Environment International 82 (2015) 69–75

Contents lists available at ScienceDirect

Environment International

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

Pyrethroid insecticide exposure and cognitive developmental disabilities in children: The PELAGIE mother–child cohort

Jean-François Viel [a,b,*], Charline Warembourg [a], Gaïd Le Maner-Idrissi [c], Agnès Lacroix [c], Gwendolina Limon [d], Florence Rouget [a,e], Christine Monfort [a], Gaël Durand [d], Sylvaine Cordier [a], Cécile Chevrier [a]

[a] INSERM-IRSET n° 1085, Epidemiological Research on Environment, Reproduction and Development, University of Rennes 1, Rennes, France
[b] Department of Epidemiology and Public Health, University Hospital, Rennes, France
[c] Research Centre for Psychology, Cognition and Communication, University of Rennes 2, Rennes, France
[d] LABOCEA Laboratory, Plouzané, France
[e] Department of Pediatrics, University Hospital, Rennes, France

ARTICLE INFO

Article history:
Received 3 December 2014
Received in revised form 20 May 2015
Accepted 21 May 2015
Available online 5 June 2015

Keywords:
Pyrethroid pesticides
Neurodevelopment
Cognitive abilities

ABSTRACT

Pyrethroid insecticides are widely used in agriculture and in homes. Despite the neurotoxicity of these insecticides at high doses, few studies have examined whether lower-level exposures could adversely affect children's neurodevelopment.

The PELAGIE cohort included 3421 pregnant women from Brittany, France between 2002 and 2006. When their children reached their sixth birthday, 428 mothers from the cohort were randomly selected, successfully contacted and found eligible. A total of 287 (67%) mothers agreed to participate with their children in the neuropsychological follow-up. Two cognitive domains were assessed by the Wechsler Intelligence Scale for Children: verbal comprehension and working memory. Five pyrethroid and two organophosphate insecticide metabolites were measured in maternal and child first-void urine samples collected between 6 and 19 gestational weeks and at 6 years of age, respectively. Linear regression models were used to estimate associations between cognitive scores and urinary pyrethroid metabolite concentrations, adjusting for organophosphate metabolite concentrations and potential confounders.

Maternal prenatal pyrethroid metabolite concentrations were not consistently associated with any children's cognitive scores. By contrast, childhood 3-PBA and *cis*-DBCA concentrations were both negatively associated with verbal comprehension scores (P-trend = 0.04 and P-trend < 0.01, respectively) and with working memory scores (P-trend = 0.05 and P-trend < 0.01, respectively). No associations were observed for the three other childhood pyrethroid metabolite concentrations (4-F-3-PBA, *cis*-DCCA, and *trans*-DCCA).

Low-level childhood exposures to deltamethrin (as *cis*-DBCA is its principal and selective metabolite), in particular, and to pyrethroid insecticides, in general (as reflected in levels of the 3-PBA metabolite) may negatively affect neurocognitive development by 6 years of age. Whatever their etiology, these cognitive deficits may be of importance educationally, because cognitive impairments in children interfere with learning and social development. Potential causes that can be prevented are of paramount public health importance.

1. Introduction

The use of pyrethroid insecticides has increased substantially throughout the world over the past several decades, replacing organophosphate and carbamate insecticides, because of their chemical potency against many pests, their relatively low mammalian toxicity and their favorable environmental profiles (Schleier and Peterson, 2011). Pyrethroid insecticides are neurotoxic agents that disrupt the normal function of the peripheral nervous system by altering the permeability of excited nerve cells to sodium ions. Repetitive nerve impulses cause incoordination, convulsions, and paralysis in insects and other pests (Soderlund and Bloomquist, 1989).

It is commonly assumed that the major exposure pathway for pyrethroid insecticides in the general population is diet (Schettgen et al., 2002). However, their use in common household products, such as household and garden insecticides, pet sprays and shampoos, lice treatments, and mosquito repellents applied to clothing, may also lead to short episodes of exposure. Young children may receive greater exposure because they are closer to the floor and surfaces where insecticides may settle, have extensive hand-to-mouth contact, and are more likely to receive head lice treatments, as suggested by their higher urinary concentrations of pyrethroid insecticide metabolites compared

* Corresponding author at: INSERM-IRSET, Department of Epidemiology and Public Health, University Hospital 2, rue Henri Le Guilloux, 35033 Rennes, France.
E-mail address: jean-francois.viel@univ-rennes1.fr (J.-F. Viel).

http://dx.doi.org/10.1016/j.envint.2015.05.009
0160-4120/

with adolescents and adults (Barr et al., 2005). The major exposure routes for children are dietary ingestion of solid foods, followed by nondietary ingestion of dust, except in homes with frequent insecticide applications, in which case dermal absorption is more important than dietary ingestion (Morgan, 2012).

In humans, pyrethroids are rapidly metabolized by ester hydrolysis and hydroxylation, primarily in the liver. The detoxified metabolites are eliminated by the kidneys for several days after exposure. Because pyrethroids are metabolized so rapidly, the concentrations of intact pyrethroids in serum or plasma are much lower than those of urinary metabolites, which are considered to reflect short-term exposures in low-exposure scenarios with the potential for misclassification (Bradman et al., 2005; Koureas et al., 2012). The major metabolites of pyrethroid insecticides detected in the urine are 3-PBA (a common metabolite of up to 20 synthetic pyrethroid insecticides), 4-F-3-PBA (a metabolite of the fluorine-substituted pyrethroid insecticide cyfluthrin), *cis*-DCCA and *trans*-DCCA (geometric isomeric metabolites of the chlorinated pyrethroid insecticides permethrin, cypermethrin, and cyfluthrin), and *cis*-DBCA (a selective metabolite of deltamethrin).

The mode of action of pyrethroid insecticides raises concerns for human health. The potential effects of low-level chronic exposure to pyrethroid insecticides on neurobehavioral functioning are particularly relevant for fetuses and young children, who may be especially vulnerable to neurotoxic agents because of their immature nervous systems and their rapid rates of brain growth and development. A limited number of epidemiologic studies have examined the associations between *maternal prenatal* pyrethroid exposure and effects on neurodevelopment (Horton et al., 2011; Shelton et al., 2014). Recently, however, Oulhote and Bouchard (2013) reported a significant association between *childhood* concentration of *cis*-DCCA in urine and behavioral difficulties. Because pyrethroid and organophosphate (OP) 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 dysfunction (Rauh et al., 2006; Eskenazi et al., 2007; Marks et al., 2010; Bouchard et al., 2011), potential confounding by OP exposure must be considered.

Using a longitudinal design, the aim of this study was to investigate associations between prenatal or childhood exposure to pyrethroid insecticides, as measured by urine pyrethroid metabolite concentrations, and cognitive abilities of 6-year-olds, after adjusting for OP insecticide metabolite levels during the corresponding measurement period.

2. Methods

2.1. Study setting and design

The PELAGIE cohort has been described previously (Petit et al., 2010; Chevrier et al., 2011). Briefly, 3421 pregnant women from Brittany, France were included from January 2002 to February 2006. Women were enrolled 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 3399 women and their newborns.

A random subcohort of 591 mothers was selected for pesticide determination in urine samples from the mothers who delivered live-born singleton infants, to obtain a final sample of size similar to those used in previous OP insecticide exposure studies (Rauh et al., 2006; Eskenazi et al., 2007). For the cognitive assessments at age 6, exclusion criteria were length of pregnancy <35 weeks of amenorrhea, neonatal abnormalities (e.g., severe hypoglycemia, low Apgar score), neonatal hospitalization, and Down syndrome. Among the 571 eligible families, 446 were successfully contacted by phone and 18 were further excluded because of previous child neuropsychological or behavioral tests (to avoid bias due to the learning effect). A total of 287 (67%) mothers agreed to participate with their child in the neuropsychological follow-up. Home visits were organized by two psychologists who were blinded to exposure status and supervised by four pediatric neuropsychologists in meetings held every two months. One of the psychologists completed child neurodevelopmental assessments, and the other psychologist was in charge of maternal interviews, maternal intelligence scoring, home environment assessments, child urine collections and dust sampling.

2.2. Cognitive assessments at age 6

The Wechsler Intelligence Scale for Children, 4th edition (WISC-IV) was used to assess cognitive abilities (Wechsler, 2003). Scores were calculated for two domains: the Verbal Comprehension Index (WISC-VCI), which measures verbal concept formation and is a good predictor of school readiness, and the Working Memory Index (WISC-WMI), which assesses child ability to memorize new information, hold it in short-term memory, concentrate and manipulate information. Higher scores indicate better neurocognitive abilities.

2.3. Maternal interviews and assessments at child age 6

Mothers completed a self-administered questionnaire to provide information on sociodemographic characteristics, lifestyle factors, their child's health, and their child's environmental exposures. Mothers were also administered the Wechsler Adult Intelligence Scale — 3rd revision (WAIS-III) (Wechsler, 1997). 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 (Caldwell and Bradley, 1979). Higher HOME scores indicate a more supportive and stimulating home environment. We adapted the scale for today's French environments. Double-blind scoring was performed on a random sample (39 homes) to monitor the accuracy of HOME inventory; 98% concordance was observed among all dichotomous items.

2.4. Laboratory methods

To measure the highest possible concentrations, first-morning-void urine samples were collected, during early pregnancy (6–19 gestational weeks) for mothers and at the visit at 6 years of age for children (Barr et al., 2010). Upon arrival at the LABOCEA laboratory (Plouzané, France), urine samples were frozen at −20 °C until analysis. To minimize analytic heterogeneity, both the mother's and the child's pyrethroid concentrations were measured in the same batch of samples.

3-PBA and 4-F-3-PBA metabolites in 1-ml urine samples were extracted using an online solid-phase extraction system Waters 2777C and Waters Oasis HLB Direct Connect cartridges with elution during the chromatography mobile phase. Separation was achieved by ultra-performance liquid chromatography (Acquity UPLC, Waters), using a Waters BEH C18 column (150 × 2.1 mm, 1.7 μm) and an elution gradient consisting of acetonitrile/formic acid 0.05% and water/formic acid 0.05%. Detection relied on ultra-performance liquid chromatography and triple quadrupole mass spectrometry (UPLC/MS–MS) (Xevo TQ-S, Waters). Reference standards were provided by Sigma-Aldrich and Dr. Ehrenstorfer. Concentration range linearity was observed from 0.02 μg/L to 2 μg/L for 3-PBA and from 0.01 to 0.20 μg/L for 4-F-PBA. The coefficients of variation ranged from 19% to 20%. Regarding *cis*-DCCA, *trans*-DCCA and *cis*-DBCA metabolites, a simultaneous extraction and derivatization was performed in 2-ml urine samples with Pentafluorobenzyl bromide (PFBBr) as alkylation reagent (Aldrich) and 1 ml dichloromethane (Carlo Erba). DCCA-D9 (Dr. Ehrenstorfer) was added as an internal standard. The organic phase was extracted