Childhood Exposure to Pyrethroids and Neurodevelopment in Canadian Preschoolers

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ABSTRACT

Background: Pyrethroid insecticides are used both residentially and agriculturally and their toxicity targets the nervous system of insects. They might also interfere with development and function of the human brain. A few epidemiological studies suggest that exposure to pyrethroids may be associated with neurobehavioral problems in children but there is little data on potential associations with cognitive outcomes. Furthermore, many studies showed that the neurotoxic effects of several pesticides are modified by sex, hence, considerations of potential sex-differences are important to investigate.

Objective: To study the cross-sectional association between urinary levels of pyrethroid metabolites and neurodevelopment, including neurobehavioral and cognitive outcomes, in preschool-age children, and to examine whether sex might modify these associations.

Methods: We used data from a follow-up examination of the Maternal-Infant Research on Environmental Chemicals (MIREC), the MIREC Child Development study (MIREC-CD Plus) on children at age 3–4 years living in 6 Canadian cities. For each participant, we collected a urine sample for measurements of pyrethroids metabolites (cis-DBCA, cis-DCCA, trans-DCCA, 3-PBA, 4-F-3-PBA). We assessed neurodevelopment with the Wechsler Primary and Preschool Scale of Intelligence–III (WPPSI-III) and two scales of the Behavior Rating Inventory of Executive Function–Preschool (BRIEF-P). Parents reported children's behavior using the Behavior Assessment System for Children–2 (BASC-2) and the Social Responsiveness Scale-2 (SRS-2). We examined associations between children's urinary pyrethroid metabolite concentrations and neurodevelopmental scores with multiple linear regression models, adjusting for confounders, in boys and girls separately.

Results: The study included 179 children (mean age: 3.2 y, range 2.8 to 4.0). The detection frequencies were high for most pyrethroid metabolites (83–100%), but lower for 4-F-3-PBA (36%). Higher concentrations of cis-DBCA were significantly associated with lower verbal, performance and full-scale IQ scores in boys (e.g., for a 2-fold increase in cis-DBCA, β = -2.0; 95% CI: -3.4, -0.6 for full-scale IQ). In girls, the only metabolite associated with cognitive scores was 3-PBA, which was associated with lower verbal IQ scores ($\beta_{\text{for a 2-fold increase}}$ = -1.3, 95% CI: -2.6, -0.1). For neurobehavioral outcomes in boys, there were associations between poorer BASC-2 Adaptive Skills scores with higher

concentrations of cis-DCCA ($\beta_{\text{for a 2-fold increase}}$ = -1.6, 95% CI: -2.3, -0.9), trans-DCCA ($\beta_{\text{for a 2-fold increase}}$ = -1.5, 95% CI: -2.2, -0.8), 3-PBA ($\beta_{\text{for a 2-fold increase}}$ = -1.7, 95% CI: -2.5, -0.9), and sum of pyrethroid metabolites ($\beta_{\text{for a 2-fold increase}}$ = -1.8, 95% CI: -2.6, -0.9). In girls, we observed a significant association between higher concentration of cis-DCCA and better BASC-2 Adaptive Skills score ($\beta_{\text{for a 2-fold increase}}$ = 1.0; 95% CI, 0.2, 1.8), but not with other urinary pyrethroids metabolites. Scores on the SRS-2 and BRIEF-P were not associated with pyrethroid metabolites.

Conclusion: There were associations between some pyrethroid pesticide metabolites and indicators of neurodevelopmental disorder, especially among boys. These associations are in agreement with previous studies and could suggest that exposure to pyrethroid pesticides represents a risk of potential toxicity for the cognitive development of children, and a risk for behavioral development. However, the cross-sectional nature of this study limits causal inferences.

KEYWORDS

Pyrethroid; insecticide; IQ; neurodevelopment; neurotoxicology

Conflict of Interest and other Ethics Statements

The authors declare they have no actual or potential competing financial interests.

HIGHLIGHTS

- Pyrethroid pesticides are widely used in Canada
- We measured pyrethroid metabolites in the urine of children from 6 Canadian cities
- Some pyrethroid metabolites were associated with lower IQ and poorer adaptive skills in boys
- These associations suggest pyrethroids may pose a risk to behavioral and cognitive development in children
- The cross-sectional nature of this study is a limitation

INTRODUCTION

Pyrethroids are a class of synthetic organic insecticides derived from the natural pyrethrins found in chrysanthemum flowers, but they are structurally modified to improve their efficacy as insecticides by increasing their environmental stability and toxicity. Their use increased over the past decade as a replacement for organophosphate pesticides, which have greater acute toxicity (U.S. EPA 2017). In Canada, pesticides are regulated by Health Canada and over 600 pesticide products containing pyrethroids were registered as of 2015, to kill insects on crops, in orchards, nurseries and greenhouses, as well as in cattle eartags and to kill mites in bee colonies (Health Canada 2016). Pyrethroids are also used as residential insecticides, for use indoors and outdoors to control insect pests, fleas and ticks on pets, and to kill mosquitoes (Health Canada 2021). Some of the more common commercial pyrethroid pesticides are permethrin, cypermethrin, deltamethrin, cyfluthrin, tetramethrin, and bifenthrin (Ye et al. 2015). For children, the diet is the main source of exposure, whereas other less common sources are residential use of pyrethroids to fight pest infestation (e.g., cockroaches) or use of pyrethroid sprays and shampoos for pets (Morgan et al. 2007; U.S. EPA 2009). Residues of several pyrethroid-based pesticides are frequently detected in some foods, and the products with the highest frequency of detected residues are fruits and vegetables (Lu et al. 2010), as well as tea and honey (Tang et al. 2018).

Several studies have shown that exposure to pyrethroids might interfere with the development of the nervous system. The potential for neurodevelopmental toxicity of pyrethroids has been shown in studies on insects and rodents, which reported that exposure to pyrethroids (at doses higher than in humans from the general population) in early life and at puberty altered neurobehavioral functioning (Soderlund 2012). The mechanisms underlying these effects could be the ability of pyrethroids to bind to and disrupt voltage-gated sodium channels of insect nerves, but also other targets in mammals, particularly voltage-gated calcium and chloride channels which have been implicated as alternative or secondary sites of action for a subset of pyrethroids (Soderlund 2012). Other mechanisms are suggested, as a persistent action on neurotransmitters or oxidative stress in various regions of the brain, with the hippocampal region exhibiting cholinergic dysfunction

(Mohammadi et al. 2019). Epidemiological studies have also examined the potential risks of low levels of exposure to pyrethroid pesticides on neurodevelopment in humans, with most studies focusing on the potential neurobehavioral toxicity of exposure to pyrethroids in children (Domingues et al. 2016; Oulhote and Bouchard 2013; Viel et al. 2017). Some studies have reported associations between higher levels of prenatal pyrethroid exposure and lower social and emotional development (Eskenazi et al. 2018; Furlong et al. 2017). Additionally, studies have shown that sex might modify the association between pyrethroids exposure and neurobehavioral outcomes (Eskenazi et al. 2018; Oulhote and Bouchard 2013; Wagner-Schuman et al. 2015). For instance, urinary pyrethroid metabolites were associated with attention deficit hyperactivity disorder (ADHD) in boys, but not in girls (8 – 15 years old) from the general population in the United States (Wagner-Schuman et al. 2015). Although some epidemiological studies have recently reported that pyrethroids can negatively affect the cognitive development of children (e.g., Viel et al. 2015), the number of studies that have analyzed this relationship remains limited.

Our objective is to study the cross-sectional association between urinary levels of pyrethroid metabolites and neurodevelopment, including neurobehavioral and cognitive outcomes, in preschool-age children, and to examine whether sex might modify these associations.

MATERIALS AND METHODS

Study population

The present study builds on the Maternal-Infant Research on Environmental Chemicals (MIREC) pregnancy cohort study. The MIREC cohort was established between 2008 and 2011 from 10 cities in 6 Canadian provinces, to document chemical level exposure in pregnant Canadian women. The eligibility criteria to be recruited into the study were: ability to consent and to communicate in English or French, be 18 years of age or older, planning to deliver at a participating study hospital, and no adverse medical history. Participants were recruited from obstetric and prenatal clinics before 14 weeks of gestation. Among the 5108 women deemed eligible, 39% consented to participate. A total of 2001 women were enrolled into the cohort (Arbuckle et al. 2013).

When children were between 36 and 48 months old, eligible mothers from the six MIREC recruitment sites with the most births (i.e., Vancouver, Toronto, Hamilton, Montreal, Kingston and Halifax) were recruited to a follow-up examination, the MIREC Child Development study (MIREC-CD Plus, n=807, 67% participation rate). Inclusion criteria were singleton birth at ≥ 28 weeks of gestation, and no major congenital birth defects, seizures nor major neurological disorders during the perinatal period. The follow-up examination included a biomonitoring and neurodevelopment visit and was completed by 610 children between 2013 and 2015. Urinary pyrethroid metabolites were measured in a subset of 198 children. The present study sample is composed of 179 children with complete data on both neurodevelopment and urinary pyrethroid metabolites.

Mothers signed informed consent forms at inclusion into the MIREC cohort and MIREC-CD Plus follow-up study. All the research protocols were reviewed and approved by the Health Canada Research Ethics Board and each of the ethics committees at the participating hospitals and research centers.

Pyrethroid metabolites measurement in urine

During the biomonitoring visit to participating families, a spot urine sample was obtained from each child (5 mL of Simport Tubes plastic). The metabolites were enzymatically hydrolyzed at 37°C followed by an extraction with hexane under acidic conditions. Then, extracts were hexafluoropropylated using hexafluoro-2-propanol and diisopropylcarbodiimide, re-extracted with hexane, and analysed by gas chromatography coupled to a mass spectrometer. The generated ions were measured following negative chemical ionization in the selected ion-monitoring mode. Five urinary pyrethroid metabolites were measured: 4-fluoro-3-phenoxybenzoic acid (4-F-3-PBA); cis -3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (cis-DBCA); cis-3- (2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (cis-DCCA); trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (trans-DCCA); and 3-phenoxybenzoic acid (3-PBA). A number of field blanks were collected to assess potential risks of contamination at collection sites, the results indicated field blanks were free of contamination.

The metabolic pathways of the different parent compounds may produce the same degradation products. Pyrethroids are metabolized primarily in the liver through the breakdown of their ester bond (Chrustek et al. 2018). These are degraded either by esterases, mainly by human carboxylesterases 1 and 2, or by oxidation by human cytochrome P450. In the case of permethrin and cypermethrin, these molecules cleave to produce three main metabolites, 3-PBA, cis-DCCA and trans-DCCA (Table 1). Cis- and trans- DCCA are metabolites of the cis and trans isomers of cypermethrin, cyfluthrin or permethrin, and 3-PBA is a metabolite common to several pyrethroids including permethrin, cypermethrin, deltamethrin, and allethrin (Demeneix et al. 2020). Cyfluthrin can turn into 4-F-3-PBA (4-fluoro-3-phenoxybenzoic acid).The metabolites of pyrethroids in urine primarily reflect recent exposure to the parent compounds, as they are rapidly metabolized and excreted, with half-lives ranging from two hours to a few days (Wessels et al. 2003).

Table 1: Pyrethroid pesticide metabolites and their parent pesticide compounds

Pyrethroid pesticide (CASRN)	**Metabolite (CASRN)**	**Metabolite abbreviations**
Cypermethrin (52315-07-8) Deltamethrin (52918-63-5) Permethrin (52645-53-1) Lambda-Cyhalothrin (91465-08-6) D-Phenothrin (26046-85-5) Fluvalinate-tau (102851-06-9)	3-phenoxybenzoic acid (3739-38-6)	3-PBA
Cyfluthrin (68359-37-5) Flumethrin (69770-45-2)	4-fluoro-3-phenoxybenzoic acid (77279-89-1)	4-F-3-PBA
Deltamethrin (52918-63-5)	cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (63597-73-9)	cis-DBCA
Cyfluthrin (68359-37-5) Permethrin (52645-53-1) Cypermethrin (52315-07-8)	cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (55701-05-8)	cis-DCCA
Cyfluthrin (68359-37-5) Permethrin (52645-53-1) Cypermethrin (52315-07-8)	trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (55701-03-6)	trans-DCCA

(Barr and Needham, 2002; CDC, 2009; Fortin et al., 2008; Starr et al., 2008)

Neurodevelopmental assessment

We used a battery of tests to assess neurodevelopment, including cognitive abilities and behavior development. First, mothers completed the Behavior Assessment System for Children-2 (BASC-2, preschool parent completed form) that assesses child's adaptive and problem behaviors. The BASC-2 is a valid and reliable 134-item assessment of children's problem behaviors in community and home settings (Reynolds and Kamphaus 2002). We analyzed the four composite BASC-2 scores: Externalizing Problems, Internalizing Problems, Adaptive Skills, and Behavioral Symptoms Index. These scales, which are the most often used in neurodevelopmental toxicology studies, were selected because they provide an overview of the child's behavioral profile rather than focusing on very specific behavioral problems. Higher scores indicate non-optimal behavior for BASC-2 scores (Externalizing Problems, Internalizing Problems, and Behavioral Symptoms Index) except for the Adaptive Skills, for which it is the reverse. Second, mothers completed the Social Responsiveness Scale-2 (SRS-2) that is aimed at identifying the presence and severity of social impairment associated with the autism spectrum. The SRS-2 is a valid and reliable 65-item assessment of reciprocal social behaviors including interpersonal behaviors, communication, and repetitive or stereotypic behaviors (Bolte et al. 2008). For the present study, we analyzed the SRS-2 total impairment score; a higher score indicates more social impairment. Third, in order to measure children's executive functioning, mothers answered the Behavior Rating Inventory of Executive Function – Preschool Version (BRIEF-P) (Gioia et al. 1996). Executive function refers to a set of processes responsible for cognitive, emotional and behavioral control. We analyzed the standardized scores for two subscales, Working Memory and Planning/Organization skills; higher score indicates poorer skills. Working memory and the capacity to plan represent central aspects of executive function, which is important for efficient cognitive functioning. Finally, children completed the Wechsler Preschool and Primary Scale of Intelligence – 3rd Edition (WPPSI-III), a valid and reliable assessment of children's cognitive abilities (Wechsler 2002). For this test, five subtests were performed in children: Receptive Vocabulary, Information, Block Design, Object Assembly and Picture Naming. Study staff from each participating study site completed a three-day training session. A single staff person from each study site administered the assessment at the children's home or in the participating study hospital

and was blind to exposure level. Software provided by the test publishers and U.S. population-based normative referent data were used to calculate standard scores of full-scale IQ, verbal IQ, and performance IQ scores. Higher scores indicate greater cognitive abilities.

Statistical analysis

For values below the limit of detection (LOD), we used machine readings and when the reading was zero, we imputed a value corresponding to half the smallest value of machine readings. Metabolite concentrations were adjusted for dilution before inclusion into the regression models. We applied this adapted formula (Just et al. 2010) : $P_c = P[(SG_m – 1)/(SG-1)]$, where P_c is the specific gravity-standardized metabolite concentration, P is the observed metabolite concentration, SG_m is the median specific gravity of the entire sample and SG is the specific gravity of the urine sample.

We used multiple linear regression models to analyze the association between metabolite urinary concentrations and neurodevelopmental test scores, adjusting for potential confounders. All analyses were stratified by sex. The distributions of urinary pyrethroid metabolites were right-skewed, so we applied log (base 2) transformation to values entered in the regression models, in order to better satisfy the assumption of constant variance and normality of residuals. We employed a directed acyclic graph to determine the minimal set of confounders to include in regression models (See Supplementary Information): child's age, maternal education, household income, study centre, and score on the Home Observation for the Measurement of the Environment (HOME) Inventory. The HOME is a semi-structured interview that measures the quality and quantity of the caregiving environment (see Table 1 for details on variable categories). For this test, the maximum score is 55. The lowest quartile is in the range of scores from 0 to 29 suggesting that the child's environment poses an increased risk to his or her development, and the highest quartile is in the range of scores from 46 to 55 suggesting an optimal environment for the child's development.

We ran regression models for each of the following exposure indicators: trans and cis-DCCA as well as their sum, cis-DBCA, 3-PBA, and the sum of all these metabolites. The association with neurodevelopment was not analyzed for the pyrethroid metabolite 4-F-3-

PBA because it was seldom detected (65% <LOD). The frequency of detection was above 83% for all other metabolites. Finally, residual plots and qq-plots were examined to verify the assumptions of constant variance and normality. There were no major violations of these assumptions. SAS EG 7.1 was used for all analyses.

RESULTS

Description of the sample, urinary pesticide metabolite concentrations

Table 2 presents the general characteristics of the 179 children and mothers included in the present study. There were slightly more girls than boys included (55.3% and 44.7%, respectively). Children had a mean age of 38.4 months (range, 33.9-47.6) at the neurodevelopment assessment. The majority of children regularly attended daycare (79.7% of children had daycare in a home or an out-of-home care setting). Scores on the HOME scale ranged from 33 to 54 (mean 47.8). The mothers of these children had a relatively high level of education (70.8% had at least an undergraduate university degree), had a family income above $ 100,000 (50.6%), were married (80.0%), and born in Canada (82.1%).

Table 2: Descriptive characteristics of participants in our study sample (subset of the MIREC-CD Plus study)

Characteristic	**Categories**	**n**	**%[1]**
Child's sex	Male	80	44.7
	Female	99	55.3
Study centre	Vancouver	16	8.9
	Toronto	31	17.3
	Hamilton	15	8.4
	Kingston	39	21.8
	Montreal	39	21.8
	Halifax	39	21.8
Maternal education	Less than college or university	21	12.0
	College, trade school diploma	30	17.2
	Undergraduate degree	69	39.4
	Graduate degree	55	31.4
	Missing	4	
Household income (Canadian $)	<=50,000	18	10.8
	50,001-100,000	64	38.6
	>100,000	84	50.6
	Missing	13	
Marital status	Married	140	80.0
	Non married	35	20.0
	Missing	4	
	Yes	147	82.1
Mother born in Canada	No	32	17.9
Daycare attendance	Does not go to daycare	36	20.3
	Care from in a home setting	68	38.4
	Care in child-care setting	73	41.3
	Missing	2	
Duration breastfeeding	No breastfeeding	16	9.1
	< 6 months	27	15.4
	6-12 months	47	26.9
	> 12 months	85	48.6
	Missing	4	
Season of urine sample collection	Winter	52	29.1
	Spring	32	17.9
	Summer	32	17.9
	Fall	63	35.1
	n	**Mean**	**Min-Max**
Child age at testing (months)	179	38.4	33.9–47.6
HOME score	179	47.8	33–54

[1] Percent calculated excluding missing values

Descriptive statistics for concentrations of urinary pyrethroid metabolites are presented in Table 3. The detection frequencies were high for most metabolites (83 – 100%). 3-PBA and trans-DCCA were the most detected, with 100% of samples above the LOD (Table 3). 4-F-3-PBA was the least frequently detected metabolite, with 65% of samples below the LOD. Urinary concentration of 3-PBA was higher than that of other metabolites [Geometric Mean (GM) = 0.39 µg/L], and was higher among boys than girls (GM = 0.43 µg/L and GM = 0.36 µg/L, respectively). Girls had higher median concentrations than boys for cis-DCCA (0.12 µg/L and 0.09 µg/L, respectively) and for trans-DCCA (0.24 µg/L and 0.21 µg/L, respectively). These concentrations were similar to those for all the children who participated in the biomonitoring visit of MIREC-CD Plus study (n=198; Table S1).

Pearson correlation coefficients between the concentrations of different pyrethroid metabolites are presented in supplementary material (Table S2). The two DCCA isomers were highly intercorrelated (r = 0.96). Cis and trans-DCCA, 3-PBA and the sum of all metabolites were also highly correlated with each other (r = 0.83 to 0.97). However, cis-DBCA and 4-F-3-PBA were less correlated with the other metabolites (r = 0.22 to 0.37).

Table 3: Descriptive statistics on concentrations of urinary pyrethroid metabolites of the entire sample, and for boys and girls separately; concentrations are standardized for specific gravity (MIREC CD-Plus Study, 2013 – 2015)

Pyrethroid metabolite	**n**	**LOD**	**n(%)<LOD**	**GM**	**min**	**P25**	**P50**	**P75**	**P95**	**max**
Entire sample										
cis-DBCA (µg/L)	178	0.006	29 (16.3)	0.02	<LOD	0.01	0.02	0.04	0.21	1.01
cis-DCCA (µg/L)	179	0.007	1 (0.6)	0.12	<LOD	0.06	0.11	0.20	1.72	5.10
trans-DCCA (µg/L)	179	0.010	0 (0)	0.27	0.02	0.11	0.22	0.46	3.33	17.34
3-PBA (µg/L)	177	0.010	0 (0)	0.39	0.04	0.17	0.32	0.70	3.41	13.62
4-F-3-PBA (µg/L)	170	0.008	111 (65.3)	NC	<LOD	<LOD	<LOD	0.01	0.12	0.62
Σ cis/trans-DCCA (nmol/L)	179	-	-	1.94	0.10	0.82	1.68	3.28	22.51	108.26
Σ metabolites (nmol/L)	168	-	-	4.10	0.39	1.80	3.51	7.00	37.02	170.93
Boys										
cis-DBCA (µg/L)	80	0.006	12 (15.0)	0.02	<LOD	0.01	0.02	0.04	0.27	1.02
cis-DCCA (µg/L)	80	0.007	0 (0)	0.12	0.02	0.06	0.09	0.23	1.41	4.13
trans-DCCA (µg/L)	80	0.010	0 (0)	0.27	0.02	0.11	0.21	0.51	3.12	9.71
3-PBA (µg/L)	80	0.010	0 (0)	0.43	0.04	0.22	0.36	0.82	2.81	6.83
4-F-3-PBA (µg/L)	77	0.008	51 (66.2)	NC	<LOD	<LOD	<LOD	0.01	0.09	0.20
Σ cis/trans-DCCA (nmol/L)	80	-	--	1.93	0.19	0.82	1.44	3.65	21.82	66.56
Σ metabolites (nmol/L)	77	-	-	4.20	0.39	2.10	3.73	7.62	35.12	101.01
Girls										
cis-DBCA (µg/L)	98	0.006	17 (17.4)	0.02	<LOD	0.01	0.02	0.03	0.16	0.34
cis-DCCA (µg/L)	99	0.007	1 (1.0)	0.12	<LOD	0.06	0.12	0.20	1.73	5.12
trans-DCCA (µg/L)	99	0.010	0 (0)	0.27	0.02	0.11	0.24	0.45	3.92	17.32
3-PBA (µg/L)	97	0.010	0 (0)	0.36	0.06	0.15	0.27	0.60	5.82	13.61
4-F-3-PBA (µg/L)	93	0.008	60 (64.5)	NC	<LOD	<LOD	<LOD	0.02	0.17	0.59
Σ cis/trans-DCCA (nmol/L)	99	-	-	1.94	0.10	0.84	1.76	3.19	27.34	108.26
Σ metabolites (nmol/L)	91	-	-	4.00	0.65	1.70	3.31	6.88	45.02	170.92

LOD: limit of detection; GM: Geometric Mean

NC: not calculated because above 50% of samples had 4-F-3-PBA concentrations below LOD

Association between urinary concentrations of pyrethroid pesticide metabolites and neurodevelopmental abilities in children

The results of multiple linear regression models stratified by sex and adjusted for selected co-variables (child age, maternal education, household income, study centre, and HOME score) are illustrated in Figure 1. These bubble charts indicate that, in boys, the associations were stronger. Thus, we note in boys, an association between cis-DBCA and lower IQ scores (verbal, performance and full scale), as well as between five exposure variables (cis-DCCA, trans-DCCA, 3-PBA, sum cis/ trans DCCA, sum of all metabolites) and lower BASC-2 Adaptive skills score. In girls, we noted significant associations between concentrations of 3-PBA and lower verbal IQ score, and between concentrations of cis-DBCA and higher BASC-2 Adaptive skills score.

The quantitative results from the regression analyses (i.e., β for 2-fold increase in exposure, and 95% CI) are presented in Table 4 for cognitive scores (WPPSI-III and BRIEF-P) and Table 5 for behavior scores (BASC-2 and SRS-2). For cognitive ability tests in boys, the magnitudes of the associations were relatively consistent between cis-DBCA and the different WPPSI-III scores, with 2 points decrease for full-scale IQ (β = -2.0, 95% CI: -3.4, -0.6), 1.7 points decrease for verbal IQ (β = -1.7, 95% CI: -2.9, -0.4) and for performance IQ (β = -1.7, 95%CI: -3.3, -0.1). In girls, higher 3-PBA concentration was associated with lower verbal IQ scores (β = -1.3, 95% CI: -2.6, -0.1). For both boys and girls, the associations of pyrethroid metabolites with BRIEF-P (i.e., working memory and planning / organizing skills) scores were not significant (Table 4).

Regarding results with tests of behavioral outcomes (Table 5), in boys, four of the five pyrethroid metabolites were significantly associated with poorer BASC-2 adaptive skills. Association estimates per 2-fold higher concentration of metabolite ranged from -1.7 points for 3-PBA (95% CI: -2.5, -0.9) to -1.5 points for trans-DCCA (95% CI: -2.2, -0.8). Only the association with cis-DBCA did not reach statistical significance (β = -0.5, 95% CI: -1.2, 0.3). There was no significant association between pyrethroid metabolites and the other BASC-2 scores in boys. In girls, high concentrations of pyrethroid metabolites showed a tendency to be associated with higher scores of BASC-2 Adaptive skills, but this

association was significant only for cis-DCCA (β = 0.9; 95% CI: 0.2, 1.8). None of the pyrethroid metabolites were associated with SRS-2 scores in boys or girls.

Figure 1: Regression summary bubble plot for log2 transformed pyrethroid metabolites and neurodevelopmental outcomes. Each circle at the intersection of an exposure and an outcome represents the result of a regression model, adjusted for covariates (child age, maternal education, household income, study centre, and HOME score). A red circle denotes a negative slope for the exposure term in the model, and a yellow circle denotes a positive slope. The size of a circle is proportional to the absolute value of the association estimate; a significant association is denoted with a thick black ring around the circle ($p<0.05$). For the first 6 outcomes (on the left of the vertical dotted line) higher scores indicate more problems, the reverse is true for the other 4 outcomes.

Table 4: Associations between urinary concentrations of pyrethroid metabolites and scores on the WPPSI-III and BRIEF-P in boys and in girls. Beta estimates are for a doubling in metabolite concentration, after adjusting for covariates (child age, maternal education, household income, study centre, and HOME score)

		Boys (n=73)			Girls (n=91)		
Pyrethroid metabolites	Test scores	Beta	Wald 95% CI	p	Beta	Wald 95% CI	p
cis-DBCA	WPPSI-III Verbal IQ	**-1.7**	**(-2.9, -0.4)**	**0.008**	-0.1	(-1.6, 1.3)	0.864
	WPPSI-III Performance IQ	**-1.7**	**(-3.3, -0.1)**	**0.039**	-1.1	(-3.1, 0.9)	0.279
	WPPSI-III Full Scale IQ	**-2.0**	**(-3.4, -0.6)**	**0.005**	-0.7	(-2.3, 0.9)	0.422
	BRIEF-P plan/organize	0.2	(-0.2, 0.6)	0.229	-0.2	(-0.6, 0.3)	0.098
	BRIEF-P working memory	0.3	(-0.3, 0.9)	0.317	-0.2	(-0.9, 0.6)	0.177
cis-DCCA	WPPSI-III Verbal IQ	0.4	(-1.2, 1.9)	0.627	-0.9	(-2.3, 0.3)	0.122
	WPPSI-III Performance IQ	-0.3	(-2.2, 1.6)	0.740	-0.4	(-2.2, 1.4)	0.647
	WPPSI-III Full Scale IQ	0.0	(-1.7, 1.7)	0.991	-0.8	(-2.2, 0.5)	0.251
	BRIEF-P plan/organize	0.3	(-0.2, 0.7)	0.245	0.0	(-0.3, 0.4)	0.106
	BRIEF-P working memory	0.2	(-0.5, 0.9)	0.516	-0.0	(-0.7, 0.6)	0.180
trans-DCCA	WPPSI-III Verbal IQ	0.8	(-0.7, 2.2)	0.299	-0.9	(-2.2, 0.3)	0.123
	WPPSI-III Performance IQ	0.1	(-1.7, 1.9)	0.886	-0.1	(-1.9, 1.6)	0.891
	WPPSI-III Full Scale IQ	0.5	(-1.1, 2.1)	0.544	-0.7	(-2.1, 0.7)	0.353
	BRIEF-P plan/organize	0.2	(-0.2, 0.6)	0.266	0.0	(-0.3, 0.4)	0.107
	BRIEF-P working memory	0.2	(-0.5, 0.8)	0.649	0.0	(-0.6, 0.6)	0.180
3-PBA	WPPSI-III Verbal IQ	0.7	(-0.9, 2.4)	0.396	**-1.3**	**(-2.6, -0.1)**	**0.044**
	WPPSI-III Performance IQ	-0.8	(-2.9, 1.3)	0.446	-0.7	(-2.6, 1.1)	0.430
	WPPSI-III Full Scale IQ	-0.0	(-1.9, 1.9)	0.981	-1.2	(-2.7, 0.3)	0.108
	BRIEF-P plan/organize	0.2	(-0.3, 0.6)	0.437	0.2	(-0.2, 0.6)	0.085
	BRIEF-P working memory	-0.2	(-1.0, 0.6)	0.605	0.4	(-0.3, 1.0)	0.144
Σ cis- and trans-DCCA	WPPSI-III Verbal IQ	0.7	(-0.9, 2.1)	0.382	-1.0	(-2.3, -0.2)	0.113
	WPPSI-III Performance IQ	-0.0	(-1.9, 1.9)	0.990	-0.3	(-2.1, 1.5)	0.780
	WPPSI-III Full Scale IQ	0.4	(-1.3, 2.0)	0.675	-0.8	(-2.2, 0.7)	0.292
	BRIEF-P plan/organize	0.2	(-0.2, 0.7)	0.265	0.0	(-0.3, 0.4)	0.106
	BRIEF-P working memory	0.2	(-0.5, 0.9)	0.613	0.0	(-0.6, 0.6)	0.180
Σ all metabolites	WPPSI-III Verbal IQ	0.8	(-0.9, 2.5)	0.353	-1.1	(-2.4, 0.3)	0.133
	WPPSI-III Performance IQ	-0.6	(-2.7, 1.6)	0.598	-0.7	(-2.6, 1.1)	0.447
	WPPSI-III Full Scale IQ	0.1	(-1.8, 2.1)	0.892	-1.1	(-2.6, 0.5)	0.188
	BRIEF-P plan/organize	0.3	(-0.2, 0.8)	0.219	0.1	(-0.3, 0.5)	0.171
	BRIEF-P working memory	0.1	(-0.7, 0.9)	0.808	0.2	(-0.5, 0.9)	0.192

BRIEF-P, Behavior Rating Inventory of Executive Function–Preschool; WPPSI-III, Wechsler Preschool and Primary Scales of Intelligence–third edition

Table 5: Associations between urinary concentrations of pyrethroid metabolites and scores on the BASC-2 and SRS-2 in boys and in girls. Beta estimates are for a doubling in metabolite concentration, after adjusting for covariates (child age, maternal education, household income, study centre, and HOME score)

		Boys (n=73)			Girls (n=91)		
Pyrethroid metabolites	Test scores	Beta	Wald 95% CI	p	Beta	Wald 95% CI	p
cis-DBCA	BASC-2 BSI	0.4	(-0.3, 1.2)	0.255	0.2	(-0.7, 1.1)	0.604
	BASC-2 Ext.	0.6	(-0.4, 1.6)	0.216	0.8	(-0.4, 1.9)	0.200
	BASC-2 Int.	0.3	(-0.7, 1.3)	0.584	-0.0	(-1.1, 1.1)	0.958
	BASC-2 Adapt. sk.	-0.5	(-1.2, 0.3)	0.213	0.4	(-0.6, 1.4)	0.431
	SRS-2	0.2	(-0.4, 0.8)	0446	-0.1	(-0.8, 0.7)	0.885
cis-DCCA	BASC-2 BSI	0.5	(-0.3, 1.4)	0.226	0.1	(-0.7, 0.9)	0.777
	BASC-2 Ext.	0.2	(-1.0, 1.3)	0.782	0.3	(-0.8, 1.3)	0.594
	BASC-2 Int.	0.8	(-0.3, 1.9)	0.154	0.1	(-0.9, 1.1)	0.903
	BASC-2 Adapt. sk.	**-1.6**	**(-2.3, -0.9)**	**0.001**	0.9	(0.2, 1.8)	0.021
	SRS-2	0.7	(-0.0, 1.4)	0.057	-0.1	(-0.7, 0.5)	0.685
trans-DCCA	BASC-2 BSI	0.4	(-0.4, 1.3)	0.314	0.1	(-0.7, 0.8)	0.897
	BASC-2 Ext.	0.1	(-1.0, 1.1)	0.921	0.2	(-0.8, 1.2)	0.703
	BASC-2 Int.	0.8	(-0.3, 1.8)	0.138	-0.1	(-1.1, 0.9)	0.878
	BASC-2 Adapt. sk.	**-1.5**	**(-2.2, -0.8)**	**0.001**	0.8	(-0.1, 1.6)	0.080
	SRS-2	0.6	(-0.1, 1.2)	0.090	-0.1	(-0.7, 0.5)	0.820
3-PBA	BASC-2 BSI	0.2	(-0.8, 1.2)	0.696	0.5	(-0.4, 1.3)	0.277
	BASC-2 Ext.	-0.0	(-1.2, 1.3)	0.895	0.8	(-0.3, 1.8)	0.151
	BASC-2 Int.	0.7	(-0.5, 2.0)	0.234	0.4	(-0.7, 1.5)	0.446
	BASC-2 Adapt. sk.	**-1.7**	**(-2.5, -0.9)**	**0.001**	0.6	(-0.4, 1.5)	0.235
	SRS-2	0.4	(-0.4, 1.2)	0.308	0.3	(-0.3, 0.9)	0.353
Σ cis- and trans-DCCA	BASC-2 BSI	0.5	(-0.4, 1.3)	0.282	0.1	(-0.7, 0.9)	0.863
	BASC-2 Ext.	0.1	(-1.0, 1.2)	0.879	0.2	(-0.8, 1.3)	0.672
	BASC-2 Int.	0.8	(-0.3, 1.8)	0.142	-0.0	(-1.0, 0.9)	0.944
	BASC-2 Adapt. sk.	**-1.5**	**(-2.2, -0.8)**	**0.001**	0.8	(-0.0, 1.7)	0.055
	SRS-2	0.6	(-0.1, 1.3)	0.076	-0.1	(-0.7, 0.5)	0.783
Σ all metabolites	BASC-2 BSI	0.4	(-0.6, 1.4)	0.458	0.3	(-0.5, 1.2)	0.454
	BASC-2 Ext.	0.2	(-1.1, 1.4)	0.815	0.6	(-0.5, 1.7)	0.258
	BASC-2 Int.	0.9	(-0.3, 2.2)	0.142	0.2	(-0.9, 1.3)	0.681
	BASC-2 Adapt. sk.	**-1.8**	**(-2.6, -0.9)**	**0.001**	0.8	(-0.2, 1.8)	0.096
	SRS-2	0.5	(-0.3, 1.3)	0.195	0.1	(-0.5, 0.8)	0.733

BASC-2, Behavioral Assessment System for Children–2; BASC-2 BSI, Behavioral Symptoms Index; BASC-2 Ext, Externalizing Problems; BASC-2 Int, Internalizing Problems; BASC-2 Adapt. Sk, Adaptive Skills; SRS-2, Social Responsiveness Scale 2nd Edition.

DISCUSSION

The present study reports associations between the concentrations of urinary metabolites of pyrethroid pesticides, especially cis-DBCA and 3-PBA, and neurodevelopment at the preschool age. Most of the associations were stronger for boys, such as those between higher concentrations of cis-DBCA and lower scores of verbal IQ, performance IQ, and full-scale IQ assessed using the WPPSI-III. We also report a significant association between higher urinary 3-PBA concentrations and lower verbal IQ scores in girls. Previous studies, although in limited number, that have examined the association between metabolites of pyrethroid pesticides in urine and neurodevelopment in children reported similar results. For instance, a study reported that concentrations of 3-PBA and cis-DBCA in 6-year old children's urine were associated with lower scores of verbal comprehension and working memory on the WISC-IV (Wechsler Intelligence Scale for Children-4th edition) (Viel et al. 2015). Urinary levels were lower than those measured in the present study (median concentration for 3-PBA was 0.02 μg/L vs 0.32 μg/L in our study). Overall, these results suggest that pyrethroids might impair cognitive function, but the specific domain (e.g., nonverbal or verbal abilities) most affected remains unclear. Furthermore, some metabolites (i.e., cis-DCCA, trans-DCCA and 3-PBA) were associated with poorer scores on the BASC-2 Adaptive skills in boys. Children with low scores on the adaptive skill scale might have difficulty adjusting to the social, school, and home environment and display communication or behavior problems (Dietrich et al. 2005).

Previous cross-sectional studies in larger population-based samples also reported results associating pyrethroid metabolites in urine with problem behaviors in children. For instance, urinary cis-DCCA was associated with more behavioral difficulties assessed with the Strengths and Difficulties Questionnaire completed by parents of 779 Canadian children 6–11 years (Oulhote and Bouchard 2013). Urinary concentrations of cis-DCCA measured in these children were similar to those measured in our study (0.05 μg/L vs 0.06 μg/L). Another study among 687 children 8–15 years in the United States reported that those with detectable levels urinary 3-PBA (median concentration 0.32 μg/L vs 0.29 μg/L in our sample) were twice as likely to have ADHD than the other children (Wagner-Schuman et al. 2015). However, in the present study pyrethroid metabolites were not

associated with SRS-2 scores, which assess social development, whereas other studies reported association with poorer social development when exposure occurred prenatally (Shelton et al. 2014, Furlong et al. 2017, Eskenazi et al. 2018).

In the present study, the strongest associations with cognitive outcomes were observed for cis-DBCA, a metabolite specific of deltamethrine. In general, *trans* isomers are less toxic than *cis* isomers because they are readily hydrolyzed by esterases (Soderlund 2012). Deltamethrin, which is only in the *cis* isoform, is less sensitive to oxidation and hydrolysis, and therefore is metabolized more slowly, hence has a longer half-life compared with other pyrethroids (Pitzer et al. 2021). The *cis* isomer of cypermethrin is also excreted more slowly than the trans isomer following oral exposure (Liu et al. 2004). This is corroborated by toxicology work on rodents reporting that the administration of the *cis* isomer of cypermethrin resulted in higher fat storage and a longer half-life of approximately 13 days for the pesticide compared to the *trans* isomer (Wolansky and Harrill 2008). Studies examining the proportion of pyrethroid metabolites excreted in urine following ingestion of cypermethrin by volunteers reported lower cis-DCCA compared to trans-DCCA (Ratelle et al. 2015; Woollen et al. 1992). In addition, other studies reported higher toxicity of the *cis* isomer of cypermethrin compared to the *trans* isomer in both humans (Furlong et al. 2017; Wagner-Schuman et al. 2015) and mice (Jin et al. 2012). In our results, higher cis-DCCA in girls was associated with higher BASC-2 Adaptive Skills scores. However, this association seems to be isolated as it was not found with the other pyrethroid metabolites, nor in other studies, which suggests that it might be a spurious association.

Sex differences have also been reported in previous epidemiological studies examining cognitive and behavioral outcomes in relation to prenatal and postnatal exposure to pyrethroid pesticides (Eskenazi et al. 2018; Wagner-Schuman et al. 2015). Human and animal data suggest that some pyrethroids might cause disruption of endocrine activity through mimicking, blocking, or synergising the effects of endogenous hormones (Brander et al. 2016; Hwang et al. 2019), which could be related to differential effects in boys and girls. Other factors might also contribute to the differential sex-associations between pyrethroid exposure and neurobehavioral development. For instance, sex-differences in liver function may play a role, because the hepatic function is essential to process many

toxic xenobiotics (Moore et all. 2023). Despite the scope and pervasiveness of sex differences in several physiological systems, there is a lack of research on the mechanisms that might be implicated in differential susceptibility of males and females to neurotoxicants (Weiss 2011). Unfortunately, the issue raison by Weiss on the predominance of males in behavioral experiments still persist today, such as in a recent study where females mice were completely excluded from an experiment on the effect of developmental pyrethroid exposure on neurobehaviors (Nguyen et al. 2023). Given the complex sex-differences in functional and structural brain characteristics, exploring how pesticides might have differential impacts is especially relevant in developmental neurotoxicology.

We observed that some pyrethroid urinary metabolites were frequently detected, especially 3-PBA and DCCA which were detected in all children of our study. Since these metabolites are cleared from the body in just a few days after exposure (Wessels et al. 2003), this high detection frequency indicates frequent exposure events. Permethrin is the most widely used pyrethroid pesticide in Canada and is found in more than 350 registered products used for a variety of agricultural, livestock, forestry, and residential insect control applications (Health Canada 2019). The urinary concentrations of 3-PBA measured in our study sample (GM = 0.39 µg/L; 2013–2015) are similar to those measured in the Canadian Health Measure Survey in children 3–5 years (GM was 0.32 µg/L in 2009-2011 and 0.40 µg/L in 2016-2017) (Health Canada 2019). In the United States, urinary concentrations of 3-PBA were higher than those reported here, with a GM of 0.70 µg/L for children 6–11 years participating in the 2011–2012 cycle of NHANES (Lehmler et al. 2020). Much lower levels were reported in 6-year old children from France, with a median of 0.02 µg/L of 3-PBA in urine samples collected in the period 2009–2012 (Viel et al. 2015).

The present study has several strengths, including a comprehensive neurodevelopmental assessment encompassing various cognitive domains, behavioral outcomes, and dimensions of socio-emotional development. Although the proportion of highly educated mothers was slightly higher in the present study subsample, participants were representative of the original MIREC cohort. To minimize confounding, several known predictors of neurodevelopment were examined and included as covariates in models. The

limitations of our study include the biomarkers used as indicators of exposure, the small sample size, and the cross-sectional design. The pyrethroid biomarkers used here to assess exposure are not specific to parent pesticides. The DCCA metabolites are derived from three pesticides (permethrin, cypermethrin, cyfluthrin), while the 3-PBA metabolite is derived from several parent pesticides (Barr et al. 2010). Due to the rapid elimination of these metabolites from the human body, measurements made in only one urine sample may not represent children's long-term exposure and could result in exposure misclassification, reducing the statistical power to detect associations. Another limitation is that urinary metabolites of pyrethroids might reflect not only exposure to the active compounds, but also exposure to metabolites formed by environmental degradation of pyrethroids (Chen et al., 2012). The inferences that can be drawn from the present findings are limited by its cross-sectional design. For instance, we cannot exclude the possibility that children with neurodevelopmental problems might display behaviors that lead to higher exposure to pesticides. The findings could also be attributable to uncontrolled confounding, although we adjusted for several important confounders. Also, we analyzed associations for multiple test scores and did not adjust for multiple comparisons. Furthermore, the evaluations by parents might not be as valid as evaluations by a professional, but the instruments used in the present study are well-validated and frequently used in developmental neurotoxicology. Finally, the mothers of children in our cohort study were older, more educated, and had higher income compared to women from the general Canadian population giving birth in the same time period (Arbuckle et al., 2013), so results might not be generalizable nationally. However, the relatively homogeneous profile of our study cohort might limit the potential for residual confounding by socioeconomic factors.

Conclusions

A growing number of epidemiological studies report associations between children's exposure to pyrethroid pesticides and poorer neurodevelopment, often with some indication of sex-differences in these associations. In the present study, we report cross-sectional associations between pyrethroid urinary metabolites and worse scores on tests of cognitive abilities and problem behaviors, especially in boys. Our results agree with previous studies and could suggest that exposure to pyrethroid pesticides represents a risk

to neurodevelopment. Our results also show that pyrethroid metabolites were detected very frequently, showing that exposure to these products is common. However, given the small and cross-sectional nature of this study, a larger prospective study would be necessary to better understand the potential effects of pyrethroids on neurodevelopment.

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SUPPLEMENTARY INFORMATION

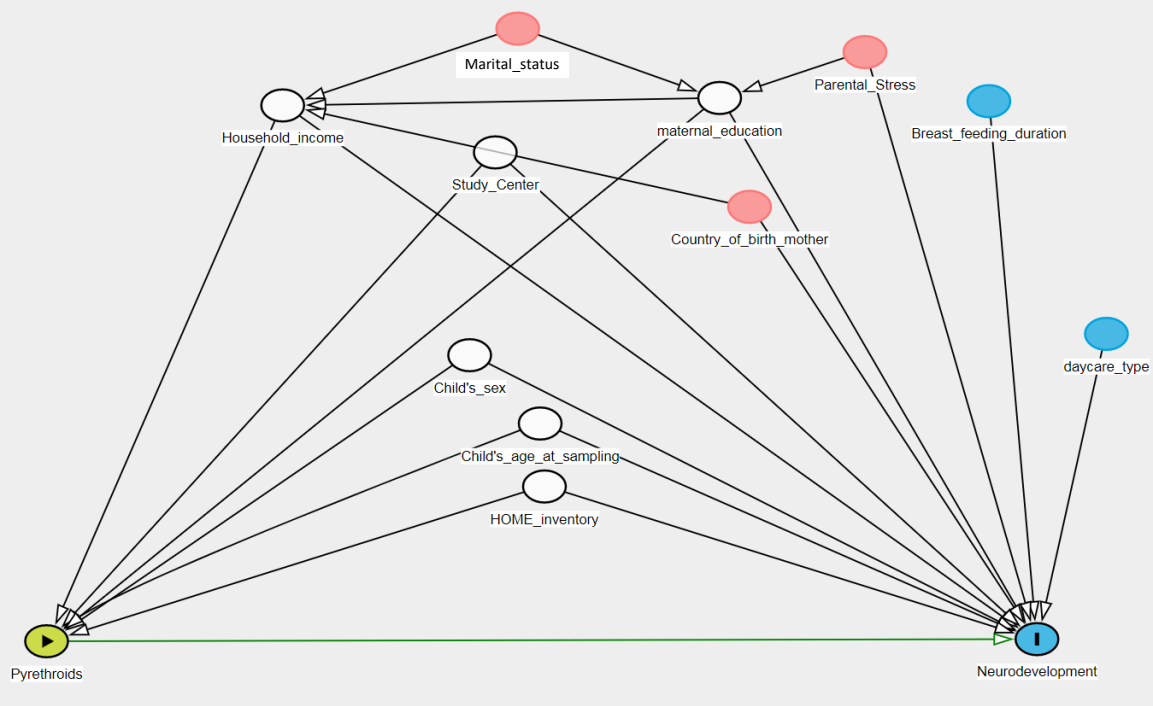

Figure S1: DAG for the association between urinary concentrations of pyrethroid metabolites and neurodevelopment

Table S1: Descriptive statistics for concentrations of urinary pyrethroid metabolites of all children in biomonitoring visit follow-up of MIREC-CD plus study; concentrations are standardized for specific gravity (MIREC CD-Plus Study, 2013 – 2015)

Pyrethroid metabolite	**n**	**LOD**	**n(%)<LOD**	**GM**	**min**	**P25**	**P50**	**P75**	**P95**	**max**
Entire sample										
cis-DBCA (µg/L)	197	0.006	37 (18.8)	0.02	<LOD	0.01	0.02	0.04	0.20	1.01
cis-DCCA (µg/L)	198	0.007	4 (2.02)	0.26	0.02	0.10	0.21	0.44	3.06	16.50
trans-DCCA (µg/L)	198	0.010	0 (0)	0.27	0.02	0.11	0.22	0.46	3.33	17.34
3-PBA (µg/L)	196	0.010	0 (0)	0.39	0.04	0.17	0.32	0.70	3.41	13.62
4-F-3-PBA (µg/L)	189	0.008	111 (65.3)	NC	<LOD	<LOD	<LOD	0.01	0.12	0.62
Σ cis/trans-DCCA (nmol/L)	198	-	-	1.94	0.10	0.82	1.68	3.28	22.51	108.26
Σ metabolites (nmol/L)	189	-	-	4.10	0.39	1.80	3.51	7.00	37.02	170.93
Boys										
cis-DBCA (µg/L)	88	0.006	15 (17.0)	0.02	<LOD	0.01	0.02	0.04	0.27	1.02
cis-DCCA (µg/L)	88	0.007	1 (1)	0.26	0.02	0.06	0.21	0.31	2.44	4.13
trans-DCCA (µg/L)	88	0.010	0 (0)	0.27	0.02	0.11	0.21	0.51	3.12	9.71
3-PBA (µg/L)	88	0.010	0 (0)	0.43	0.04	0.22	0.36	0.82	2.81	6.83
4-F-3-PBA (µg/L)	85	0.008	51 (59.9)	NC	<LOD	<LOD	<LOD	0.01	0.09	0.20
Σ cis/trans-DCCA (nmol/L)	88	-	--	1.93	0.19	0.82	1.44	3.65	21.82	66.56
Σ metabolites (nmol/L)	85	-	-	4.20	0.39	2.10	3.73	7.62	35.12	101.01
Girls										
cis-DBCA (µg/L)	109	0.006	22 (20.)	0.02	<LOD	0.01	0.02	0.03	0.16	0.34
cis-DCCA (µg/L)	110	0.007	3 (2.7)	0.26	<LOD	0.06	0.22	0.35	2.97	16.50
trans-DCCA (µg/L)	110	0.010	0 (0)	0.27	0.02	0.11	0.24	0.45	3.92	17.32
3-PBA (µg/L)	108	0.010	0 (0)	0.36	0.06	0.15	0.27	0.60	5.82	13.61
4-F-3-PBA (µg/L)	104	0.008	60 (57.6)	NC	<LOD	<LOD	<LOD	0.02	0.17	0.59
Σ cis/trans-DCCA (nmol/L)	110	-	-	1.94	0.10	0.84	1.76	3.19	27.34	108.26
Σ metabolites (nmol/L)	104	-	-	4.00	0.65	1.70	3.31	6.88	45.02	170.92

LOD: limit of detection

NC: not calculated because above 50% of samples had 4-F-3-PBA concentrations below LOD

Table S2: Pearson correlation coefficients between log-transformed pyrethroid metabolites (r, p-value and n); concentrations are standardized for specific gravity.

	cis-DBCA	cis-DCCA	trans-DCCA	3-PBA	4-F-3-PBA	Σ cis- and trans-DCCA	Σ all metabolites
cis-DBCA		0.31 <0.000 197	0.31 <0.001 197	0.37 <0.001 195	0.28 <0.001 189	0.31 <0.001 197	0.37 <0.001 187
cis-DCCA	0.31 <0.001 197		**0.96** **<0.001** **198**	**0.83** **<0.001** **196**	0.22 0.003 189	**0.98** **<0.001** **198**	**0.93** **<0.001** **187**
trans-DCCA	0.31 <0.001 197	**0.96** **<0.001** **198**		**0.85** **<0.001** **196**	0.25 0.001 189	**0.99** **<0.001** **198**	**0.94** **<0.001** **187**
3-PBA	0.37 <0.001 195	**0.83** **<0.001** **196**	**0.85** **<0.001** **196**		0.22 0.002 187	**0.85** **<0.001** **196**	**0.97** **<0.001** **187**
4-F-3-PBA	0.28 <0.001 189	0.22 0.003 189	0.25 0.001 189	0.22 0.002 187		0.24 0.001 189	0.27 0.001 187
Σ cis- and trans-DCCA	0.31 <0.001 197	**0.98** **<0.001** **198**	**0.99** **<0.001** **198**	**0.85** **<0.001** **196**	0.24 0.001 189		**0.95** **<0.001** **187**
Σ all metabolites	0.37 <0.001 187	**0.93** **<0.001** **187**	**0.94** **<0.001** **187**	**0.97** **<0.001** **187**	0.27 0.001 187	**0.95** **<0.001** **187**	