
Gender									
Male	247	49.3	151	50.3	38	50.0	58	46.4	
Female	254	50.7	149	49.7	38	50.0	67	53.6	0.754
Age Group									
5	61	12.2	41	13.7	6	7.9	14	11.2	
6–7	186	37.1	116	38.6	33	43.4	37	29.6	
8–9	158	31.5	89	29.7	27	35.5	42	33.6	
10–14	96	19.2	54	18.0	10	13.2	32	25.6	0.128
Ethnicity (Prioritised)									
Māori	47	9.4	31	10.3	7	9.2	9	7.2	
Pacific	9	1.8	8	2.7	0	0.0	1	0.8	
European	438	87.4	256	85.3	68	89.5	114	91.2	
Other	7	1.4	5	1.7	1	1.3	1	0.8	0.665
Region									
Wellington-Porirua-Hutt	169	33.7	151	50.3	8	10.5	10	8.0	
Otaki-Manawatu-Upper North Island	34	6.8	0	0.0	13	17.1	21	16.8	
Wairarapa-Hawkes Bay	191	38.1	105	35.0	37	48.7	49	39.2	
South Island	107	21.4	44	14.7	18	23.7	45	36.0	<0.0001
Weight Status									
Underweight	21	4.7	15	5.8	2	3.0	4	3.4	
Normal	320	71.9	182	69.7	57	85.1	81	69.2	
Overweight	77	17.3	48	18.4	5	7.4	24	20.5	
Obese	27	6.1	16	6.1	3	4.5	8	6.9	0.213
- Not available (11.18%)	56								
Deprivation quintile (Q)									
Q1 (lowest deprivation)	168	33.5	100	33.3	26	34.2	42	33.6	
Q2	155	30.9	89	29.7	23	30.3	43	34.4	
Q3	87	16.8	47	15.7	13	17.1	24	19.2	
Q4	66	13.2	43	14.3	9	11.8	14	11.2	
Q5 (highest deprivation)	28	5.6	21	7.0	5	6.6	2	1.6	0.551

Ethnicity: Ethnicity is prioritised as follows: Māori before Pacific Islander before European New Zealander before Other). **Region:** We combined Upper North Island and Otaki-Manawatu-UpperNI as one group since the number of participants living in Upper North Island is very small. **Weight Status:** Weight status was computed using centiles and z-scores for height/weight-for-age according to WHO criteria – for age 5–19 years (WHO, 2007). **Deprivation quintile:** This is based on NZDep 2013, which is an area-based index of socioeconomic deprivation in New Zealand (Atkinson et al., 2014). It measures the level of deprivation for people in each small area and is based on nine Census variables (for the purpose of this study these were presented as quintiles with the lowest quintile representing the least deprived participants).

was no clear difference in the detection frequency for each compound between the farm, urban and rural groups.

The highest median concentration was observed for TCPy (13 μg/g creatinine) and DMP (11 μg/g creatinine). Correlations between biomarkers are presented in Table S7. The correlation coefficients were lower than 0.6 for almost all analyses, with the exception of the correlation between 3-PBA and *trans*-DCCA ($r = 0.82$; $p < 0.01$).

Compared to other countries, levels of most DAPs and PNP in New Zealand Children were relatively low, while levels of TCPy and pyrethroid metabolites were generally higher (Table 4).

Concentrations of DMTP, TCPy, 3-PBA, DCCA and 2,4-D in urine collected in the low spray season were significantly higher than in the high spray season (Table 5), mainly for the urban group. For PNP we found the opposite pattern, with higher levels in the high spray season, in both the farm and urban groups, as well as for DEP in the farm group only.

3.4. Associations with demographic and life-style factors

For most of the pesticide biomarkers the creatinine adjusted concentrations were inversely associated with age (Table 6). Differences in biomarker concentrations between males and females occurred only for urinary DEAP (higher in males) and PNP (higher in females), and were not consistently observed for both seasons. Body weight was generally not associated with urine concentrations, but a positive association with DEAP in the high season was observed, although no such association was found when using crude concentration (ng/mL) of DEAP (data not shown). Ethnicity was not associated with urine concentrations for any of the pesticide biomarkers. Levels of PNP and *trans*-DCCA were higher in participants from more socioeconomically deprived households, but only during the spray season.

Urine concentrations did not differ between children living on a farm and children living in urban areas, with the exception of TCPy, for which concentrations were 40% higher in farm children during the high spray season. Living in rural areas was associated with higher concentrations of 3-PBA and *trans*-DCCA in both seasons, but particularly in the high spray season (Table 6).

The use of pesticides inside the home and the frequency of farm visits were not associated with urine concentrations, but urinary *trans*-DCCA was elevated in children who were inside the house when in-home pest control was applied. Organic fruit consumption was not associated with lower urine concentrations, while washing fruit before eating and organic vegetable consumption were associated with lower urine PNP concentration. Consumption of organic food other than fruit or vegetables was associated with lower concentration of TCPy in the high spray season. Having a dog in the home was associated with higher

Table 3
Descriptive statistics for pesticide metabolite/biomarker concentrations in urine (μg/g creatinine; only showing data with DF ≥ 10%). See full dataset in Table S5 in the SM).

Chemicals	Group	LOD (ng/mL)	N	DF%	Mean (SD)	GM (GSD)	Median (P25-P75)
Organophosphates							
DMP	All	0.23	851	95.7	22 (37)	9.6 (4.4)	11 (5.0–25)
	Urban		511	95.3	22 (34)	9.8 (4.5)	12 (5.7–24)
	Rural		124	98.2	25 (39)	10 (5)	11 (4.8–30)
	Farm		216	95.9	19 (42)	8.7 (4.0)	10 (4.1–22)
DMTP	All	0.089	934	98.7	6.9 (18.0)	3.3 (3.5)	3.7 (1.7–7.4)
	Urban		559	98.7	7.1 (20.0)	3.4 (3.5)	4.0 (1.8–7.7)
	Rural		140	100.0	7.0 (17.0)	3.0 (3.6)	3.0 (1.7–6.4)
	Farm		235	98.3	6.3 (14.0)	3.1 (3.5)	3.5 (1.5–7.3)
DMDTP	All	0.018	937	66.6	0.23 (0.72)	0.050 (5.439)	0.044 (0.011–0.18)
	Urban		559	65.6	0.23 (0.81)	0.050 (5.573)	0.044 (0.010–0.19)
	Rural		141	74.6	0.21 (0.54)	0.049 (5.398)	0.051 (0.0097–0.16)
	Farm		237	67.4	0.22 (0.59)	0.052 (5.188)	0.043 (0.012–0.18)
DEP	All	0.17	791	84.3	3.4 (8.9)	1.1 (5.0)	1.3 (0.39–3.4)
	Urban		462	86.3	3.7 (11.0)	1.2 (4.9)	1.3 (0.48–3.8)
	Rural		121	84.3	3.3 (4.9)	1.1 (5.5)	1.5 (0.31–4.0)
	Farm		208	79.4	2.8 (6.5)	0.89 (4.93)	1.1 (0.26–2.6)
DETP	All	0.032	936	86.0	1.4 (4.2)	0.38 (5.86)	0.50 (0.16–1.3)
	Urban		558	86.4	1.5 (4.9)	0.41 (5.83)	0.52 (0.18–1.3)
	Rural		141	87.3	1.3 (3.5)	0.32 (6.30)	0.45 (0.12–1.1)
	Farm		237	84.7	1.2 (2.8)	0.36 (5.65)	0.47 (0.14–1.2)
DEDTP	All	0.0050	936	15.8	NC	NC	NC
	Urban		558	14.9	NC	NC	NC
	Rural		141	15.9	NC	NC	NC
	Farm		237	18.2	NC	NC	NC
TCPy	All	0.13	941	100.0	19 (38)	13 (2)	13 (7.7–23)
	Urban		557	100.0	18 (16)	13 (2)	13 (7.8–24)
	Rural		144	100.0	17 (15)	13 (2)	12 (8.5–21)
	Farm		240	100.0	22 (71)	13 (2)	14 (7.0–23)
PNP	All	0.076	941	99.8	0.67 (1.20)	0.48 (2.11)	0.46 (0.29–0.76)
	Urban		557	99.8	0.67 (0.66)	0.49 (2.14)	0.47 (0.29–0.80)
	Rural		144	98.4	0.61 (0.56)	0.46 (2.06)	0.41 (0.29–0.72)
	Farm		240	100.0	0.72 (2.10)	0.47 (2.09)	0.47 (0.29–0.73)
Pyrethroids							
3-PBA	All	0.012	941	99.3	2.3 (6.8)	1.3 (2.8)	1.3 (0.71–2.3)
	Urban		557	98.9	2.1 (4.0)	1.1 (3.1)	1.2 (0.62–2.2)
	Rural		144	100.0	2.5 (2.4)	1.7 (2.5)	1.7 (0.93–3.2)
	Farm		240	100.0	2.6 (12.0)	1.4 (2.4)	1.4 (0.89–2.3)
***trans*-DCCA**	All	0.0083	941	99.7	2.4 (5.0)	1.2 (3.0)	1.1 (0.56–2.2)
	Urban		557	99.5	2.4 (5.7)	1.0 (3.3)	1.0 (0.48–2.0)
	Rural		144	100.0	2.7 (3.4)	1.7 (2.7)	1.7 (0.84–3.1)
	Farm		240	100.0	2.2 (4.2)	1.3 (2.6)	1.1 (0.68–2.2)
Herbicides							
2,4-D	All	0.12	944	85.7	0.30 (0.61)	0.21 (2.23)	0.21 (0.13–0.34)
	Urban		560	86.0	0.29 (0.31)	0.21 (2.24)	0.20 (0.12–0.34)
	Rural		144	89.1	0.27 (0.35)	0.20 (2.01)	0.18 (0.13–0.31)
	Farm		240	84.1	0.36 (1.10)	0.21 (2.35)	0.22 (0.13–0.34)

N: the number of samples that were available to be analysed for the specific chemicals and have passed the QC procedures during chemical analysis; LOD: limit of detection, DF: detection frequency, P25-P95: percentile values, NC: not calculated due to a low DF.

concentrations of urinary *trans*-DCCA for both seasons (Table 6).

4. Discussion

4.1. Pesticide metabolites/biomarkers in New Zealand children

The frequent detection of the pesticide metabolites/biomarkers measured indicate ubiquitous exposure to OPs, PYRs and 2,4-D in school-aged children in New Zealand.

DAPs. Urinary DAPs provide useful information about exposure to OP pesticides as a class (Barr et al., 2004). Compared to other countries/regions, DAP concentrations in New Zealand school-aged children were generally at the lower end (Table 4). The exception was DMP, for which concentrations in New Zealand (and Australian (Heffernan et al., 2016)) children was higher than that observed in other countries. The specific profiles of DAPs, i.e. the concentration of DMAP (i.e. DMP, DMTP and DMDTP) compared to DEAP (i.e. DEP, DETP and DEDTP), appear different among countries. In particular, in New Zealand, Australia, Canada and Israel the concentration of DMAP is generally higher than DEAP (Heffernan et al., 2016; Berman et al., 2020; Health Canada. Cycle 5, 2016–2017) whereas in Chile and Malaysia the opposite is found (Munoz-Quezada et al., 2012; Mat Sutris et al., 2016), likely reflecting differences in specific OP-use patterns between countries.

TCPy. TCPy is a metabolite of chlorpyrifos, chlorpyrifos-methyl, and triclopyr. According to Registered Veterinary Medicines, Agricultural Chemicals and Vertebrate Toxic Agents (ACVM register), chlorpyrifos is currently used as an active ingredient in 18 products and triclopyr is used in 37 products whereas chlorpyrifos-methyl is not registered as an active ingredient for any current pesticide products in New Zealand (New Zealand Food Safety Website. Accessed Mar 2020). The concentration of TCPy in New Zealand children was lower than that in Australian children of similar ages, but 2.2–7.3 times higher than other countries such as the US, Spain and Thailand. This was unlikely because rural and farm children were oversampled (see methods) as median levels were very similar for urban, rural and farm children (13 μg/g, 12 μg/g and 14 μg/g creatinine, respectively; Table 3). Also, even when we excluded farm children who had the highest levels (Table 6), the TCPy concentration in New Zealand children was still higher than those

Table 4
A summary of concentrations (μg/g creatinine; median value unless indicated otherwise) of selected biomarkers from different studies on school-aged children. To increase comparability, only data from samples collected in 2007 and onwards are shown and discussed here.

DAPs								
Country	**Age (y)**	**N**	**Collection year**	**DEP**	**DETP**	**DMP**	**DMTP**	**Ref**
NZ	6–11	~900	2015/17	1.3	0.50	11	3.7	This study
Australia[a,b,c]	5–14	4	2012/13	6.5	2.7	16	12	Heffernan et al. 2016
Spain	6–11	125	2010	2.28	<0.40[i]	<1.6[i]	<0.40[i]	Roca et al., 2014
U.S.	6–11	~380	2007/08[d]	< 0.37[j]	< 0.56[j]	< 0.47[j]	4.26	USCDC, 2019
Canada	6–11	~500	2016/17	3.5	0.64	3.1	2.7	Health Canada. Cycle 5, 2016–2017
Chile[e]	6–12	190	2010 (summer)	8.7	7.7	5.1	5.3	Munoz-Quezada et al., 2012
Chile[e]	6–12	190	2011 (fall)	18.6	3.7	4.4	4.4	Munoz-Quezada et al., 2012
Thailand[e,f]	6–8	53	2011	2.3/0.5	1.7/0.4	4.3/3.3	0.6/0.6	Rohitrattana et al., 2014
Malaysia[c]	7–12	180	Unclear	11.57	< 0.10[j]	2.15	< 0.10[j]	Mat Sutris et al., 2016
Norway[c]	6–12	112	2012	3.8	0.43	< 2.2 [k]	5.3	Cequier et al., 2017
Israel[c]	4–11	103	2015/6	2.4	0.4	5.8	6.2	Berman et al., 2020
Other biomarkers								
Country	**Age (y)**	**N**	**Collection year**	**TCPy**	**PNP**	**3-PBA**	***trans*-DCCA**	**2,4-D**	**Ref**
NZ	6–11	~900	2015/17	13	0.46	1.3	1.1	0.21	This study
Australia[a,b,c]	5–14	4	2012/13	26	1.6	2	3.1	0.49	Heffernan et al. 2016
Spain	6–11	125	2010	3.4	0.93	<0.80[i]	<0.40[i]	<0.40[i]	Roca et al., 2014
U.S.	6–11	~380	2009/10[d]	1.77	0.717	0.667	< 0.60[j]	0.478	USCDC, 2019
Canada	6–11	~500	2016/17	1.5 [g]	NR	0.41	0.20	0.28 [h]	Health Canada. Cycle 5, 2016–2017
Poland	<18	184	2012	NR	NR	0.171	< 0.10[j]	NR	Wielgomas and Piskunowicz, 2013
Thailand[e,f]	6–8	53	2011	6.0/2.6	NR	NR	NR	NR	Rohitrattana et al., 2014
South Korea[e]	6–12	70	2011	NR	NR	1.46	NR	NR	Jo et al., 2015

NB: a: mean concentration; b: pool samples (100 individual samples contributing to one pool); c: ng/mL urine; d: these data were the most recent published in USCDC 2019 report; e: geometric mean; f: concentrations for rice farming communities/aquacultural farming communities; g: data from 2014/15; h: data from 2009/11; i: limit of quantification (LOQ) as reported in ng/mL urine; j: limit of detection (LOD) as in ng/mL urine; k: method detection limit (MDL) as in ng/mL.

Table 5
Comparison of concentrations of pesticide exposure biomarkers in children's urine collected from high and low spray seasons.

Analyte	**Group**	**High spray season**				**Low spray season**				**p value (*t* test)**	
		N	**Mean (SD)**	**GM (GSD)**	**Median (P25-P75)**	**N**	**Mean (SD)**	**GM (GSD)**	**Median (P25-P75)**	**P value**	**Pairs (N)**
DMP	All	432	21 (38)	11 (3)	12 (5.8–21)	419	22 (36)	8.6 (5.5)	11 (4.2–26)	0.6322	367
	Farm	109	20 (54)	9.0 (4.1)	10 (4.3–24)	107	17 (23)	8.4 (4.0)	11 (3.8–22)	0.5407	95
	Urban	258	20 (26)	11 (3)	12 (6.3–20)	253	24 (40)	8.6 (5.9)	12 (4.3–28)	0.7726	221
	Rural	65	25 (43)	12 (3)	12 (5.6–26)	59	25 (35)	8.6 (6.5)	10 (4.4–33)	0.3738	51
DMTP	All	457	5.9 (13.9)	2.9 (3.4)	3.2 (1.5–6.0)	477	7.8 (21.6)	3.7 (3.6)	4.3 (1.9–8.6)	**0.0001 (L > H)**	434
	Farm	115	6.1 (19.0)	2.6 (3.7)	3.0 (1.5–5.2)	120	6.4 (6.8)	3.6 (3.4)	4.5 (1.7–8.3)	**0.0235 (L > H)**	111
	Urban	270	5.6 (7.5)	3.0 (3.3)	3.6 (1.5–6.5)	289	8.6 (26.8)	3.9 (3.6)	4.6 (1.9–8.8)	**0.0086 (L > H)**	258
	Rural	72	6.8 (21.0)	2.8 (3.3)	2.8 (1.8–4.9)	68	7.2 (11.0)	3.3 (3.8)	3.3 (1.6–9.7)	0.1040	65
DEP	All	414	3.9 (11.3)	1.4 (4.1)	1.5 (0.64–3.6)	377	2.9 (5.1)	0.84 (5.74)	0.95 (0.23–3.3)	0.0902	309
	Farm	106	4.1 (8.6)	1.5 (4.5)	1.6 (0.66–3.5)	102	1.5 (2.5)	0.53 (4.64)	0.57 (0.12–1.6)	**0.0002 (H > L)**	88
	Urban	245	4.1 (13.4)	1.5 (3.9)	1.5 (0.65–3.7)	217	3.4 (5.8)	0.98 (5.85)	1.1 (0.26–3.9)	0.7359	174
	Rural	63	2.9 (4.7)	1.2 (4.5)	1.4 (0.34–3.2)	58	3.7 (5.1)	1.0 (6.7)	1.6 (0.14–5.1)	0.8135	47
DETP	All	456	1.4 (4.1)	0.36 (5.79)	0.47 (0.15–1.2)	480	1.4 (4.3)	0.40 (5.92)	0.51 (0.17–1.3)	0.1907	426
	Farm	115	1.4 (3.7)	0.41 (5.77)	0.58 (0.14–1.3)	122	0.93 (1.37)	0.33 (5.54)	0.39 (0.14–1.1)	0.5507	113
	Urban	270	1.4 (4.3)	0.39 (5.69)	0.47 (0.17–1.3)	288	1.7 (5.4)	0.43 (5.97)	0.56 (0.18–1.3)	0.1615	257
	Rural	71	1.1 (4.4)	0.25 (6.11)	0.33 (0.083–0.98)	70	1.4 (2.3)	0.41 (6.36)	0.54 (0.15–1.6)	0.2097	66
TCPy	All	457	17 (52)	11 (2)	11 (6.2–19)	484	21 (17)	15 (2)	16 (8.9–27)	**<0.0001 (L > H)**	441
	Farm	117	26 (100)	13 (2)	13 (6.8–20)	123	18 (14)	13 (2)	14 (7.1–24)	0.3238	115
	Urban	266	14 (13)	9.8 (2.4)	11 (5.2–18)	291	22 (18)	17 (2)	17 (9.9–29)	**<0.0001 (L > H)**	257
	Rural	74	15 (13)	11 (2)	11 (6.8–17)	70	20 (17)	15 (2)	15 (9.0–24)	**0.0398 (L > H)**	69
PNP	All	457	0.79 (1.65)	0.55 (2.12)	0.51 (0.33–0.87)	484	0.56 (0.53)	0.43 (2.06)	0.40 (0.26–0.70)	**<0.0001 (H > L)**	441
	Farm	117	0.92 (3.03)	0.56 (2.05)	0.53 (0.32–0.90)	123	0.53 (0.47)	0.40 (2.07)	0.40 (0.24–0.63)	**0.0023 (H > L)**	115
	Urban	266	0.77 (0.76)	0.56 (2.17)	0.51 (0.34–0.89)	291	0.57 (0.54)	0.43 (2.06)	0.40 (0.25–0.73)	**0.0002 (H > L)**	257
	Rural	74	0.63 (0.56)	0.48 (2.05)	0.44 (0.31–0.75)	70	0.58 (0.56)	0.44 (2.07)	0.40 (0.29–0.63)	0.2454	69
3-PBA	All	457	2.4 (9.1)	1.2 (3.1)	1.2 (0.65–2.3)	484	2.2 (3.4)	1.4 (2.6)	1.4 (0.81–2.4)	**0.0398 (L > H)**	441
	Farm	117	3.3 (16.8)	1.3 (2.6)	1.3 (0.86–2.1)	123	2.0 (1.8)	1.5 (2.2)	1.5 (0.94–2.6)	0.3349	115
	Urban	266	1.9 (3.8)	0.99 (3.31)	1.1 (0.51–2.1)	291	2.2 (4.2)	1.3 (2.9)	1.3 (0.69–2.2)	**0.0029 (L > H)**	257
	Rural	74	2.7 (2.8)	1.6 (2.9)	1.6 (0.72–3.6)	70	2.4 (1.8)	1.8 (2.2)	1.9 (1.1–3.1)	0.7801	69
Trans-DCCA	All	457	2.4 (5.8)	1.1 (3.2)	1.0 (0.50–2.0)	484	2.4 (4.2)	1.3 (2.8)	1.2 (0.61–2.3)	**<0.0001 (L > H)**	441
	Farm	117	2.4 (5.3)	1.2 (2.7)	1.1 (0.67–2.2)	123	2.0 (2.6)	1.3 (2.4)	1.2 (0.71–2.2)	0.0541	115
	Urban	266	2.2 (6.3)	0.91 (3.42)	0.84 (0.44–1.8)	291	2.6 (5.0)	1.2 (3.1)	1.0 (0.51–2.2)	**<0.0001 (L > H)**	257
	Rural	74	3.0 (4.2)	1.6 (2.9)	1.5 (0.75–3.1)	70	2.5 (2.3)	1.7 (2.4)	1.8 (0.91–3.1)	0.8632	69
24-D	All	458	0.30 (0.83)	0.18 (2.40)	0.17 (0.10–0.31)	486	0.31 (0.30)	0.24 (2.02)	0.23 (0.15–0.35)	**<0.0001 (L > H)**	441
	Farm	117	0.43 (1.53)	0.20 (2.72)	0.20 (0.11–0.38)	123	0.29 (0.27)	0.23 (1.98)	0.23 (0.15–0.32)	0.6196	115
	Urban	267	0.25 (0.28)	0.17 (2.30)	0.16 (0.098–0.28)	293	0.33 (0.33)	0.25 (2.10)	0.24 (0.15–0.38)	**<0.0001 (L > H)**	260
	Rural	74	0.29 (0.47)	0.19 (2.27)	0.16 (0.12–0.32)	70	0.24 (0.14)	0.21 (1.71)	0.21 (0.14–0.31)	0.6558	69

Table 6
Associations between demographic and life-style factors and biomarker concentrations in urine (μg/g creatinine log-transformed), expressed as exposure ratios with 95% CI.

	DMAP		DEAP		TCPy		PNP		3-PBA		*Trans*-DCCA		2,4-D	
	High season (n = 325)	Low season (n = 346)	High season (n = 324)	Low season (n = 344)	High season (n = 325)	Low season (n = 347)	High season (n = 325)	Low season (n = 347)	High season (n = 325)	Low season (n = 347)	High season (n = 325)	Low season (n = 347)	High season (n = 326)	Low season (n = 349)
R	0.28	0.26	0.26	0.26	0.36	0.29	0.36	0.21	0.39	0.35	0.42	0.39	0.29	0.24
Age	**−1.1 (−1.2; −1.0)****	**−1.1 (−1.2; −1.0)***	−1.0 (−1.1; 1.1)	−1.1 (−1.2; 1.0)	**−1.1 (−1.1; −1.0)****	**−1.1 (−1.1; −1.0)***	**−1.1 (−1.1; −1.0)****	−1.0 (−1.0; 1.0)	**−1.1 (−1.2; −1.0)****	**−1.1 (−1.2; −1.1)*****	**−1.1 (−1.2; −1.0)****	**−1.1 (−1.2; −1.1)*****	**−1.1 (−1.1; −1.0)***	**−1.0 (−1.1; −1.0)***
Gender (female vs male)	1.0 (−1.2; 1.3)	−1.1 (−1.4; 1.2)	**−1.4 (−2.0; −1.0)***	1.3 (−1.1; 1.9)	−1.1 (−1.3; 1.1)	−1.1 (−1.2; 1.1)	**1.2 (1.0; 1.4)***	1.1 (−1.1; 1.3)	−1.2 (−1.5; 1.1)	−1.0 (−1.2; 1.2)	−1.1 (−1.3; 1.2)	−1.0 (−1.2; 1.2)	−1.1 (−1.3; 1.2)	−1.1 (−1.3; 1.0)
Weight Status (heavier)[#]	−1.0 (−1.2; 1.2)	−1.1 (−1.4; 1.1)	**1.4 (1.1; 1.8)***	1.0 (−1.3; 1.4)	−1.1 (−1.3; 1.1)	−1.1 (−1.3; 1.0)	−1.0 (−1.1; 1.1)	−1.1 (−1.2; 1.1)	−1.1 (−1.3; 1.1)	−1.1 (−1.2; 1.1)	−1.0 (−1.2; 1.2)	−1.1 (−1.3; 1.1)	−1.1 (−1.3; 1.1)	−1.1 (−1.2; 1.1)
Ethnicity (European vs others)	−1.3 (−1.9; 1.2)	−1.0 (−1.7; 1.6)	1.3 (−1.3; 2.3)	1.0 (−1.9; 2.0)	1.3 (−1.0; 1.8)	−1.0 (−1.4; 1.3)	1.2 (−1.1; 1.5)	1.0 (−1.3; 1.3)	1.0 (−1.4; 1.6)	−1.1 (−1.4; 1.3)	1.1 (−1.3; 1.7)	−1.0 (−1.4; 1.4)	1.2 (−1.2; 1.6)	−1.1 (−1.4; 1.2)
Deprivation Index (More deprived)[#]	−1.1 (−1.2; 1.1)	−1.1 (−1.3; 1.0)	−1.1 (−1.2; 1.1)	1.0 (−1.1; 1.2)	1.0 (−1.0; 1.1)	1.1 (−1.0; 1.1)	**1.1 (1.0; 1.2)***	1.0 (−1.1; 1.1)	1.1 (−1.0; 1.2)	1.0 (−1.1; 1.1)	**1.2 (1.0; 1.3)****	1.0 (−1.1; 1.1)	1.0 (−1.1; 1.1)	−1.0 (−1.1; 1.1)
Living on a farm (vs urban)	1.1 (−1.3; 1.6)	1.1 (−1.4; 1.7)	1.4 (−1.2; 2.2)	−1.3 (−2.3; 1.3)	**1.4 (1.1; 1.8)***	−1.1 (−1.4; 1.1)	1.1 (−1.1; 1.4)	1.0 (−1.2; 1.3)	1.2 (−1.2; 1.7)	1.1 (−1.2; 1.3)	1.3 (−1.1; 1.8)	1.1 (−1.2; 1.5)	1.1 (−1.2; 1.5)	−1.2 (−1.5; 1.1)
Living in rural areas (vs urban)	1.2 (−1.3; 1.7)	1.0 (−1.5; 1.6)	−1.1 (−1.8; 1.5)	1.2 (−1.4; 2.2)	1.0 (−1.3; 1.4)	−1.2 (−1.5; 1.1)	−1.1 (−1.4; 1.2)	1.0 (−1.3; 1.3)	**1.7 (1.1; 2.3)****	**1.3 (1.0; 1.8)***	**1.9 (1.3; 2.7)*****	**1.5 (1.1; 2.0)***	−1.0 (−1.4; 1.3)	−1.2 (−1.5; 1.1)
No. of types of pesticides used in home (increased)	−1.1 (−1.3; 1.0)	1.0 (−1.1; 1.2)	−1.0 (−1.2; 1.1)	−1.1 (−1.3; 1.0)	1.1 (−1.0; 1.1)	−1.0 (−1.1; 1.0)	−1.1 (−1.1; 1.0)	−1.0 (−1.1; 1.0)	1.0 (−1.1; 1.2)	1.0 (−1.1; 1.1)	1.0 (−1.1; 1.1)	1.0 (−1.1; 1.1)	1.0 (−1.1; 1.1)	1.0 (−1.1; 1.1)
Inside home when pest control (increased frequency)	1.1 (−1.1; 1.2)	−1.1 (−1.3; 1.0)	−1.0 (−1.2; 1.1)	1.1 (−1.1; 1.3)	1.1 (−1.0; 1.2)	1.0 (−1.1; 1.1)	−1.0 (−1.1; 1.0)	1.0 (−1.0; 1.1)	1.1 (−1.1; 1.2)	1.0 (−1.0; 1.1)	1.1 (−1.0; 1.2)	**1.1 (1.0; 1.2)***	−1.0 (−1.1; 1.1)	1.0 (−1.0; 1.1)
Farm visiting (increased frequency)	1.0 (−1.1; 1.2)	−1.1 (−1.2; 1.1)	1.0 (−1.1; 1.2)	1.0 (−1.2; 1.2)	1.1 (−1.0; 1.2)	1.0 (−1.1; 1.1)	1.0 (−1.0; 1.1)	−1.0 (−1.1; 1.0)	1.0 (−1.1; 1.2)	−1.0 (−1.1; 1.1)	1.0 (−1.1; 1.1)	−1.0 (−1.1; 1.1)	1.1 (−1.0; 1.2)	1.0 (−1.0; 1.1)
Consumption of fruit (increased portions per day)	1.0 (−1.1; 1.2)	−1.1 (−1.3; 1.1)	1.1 (−1.1; 1.3)	−1.0 (−1.3; 1.2)	1.1 (−1.0; 1.2)	1.0 (−1.1; 1.1)	−1.0 (−1.1; 1.1)	1.0 (−1.1; 1.1)	1.1 (−1.1; 1.2)	−1.0 (−1.1; 1.1)	−1.0 (−1.2; 1.1)	−1.0 (−1.1; 1.1)	−1.0 (−1.1; 1.1)	1.0 (−1.1; 1.1)
Fruit washing before eating (decreased frequency)	1.0 (−1.2; 1.2)	−1.0 (−1.2; 1.2)	1.0 (−1.2; 1.3)	−1.0 (−1.3; 1.2)	1.0 (−1.1; 1.2)	1.1 (−1.0; 1.2)	**1.1 (1.0; 1.2)***	1.1 (−1.0; 1.2)	1.0 (−1.1; 1.2)	1.0 (−1.1; 1.2)	1.0 (−1.1; 1.2)	1.1 (−1.1; 1.2)	1.1 (−1.1; 1. (−2)	−1.0 (−1.1; 1.1)
Organic fruit consumption (decreased frequency)	−1.1 (−1.4; 1.2)	1.1 (−1.2; 1.5)	−1.1 (−1.5; 1.2)	−1.1 (−1.5; 1.3)	−1.0 (−1.2; 1.2)	1.1 (−1.1; 1.3)	1.1 (−1.1; 1.3)	1.1 (−1.0; 1.3)	−1.0 (−1.3; 1.2)	−1.1 (−1.3; 1.1)	−1.1 (−1.4; 1.1)	−1.1 (−1.3; 1.2)	−1.1 (−1.4; 1.1)	1.1 (−1.1; 1.2)
Consumption of vegetable (increased portions per day)	−1.1 (−1.2; 1.1)	1.1 (−1.1; 1.3)	−1.1 (−1.3; 1.1)	1.1 (−1.1; 1.4)	1.1 (−1.1; 1.2)	1.1 (−1.0; 1.2)	−1.0 (−1.1; 1.1)	−1.0 (−1.1; 1.1)	**−1.3 (−1.4; −1.1)****	−1.1 (−1.2; 1.0)	**−1.2 (−1.4; −1.1)****	−1.1 (−1.3; 1.0)	1.1 (−1.0; 1.2)	1.1 (−1.0; 1.2)
Vegetable washing before eating (decreased frequency)	1.1 (−1.1; 1.4)	1.1 (−1.2; 1.4)	−1.1 (−1.5; 1.2)	1.3 (−1.1; 1.7)	−1.0 (−1.2; 1.1)	−1.0 (−1.2; 1.1)	−1.1 (−1.3; 1.0)	1.0 (−1.1; 1.2)	1.0 (−1.2; 1.2)	1.1 (−1.1; 1.3)	1.1 (−1.1; 1.3)	1.1 (−1.1; 1.3)	**−1.2 (−1.5; −1.1)***	1.0 (−1.1; 1.1)
Organic vegetable consumption (decreased frequency)	−1.1 s (−1.4; 1.1)	−1.1 (−1.5; 1.2)	1.0 (−1.3; 1.3)	1.1 (−1.3; 1.5)	−1.1 (−1.3; 1.1)	−1.1 (−1.3; 1.1)	−1.1 (−1.3; 1.0)	**−1.2 (−1.3; −1.0)***	1.1 (−1.2; 1.4)	1.1 (−1.1; 1.2)	1.0 (−1.2; 1.3)	−1.0 (−1.2; 1.2)	1.1 (−1.1; 1.3)	−1.1 (−1.3; 1.1)

(*continued on next page*)

Table 6 (*continued*)

	DMAP		DEAP		TCPy		PNP		3-PBA		*Trans*-DCCA		2,4-D	
	High season (n = 325)	Low season (n = 346)	High season (n = 324)	Low season (n = 344)	High season (n = 325)	Low season (n = 347)	High season (n = 325)	Low season (n = 347)	High season (n = 325)	Low season (n = 347)	High season (n = 325)	Low season (n = 347)	High season (n = 326)	Low season (n = 349)
Other organic food consumption (decreased frequency)	1.2 (−1.0; 1.4)	1.1 (−1.1; 1.3)	1.1 (−1.2; 1.3)	−1.1 (−1.4; 1.1)	**1.2 (1.0; 1.3)***	1.0 (−1.1; 1.2)	1.0 (−1.1; 1.1)	−1.0 (−1.1; 1.1)	−1.0 (−1.2; 1.1)	1.1 (−1.1; 1.2)	1.0 (−1.1; 1.2)	1.1 (−1.0; 1.3)	1.1 (−1.0; 1.2)	1.0 (−1.1; 1.1)
Having a dog at home	−1.1 (−1.5; 1.2)	−1.3 (−1.9; 1.1)	−1.1 (−1.6; 1.3)	1.2 (−1.3; 1.8)	**−1.3 (−1.7; −1.0)***	−1.1 (−1.3; 1.2)	**−1.3 (−1.5; −1.1)***	−1.1 (−1.3; 1.1)	1.3 (−1.0; 1.8)	1.2 (−1.1; 1.4)	**1.4 (1.1; 1.9)***	**1.3 (1.0; 1.7)***	−1.1 (−1.4; 1.2)	−1.0 (−1.2; 1.2)
Having a cat at home	1.1 (−1.3; 1.5)	1.2 (−1.2; 1.7)	−1.3 (−1.9; 1.2)	−1.4 (−2.3; 1.1)	−1.2 (−1.5; 1.1)	**−1.3 (−1.6; −1.1)***	−1.0 (−1.2; 1.2)	−1.1 (−1.3; 1.2)	1.2 (−1.1; 1.7)	1.0 (−1.2; 1.3)	1.1 (−1.2; 1.5)	1.2 (−1.1; 1.5)	−1.1 (−1.4; 1.2)	−1.1 (−1.3; 1.1)
Pets treated for fleas, ticks or mites (increased frequency)	−1.0 (−1.2; 1.2)	−1.1 (−1.3; 1.1)	1.2 (−1.1; 1.5)	1.1 (−1.2; 1.4)	1.1 (−1.0; 1.2)	1.0 (−1.1; 1.1)	1.0 (−1.1; 1.2)	1.0 (−1.1; 1.1)	1.0 (−1.1; 1.2)	−1.1 (−1.2; 1.1)	−1.0 (−1.2; 1.2)	−1.1 (−1.3; 1.0)	−1.1 (−1.2; 1.1)	1.0 (−1.1; 1.1)

NB: * P < 0.05; ** P < 0.001. Associations with a P value < 0.05 are marked in bold. See Table S4 in the SM for factor explanation; # see Table 2 for the categories for weight Status and Deprivation Index.

observed in other countries except for Australia, which also reported higher TCPy levels (data not shown). As chlorpyrifos and triclopyr can degrade into TCPy in the environment which is even more persistent than the parent compounds (Spain, 2017; Stockholm Convention Website, 2022), environmental exposure to the metabolite itself may also contribute to urinary concentrations (Li et al., 2019). Therefore the high concentration of TCPy measured in New Zealand children may reflect both exposure to environmental degradation residues due to long-term use of chlorpyrifos and/or triclopyr, and direct exposure to these pesticides and metabolites. As DEP and DETP are non-specific metabolites for a wide range of organophosphates, their detection in urine samples could be due to the exposure to multiple parent compounds, not only chlorpyrifos. The fact that the correlation coefficient was low between TCPy-DEP and TCPy-DETP suggested that exposure to other organophosphates may have also contributed to the concentration of urinary DEP and DETP.

PNP. Urinary PNP concentrations in New Zealand children were generally lower than in other countries. PNP is a metabolite of pesticides parathion and parathion-methyl, which are no longer used in New Zealand, and a metabolite of nitrobenzene, which is widely used for chemical synthesis (e.g. aniline) and petroleum refining (Berner et al., 2009). PNP is also used to manufacture drugs, fungicides, and dyes and to darken leather (ATSDR, 1992). The low concentrations of PNP in this study indicate that the combined exposure to parathion, parathion-methyl, nitrobenzene and PNP itself were low relative to other countries.

3-PBA and trans-DCCA. 3-PBA and *trans*-DCCA were significantly correlated ($r = 0.82$, $p < 0.01$; Table S7), with 3-PBA being a metabolite of several pyrethroids including permethrin and cypermethrin and *trans*-DCCA a metabolite of cyfluthrin, permethrin and cypermethrin. The other metabolite of cyfluthrin, 4-F-3PBA, had a very low detection frequency (DF) (<10%). Indeed permethrin and cypermethrin are registered as an active ingredient in 26 and 16 products used in New Zealand, respectively, while cyfluthrin is not registered (New Zealand Food Safety Website. Accessed Mar 2020). Furthermore, in humans only permethrin and cypermethrin are converted to DCCA and 3-PBA, while remaining pyrethroids generate mainly 3-PBA and/or other metabolites (Wielgomas and Piskunowicz, 2013). Therefore, our results suggest an important role of permethrin and cypermethrin as sources for exposure to pyrethroids in New Zealand children, similar to previous Australian (Li et al., 2019) and Polish studies (Wielgomas and Piskunowicz, 2013) and the NHANES study in the US (USCDC, 2018).

Concentrations of these PYR exposure biomarkers in New Zealand school-aged children were comparable with levels measured in Australia and South Korea but higher than those seen in North America and Poland. This trend remained even after excluding rural children who had higher concentrations of 3-PBA and *trans*-DCCA (Table 6) compared to urban children (data not shown). It is therefore unlikely that these higher levels are the result of oversampling rural and farm children. In our study rural residency, in-home pest control, and dog ownership were associated with higher levels of pyrethroid metabolites. This is consistent with previous Australian (Babina et al., 2012) and Polish studies (Wielgomas and Piskunowicz, 2013) suggesting that children from rural areas may have greater environmental exposure to PYR, with diet the most important source for children from urban areas (Wielgomas and Piskunowicz, 2013). The use of pet-care products has also been found to increase exposure (Wielgomas and Piskunowicz, 2013). Pyrethroid exposure can also occur from spraying of pyrethroids against mosquitoes, from pyrethroid treated textile floor coverings or from in-home pest control operations (Leng et al., 2003).

2,4-D. 2,4-D is the third most commonly used herbicide in New Zealand agriculture (Manktelow et al., 2005), constituting 6.62% of the overall national pesticide usage, and is also used in residential settings. Urinary levels in New Zealand were relatively low or similar compared to other countries. Levels were the same for farm children, rural children and urban children, although the highest individual levels were measured among farm children during the high spray season (as illustrated by the high SD for this group). 2,4-D exposure can result from the diet, and from 2,4-D used in outdoor applications being tracked into homes, as illustrated by high 2,4-D detection frequencies in carpet dust (Morgan et al., 2008).

4.2. Seasonal difference

We measured the urinary pesticide biomarkers in the pesticide high and low season, to be able to discern whether spray drift or proximity to spraying activities could have a significant impact on children's exposure to pesticides. This did not appear to be the case: urine levels of DMTP, TCPy, 3-PBA, DCCA and 2,4-D were significantly higher in the low spray season (Table 5). The opposite, for example for 3-PBA, was shown in a recent Australian study where a higher concentration was observed in spring/summer (high spray season) (English et al., 2019). The seasonal trend observed in our study is therefore not likely related to spraying in the proximity of the children's residence but more likely the result of seasonal variations in food sources (Attfield et al., 2014), and is

in line with dietary intake as an important exposure pathway for pesticides (ATSDR, 2003; Attfield et al., 2014; Bradman et al., 2011; Lu et al., 2009; Lu et al., 2008; Morgan et al., 2007; Morgan et al., 2011; Panuwet et al., 2009) in this study population. In the low spray season, during the colder months in New Zealand, more fruit and vegetables are imported from overseas, (Stats NZ website., Accessed Mar 2020) particularly from Australia and the USA (Freshfacts website, accessed March 2020), which may be associated with higher pesticide residues. It should be noted that the seasons in Australia and New Zealand are opposite to the Northern Hemisphere so during this period the pesticide usage in the USA maybe relatively high. Interestingly, however, urinary levels of some pesticide metabolites in the US population (Table 4) are lower (particularly for TCPy) than what we observed in the current study among New Zealand children, which, if we assume similar dietary patterns between both populations, suggest that fruit and vegetable imports from the USA may not fully explain the seasonal patterns observed in this study. The post-harvest use of pesticides for transportation and storage is another potential contributor to the concentration of pesticides on fruit/vegetable products, and therefore urinary levels of pesticide metabolites. However, due to the very limited data on pesticide-usage and residues in foodstuffs in New Zealand, the specific reasons for the observed seasonal patterns remain unclear.

PNP levels were higher in the high spray season. Again, this pattern is not likely the result of local spraying practices, because parathion and parathion-methyl are no longer used in New Zealand (see above).

Our results do suggest that for children living on a farm, local spraying may contribute to their overall pesticide exposure. In particular, farm children had significantly higher TCPy levels compared to urban children or rural children not living on a farm, which was only observed for the high spray season, suggesting that local spraying and its residues may contribute to farm children's exposure to chlorpyriphos, the parent compound of TCPy. In addition, farm children's DEP concentrations during the high spray season were three times higher compared the low season, while this pattern was absent for urban children or rural children not living on a farm. This may be associated with multiple OPs used in this season in farm areas, as suggested by Becker et al. (2006).

4.3. Exposure risk assessment

For the purpose of risk assessment, urinary pesticide metabolites/biomarkers may be compared to Biomonitoring Equivalents (BEs), an estimated urinary concentration of a chemical metabolite corresponding to specific guideline values (Aylward et al., 2018). An alternative is Biomonitoring Guidance Values (BGVs), which is developed using PBPK/PD model of interindividual differences in human metabolism and physiology (Arnold et al., 2015). BEs and BGVs serve as screening values for the assessment of biomonitoring data. BEs provide an initial evaluation of whether the detected concentrations are well below, near, or above the concentrations corresponding to current exposure guidelines (Aylward et al., 2018); For pesticides BGVs assess the relationship between metabolite concentrations and red blood cell cholinesterase inhibition (Arnold et al., 2015). Currently available data relevant to this study include BGVs for TCPy (corresponding to chlorpyrifos) (Arnold et al., 2015), and BEs for 3-PBA as a non-specific marker of cumulative exposure to selected pyrethroids (Aylward et al., 2018) and 2,4-D (Aylward and Hays, 2015).

In this study, median and P95 concentrations of TCPy were 13 and 49 μg/g creatinine (Table 3), and 17 and 63 ng/mL urine (Table S6). These values were two orders of magnitude below the TCPy BGV for adults (2100 ng/mL) and one order of magnitude below the BE for infants (520 ng/mL). Comparing our data against BGVs have limitations. In particular, several studies have reported that alterations in neurodevelopment occurred at exposures to chlorpyrifos below the threshold for cholinesterase inhibition, which suggests the arbitrary use of BGVs may underestimate the potential toxicity of chlorpyrifos (Slotkin et al., 2006; Aldridge et al., 2003; Qiao et al., 2002).

The median and P95 levels of 3-PBA measured in New Zealand children were 1.3 and 5.7 μg/g creatinine (Table 3), and 1.6 and 8.0 ng/mL urine (Table S6), respectively. These are in the same order of magnitude as the reported Tier 1 BE (1.7 ng/mL), which is a highly conservative screening value for assessment of population urinary 3-PBA data assuming all of the source exposure is from the most toxic pyrethroid compound. Our results were considerably lower than the reported Tier 2 BE (87 ng/mL), which is based on relative exposure weights for individual pyrethroid compounds. The highest 3-PBA concentration was 74 ng/mL, measured in the urban area, and was close to the Tier 2 BE.

For 2,4-D, levels measured in this study were generally below 1 μg/g creatinine and the max was 5.9 ng/mL urine in farm areas. These were several orders of magnitude below the BE values of 10,500 and 7,000 ng/mL for adults and children aged 3–6, respectively, estimated based on the US-EPA reference dose (RfD) and chronic exposure (Aylward and Hays, 2015).

4.4. Predictors/factors for pesticide exposure

Demographic factors. Similar to previous reports in German children (Becker et al., 2006), age was inversely associated with the creatinine-adjusted concentrations of most biomarkers (Table 6). This may be mainly due to lower levels of creatinine in younger children and/or that dietary exposure to pesticides decreases with age (Heudorf and Angerer, 2001). In fact, when examining the association with age using crude concentration of these biomarkers in urine, the inversed association was no longer consistently observed, although it remained for DMAP (high season), 3-PBA (low season), and *trans*-DCCA (high season). Location (defined by urban/farm/rural classifications) was another important predictor for some metabolites/biomarkers as discussed in Section 4.1. Girls had a higher concentration of PNP than boys during the high spray season, which may be related to nitrobenzene or PNP in dyes and flavouring agents in cosmetics such as nail polish that girls use more than boys, although it is not clear why this was not found in the low spray season. Higher urinary levels of PNP and *trans*-DCCA were found in children living in more deprived areas, which is consistent with previous studies that have shown higher exposures to environmental chemicals in children with low socioeconomic backgrounds (Sexton and Ryan, 2012).

Lifestyle factors. Children who were inside the house when pest control was conducted had higher pyrethroids exposures as indicated by significantly elevated urinary *trans*-DCCA, which is consistent with previous studies (English et al., 2019; Naeher et al., 2010; Wessels et al., 2003; Wielgomas and Piskunowicz, 2013; Rauch et al., 2018). We also found that having a dog in the home was associated with higher concentrations of *trans*-DCCA, similar to our previous study in Australian infants (0-2y) (English et al., 2019). This may be associated with flea treatments and track-in of pesticides from outside to the home by the dog (English et al., 2019), although our current data did not show an association between flea treatment and pyrethroid biomarkers (Table 6).

Dietary intake has been identified in previous studies (Lu et al., 2008; Panuwet et al., 2009; Roca et al., 2014) as the most important pathway for general population exposure to pesticides, and our finding that many exposures during the high spray season were not elevated appear to confirm this. Other observations consistent with this are that consumption of organic food other than vegetables and fruits was associated with lower concentrations of TCPy (in the high spray season). Organic diets have been found to reduce children's exposure to OPs including chlorpyrifos (Lu et al., 2006). Lower frequency of fruit washing before eating was associated with higher levels of PNP (in the high spray season). However, for other metabolites no association with fruit and vegetable consumption, and consumption of organic foods, were found.

4.5. Limitations and future perspectives

Due to short biological half-lives in the human body (<3 days) for most currently used pesticides, biomarkers in urine samples mostly represent recent exposures. As a consequence, and given the potentially large temporal variance of exposure, one-off urine samples only provide a snapshot of an individual's recent exposure which may not be representative of their ongoing/longer-term exposure. However, this is less of an issue when reporting average levels for larger groups as we have done in this study. Similarly, questionnaire data are more representative of behaviour over a longer period and associations with one-off urine samples indicative of only relatively recent exposure may therefore not be entirely valid. As noted above, metabolites/biomarkers measured in urine samples may also partly come from the metabolites themselves that humans are exposed to. Therefore, using the levels of metabolites/biomarkers measured in urine may be an overestimate of the actual pesticide exposure. In future studies additional metabolites should be included and analysed to better assess the exposure to pesticides that have multiple degradation products. For example, we analysed atrazine mercapturate to assess the exposure for atrazine. In future research, other metabolites such as diaminochlorotriazine (DACT) and desethylatrazine (DEA) should also be considered due to their prevalence in urine samples in exposed cohorts (Barr et al., 2007). Chemical compositions of pesticides continue to change, which leads to changes in human exposures. Future research is warranted to establish sensitive and reliable analytical methods to measure a wider range of pesticide chemicals and metabolites.

In addition, this study involved multiple comparisons, which may have contributed to some chance findings. However, for our regression analyses in Table 5 (comparing urinary concentration between high and low spray seasons) we found 16 (44%) statistically significant ($p < 0.05$) findings across 36 comparisons, which is considerably more than expected based on chance alone (1.8; 5%). We found 33 (11.8%) significant findings across 280 comparisons in Table 6, which again is substantially more than what would be expected based on chance alone: 14 (5%). Therefore, we do not consider it likely that our main results and conclusions are based on erroneous chance findings; however, we cannot exclude that some of the statistically significant findings may be due to chance.

5. Conclusions

Biomarkers for pesticides in multiple classes including organophosphate pesticides and pyrethroids, and a phenoxyacetic herbicide 2,4-D were frequently detected. While a comprehensive risk assessment cannot be conducted for all biomarkers, for those with available Biomonitoring Guidance Values (2,4-D, 3-PBA, and TCPy), urinary levels indicate that exposures for these compounds were below relevant exposure guidance values, although, as noted above, these guidance values have limitations. Compared to peers in the US, New Zealand children may have higher exposures to chlorpyrifos/triclopyr and pyrethroids. Factors associated with pesticides exposures include age, season (potentially related to imported food), living location, diet, in-home pest control, and pets. As we did not consistently observe higher levels during the spray season, nor consistently higher levels for farm or rural children compared to urban children, these results suggested that pesticide exposure sources such as spray drift, exposure on the farm, or exposure from living in a rural area, are not major contributors to pesticide exposure for children in the New Zealand population. To reduce children's population levels of pesticide exposure, most impact can likely be made by reducing pesticide residues in food, targeting both locally produced and imported products.

CRediT authorship contribution statement

Yan Li: Conceptualization, Methodology, Validation, Writing – original draft, Writing – review & editing. **Xianyu Wang:** Conceptualization, Methodology, Validation, Writing – original draft, Writing – review & editing, Visualization, Project administration. **Jean Feary McKenzie:** Investigation, Writing – review & editing, Project administration. **Andrea 't Mannetje:** Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing. **Soo Cheng:** Methodology, Validation, Writing – original draft. **Chang He:** Methodology, Validation, Writing – review & editing. **Janet Leathem:** Methodology, Validation, Writing – review & editing. **Neil Pearce:** Methodology, Validation, Writing – review & editing. **Jordi Sunyer:** Methodology, Validation, Writing – review & editing. **Brenda Eskenazi:** Methodology, Validation, Writing – review & editing. **Ruby Yeh:** Methodology, Writing – original draft. **Lesa L. Aylward:** Conceptualization, Validation, Writing – original draft, Writing – review & editing, Visualization, Supervision. **Geoffrey Donovan:** Methodology, Investigation, Writing – review & editing. **Jochen F. Mueller:** Writing – original draft, Writing – review & editing, Supervision. **Jeroen Douwes:** Conceptualization, Methodology, Validation, Writing – original draft, Writing – review & editing, Funding acquisition, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors gratefully acknowledge the participants and parents who are involved in this study. The authors thank Drs Naomi Brewer and Amanda Eng for assisting with the development of study materials; Michelle Gray and Tracey Whaanga for assisting with field work; Nhung Dong and Zachary Paxman (The University of Queensland), and Shaoyu Tang (Dongguan University of Technology) for their assistance in sample analysis and Michael Gallen, Jake O'Brien, and Geoff Eaglesham (The University of Queensland) for analytical support. This study was funded by the New Zealand Health Research Council. Yan Li is supported by a University of Queensland International Scholarship (UQI). Xianyu Wang is partly funded by The National Health and Medical Research Council (NHMRC). Jochen F. Mueller is funded by a UQ Fellowship. We thank the Health Research Council New Zealand for funding this study through grant number HRC13/235.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2022.107206.

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