Behavioural disorders in 6-year-old children and pyrethroid insecticide exposure: the PELAGIE mother-child cohort

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ABSTRACT

Objective The potential impact of environmental exposure to pyrethroid insecticides on child neurodevelopment has just started to receive attention, despite their widespread use. We investigated the associations between prenatal and childhood exposure to pyrethroid insecticides and behavioral skills in 6-year-olds.

Methods The PELAGIE cohort enrolled 3,421 pregnant women from Brittany, France between 2002 and 2006. When their children turned six, 428 mothers were randomly selected for the study, and 287 (67%) agreed to participate. Children's behavior was assessed using the Strengths and Difficulties Questionnaire (SDQ). Three subscales (prosocial behavior, internalizing disorders and externalizing disorders) were considered. Five pyrethroid metabolites were measured in maternal and child urine samples collected between 6 and 19 gestational weeks and at 6 years of age, respectively. Logistic regression and reverse-scale Cox regression models were used to estimate the associations between SDQ scores and urinary pyrethroid metabolite concentrations, adjusting for organophosphate metabolite concentrations and potential confounders.

Results Increased prenatal *cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (DCCA) concentrations were associated with internalizing difficulties (Cox p-value=0.05). For childhood 3‑phenoxybenzoic acid (PBA) concentrations, a positive association was observed with externalizing difficulties (Cox p-value=0.04) and high odds ratios (ORs) were found for abnormal or borderline social behavior (OR=2.93, 95% CI: 1.27, 6.78, and OR=1.91, 95% CI: 0.80, 4.57, for the intermediate and highest metabolite categories, respectively). High childhood *trans*-DCCA concentrations were associated with reduced externalizing disorders (Cox p-value=0.03).

Conclusions The present study suggests that exposure to certain pyrethroids, at environmental levels, may negatively affect neurobehavioral development by 6 years of age.

Keywords Behavioral disorders; Pyrethroid insecticides; Urine concentrations; Prenatal exposure; Childhood exposure

What this paper adds

- Pyrethroid insecticides are widely used in agriculture and in homes.
- The neurobehavioral effects of environmental exposure to pyrethroids in children have just started to receive attention.
- Increased prenatal *cis*-DCCA pyrethroid metabolite concentrations were associated with internalizing difficulties at age 6 (as assessed using the Strengths and Difficulties Questionnaire).
- A positive association was observed between childhood 3-PBA pyrethroid metabolite concentrations and externalizing difficulties at age 6.
- This study suggests that exposure to certain pyrethroids at the low environmental doses encountered by the general public may be associated with behavioral disorders in children.

INTRODUCTION

Pesticide-monitoring studies carried out in the European Union and the United States have indicated a shift in residential pesticide exposure from organophosphate (OP) insecticides to pyrethroid insecticides in recent decades, with detectable amounts of pyrethroid metabolites in urine samples from the general population.[1-3] This shift results from the increasing concern about the adverse health consequences of OP insecticides, while pyrethroids were purportedly a safer alternative for humans and the environment.[4] Like many other classes of insecticides, pyrethroids are neurotoxicants. They allow more sodium ions to cross and depolarize the neuronal membrane and cause repetitive nerve impulses in insects and other pests.[5] Because of increasing pesticide regulations to protect health and the environment, pyrethroids have become the predominant insecticide class (to control pests in residential and agricultural settings, and to treat head lice and scabies in humans and fleas in pets),[6] yet animal studies suggest the potential for neurodevelopmental toxicity.[5]

Exposure to pyrethroids in the general population is widespread, mostly through diet and indoor residential uses (via ingestion, dermal, and inhalation pathways).[7] After uptake in the human body, pyrethroids are rapidly metabolized and mainly excreted in urine. Many pyrethroids are enzymatically transformed into the relatively non-class-specific metabolite 3‑phenoxybenzoic acid (3‑PBA). Permethrin, cypermethrin and cyfluthrin are also transformed into *cis* or *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (*cis*- or *trans*-DCCA) metabolites. 4-fluoro-3-phenoxybenzoic acid (4-F-3PBA) is a specific metabolite of cyfluthrin and cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid (cis-DBCA) a specific metabolite of deltamethrin. Pyrethroid compounds can cross the placental barrier as permethrin was detected in human cord plasma samples, collected at or immediately postpartum.[8]

The potential neurobehavioral toxicity of pyrethroid exposure in children has just started to receive attention. Shelton et al. showed that children (aged 2 to 5 years) of mothers residing near pyrethroid insecticide agricultural applications just prior to conception or during their third trimester were at greater risk for autism spectrum disorders.[9] Regarding childhood exposures, Oulhote and Bouchard observed a significant association between childhood (6-11 years of age) concentration of the *cis*-DCCA pyrethroid metabolite in the urine (but not 3-PBA or *trans*-DCCA concentrations) and high scores for total behavioral difficulties on the Strengths and Difficulties Questionnaire (SDQ).[10] Wagner-Schuman et al. found an association among 8- to 15-year-old children between increased urinary levels of the pyrethroid metabolite 3-PBA (the only metabolite

considered) with attention deficit hyperactivity disorder (ADHD) and hyperactive-impulsive symptoms.[11] Domingues et al. showed that levels of 3-PBA were higher in the urine of a group of children (aged 5 to 12 years) affected by autism spectrum disorders in comparison with those of control children.[12] On the other hand, Quirós-Alcalá et al. reported that childhood (6 to 15 years of age) 3-PBA, *cis*-DCCA and *trans*-DCCA pyrethroid metabolite concentrations were not associated with parental reports of ADHD.[13] However, neither of these studies assessed exposure to pyrethroid insecticides both prenatally and during childhood.

Using a longitudinal design, we recently showed with the French PELAGIE mother-child cohort data that childhood exposure to pyrethroid insecticides, as measured by urinary 3-PBA and *cis*-DBCA metabolite concentrations, was associated with poorer neurocognitive abilities in children at 6 years of age, after adjusting for prenatal pyrethroid exposure, various potential confounders and childhood OP insecticide metabolite levels.[14] Conversely, we observed no consistent associations between neurocognitive abilities and prenatal urinary pyrethroid metabolite concentrations. Our goal is now to investigate the associations between prenatal or childhood exposure to pyrethroid insecticides and mental and behavioral difficulties in 6-year-olds, as assessed by the SDQ.

METHODS

Study setting and design

Subjects in this report were selected from the French PELAGIE mother-child cohort that was extensively described previously.[15-16] Briefly, 3,421 pregnant women from Brittany, France were included from January 2002 to February 2006. Women were enrolled during their first prenatal visit before the 19th week of gestation after completing a questionnaire at home concerning family, social and demographic characteristics, diet and lifestyle. Midwives and pediatricians at the maternity units provided the study staff with medical information about the pregnancy, delivery, birth weight and neonatal health for 3,399 women and their newborns.

As described in Viel et al., a random subcohort of 571 mothers was selected for neuropsychological follow-up and potential pesticide determination in prenatal urine sample from the mothers who delivered live-born singleton infants (without severe neonatal abnormalities or hospitalization), to obtain a final sample of size similar to those used in previous insecticide exposure studies.[14] Among these mothers, 446 were successfully contacted by phone, and 18 were further excluded because their child had already undergone neuropsychological or behavioral tests

(to avoid bias due to learning effect and benefits of possible psychological care). A total of 287 (67%) mothers agreed to participate with their 6-year-old child in the neuropsychological follow-up that took place between October 2009 and September 2012. Mothers completed a self-administered questionnaire to provide information on sociodemographic characteristics, lifestyle factors and their child's health, behavior and environmental exposures. Home visits were organized by two psychologists who were blinded to exposure levels. They were in charge of maternal intelligence scoring, child neurodevelopmental assessments, maternal interviews for home environment assessments, child urine collections and dust sampling.

Assessment of child behavior at age 6

Behavior was assessed using the French parent version of the SDQ,[17] a validated screening instrument for epidemiologic research in children 4 to 16 years of age.[18] Parents, mostly mothers, completed a list of 25 questions to describe their child's behavior in the previous 6 months. The responses for each item were coded 0 for "not true", 1 for "somewhat true" or 2 for "certainly true". These 25 core attributes were divided into five subscales (emotional, conduct, hyperactivity, peer and prosocial behavior) with five items each. Each subscale had a summed score ranging from 0 to 10. Children with higher scores are more likely to have behavioral problems, except on the prosocial subscale, where higher scores indicate positive social behavior.

The SDQ was not completed by 3 children in the study. In the remaining 284 children, thirteen missing data values for the neurobehavioral scores (1 emotional, 3 conduct, 5 hyperactivity and 4 peer) were replaced, for a given subscale, by the mode estimated from the children with an exact match on the three other subscales (as missingness was fairly limited we did not use multiple imputation).

Following Goodman et al.,[19] we calculated two alternative ten-item "internalizing" (emotional and peer items) and "externalizing" (behavioral and hyperactivity items) SDQ subscales that each ranged from 0 to 20 because they are more appropriate when selecting outcome variables in low-risk, epidemiological samples. Prosocial behavior was classified as normal, borderline or abnormal using French normative cut-off points (7 to 10 = normal, 6 = borderline and 0 to 5 = abnormal, because high scores are desirable for the prosocial scale).[17] No cut-off points on the internalizing and externalizing subscales were available from a French population sample; however, the French cut-off points on the original items were very similar to those of the UK and US.[17] Therefore, we

used the UK cut-offs for the remaining two scores: internalizing subscale (0 to 5 = normal, 6 to 7 = borderline and 8 to 20 = abnormal) and externalizing subscale (0 to 8 = normal, 9 to 10 = borderline and 11 to 20 = abnormal).[19] Then, the abnormal and borderline categories were a priori aggregated to create a dichotomous dependent variable with sufficient outcomes for analysis: 49 (17.3%) for reverse-scored prosocial behavior, 67 (23.6%) for internalizing subscale and 51 (18.0%) for externalizing subscale.

Maternal interviews and home assessments at child age 6

The Wechsler Adult Intelligence Scale – 3rd revision (WAIS-III) was administered to mothers.[20] The Verbal Intelligence Quotient (VIQ) score was used to assess general knowledge, language, reasoning and memory skills. To evaluate the quality and extent of stimulation available to the child in the home environment, the HOME (Home Observation for Measurement of the Environment) inventory was used, as in many studies of neurotoxicity.[21] Higher HOME scores indicate a more supportive and stimulating home environment.

Pyrethroid and other neurotoxicants exposure assessments

To measure the highest possible pyrethroid concentrations, first-morning-void urine samples were collected, during early pregnancy (6-19 gestational weeks) for mothers and during the 6-year visit for children (at age ranging from 5.99 to 6.27 years). Samples were kept frozen in storage at -20°C until analysis at the LABOCEA laboratory (Plouzané, France).

We measured the five major metabolites of pyrethroid insecticides detected in the urine: 3-PBA, 4-F-3-PBA, *cis*-DCCA and *trans*-DCCA, and *cis*-DBCA. Six nonspecific OP dialkylphosphate metabolites were also measured. Concentrations were summed to obtain overall concentrations of diethylphosphate metabolites (DE; sum of diethylphosphate, diethylthiophosphate and diethyldithiophosphate) and dimethylphosphate metabolites (DM; sum of dimethylphosphate, dimethylthiophosphate and dimethyldithiophosphate). Extensive details on the laboratory methods can be found elsewhere.[14]

Eighty-two mothers were missing measures for all pyrethroid metabolites, and 55 mothers were missing measures for DM and DE phosphate metabolites, mostly because the entire samples were

used for other urine assays. Three children had missing 3-PBA, 4-F-3PBA, DM and DE phosphate levels, and four had missing *trans*-DCCA, *cis*-DCCA and *cis*-DBCA measures.

Lead, which we considered to be a potential confounder, was measured using a standard protocol whereby wipe samples of floor dust were collected from the living room.[22] No blood lead levels were available.

Statistical analyses

Associations between dichotomized behavioral subscales (abnormal/borderline vs. normal) as outcomes and prenatal urinary pyrethroid metabolite concentrations were examined using multiple logistic regression models with the following selection and analysis strategy. Metabolite concentrations were categorized as following: if the proportion of non-detected values (i.e., values lower than the limit of detection - LOD) was greater than 50% then two groups were defined (< LOD and ≥ LOD); if the proportion of non-detected values was within the range of 30-50% then three groups were defined (< LOD, and for those with a detectable level, subdivided below and above the median); in the remaining situation, concentrations were divided into tertiles.

The following maternal factors were considered: age at the beginning of pregnancy (continuous), place of residence (rural or urban), parity (0 or ≥1), pre-pregnancy body mass index (≤25 or >25 kg/m²), education (≤12 or >12 years), WAIS-III VIQ (continuous), tobacco smoking at the beginning of pregnancy (no or yes), usual fish consumption before pregnancy (<2 or ≥2 times a week), length of pregnancy (continuous) and breastfeeding, whether exclusive or not (none, ≤16 or >16 weeks). The following variables were considered for the 6-year-old children: sex, birth weight (continuous), education (nursery or primary school), number of siblings at age 6 (continuous), sleep duration (<10.5, 10.5-11 or >11 hours per day), duration of television watching (<2.5, 2.5-4.5 or >4.5 hours per week), duration of video game playing (0, 0-1.5 or ≥1.5 hours per week), regular extra-curricular sport activities (no, yes) and urinary cotinine concentration measured in the same urine samples as the pesticides (<6 or ≥6 µg/L). Finally, several environmental factors and co-exposures were also examined: HOME score when the child was 6 years of age (continuous), acid-leachable lead in the living room (≤1, 1-3 or >3 µg/m²), number of smokers at home (0, 1 or ≥2), and cigarettes smoked at home (0, 0-10 or >10 per day). The psychologist who administered the psychological tests was also investigated as a potential source of measurement errors. To preserve the size of the analytic sample, missing values for covariates were replaced by the modal value from participants with non-

missing values. Imputation was required for 6 mothers (6 missing data values, i.e., 0.2%) and 14 children (40 missing data values, i.e., 1.5%).

We included the child's sex and maternal education in models a priori because they are important determinants of children's behavior. Because pyrethroid and organophosphate insecticides are frequently encountered in the same environments, and because recent studies have provided compelling evidence for an association between prenatal OP insecticide exposure and neurodevelopmental deficits,[23-24] potential confounding by OP exposure was considered by forcing DE and DM phosphate metabolites in maternal urine samples into the models. For each pyrethroid metabolite measured in the prenatal period, the corresponding childhood concentration was similarly included in the models to account for its potential competing influence. In addition, we included urinary creatinine concentrations (for mothers and children) to account for urinary dilution.[25] The remaining variables that predicted both the behavioral scores and the pyrethroid metabolite levels with p-value < 0.2 were retained as model covariates. Separate models were used to estimate associations with the five pyrethroid metabolites.

Moreover, a reverse-scale Cox regression model recently proposed by Dinse et al. was performed to handle non-detected values. In this alternative method, the measured metabolite is treated as the modelled outcome, switching the roles of exposure and health effect.[26] The method begins by reversing the concentration scale and then applying Cox regression analysis with adjustment for potential confounders (the same confounding variables that were used in the logistic regression-based approach). The method makes full use of quantifiable metabolite measurements and appropriately treats non-detected values as censored. The corresponding hazard ratio parameter is interpretable as an odds ratio (OR), but in a different way from the OR obtained in logistic regression models. This OR is the odds of the health outcome at concentration *t* divided by the odds of the health outcome for the aggregate of concentrations *below t*, assuming that this OR is the same across all concentrations. In other words, for 2 children whose scores differ by 1 point, it represents the OR for the higher-scoring child having a given pyrethroid metabolite concentration versus *all* lower concentrations. As this interpretation is not straightforward and could cause confusion with logistic regression-derived ORs, we decided not to report hazard ratio parameters but their corresponding p-values, all the more as associations detected by reverse-scale Cox regression models were always of the same sign as trends indicated by logistic regression models.

In separate analyses, we explored possible sex-related differences in the association between urinary pyrethroid metabolites and the outcomes of interest by introducing a term for sex * urinary concentration into the final models. We set the threshold for statistical significance at p-value < 0.15 for interaction.

For childhood exposure, we examined the cross-sectional association of behavioral scores with pyrethroid metabolite concentrations using the same confounder selection strategy. The metabolite concentration categories slightly differed because the limits were based on childhood (and not prenatal) metabolite distributions. Childhood DE and DM concentrations were forced into the models. As with prenatal concentrations, childhood pyrethroid metabolite concentrations were treated as categorical (logistic regression model) and as continuous (reverse-scale Cox regression model) variables.

For each outcome of interest, adjusted ORs and 95% confidence intervals (CIs) were estimated from the logistic models. P-values < 0.05 were considered statistically significant, and all tests were two-sided. All statistical analyses were performed using R software (R Development Core Team 2015).

Ethics statement

This study was approved by the French Consulting Committee for the Treatment of Information in Medical Research (no. 09.485) and by the French National Commission for the Confidentiality of Computerized Data (no. 909347). Written informed consent was obtained from each mother. The children provided verbal and witnessed assent.

RESULTS

Description of the population

Demographic characteristics and lifestyle factors for the 287 mother-child pairs studied are reported in Table 1. At the beginning of their pregnancies, most mothers were greater than 27 years of age, multiparous, of healthy weight, college graduates and non-smokers. The 6-year-old children predominantly attended nursery school, lived in a non-smoking environment, slept at least 10.5 hours per day, and participated in regular extra-curricular sport activities.

Levels of urinary pyrethroid metabolites

Table 2 presents the detection frequencies and distributions of the five pyrethroid metabolites measured in the maternal and child first-morning-void urine samples. *Trans*-DCCA, *cis*-DBCA and *cis*-DCCA metabolites were the most frequently detected species in both the mothers (99.9%, 68.3% and 64.9%, respectively) and the children (96.5%, 85.2% and 64.7%, respectively). Median concentrations followed broadly similar patterns.

Correlation coefficients between pyrethroid metabolites have been fully reported elsewhere.[14] Briefly, coefficients were high in maternal urine for the two DCCA isomers (r = 0.61) and moderate for all other metabolite pairs (r ≤ 0.39). A similar pattern was observed in child urine (r = 0.74, and r ≤ 0.39, respectively). Mother *trans*-DCCA concentrations were moderately correlated (r = 0.24) with their child counterparts, whereas the remaining four mother pyrethroid metabolite concentrations were uncorrelated with their child counterparts (r ≤ 0.04).

Associations between maternal prenatal urinary levels of pyrethroid metabolites and child neurobehavioral scores

Table 3 presents the associations between prenatal pyrethroid metabolite concentrations and SDQ scores, after adjusting for potential confounders, urinary creatinine levels, DM and DE prenatal concentrations and the corresponding childhood pyrethroid metabolite concentrations. None of the ORs differed significantly from unity. Reverse-scale Cox analyses showed that *cis*-DCCA concentrations were positively associated with internalizing difficulties (Cox p-value = 0.05).

There was an interaction between 3-PBA and sex for the association with abnormal or borderline social behavior (p interaction = 0.11). The inverse association was stronger for girls (OR=0.11, 95% CI 0.01 to 1.24) than for boys (OR=0.70, 95% CI 0.16 to 2.96), although both associations were not statistically significant.

Associations between childhood urinary levels of pyrethroid metabolites and child neurobehavioral scores

Table 4 reports the results for childhood pyrethroid metabolite concentrations. No consistent association was found between the internalizing score and any metabolite concentration. For childhood 3-PBA concentrations, a positive association was observed with externalizing difficulties

(Cox p-value = 0.04) and high ORs were found for abnormal or borderline social behavior (OR=2.93, 95% CI 1.27 to 6.78, and OR=1.91, 95% CI 0.80 to 4.57 for the intermediate and highest metabolite categories - i.e., detectable values, respectively). Increased childhood *trans*-DCCA concentrations were associated with reduced externalizing disorders (Cox p-value = 0.03).

No association differed by sex for any metabolite concentration or any behavioral subscale (p interaction > 0.15).

DISCUSSION

Two substantive findings emerged from this study. First, a positive association was observed between prenatal urinary *cis*-DCCA concentrations and internalizing difficulties as assessed with SDQ scores measured at 6 years of age. Second, we found that childhood exposure to pyrethroid insecticides, in general (as reflected by 3-PBA concentrations in the urine of 6-year-old children), was associated with increased odds of behavioral disorders for the externalizing and reverse-scored prosocial behavior subscales. The latter results were consistent with the biosocial model of externalizing behavior, as externalizing disorders manifest as defiant and disruptive behavior and reflect the child negatively acting on the external environment.[27] We have no current explanation for the counter-intuitive association observed between childhood high *trans*-DCCA concentrations and reduced externalizing disorders. We only note that similar inverse associations with SDQ scores were reported for prenatal exposure to perfluorinated chemicals.[28] Finally, we found little evidence of effect modification by sex of the child.

As reported by Oulhote and Bouchard, several mechanisms could underlie the association between pyrethroid insecticides and behavioral disorders in children.[10] The increase in sodium influx caused by pyrethroid insecticides could affect neuronal synaptic plasticity through modulation of the brain derived neurotrophic factor. Moreover, exposure to pyrethroid insecticides could induce alterations in dopamine transporter function and influence brain microanatomy and cholinergic/ dopaminergic neurochemistry.

The present study has many strengths, including its longitudinal design with pyrethroid exposure assessments both prenatally and during childhood, the mental health assessment tool (SDQ), extensive information on covariates, and a thorough confounder identification and control strategy. The SDQ is a brief screening device for identifying children at high-risk for mental health problems in epidemiologic research. Its reliability has been well documented, and its sensitivity to subtle

neurodevelopment changes with environmental exposures has been demonstrated.[18 19 28] We dichotomized SDQ subscales to denote clinical significance and allow easy interpretation (based on ORs). To minimize residual confounding, we deliberately examined or adjusted for numerous risk factors, including known predictors of neurodevelopment factors. We also considered information about additional environmental neurotoxic exposures from substances such as OP insecticides and lead. Participants were representative of the PELAGIE cohort, although highly educated mothers were slightly more numerous (68% vs. 62%).[15] Moreover, their homogeneous socioeconomic profile (rather wealthy families, reflective of the whole cohort) may be observed as strength because it reduced the potential for uncontrolled confounding. We used a sound and flexible statistical technique to handle biomarker values falling below LODs. The reverse-scale Cox regression model allows full use of the available data, is valid even with extreme LOD censoring, and does not assume any parametric distribution.[26] It was reassuring that both logistic and reverse-scale Cox models produced fairly consistent results; in our opinion, the latter was more convincing because of its quantitative nature.

Several limitations of this study should be noted. Assessing pyrethroid exposures in urine samples is challenging because they are cleared from the body in just a few days, with substantial within-child variability.[29] Consequently, pyrethroid metabolites from spot urine samples may not represent a child's average exposure over time and may result in misclassification, reducing the statistical power to detect associations. An additional concern about urinary biomarkers is that the metabolites detected in urine may not be due entirely to exposure to parent compounds, as there may also be a minor contribution from exposure to the metabolites themselves in the environment.[11] Another limitation is that we did not correct for multiple comparisons in these exploratory analyses, considering the limited evidence of association between neurodevelopment and exposure to pyrethroid insecticides. Moreover, as both maternal and child pyrethroid metabolite levels were measured in one batch, we cannot rule out the possibility that a degradation of pyrethroid metabolites may have occurred during the years in which the maternal urine samples were stored at -20°C. Finally, because child metabolite concentrations reflect concurrent exposures, the temporal relation with the outcome is unclear; reverse causality becomes conceivable as children with behavioral problems (e.g., hyperactivity) might increase their exposure to pesticides.[10 13]

To our knowledge, only one study had previously assessed the association between postnatal pyrethroid exposure and child behavioral development using the SDQ. Some differences between

our results and those of Oulhote and Bouchard are worth noting.[10] Some differences between the two studies. The median concentration of 3-PBA was lower in French PELAGIE children (0.018 µg/L) compared to Canadian children (0.200 µg/L), but *cis*-DCCA values were similar in both groups (0.099 µg/L and 0.05 µg/L, respectively). We considered three SDQ subscales (internalizing, externalizing and reverse-scored prosocial behavior), while Oulhote and Bouchard studied the total difficulties and four of the five dimension scales as too few children in their study had high scores on the prosocial behavior to estimate association.[10] Moreover, only the abnormal category was considered as an outcome in the Canadian study (i.e., the borderline and normal categories were aggregated). Like Oulhote and Bouchard, we found an interaction between 3-PBA and sex but for a different period of exposure (pregnancy vs. childhood), a different SDQ subscale (prosocial behavior vs. conduct disorder), and with opposite effects (boys with higher OR vs. girls with higher OR).

CONCLUSION

The current study suggests that exposure to certain pyrethroids at the low environmental doses encountered by the general public may be associated with behavioral disorders in children. Together with our previous report on cognitive disabilities, this study contributes to a broader understanding of the potential risk to neurodevelopment from pyrethroid insecticides. Whatever their etiology, awareness of neurodevelopmental deficits might be socially and educationally meaningful. Identifying the potential causes that can be remediated is, therefore, of paramount public health importance.

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Contributors JFV performed the statistical analyses, and drafted the manuscript, assisted by CC. CC conceived and planned the study, assisted by SC. All authors were involved in the interpretation of the data, revision of the manuscript for important intellectual content, and the final approval of the manuscript.

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Competing interests None declared.

Patient consent Written informed consent was obtained from each mother. The children provided verbal and witnessed assent.

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Table 1 Sociodemographic and lifestyle factors of the study's mother-child pairs (n=287, PELAGIE cohort, France) (from Viel et al. 2015)

Characteristics	No.	%
Maternal factors		
Age (years)[a]		
≤ 27	62	21.6
28 - 31	131	45.6
≥ 32	94	32.8
Place of residence		
rural	158	55.1
urban	129	44.9
Parity		
0	122	42.5
≥ 1	165	57.5
Body mass index (kg/m²)		
≤ 25	236	82.2
> 25	51	17.8
Education (years)		
≤12	91	31.7
>12	196	68.3
Tobacco smoking at the beginning of pregnancy		
No	216	75.3
Yes	71	24.7
Child factors		
Sex		
Boy	139	48.4
Girl	148	51.6
Birth weight (grams)[a]		
< 3,380	143	49.8
≥ 3,380	144	50.2
Education		
Nursery school	214	74.6
Primary school	73	25.4
Smokers at home		
0	169	58.9
> 0	118	41.1
Sleep duration (hours per day)		
< 10.5	74	25.8

10.5 - 11	129	44.9
> 11	84	29.3
Regular extra-curricular sport activities		
No	81	28.2
Yes	206	71.8

[a] For the sake of clarity, this variable is categorized in the table, but it was introduced into regression models as a continuous variable.

Table 2 Concentrations of pyrethroid insecticide urinary metabolites (µg/L) (PELAGIE cohort, France) (modified from Viel et al. 2015)

Exposure	No.	LOD	Percent <LOD	50th percentile	75th percentile	90th percentile
Prenatal (before the 19th week of gestation)						
3-PBA	205	0.008	69.8	< LOD	0.018	0.075
4-F-3-PBA	205	0.003	91.2	< LOD	< LOD	< LOD
cis-DCCA	205	0.067	35.1	0.090	0.174	0.302
trans-DCCA	205	0.010	2.0	0.140	0.270	0.568
cis-DBCA	205	0.067	31.7	0.105	0.184	0.390
Childhood (at 6 years of age)						
3-PBA	284	0.008	36.3	0.018	0.047	0.089
4-F-3-PBA	284	0.003	84.2	< LOD	< LOD	0.008
cis-DCCA	283	0.067	35.3	0.099	0.189	0.312
trans-DCCA	283	0.010	3.5	0.222	0.583	1.159
cis-DBCA	283	0.067	14.8	0.220	0.428	0.922

Abbreviation: LOD, limit of detection.

Table 3 Adjusted ORs[a] (95% CI) and Cox *p*-values for abnormal or borderline scores on the Strengths and Difficulties Questionnaire (SDQ) and prenatal concentrations of urinary pyrethroid metabolites (n=204, PELAGIE cohort, France)

Metabolites (µg/L)	Internalizing score	Externalizing score	Reverse-scored prosocial behavior
	OR (95% CI)	OR (95% CI)	OR (95% CI)
3-PBA			
<0.008	Ref.	Ref.	Ref.
≥0.008	0.79 (0.32 to 1.97)	0.64 (0.23 to 1.80)	0.37 (0.12 to 1.11)
Cox p-value[c]	0.76	0.43	0.10
4-F-3-PBA			
<0.003[b]	Ref.	Ref.	Ref.
≥0.003	1.43[d] (0.29 to 7.00)	4.75 (0.73 to 31.01)	0.63[d] (0.06 to 5.98)
Cox p-value[c]	0.72	0.07	0.93
cis-DCCA			
<0.067[b]	Ref.	Ref.	Ref.
0.067-0.137	1.47 (0.50 to 4.28)	1.24 (0.40 to 3.88)	0.53 (0.17 to 1.63)
≥0.138	2.33 (0.76 to 7.17)	1.79 (0.51 to 6.30)	0.76 (0.23 to 2.58)
Cox p-value[c]	0.05	0.26	0.22
trans-DCCA			
<0.086	Ref.	Ref.	Ref.
0.086-0.209	1.44 (0.47 to 4.43)[d]	0.39 (0.12 to 1.26)	0.61 (0.20 to 1.87)[d]
≥0.210	1.19 (0.40 to 3.53)[d]	0.60 (0.19 to 1.91)	0.60 (0.20 to 1.82)[d]
Cox p-value[c]	0.35	0.20	0.58
cis-DBCA			
<0.067[b]	Ref.	Ref.	Ref.
0.067-0.154	1.91 (0.64 to 5.68)	2.55 (0.71 to 9.14)	0.73 (0.24 to 2.27)
≥0.155	1.10 (0.35 to 3.51)	1.74 (0.45 to 6.64)	0.61 (0.19 to 2.02)
Cox p-value[c]	0.99	0.22	0.39

[a] Maternal education, child sex, corresponding childhood pyrethroid metabolite concentration, urinary creatinine concentrations (mother and child), and detection of dimethyl (DM) and diethyl (DE) phosphates in maternal urine samples were forced into all models.

[b] Limit of detection.

[c] P-value for association between SDQ score and metabolite concentration provided by reverse-scale Cox regression models.

[d] Adjusted for maternal tobacco smoking at the beginning of pregnancy.

Table 4 Adjusted ORs[a] (95% CI) and Cox *p*-values for abnormal or borderline scores on the Strengths and Difficulties Questionnaire (SDQ) and child concentrations of urinary pyrethroid metabolites (n=282, PELAGIE cohort, France)

Metabolites (µg/L)	Internalizing score	Externalizing score	Reverse-scored prosocial behavior
	OR (95% CI)	OR (95% CI)	OR (95% CI)
3-PBA			
<0.008[b]	Ref.	Ref.	Ref.
0.008-0.037	1.41 (0.73 to 2.73)	1.52 (0.67 to 3.42)	2.93 (1.27 to 6.78)
≥0.038	0.70 (0.34 to 1.46)	1.96 (0.90 to 4.30)	1.91 (0.80 to 4.57)
Cox p-value[c]	0.94	0.04	0.07
4-F-3-PBA			
<0.003[b]	Ref.	Ref.	Ref.
≥0.003	0.86 (0.07 to 1.28)[d]	0.55 (0.21 to 1.41)[e]	1.35 (0.59 to 3.07)
Cox p-value[c]	0.71	0.27	0.34
cis-DCCA			
<0.067[b]	Ref.	Ref.	Ref.
0.067-0.158	1.06 (0.52 to 2.15)	0.63 (0.27 to 1.45)[d]	1.20 (0.53 to 2.71)[f]
≥0.159	0.97 (0.47 to 2.03)	0.97 (0.44 to 2.15)[d]	1.05 (0.45 to 2.56)[f]
Cox p-value[c]	0.95	0.80	0.68
trans-DCCA			
<0.136	Ref.	Ref.	Ref.
0.136-0.409	1.22 (0.59 to 2.51)[d]	0.60 (0.27 to 1.33)	0.71 (0.30 to 1.64)[g]
≥0.410	0.99 (0.47 to 2.10)[d]	0.57 (0.25 to 1.30)	0.76 (0.32 to 1.82)[g]
Cox p-value[c]	0.91	0.03	0.06
cis-DBCA			

<0.134	Ref.	Ref.	Ref.
0.134-0.345	0.49 (0.22 to 1.13)[h]	1.92 (0.29 to 1.57)[h]	0.91 (0.35 to 2.34)[g]
≥0.346	1.49 (0.73 to 3.06)[h]	0.82 (0.36 to 1.86)[h]	2.14 (0.89 to 5.18)[g]
Cox p-value[c]	0.49	0.55	0.23

[a] Maternal education to child sex to child urinary creatinine concentration to and detection of dimethyl (DM) and diethyl (DE) phosphates in child urine samples were forced into all models.
[b] Limit of detection.
[c] P-value for association between SDQ score and metabolite concentration provided by reverse-scale Cox regression models.
[d] Adjusted for maternal tobacco smoking at the beginning of pregnancy.
[e] Adjusted for HOME score.
[f] Adjusted for child extra-curricular sport activities.
[g] Adjusted for child extra-curricular sport activities and child duration of television watching.
[h] Adjusted for parity.