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# Prenatal exposure to Organophosphate Pesticides and infant respiratory and allergic outcomes in the PELAGIE cohort

Quentin Vieille

► **To cite this version:**

Quentin Vieille. Prenatal exposure to Organophosphate Pesticides and infant respiratory and allergic outcomes in the PELAGIE cohort. Life Sciences [q-bio]. 2016. dumas-01745495

**HAL Id: dumas-01745495**

**<https://dumas.ccsd.cnrs.fr/dumas-01745495v1>**

Submitted on 5 Jun 2018

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**THÈSE D'EXERCICE / UNIVERSITÉ DE RENNES 1  
FACULTÉ DE MEDECINE**

*Sous le sceau de l'Université Européenne de Bretagne*

**THÈSE EN VUE DU  
DIPLOME D'ÉTAT DE DOCTEUR EN MEDECINE**

Présentée par

**Quentin Vieille**

Né le 11 mars 1988 à Mouchamps

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**Intitulé de la thèse :**

*Exposition prénatale aux insecticides  
organophosphorés et manifestations  
respiratoires et allergiques chez l'enfant  
avant 2ans dans la cohorte PELAGIE.*

**Thèse soutenue à RENNES  
le 7 juin 2016**

devant le jury composé de :

**Vincent LAVOUE**

PU-PH – CHU Rennes / *Président*

**Françoise TATTEVIN**

MCA – Université de Rennes 1 / *Juge*

**Ronan GARLANTEZEC**

MCU-PH – CHU Rennes / *Juge*

**Fabienne PELE**

CCA – Université de Rennes 1 / *Directrice de thèse*

## Remerciements

Je remercie tout d'abord les membres du jury, son président Professeur Vincent Lavoué ainsi que Docteur Ronan Garlantezec et Docteur Françoise Tattevin qui me font l'honneur d'accepter d'évaluer mon travail de thèse.

Je remercie particulièrement la directrice de cette thèse et membre du jury, Docteur Fabienne Pelé qui m'a proposé ce travail il y a 1,5 an puis qui m'a accompagné patiemment, aidé dans ses étapes difficiles jusqu'à cette soutenance et encore après. Je n'aurai rien pu faire sans elle.

Je remercie mes collègues de quelques mois, dans cette belle équipe de l'IRSET, sur le thème qui me tient à cœur de la « santé, environnement et travail ».

Je remercie le soutien du quotidien, mon amie Alice ainsi que ma famille et mes amis.

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**Titre – Title**

**Exposition prénatale aux insecticides organophosphorés et manifestations respiratoires et allergiques chez l'enfant avant 2ans dans la cohorte PELAGIE.**

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**Prenatal exposure to Organophosphate Pesticides and infant respiratory and allergic outcomes in the PELAGIE cohort.**

## **Valorisation Scientifique**

### **Communication orale avec discussion**

**Vieille Q**, Chevrier C, Rouget F, Monfort C, Cordier S, Pelé F : Exposition prénatale aux insecticides organophosphorés et manifestations respiratoires et allergiques chez l'enfant avant 2ans dans la cohorte PELAGIE. 6<sup>ème</sup> Journée de Recherche en Médecine Générale Grand Ouest, 11 juin 2016, Rennes.

## Résumé – Abstract

### Résumé

Introduction : Les systèmes respiratoire et immunitaire en développement (en particulier pendant la vie intra-utérine) sont très sensibles à l'environnement. Des perturbations mineures pendant cette phase peuvent induire chez le nourrisson une résistance diminuée aux maladies infectieuses, des maladies allergiques ou de l'asthme, pathologies particulièrement fréquentes en médecine générale. Des recherches épidémiologiques mettent en évidence que les insecticides organophosphorés (OPs), très présents dans nos environnements, pourraient être toxiques pour ces systèmes. Ainsi cette étude vise à estimer l'effet de l'exposition prénatale aux OPs sur la survenue de manifestations respiratoires ou allergiques du jeune enfant.

Méthodes : Ce travail, basé sur la cohorte PELAGIE, inclut 256 couples mère-enfant. L'exposition prénatale aux OPs a été estimée par la mesure des concentrations en diéthyl- (DEP) et diméthyl- (DMP) phosphates (métabolites des OPs), dans les urines maternelles en début de grossesse. Les concentrations en DMP et DEP étaient classées en haute, moyenne ou faible selon les terciles. Les manifestations cliniques - sifflements, nombre de bronchiolite/bronchite, allergie alimentaire, eczéma et rhinite – étaient recueillies par questionnaires adressés aux familles aux 2 ans de l'enfant. Les associations ont été estimées par régression logistique multivariée.

Résultats : De plus hauts niveaux urinaires en DMP étaient associés à une augmentation du risque de manifester 1 à 2 épisodes de *bronchiolite/bronchite*, avec une relation dose-réponse ( $p$  de tendance = 0,01). Les *sifflements* ont par contre semblé moins fréquents parmi le tercile moyennement exposées par rapport aux non-exposées ( $p$ -value = 0,02). Il n'existait pas d'association avec les DEP, ni avec les autres manifestations cliniques.

Discussion : Ces résultats sont en partie cohérents avec les précédentes études, suggérant une influence de l'exposition prénatale aux OPs sur le développement immunitaire et respiratoire. Le mécanisme de toxicité potentielle des OPs n'est pas clairement établi et nécessitera d'autres études spécifiques.

## Abstract

**Introduction:** The prenatal period is an important window of vulnerability for the developing immune and respiratory systems. Slight environmental perturbations during this period may induce several diseases. In infancy, these diseases include decreased resistance to infectious diseases, hypersensitivity reactions or asthma. The immune and respiratory systems are both sensitive to developmental exposure to chemicals such as organophosphate pesticides (OPs). This study aimed at exploring the effect of prenatal exposure to OPs on respiratory and immunological health in infants.

**Methods:** This study is based on the PELAGIE cohort which included 256 mother-child pairs. Dimethyl (DMP) and diethyl phosphates (DEP), nonspecific OP metabolites, were quantified in maternal urine collected at the beginning of pregnancy and disease outcomes were recorded for the first two years of child life. Maternal DMP and DEP urinary levels were categorized as low, medium or high. Outcomes – bronchiolitis/bronchitis, wheezing, eczema, rhinitis and food allergy - were assessed by self-administered questionnaire at age two. Associations between exposures and outcomes were estimated by logistic regression models, adjusting for potential confounders.

**Results:** High DMP metabolite levels in maternal urine were associated with an increased risk of occurrence of 1 or 2 bronchiolitis/bronchitis episodes with a dose-response relationship ( $p$ -trend=0.01) while no association was observed with the occurrence of three or more bronchiolitis/bronchitis episodes. Medium DMP metabolite levels were negatively associated with wheezing ( $p$ -value=0.02). No association with DMP levels was observed for the three other outcomes. No association was observed between DEP urinary levels and any disease outcomes.

**Conclusions:** Two previous epidemiological studies identified higher risks of recurrent otitis media in infants, and respiratory symptoms around 6 years of age, associated with pregnant maternal urinary OP metabolites levels. Our results complete those observations suggesting a potential developmental effect of prenatal OP exposure on respiratory and immunological health.



## **Keywords – Highlights - Abbreviations**

### **Keywords**

Organophosphate pesticides, prenatal exposure, respiratory infections, Allergy, infant, cohort.

### **Highlights**

Organophosphates prenatal exposure could expose to infant bronchitis and bronchiolitis.

### **Abbreviations**

aOR: adjusted Odd-ratio

BMI: body mass index

DAP: dialkyl phosphate

DEP: diethyl phosphate

DMP: dimethyl phosphate

ISAAC: international study on asthma and allergies in childhood

OPs: organophosphates pesticides

# ARTICLE

## 1. Introduction

Asthma and allergies are frequent children's diseases. Many studies during the 1990's showed an increase of their frequency and a geographic variability (1–4). Genetic participation cannot alone explain those variations in time and places. Only 30% of atopic' children come from atopic' families (5). None of variation in diagnostic efficacy, allergens diversity and exposure frequency seem to be responsible for this effect (5). Besides, respiratory infections are common childhood diseases (6,7). Both infectious and allergic diseases depend on the functional state of the immune and respiratory systems. Thus the occurrence of these diseases can be influenced by immune or respiratory systems perturbations.

The prenatal period is an important window of vulnerability for the developing immune and respiratory systems (8–12). Slight perturbations during this period may expose to long-term dysfunction (13,14) and contribute to several diseases. In infancy, it includes decreased resistance to infectious diseases, hypersensitivity reactions or asthma. To dates, some environmental chemicals showed a potential toxicity on developing immune or respiratory systems after prenatal exposure: e.g. Polychlorobiphenyls (PCBs), dioxins, tobacco smoke, arsenic, atrazine or dichlorodiphenyldichloroethylene (DDE) (15–17).

France is the third European pesticide consumer per hectare, mainly on croplands (18). Organophosphates pesticides (OPs) are one of the major classes of pesticides. The principal way of exposure is oral by alimentation (19). In France, several OPs were banned for agricultural and veterinary use in 2007 (20). But in 2016, many of other OPs are still authorized (e.g. chlorpyrifos, malathion, dimethoate, phosmet, pirimiphos-methyl) (21).

OP toxicity on immune or respiratory systems remains unclear. In adults, epidemiological studies showed an association between occupational non acute OP exposure in farm and the risk of asthma symptoms of farmers (22), particularly atopic asthma (23,24). A direct toxicity of OP exposure to the respiratory tract seems biologically plausible. OPs were showed to increase airway hyperreactivity in guinea pigs at chronic doses below those causing acetylcholinesterase inhibition (which is their principal mechanism of action) (25–27). Moreover, this effect was found enhanced with allergen-sensitized compared to non-sensitized animals (27,28). Besides, immunological changes have been reported after *human adult* chronic OP exposure (29). Concerning developmental toxicity, OP exposure during the entire pregnancy to

weaning period in mice has been shown to expose to long-term modulations of the immune system in their offspring (13,14). In Human, several studies showed the ability for OPs to cross placenta barrier resulting in fetus exposure (30,31). Moreover, epidemiological studies suggested a potential effect of OP prenatal exposure on the developing immune and/or respiratory systems. Weselak et al observed in 2007 that children born from mothers who lived during pregnancy in farms that used OPs, had increased allergies or hay fever risks compared to children of unexposed mothers (32), whereas Duramad et al. showed an association between OP exposure in women during pregnancy and increase of a biologic marker of atopic disposition at 2years old in their children (33). More recently, in the CHAMACOS cohort, higher OP metabolites urinary levels were associated with asthma-like symptoms at age five and seven (34) and to a decreased lung function at age seven (35).

Our study objective here was to simultaneously determine whether several infectious, respiratory and allergic symptoms until age two were correlated to OPs fetal exposure.

## 2. Methods

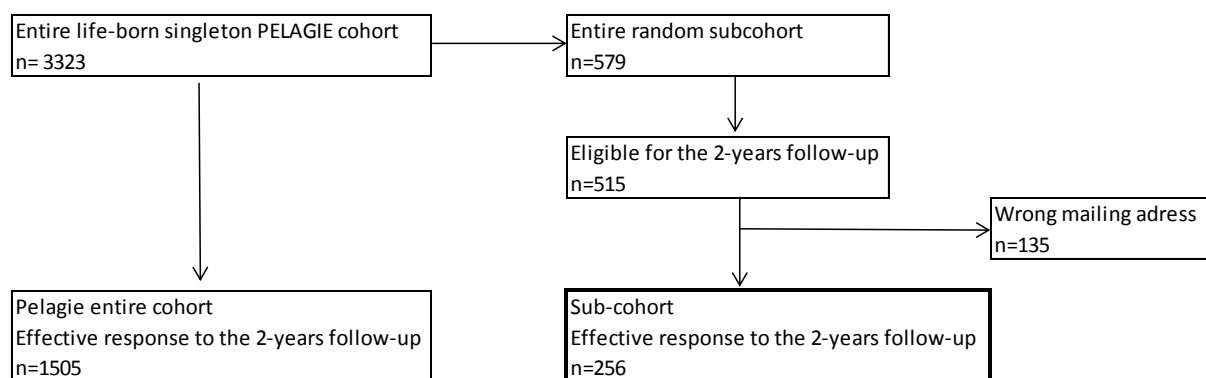
### 2.1. Study population and follow-up

The PELAGIE (Endocrine Disruptors: Longitudinal Study on pregnancy abnormalities, Infertility and Childhood) mother-child cohort, included 3421 women in early pregnancy (before 19 gestational weeks) in Brittany (Ille-et-Vilaine, Finistère and Côtes-d'Armor) between May 2002 and February 2006. The study was designed to assess the potential impact of environmental and occupational exposure to chemicals on pregnancy and child development. Pregnant women were enrolled by obstetricians, gynecologists, or ultrasonographers during early prenatal care visits. Health practitioners explained the nature and objectives of the study, and women who agreed to participate signed an informed consent form. Several follow-up were organized for the PELAGIE mother-child cohort. For the present study we interested in the birth time and the follow-up organized at the age of two of the child.

At inclusion, women were asked to collect, at home, the first morning void urine sample; and to complete a self-administered questionnaire including home address, social, demographic, occupational, and medical characteristics, as well as dietary habits and lifestyle. They returned the questionnaire and the urine sample to the research laboratory by local mail using a self-addressed stamped package provided to them at the time of enrollment.

At birth, midwives collected information on pregnancy and birth conditions. The infant's health status was assessed by the pediatric perinatologist. Women gave birth to 3323 live born singleton. From this group, 579 mother-child pairs were randomly selected for chemical analysis of the urine sample collected at inclusion. Five hundred and fifteen pairs were eligible for the follow-up organized at age two (excluding families whose child or mother had died in the meantime, and the children of the 2002 cohort who were already over 2-year old at the time the follow-up began). Among them, 135 never received the two-year follow-up questionnaire because of wrong or no longer valid mailing addresses (that is, the post office returned the questionnaires to us). Among the remaining 380 mother-child pairs, who presumably received the questionnaire, 256 (67%) mailed it back completed. The flow chart is presented in [Figure 1](#).

When their child reached age two, families completed a self-administered questionnaire which collected data on child's environment and health since birth, including allergic, respiratory and infectious disease symptoms. The study procedures were approved by the appropriate French ethics committees.

**Figure 1: Flow chart : Pelagie sub-cohort for Ops exposure assesment**

## 2.2. Exposure measurement

At inclusion, each woman sent by mail to our laboratory a urine sample (first morning void) in a 10-mL test tube (95 × 16-mm polypropylene, with wing plug). Upon arrival at the laboratory, urine samples were frozen at  $-20^{\circ}\text{C}$  until analysis. Exposure to OPs was assessed by measuring six dialkyl phosphate metabolites (DAP) (dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP)). Chemical analyses were performed by the Idhesa Institute (Plouzané, France) with liquid chromatography/triple-quadrupole mass spectrometry (LC/MS-MS) after solid-phase extraction (SPE). The limit of quantification (LOQ) was 0.2  $\mu\text{g/l}$  for DMP, 1  $\mu\text{g/l}$  for DMTP, 0.45  $\mu\text{g/l}$  for DMDTP, 1.25  $\mu\text{g/l}$  for DEP, 1.7  $\mu\text{g/l}$  for DETP and 0.02  $\mu\text{g/l}$  for DEDTP. Creatinine concentrations in urine were determined.

Because dialkyl phosphates originate from multiple OPs, quantities of the six metabolites were converted to molar concentration (nanomoles per liter). The three dimethyl phosphate metabolites and three diethyl phosphate metabolites were summed up to obtain total concentrations of dimethyl ( $\Sigma\text{DMP}$ ) and diethyl ( $\Sigma\text{DEP}$ ) phosphate metabolites for each woman. For this computation, left censored values (i.e.,  $<\text{LOQ}$ ) were replaced by 0 because of high variations in LOQ concentrations between metabolites. Maternal urinary concentrations of OP metabolites were then categorized as low, medium or high using the terciles of the  $\Sigma\text{DMP}$  distribution. For  $\Sigma\text{DEP}$ , the low exposure category included all samples with concentrations not quantified for all the 3 diethylphosphate metabolites, the median computed among quantified concentrations was used to define the medium (below the median), and the high exposure group (above the median), respectively.

### 2.3. Outcome assessment

At the 2-year follow-up, the child's principal caregiver, usually the mother (98%) completed a self-administered questionnaire aimed at evaluating the child's health since birth. Five outcomes were considered: *bronchiolitis/bronchitis*, *wheeze*, *eczema*, *food allergy* and *rhinitis*.

We classified the child as having had 0, 1 to 2, or  $\geq 3$  *Bronchiolitis/Bronchitis* episodes according to the responses to the following questions: "Has your child ever had bronchiolitis or bronchitis?" and "how many times?".

Children were considered as having *Wheeze* in case of positive responses to the following questions: "Has your child ever had wheezing or whistling in the chest at any time in the past?" or "Has your child ever had an asthma attack since birth?".

*Eczema* was identified by a positive answer to both of the following questions (from the International Study on Asthma and Allergies in Childhood (ISAAC) questionnaire (36)): (1) "Has your child ever had an itchy skin rash, which was coming and going?" and if yes (2) "Has this itchy rash affected any of the following places - the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears, or eyes?" A positive answer to the single question "Has your child ever had a medical diagnosis of eczema?" also identified the presence of *eczema*.

The presence of a *food allergy* was identified by a positive answer to any one of the following three questions: "Has your child ever had a medical diagnosis of cow's milk allergy?", "Has your child ever had a medical diagnosis of any food allergy?", and "Has your child ever had an allergic reaction after eating food (swollen lips or face, gastrointestinal response)?" For the last question, time to the allergic reaction and the type of food involved were verified.

*Rhinitis* was identified with two questions: (1) "Has your child ever had sneezing, runny nose or stuffy nose, without infection?" and (2) "During last year, was this nose affection associate with watering or itching eyes?". Positive response to both questions classified the child in "certain rhinitis". The "probable rhinitis" included all children with positive response to the first unique question. "No rhinitis" included the rest of children with negative response to the first unique question.

## 2.4. Statistical analysis

The associations between prenatal OP exposure and outcomes were estimated using binomial logistic regression for *wheeze*, *eczema* and *food allergy*. Polytomous logistic regression was used for *bronchiolitis/bronchitis* and *rhinitis*. To estimate the adjusted effect of OP exposure, the following known or suspected risk factors were considered for inclusion in the models: mother's age (continuous), maternal education ( $\leq 12$  years,  $> 12$  years), prenatal exposure to tobacco (yes, no), mother or grandparents history of asthma/allergy (yes, no), child's sex, cesarean delivery (yes, no), term birth (continuous), feeding method during the first 3 months of life (exclusive formula feeding, mixed, exclusive breastfeeding), number of siblings at birth (0, 1,  $\geq 2$ ), contact with farm animals (yes, no), contact with domestic animals (yes, no), attendance at group daycare (yes, no), postnatal exposure to tobacco (0, 1 to 10 cigarettes/day,  $\geq 10$  cigarettes/day). All potential confounders that were associated with one of the two exposure variables or one of the fifth outcome variables with a p-value  $< 0.25$  in the univariate analysis were integrated at all models. Due to a poor number of positive mold and/or dampness (yes, no) (account for 3 children on total 256 sample size) and small-for-gestational age ( $< 10^{\text{th}}$  percentile) (account for 10 children) record, those variables were not integrated. Missing data on the covariates (30% in the data set) were imputed using multiple imputations, under the missing at random assumption (37). Ten data sets were generated, multivariate regression were run within each of the data set, and the results were combined to produce estimates with standard error that incorporate missing data.

### 3. Results

#### 3.1. Population Study

Mothers of the study population were more educated ( $p=0.03$ ) and more likely to smoke during pregnancy ( $p=0.04$ ) compared to non-participant. There were no difference regarding child's sex, weight and height at birth, term at birth, caesarean delivery, and small-for-gestational age (10th). Concerning pesticide exposure,  $\sum$ DMP urinary concentration was significantly higher in participant mothers compared to non-participant ( $p=0.02$ ). There was no difference for  $\sum$ DEP urinary concentration.

In summary, mothers of the study population had a high educational level; 17.5% reported smoking 1 to 10 cigarettes per day and 5.2% more than 10 cigarettes per day at the beginning of pregnancy. Regarding the child, one third had a family history of asthma or allergy; most of them were born at term, by vaginal delivery, with a normal birth weight for gestational age. Two thirds were breast feed during first 3months (Table 1).

**Table 1: Characteristics of the study population (n=256)**

<b>Maternal characteristics</b>	<b>n</b>	<b>(%)</b>
Age (years)		
<30	118	46.1
30≤ - <35	94	36.7
≥35	44	17.2
Education (≤12 years)	84	32.8
Nulliparous (yes)	109	42.7
Mother height (cm) *	164.8	5.9
Prepregnancy BMI (kg/m <sup>2</sup> )		
<18.5	18	7.0
18.5-25	194	75.8
25-30	32	12.5
>30	12	4.7
Tobacco smoke at the beginning of pregnancy		
0 cig/day	194	77.3
0< - <10	44	17.5
≥10	13	5.2
Family history of asthma or allergy (yes)	62	29.8
Alcohol consumption during pregnancy (yes)	39	15.7
<b>Child characteristics</b>		
Child's sex (Male)	136	53.1
Birth weight *	3388.6	484.7
Birth height *	49.8	2.3
Term (SA) *	39.4	1.6
Preterm birth (<37SA)	7	2.7
Caesarean delivery (yes)	43	17.2
Small-for-gestational age (<10th percentile)	10	3.9
<b>Environment characteristics</b>		
Feeding during the first 3 months		
Formula only	80	31.9
Mixed feeding	80	31.9
Breast feeding	91	36.3
Siblings		
1 (Pelagic's child)	109	42.8
2	86	33.7
3 or more	60	23.5
Collective daycare attendance (yes)	30	12.9
Contact with farm animal (yes)	77	30.3
Pets at home (yes)	141	56.4
Mould and/or dampness at home (yes)	3	1.2
Postnatal Tobacco		
0 cig/day	151	61.6
1 to 10	51	20.8
> 10	43	17.6

\* mean (standard deviation).

Missing values : Parity (1), asthma family history (48), alcohol consumption (8), ceasarian delivery (6), daycare attendance (23), farm animal (2), pets (6), mould/dampness (10).



### 3.2. Diethyl and Dimethyl phosphates urinary concentrations

The  $\Sigma$ DEP and  $\Sigma$ DMP urinary concentrations showed no association with the season of urine collect, respectively. The  $\Sigma$ DEP median was 13.6nmol/L and  $\Sigma$ DMP median was 47.8nmol/L. Factors associated with exposures are presented in Annexe 1.

### 3.3. Description of the Outcomes

Thirty-eight per cent (n=96), 39% (n=99) and 23% (n=57) of children had manifested *none, 1 to 2, or 3 or more bronchiolitis/bronchitis* episodes during their first two years of life, respectively. 20% (n=51) had presented *wheeze* symptoms. 33% (n=83) of the children were reported to have had *eczema* symptoms, 9% (n=22) had *food allergy*. 22% (n=56) had rhinitis symptoms without conjunctivitis and 7% (n=18) had presented rhinitis and conjunctivitis associated.

We observed positive association between several of the five outcomes as presented in Table 2. Potential risk factors associated with outcomes are presented in Annexe 2.

**Table 2: Outcome crossing (Pelagie sub-cohort. France) : p-value<sup>⌘</sup>**

	Bronchiolitis/ bronchitis	Wheeze	Eczema	Food allergy
Bronchiolitis/bronchitis	X			
Weeze	<0.001	X		
Eczema	NS (0.6)	NS (0.24)	X	
Food allergy	0.03	NS (0.37)	<0.001	X
Rhinitis	NS (0.55)	<0.001 \$	0.05	NS (0.19)

⌘ Basis statistical test was Chi-squared Test

\$ Fisher's exact Test was realized when expected counts <5

Abbreviations : NS (non-statistically significant).

### 3.4. Associations between exposure and outcomes

Higher  $\Sigma$ DMP concentration in maternal urine was associated with an increased risk of occurrence of 1 to 2 bronchiolitis/bronchitis episodes (p-trend=0.01). No association was observed between  $\Sigma$ DMP and 3 or more bronchiolitis/bronchitis episodes. Medium versus low  $\Sigma$ DMP concentration was associated with a decreased risk of wheeze (0.02). No association was observed between  $\Sigma$ DMP levels and the others outcomes (Table 3). Concerning  $\Sigma$ DEP urinary concentration, no association was observed with none of the five outcomes (Table 3).

**Table 3 : Associations [aOR (95% CI)]<sup>2</sup> between dimethyl-, diethyl phosphate urinary concentrations and outcomes (PELAGIE cohort, France)**

	Bronchitis and bronchiolitis		Wheeze	Eczema	Food Allergy	Rhinitis	
	1 to 2	$\geq 3$				Possible	Certain
	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)
<b>dimethylphosphates (<math>\Sigma</math>DMP)</b>							
Low ( $\leq 18.32$ nmol/L)	ref.	ref.	ref.	ref.	ref.	ref.	ref.
Medium ( $18.32 < \leq 63.61$ nmol/L)	1.81 (0.81 to 4.04)	0.63 (0.25 to 1.58)	<b>0.30 (0.11 to 0.81)</b>	0.83 (0.38 to 1.79)	0.59 (0.15 to 2.37)	0.64 (0.27 to 1.51)	0.40 (0.1 to 1.57)
High ( $> 63.61$ nmol/L)	<b>2.46 (1.12 to 5.39)</b>	0.68 (0.27 to 1.70)	0.98 (0.45 to 2.16)	1.06 (0.51 to 2.18)	0.69 (0.20 to 2.39)	0.72 (0.32 to 1.63)	0.42 (0.11 to 1.53)
<b>diethylphosphates (<math>\Sigma</math>DEP)</b>							
Low (<LOQ)	ref.	ref.	ref.	ref.	ref.	ref.	ref.
Medium ( $LOQ \leq < 14.84$ nmol/L)	1.16 (0.55 to 2.47)	1.69 (0.72 to 4.00)	2.10 (0.93 to 4.7)	1.24 (0.61 to 2.52)	0.18 (0.03 to 1.16)	0.97 (0.43 to 2.21)	0.92 (0.27 to 3.15)
High ( $\geq 14.84$ nmol/L)	1.39 (0.68 to 2.87)	1.25 (0.50 to 3.08)	1.13 (0.47 to 2.69)	1.32 (0.65 to 2.65)	1.07 (0.35 to 3.30)	1.37 (0.63 to 2.98)	0.47 (0.11 to 2.09)

aOR : Adjusted Odds Ratio; CI : Confidence Interval; LOQ : Limit of quantification.

<sup>2</sup> Adjusted for mother education level, parity, prenatal and postnatal tobacco exposure, atopy in mother or grandparents history, child sex, caesarean delivery, term delivery, feeding mode, mother's height, weight and age, high traffic road proximity, pets at home, daycare attendance, contact with farm animal

Effective: bronchitis and bronchiolitis (252 mother-child pairs), wheeze (256), rhinitis (254), food allergy (255), eczema (251)

## 4. Discussion

Higher prenatal maternal urinary level of dimethyl phosphate metabolites was associated with an increased risk of manifesting 1 to 2 bronchiolitis/bronchitis episodes with a dose-response tendency; A medium urinary level of  $\Sigma$ DMP was associated with a lower risk of wheezing. For those two outcomes no association was observed with  $\Sigma$ DEP. Finally no association was noted with eczema, food allergy and rhinitis.

Our study has several interests. The longitudinal design allowed to prospectively estimate prenatal OP exposure by measuring concentrations of dimethyl and diethyl phosphate metabolites in urine sample which reflect the aggregate of exposure to OPs from multiple sources and routes. Furthermore we recorded at one time infectious, respiratory and allergic symptoms. The sample size is relatively consequent with 256 pairs. Many highlighted potential confounding factors were taken into account.

The choice of Brittany is relevant because it is a rural territory with intensive pesticides use. Compared to earlier American or German cohorts, OP metabolites concentrations measured in the PELAGIE sub-cohort were lower (38). Exposure to OPs in this study was not occupational, acute, or accidental; it corresponded to the general population exposure in Brittany between 2002 and 2006.

Our study has also some limitations. The questionnaire was self-administered, sent by the postal service, and quite long to fill out (about 2 hours). Each of these factors may have discouraged participation and induced attrition at follow-up with participants more educated and more exposed (higher urinary concentration of  $\Sigma$ DMP in early pregnancy than non-participants). The indirect data collection could have limited information reliability of outcomes. Concerning exposure, we used urinary non-specific OP metabolites to estimate prenatal OP exposure. It is the principal method used in current epidemiologic studies. This method has the advantage that it can track several OP chemicals at the same time. But diet (with its intrinsic variability) as the main source of OP exposure and the OPs' short biologic half-life generate an important within-subject variability (39). Moreover, differences in metabolization and excretion capacity cause an inter-subject variability (40). In view of this, an only one single-day urine sample collection would not accurately reflect exposures over the entire pregnancy but only a short-term window of exposure in early pregnancy (41,42). In the same way, because we did not measure later prenatal or postnatal OP exposure, it is difficult to distinguish the independent role of prenatal exposure from later ones that may proxy measured early prenatal exposures. Furthermore, several studies showed

the presence of OP metabolites in the environment, as natural degradation products of pesticides molecules (43,44). These “natural” metabolites could be absorbed and be part of the urine OP metabolites measured in our study, and thus reduce their reliability to reflect OP exposure. Then, although we have adjusted the Odd-ratios with many covariates, we did not integrate others environmental toxicants and some residual confounding could remain. Finally, as our study is exploratory in nature, another limitation comes from the possibility of chance findings due to multiple comparisons. We may have rejected the null hypothesis too readily, but we point out that our main finding (positive association between prenatal OP exposure and occurrence of 1 to 2 bronchiolitis/bronchitis episodes) was significant at the 0.01 level. These limitations implicate some reserve on results interpretation.

To our knowledge, only one epidemiological study interested in prenatal role of OPs on infant bronchitis: Weselak et al. observed no association between prenatal OP exposure and “chronic bronchitis or cough” from birth to twelve years and over age. The study population was offspring of farmer women exposed during pregnancy; OP exposure and outcomes were assessed by self-administered questionnaires, retrospectively on more than ten years (32). If we broaden to the whole respiratory tract infections, one epidemiological study observed an increased risk of recurrent otitis media associated with prenatal OP exposure (estimated by measure of concentrations of dimethyl and diethyl phosphates metabolites in maternal urines in early pregnancy) (45). The association we observed between OP prenatal exposure and the occurrence of 1 to 2 bronchiolitis/bronchitis episodes, or Buscail et al with recurrent otitis media could potentially be an illustration of susceptibility to infections as a clinical manifestation of immune disruption due to OP toxicity. This developmental immunotoxicity was suggested by Watanabe et al in 2013. Actually, they observed that perinatal exposure to methamidophos (an OP) suppressed the production of proinflammatory cytokines in response to respiratory syncytial virus infection in the lung tissues in the offspring generation of mice. Besides, treatment with methamidophos did not affect the pulmonary viral titers but suppressed moderately the inflammation of lung tissues of RSV-infected offspring, histopathologically. They concluded to an irregular immune response following OP exposure. However, concerning recurrent otitis media it must be underline that both atopic condition and upper respiratory tract infection seem to be well-known risk factors (46,47).

The reason for the second finding of lower risk of wheezing in infants of mothers with medium urinary  $\Sigma$ DMP levels remains elusive. Due to the young age of participants and the diversity of etiology of wheeze at this age, the questionnaire cannot readily distinguish if wheeze symptoms are from allergic, respiratory or infectious origin. In the same way, with neither atopic (skin or blood test) nor lung function tests

conducted, expectation on asthma predictivity or other OP respiratory effect is difficult to do. So the interpretation of the association of OP exposure to wheeze needs some caution. To our knowledge, few studies interested in analyzing the effect of prenatal OP exposure on wheeze in children (32–35,48). Weselak et al. observed no association with asthma outcome, at all age ((32) – methods of this study detailed above). Besides, our finding is consistent with Reardon et al. longitudinal study which showed a *negative* association between diazinon level (an OP) measured in air during 48hours in last trimester of pregnancy and wheeze, cough without a cold and allergen sensitization by age 5, whereas no association was found with chlorpyrifos air measures (48). But it contrast with Raanan et al.’s studies on the CHAMACOS cohort that recently observed a strong *positive* association between OP metabolites and asthma-like symptoms at age five and seven, and decreased lung function at age seven. These positive associations concerned the second half of pregnancy (and separately children under age five) window of exposure whereas no association was found with OP exposure during first half of pregnancy. No atopic test was conducted (34,35).

Concerning allergic outcomes, Duramad et al. observed an association between prenatal OP exposure and an increase of a biological marker for atopic disposition at age two. In fact, they showed that lymphocyte T-helper 2 levels were higher in children with mothers who performed agricultural fieldwork or had other agricultural workers living in the household. But OP exposure was only presumed to be high due to the agricultural field work status; and other exposures, such as dust, coarse particulate matter, endotoxin, and other microbial products that are part of the agricultural environment, which could have been confounding factors, were not assessed. Weselak et al. also observed a *positive* association with “allergies or hayfever”, with the limitations described above (32). On another way, Reardon et al. observed a *negative* association between prenatal diazinon air exposure and allergen sensitization, as mentioned above (48). One animal study and one in vitro on human blood study also suggested an increased type 1 immune response following OP administration, in contrast to an atopic stimulation (49,50). In view of this, it is uneasy to conclude concerning prenatal OP role on allergic outcomes.

To the best of our knowledge, OP toxicity mechanism(s) is not clearly established, and our study could have raised a non-classical toxicity mechanism for the susceptibility to infant respiratory infections. However, results from this observational epidemiological study need to be taken with caution. Further work is needed to assess uncommon OP toxicity mechanism.

## 5. Conclusion

In line with our previous work on the PELAGIE cohort that suggested that prenatal OP exposure is associated to increased risk of recurrent media otitis (45), this study suggests an association between prenatal OP exposure and increased *bronchiolitis/bronchitis* frequency. However, this is an observational epidemiological study and with some reserve on the results we observed, this need some further work designed to assess uncommon toxicity suggested.

### Interest link

None

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## **Supplemental Files**

**Annexe 1: Population characteristics by tercile groups of dimethyl and diethyl phosphate metabolites.**

**Annexe 2: Population characteristics by respiratory, allergic and infectious symptoms.**

**Annexe 1: Population characteristics by tertile groups of dimethyl and diethyl phosphate metabolites.**

	ΣDMP						p-value <sup>α</sup>	ΣDEP						p-value <sup>α</sup>
	1st tertile (<18.6nmole/L)		2nd tertile (18.6≤<72.1nmole/L)		3rd tertile (≥72.1nmole/L)			1st tertile (<LOQ)		2nd tertile (LOQ≤<14.2nmole/L)		3rd tertile (≥14.2nmole/L)		
	n	%	n	%	n	%		n	%	n	%	n	%	
<b>Urine collect season</b>														
Spring	18	27.3	25	37.9	23	34.8	0.27	39	59.1	12	18.2	15	22.7	0.4
Summer	14	27.5	13	25.5	24	47.1		25	49	16	31.4	10	19.6	
Autumn	25	39.7	22	34.9	16	25.4		26	41.3	17	27	20	31.7	
Winter	27	35.5	25	32.9	24	31.6		38	50	18	23.7	20	26.3	
<b>Diethyl phosphate metabolites</b>														
1st tertile (<LOQ)	50	39.1	46	35.9	32	25.0	<0.01							
2nd tertile (LOQ≤<14.2nmole/L)	20	31.7	21	33.3	21	34.9				X				
3rd tertile (≥14.2nmole/L)	14	21.5	18	27.7	33	50.8								
<b>Maternal characteristics</b>														
<b>Age (years)</b>														
<30	35	29.7	41	34.7	42	35.6	0.43	60	50.8	29	24.6	29	24.6	0.93
30≤ - <35	31	33.0	28	29.8	35	37.2		46	48.9	25	26.6	23	24.5	
≥35	18	40.9	16	36.4	10	22.7		22	50.0	9	20.5	13	29.5	
<b>Education</b>														
≤12 years	24	28.6	30	35.7	30	35.7	0.6	49	58.3	18	21.4	17	20.2	0.17
>12 years	60	34.9	55	32.0	57	33.1		79	45.9	45	26.2	48	27.9	
<b>Tobacco smoke at the beginning of pregnancy (No of cig/day)</b>														
0	65	33.5	65	33.5	64	33.0	0.3	92	47.4	53	27.3	49	25.3	0.56
0< - <10	14	31.8	16	36.4	14	31.8		26	59.1	8	18.2	10	22.7	
≥10	3	23.1	2	15.4	8	61.5		7	53.8	2	15.4	4	30.8	
<b>History of Allergy (mother or GDP)</b>														
No	55	37.7	44	30.1	47	32.2	0.45	74	50.7	38	26.0	34	23.3	0.29
Yes	18	29.0	23	37.1	21	33.9		27	43.5	14	22.6	21	33.9	
<b>Birth characteristics</b>														
<b>Child's sex</b>														
Male	47	34.6	37	27.2	52	38.2	0.08	70	51.5	30	22.1	36	26.5	0.6
Female	37	30.8	48	40.0	35	29.2		58	48.3	33	27.5	29	24.2	
<b>Caesarean delivery</b>														
No	75	36.2	62	30.0	70	33.8	0.03	104	50.2	53	25.6	50	24.2	0.43
Yes	8	18.6	21	48.8	14	32.6		21	48.8	8	18.6	14	32.6	
Term *	39.71 (1.18)		39.35 (1.32)		39.11 (2.05)		0.05	39.45 (1.74)		39.17 (1.66)		39.48 (1.12)		0.46
<b>Environment characteristics</b>														
<b>Feeding during the first 3 months</b>														
Formula only	21	26.2	29	36.2	30	37.5	0.08	44	55.0	16	20.0	20	25.0	0.38
Mixed feeding	26	32.5	21	26.2	33	41.2		37	46.2	19	23.8	24	30.0	
Breast feeding	37	40.7	32	35.2	22	24.2		44	48.4	28	30.8	19	20.9	
<b>Siblings</b>														
1 (Pelagic's child)	38	34.9	35	32.1	36	33	0.75	59	54.1	25	22.9	25	22.9	0.8
2	27	31.4	32	37.2	27	31.4		41	47.7	23	26.7	22	25.6	
3 or more	19	31.7	17	28.3	24	40		28	46.7	14	23.3	18	30.0	
<b>Daycare attendance</b>														
No	61	30.0	71	35.0	71	35.0	0.17	95	46.8	53	26.1	55	27.1	0.73
Yes	14	46.7	7	23.3	9	30.0		16	53.3	6	20.0	8	26.7	
<b>Number of cigarettes/day at home after birth</b>														
0	56	37.1	46	30.5	49	32.5	0.05	75	49.7	42	27.8	34	22.5	0.51
1 to 10	9	17.6	24	47.1	18	35.3		24	47.1	10	19.6	17	33.3	
> 10	17	39.5	10	23.3	16	37.2		19	44.2	11	25.6	13	30.2	
<b>Contact with domestic animal</b>														
No	36	33.0	34	31.2	39	35.8	0.81	56	51.4	26	23.9	27	24.8	0.96
Yes	46	32.6	49	34.8	46	32.6		70	49.6	34	24.1	37	26.2	
<b>Contact with farm animal</b>														
No	58	32.8	56	31.6	63	35.6	0.64	80	45.2	49	27.7	48	27.1	0.09
Yes	26	33.8	28	36.4	23	29.9		46	59.7	14	18.2	17	22.1	

\* mean (standard deviation).

<sup>α</sup> Basis statistical test was Chi-squared Test<sup>§</sup> Fisher's exact Test was realised when expected counts were <5.

**Annexe 2: Population characteristics by respiratory, allergic and infectious symptoms**

Maternal characteristics	Bronchiolitis/Bronchitis (number of)						Wheezing						Eczema						Food Allergy						Rhinitis									
	0		1 to 2		≥3		Yes		No		p-value		Yes		No		p-value		Yes		No		p-value		Probable		Certain		p-value					
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%				
<b>Maternal characteristics</b>																																		
Age (years)																																		
<30	45	38.8	43	37.1	28	24.1	0.69	25	21.2	82	31.0	0.75	109	93.2	8	6.8	0.46 \$	69.5	27	22.9	9	7.6	0.98 \$											
30 ≤ <35	31	33.7	40	43.5	21	22.8	19.1	18	19.1	61	66.3	31	33.7	83	88.3	11	11.7		68	73.1	19	20.4	6	6.5										
≥35	20	45.5	16	36.4	8	18.2		8	18.2	27	62.8	16	37.2	41	93.2	3	6.8		30	69.8	10	23.3	3	7.0										
Education																																		
≤12 years	26	31.3	34	41.0	23	27.7	0.22	65	77.4	55	67.1	27	32.9	77	92.8	6	7.2	0.58	52	62.7	26	31.3	5	6.0	<b>0.04</b>									
>12 years	70	41.4	65	38.5	34	20.1		32	18.6	113	66.9	56	33.1	156	90.7	16	9.3		128	74.9	30	17.5	13	7.6										
Tobacco smoke at the beginning of pregnancy																																		
0 sig/day	72	37.9	70	36.8	48	25.3	0.36 \$	156	80.4	126	66.3	64	33.7	177	91.2	17	8.8	0.82 \$	137	71.4	39	20.3	16	8.3	0.65 \$									
0 ≤ <10	19	43.2	20	45.5	5	11.4		34	77.3	31	72.1	12	27.9	39	90.7	4	9.3		32	72.7	11	25.0	1	2.3										
≥10	5	38.5	5	38.5	3	23.1		2	15.4	7	53.8	6	46.2	13	100	0	0.0		9	69.2	3	23.14	1	7.7										
History of Allergy (mother or GDP)																																		
no	62	43.4	53	37.1	28	19.6	0.21	121	82.9	99	69.2	44	30.8	134	92.4	11	7.6	0.12	106	73.1	30	20.7	9	6.2	0.30 \$									
yes	19	31.1	25	41.0	17	27.9		16	25.8	35	37.4	26	42.6	53	85.5	9	14.5		39	63.9	15	24.6	7	11.5										
<b>Birth characteristics</b>																																		
<b>Child's sex</b>																																		
Male	48	35.8	54	40.3	32	23.9	0.72	102	75.0	85	63.4	49	36.6	123	91.1	12	8.9	0.87	91	66.9	36	26.5	9	6.6	0.19									
Female	48	40.7	45	38.1	25	21.2		17	14.2	83	70.9	34	29.1	110	91.7	10	8.3		89	75.4	20	16.9	9	7.6										
Caesarean delivery																																		
No	80	39.2	80	39.2	44	21.6	0.5	164	79.2	136	67.0	67	33.0	187	90.8	19	9.2	0.55 \$	148	72.2	41	20.0	16	7.8	0.14 \$									
Yes	13	31.0	17	40.5	12	28.6		6	14.0	30	71.4	12	28.6	41	95.3	2	4.7		28	65.1	14	32.6	1	2.3										
Term *	39.6	1.12	39.3	1.84	39.3	1.64	0.21	39.4	1.53	39.4	1.68	39.3	1.4	39.4	1.37	39.1	3.07	0.36	39.4	1.43	39.3	2.12	39.4	1.2	0.93									
Birth weight *	3458.9	493	3390.8	3151	3294.5	467.4	0.13	3397.6	473.5	3352.5	530.6	0.55	3416.6	498.6	3331.4	48.3	0.19	3403.9	664.3	3236.6	664.8	0.12	3395.3	488.0	3328.4	523.8	3485.0	347.9	0.45					
<b>Environment characteristics</b>																																		
<b>Feeding during the first 3 months</b>																																		
Formula only	29	36.3	33	41.3	18	22.5	0.77	62	77.5	46	59.7	31	40.3	67	84.8	12	15.2	0.052	52	65.0	24	30.0	4	5.0	<b>0.11</b>									
Mixed feeding	27	34.2	31	39.2	21	26.6		19	23.8	51	63.7	29	36.2	75	93.8	5	6.2		54	68.4	19	24.1	6	7.6										
Breast feeding	37	42.0	34	38.6	17	19.3		14	15.4	66	74.2	23	25.8	86	94.5	5	5.5		70	77.8	15	13.3	8	8.9										
Siblings																																		
1 (Pélagé's child)	43	39.8	36	33.3	29	26.9	0.42	91	83.5	80	74.8	27	25.2	100	92.6	8	7.4	<b>0.07</b>	79	72.5	25	22.9	5	4.6	0.42 \$									
2	29	34.5	37	44.0	18	21.4		22	25.6	41	49.4	42	50.6	74	86.0	12	14.0		58	68.2	17	20.0	10	11.8										
3 or more	23	39.0	26	44.1	10	16.9		11	18.3	47	78.3	13	21.7	58	96.7	2	3.3		42	71.2	14	23.7	3	5.1										
Daycare attendance																																		
no	80	40.2	78	39.2	41	20.6	0.27	164	80.8	138	69.0	62	31.0	188	93.1	14	6.9	<b>0.03 \$</b>	143	71.1	45	22.4	13	6.5	0.65 \$									
yes	9	30.0	11	36.7	10	33.3		9	30.0	15	51.7	14	48.3	24	80.0	6	20.0		20	66.7	7	23.3	3	10.0										
Number of cigarettes/day at home after birth																																		
0	52	35.4	58	39.5	37	25.2	0.15	117	77.5	96	65.3	51	34.7	138	91.4	13	8.6	0.28 \$	103	68.7	35	23.3	12	8.0	0.90 \$									
1 to 10	19	37.3	23	45.1	9	17.6		6	11.8	33	66.0	17	34.0	43	86.0	7	14.0		38	76.0	9	18.0	3	6.0										
> 10	23	53.5	15	34.9	5	11.6		9	20.9	32	74.4	11	25.6	41	95.3	2	4.7		32	74.4	8	18.6	3	7.0										
Contact with domestic animal																																		
No	36	33.3	43	39.8	29	26.9	0.23	88	80.7	69	64.5	38	35.5	96	88.1	13	11.9	0.13	78	72.2	22	20.4	8	7.4	0.95									
Yes	58	42.0	54	39.1	26	18.8		28	19.9	96	69.1	43	30.9	132	93.6	9	6.4		100	70.9	31	22.0	10	7.1										
Contact with farm animal																																		
No	63	36.2	68	39.1	43	24.7	0.44	141	79.7	115	66.1	59	33.9	159	90.3	17	9.7	0.24	126	72.0	38	21.7	11	6.3	0.67									
Yes	33	43.4	29	38.2	14	18.4		15	19.5	62	80.5	15	19.5	73	94.8	4	5.2		52	67.5	18	23.4	7	9.1										

\* mean (standard deviation)  
 † Basis statistical test was Chi-squared Test  
 \$ Fisher's exact Test was realized when expected counts were <5.

**VIEILLE, Quentin**

**Sujet : Exposition prénatale aux insecticides organophosphorés et manifestations respiratoires et allergiques chez l'enfant avant 2ans dans la cohorte PELAGIE.**

44 feuilles, 1 illustration, 5 tableaux. 30 cm.- Thèse : Médecine Générale ; Rennes 1 : 07/07/2016 ; N°

**Résumé – français**

Introduction : Les systèmes respiratoire et immunitaire en développement (en particulier pendant la vie intra-utérine) sont très sensibles à l'environnement. Des perturbations mineures pendant cette phase peuvent induire chez le nourrisson une résistance diminuée aux maladies infectieuses, des maladies allergiques ou de l'asthme, pathologies particulièrement fréquentes en médecine générale. Des recherches épidémiologiques mettent en évidence que les insecticides organophosphorés (OPs), très présents dans nos environnements, pourraient être toxiques pour ces systèmes. Ainsi cette étude vise à estimer l'effet de l'exposition prénatale aux OPs sur la survenue de manifestations respiratoires ou allergiques du jeune enfant.

Méthodes : Ce travail, basé sur la cohorte PELAGIE, inclut 256 couples mère-enfant. L'exposition prénatale aux OPs a été estimée par la mesure des concentrations en diéthyl- (DEP) et diméthyl- (DMP) phosphates (métabolites des OPs), dans les urines maternelles en début de grossesse. Les concentrations en DMP et DEP étaient classées en haute, moyenne ou faible selon les terciles. Les manifestations cliniques - sifflements, nombre de bronchiolite/bronchite, allergie alimentaire, eczéma et rhinite – étaient recueillies par questionnaires adressés aux familles aux 2 ans de l'enfant. Les associations ont été estimées par régression logistique multivariée.

Résultats : De plus hauts niveaux urinaires en DMP étaient associés à une augmentation du risque de manifester 1 à 2 épisodes de bronchiolite/bronchite, avec une relation dose-réponse (p de tendance = 0,01). Les sifflements ont par contre semblé moins fréquents parmi le tercile moyennement exposées par rapport aux non-exposées (p-value = 0,02). Il n'existait pas d'association avec les DEP, ni avec les autres manifestations cliniques.

Discussion : Ces résultats sont en partie cohérents avec les précédentes études, suggérant une influence de l'exposition prénatale aux OPs sur le développement immunitaire et respiratoire. Le mécanisme de toxicité potentielle des OPs n'est pas clairement établi et nécessitera d'autres études spécifiques.

**Abstract - english**

**Introduction:** The prenatal period is an important window of vulnerability for the developing immune and respiratory systems. Slight environmental perturbations during this period may induce several diseases. In infancy, these diseases include decreased resistance to infectious diseases, hypersensitivity reactions or asthma. The immune and respiratory systems are both sensitive to developmental exposure to chemicals such as organophosphate pesticides (OPs). This study aimed at exploring the effect of prenatal exposure to OPs on respiratory and immunological health in infants.

**Methods:** This study is based on the PELAGIE cohort which included 256 mother-child pairs. Dimethyl (DMP) and diethyl phosphates (DEP), nonspecific OP metabolites, were quantified in maternal urine collected at the beginning of pregnancy and disease outcomes were recorded for the first two years of child life. Maternal DMP and DEP urinary levels were categorized as low, medium or high. Outcomes – bronchiolitis/bronchitis, wheezing, eczema, rhinitis and food allergy - were assessed by self-administered questionnaire at age two. Associations between exposures and outcomes were estimated by logistic regression models, adjusting for potential confounders.

**Results:** High DMP metabolite levels in maternal urine were associated with an increased risk of occurrence of 1 or 2 bronchiolitis/bronchitis episodes with a dose-response relationship ( $p$ -trend=0.01) while no association was observed with the occurrence of three or more bronchiolitis/bronchitis episodes. Medium DMP metabolite levels were negatively associated with wheezing ( $p$ -value=0.02). No association with DMP levels was observed for the three other outcomes. No association was observed between DEP urinary levels and any disease outcomes.

**Conclusions:** Two previous epidemiological studies identified higher risks of recurrent otitis media in infants, and respiratory symptoms around 6 years of age, associated with pregnant maternal urinary OP metabolites levels. Our results complete those observations suggesting a potential developmental effect of prenatal OP exposure on respiratory and immunological health.

Rubrique de classement :	Epidemiologie Environnementale
Mots-clés :	Pesticides organophosphorés, exposition prénatale, infections respiratoires, allergie, eczéma, allergie alimentaire, enfant.
Mots-clés anglais MeSH :	Organophosphate pesticides, prenatal exposure, respiratory infections, Allergy, infant, cohort.
JURY :	Professeur Vincent Lavoué [président du jury] Docteur Fabienne Pelé [directeur de thèse]
Asseseurs :	Docteur Ronan Garlantezec [juge] Docteur Françoise Tattevin [juge]