

Decreased lung function in 7-year-old children with early-life organophosphate exposure

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ABSTRACT

Background Organophosphate pesticides are heavily used in agriculture, and adverse associations with respiratory health in occupational settings have been reported. However, most of the evidence comes from studies where there were no biomarkers of exposure and no objective outcome measurement. Non-occupational chronic effects among residents living in agricultural communities are less well described.

Objective To evaluate associations between early-life organophosphate exposure and lung function of children living in an agricultural community.

Methods Participants were 279 children from the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) longitudinal birth cohort. The area under the curve for organophosphate exposure was determined by urinary diethyl and dimethyl dialkylphosphate metabolites of organophosphate pesticides, which were measured five times during childhood (6–60 months). Spirometry was performed at age 7 years. Regression models controlled for maternal smoking during pregnancy, season of birth, particulate matter concentrations with aerodynamic diameter $\leq 2.5 \mu\text{m}$ ($\text{PM}_{2.5}$), breast feeding duration, mould and pets at home, distance of home from a highway, food insecurity, maternal education, season of spirometry, sex, height and technician.

Results Childhood diethyl, dimethyl and total dialkylphosphate concentrations were associated with significant decreases in lung function at age 7. Specifically, we found lower FEV_1 , (L/s) ($\beta = -0.16$, 95% CI -0.30 to -0.02 , $p = 0.03$) and FVC (L) ($\beta = -0.17$, 95% CI -0.34 to 0.01 , $p = 0.06$) per 10-fold increase of total dialkylphosphate levels.

Conclusions Early-life organophosphate exposure as assessed by dialkylphosphate concentrations was adversely associated with 7-year-old children's lung function.

INTRODUCTION

The aetiology of chronic paediatric respiratory disease, such as asthma, is not fully understood.^{1–3} Some environmental agents such as air pollution and tobacco smoke have been reported to be adversely associated with respiratory symptoms and impaired lung function,^{1 2} and others such as pesticides and household chemicals have been implicated.^{2 3}

Although most residential uses of organophosphate pesticides (OPs) were phased out in the mid-2000s, they are still heavily applied in US agriculture with 15 million kilograms used in 2007

Key messages

What is the key question?

- Does prolonged early-life exposure to organophosphate pesticides (OPs), the most commonly used pesticide in agriculture, have an adverse effect on paediatric lung function?

What is the bottom line?

- This study presents the first scientific evidence that non-occupational early-life exposure to OPs (ie, chronic low-level exposure to OPs at environmental relevant concentrations) is associated with decreased pulmonary function and may have a restrictive effect on children's lungs.

Why read on?

- The causes for much paediatric respiratory disease are not fully understood; we present a new link between pesticides that are heavily used in agriculture and paediatric respiratory health that may be important to clinicians and researchers.

(36% of total insecticide use).^{4 5} This is reflected in the widespread detection of OP metabolites—dialkyl phosphate metabolites (DAPs)—in urine samples from the general US population.⁶

OPs can affect respiratory function by inhibition of acetylcholinesterase (AChE).⁷ Additionally, animal studies have reported that at doses below those that can cause AChE inhibition, OPs can induce airway hyper-reactivity.⁸

Occupational investigations have reported adverse associations between pesticides and respiratory health, including rhinitis, asthma, chronic bronchitis, cough and wheeze.^{9 10} A comparison of 89 pesticide sprayers with 25 controls found an inverse association between self-reported long-term pesticide exposure and forced expiratory flow at 25–75% (FEF_{25-75}), and between short-term exposure as measured by serum cholinesterase and FEV_1 .¹¹ Working for the agricultural sector was inversely associated with FEV_1 (in ex-smokers) in 13 253 subjects in the European Community Respiratory Health Survey study.¹² Reported occupational exposure to unspecified pesticides was inversely associated with FEV_1 and FEV_1/FVC in 11 851 subjects in the LifeLines cohort.¹³



A reduction of FEV₁ and FVC was demonstrated among 25 Sri Lankan farmers occupationally exposed to OPs.¹⁴ In a non-occupational survey of 213 households in South Africa an adverse association between wheeze and use of insecticide in the home was reported,¹⁵ and in random household samples in rural Ethiopia, wheeze and the use of malathion (an OP pesticide) were associated.¹⁶

Reported paediatric respiratory symptoms were associated in a few studies with early-life, non-specific pesticide exposure in non-longitudinal designs.^{17, 18} A study based on 14 065 participants aged ≤18 years from the National Health and Nutrition Examination Survey reported ambiguous results—an association between wheezing and dry cough and pesticide use in the kitchen and dining room, but no association with overall domestic use.¹⁹

We previously reported an association between respiratory symptoms at age 5 and 7 and early-life exposure to OP pesticides as assessed by urinary DAPs measured during pregnancy and childhood.²⁰ These children were participants in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) study, a longitudinal birth cohort investigating the health effects of environmental exposures on pregnant women and children living in the Salinas Valley, California.²¹ In this study, we examine the association of urinary DAP concentrations in pregnant women and their children in early childhood with spirometry at 7 years of age. Here we present the first report³ on an association between exposure to OP pesticides, as measured by DAPs and pulmonary lung function tests in children.

METHODS

Study setting and design

Pregnant mothers were recruited to the CHAMACOS study during October 1999–October 2000. Women were eligible for the study if they were ≥18 years of age, at <20 weeks gestation and were planning to deliver at the county hospital, English or Spanish speaking and Medi-Cal (a state health insurance programme based on economic need) eligible.

Participants

Six hundred and one pregnant mothers were recruited to the cohort; 526 delivered live-born surviving singletons. Detailed methods are described elsewhere.²¹ Research protocols were approved by the University of California, Berkeley, Committee for the Protection of Human Subjects. Written informed consent was obtained from the mothers. Child assent was obtained at age 7.

We performed spirometry on 279 of these children at age 7. FEV₁, FVC and FEF_{25–75} were available for 279, 250 and 250 children, respectively. Of the children for whom we obtained FEV₁, FVC and FEF_{25–75}, we had prenatal urinary DAPs for 275, 246 and 246 mothers and early childhood DAPs for 231, 206 and 206 children, respectively.

Maternal interviews

Information on covariates was obtained by maternal interviews, home visits conducted by trained personnel when the children were 6 and 12 months old and abstraction of prenatal and delivery records by a registered nurse. Mothers were also asked whether the child had been prescribed any medication for asthma or wheezing/whistling, or tightness in the chest. Women were interviewed twice during pregnancy (mean±SD=13.4±4.7, 26.5±2.6 weeks' gestation), after delivery and when their children were 0.5, 1, 2, 3.5, 5 and 7 years old.

Spirometry

A clinical examination was performed when the child was 7, including maximum expiratory flow volume tests and height and weight measurements. Identical dry-seal spirometers were used in the study. Routine calibration was performed every morning. Tests were performed by bilingual and bicultural technicians specifically trained for the study. Three identical EasyOne spirometers were used by two technicians; 92% of the tests were performed by the same technician. Quality control was supervised by two physicians experienced in paediatric spirometry.

Each child performed a maximum of eight expiratory manoeuvres and up to three best acceptable tests were kept by the spirometric software. Each acceptable blow lasted at least 3 s. The children were coached to blow until the EasyOne signalled that the test had ended. All attempts were coached. Quality control was also achieved by verifying that the child did not lean forward; air did not leak out of the side; the child took enough air in and took a deep breath right from the beginning; blew enough air out; did not stop blowing too soon; and took a big smooth breath. All manoeuvres were reviewed again and verified by the two physicians to assure quality control. See online supplementary methods for more details.

Pesticide exposure: DAP metabolites

Six urinary DAP metabolites of OP pesticides comprised three diethyl (DEs: diethylphosphate, diethylthiophosphate and diethyldithiophosphate) and three dimethyl (DMs: dimethylphosphate, dimethylthiophosphate and dimethyldithiophosphate) phosphates were measured twice during pregnancy (M±SD=13.4±4.7 and 26.5±2.6 weeks, respectively) and five times during childhood when children were approximately 0.5, 1, 2, 3.5 and 5 years old. Maternal and child urine samples were stored at −80°C. Non-specific DAP metabolites were summed on a molar basis to calculate total DEs, total DMs and total DAPs.²² Analyses were conducted by the Division of Laboratory Science at the Centers for Disease Control and Prevention using gas chromatography-tandem mass spectrometry and quantified using isotope dilution calibration.²³

Data analysis

For prenatal analysis, we examined diethyl phosphates (DEs), dimethyl phosphates (DMs) and total DAP concentrations (nmol/L) from maternal urinary samples collected at the first and second halves of pregnancy (0–20, 21–40 weeks' gestation), as well as the average of these two measurements. For postnatal analysis, we calculated the area under the curve (AUC) from the five urinary metabolite measurements conducted during childhood using the trapezoidal method.²⁰ For each time interval, we calculated the time-weighted average concentration by multiplication of time between measurements (years) by the average of the two measured concentrations. Time-weighted averages from each time interval were summed to calculate the AUC. Forty-three children were excluded from the AUC analysis owing to lack of DAP measurements at either 0.5 or 5 years, or lack of more than one measurement at ages 1, 2 and 3.5 years. If a child had only one missing DAP measurement at 1, 2, or 3.5 years (n=5), we imputed the mean of the two closest measures. DAP concentrations (in the first and second halves of pregnancy; the arithmetic average of the two pregnancy measurements; and the AUC of the childhood measurements) were log₁₀ transformed. DAP concentrations were corrected for urinary dilution in maternal samples by dividing the DAP

concentration by urinary specific gravity and in child samples by dividing by urinary creatinine.

We used general linear models (GLMs) to estimate the associations of prenatal and early childhood DAPs with FEV₁, FVC, FEV₁/FVC and FEF_{25–75}. We estimated the associations between the highest spirometric measures for children who had one, two or three manoeuvres and for children who had at least two manoeuvres (ie, two or three manoeuvres). Covariates were selected based on directed acyclic graphs. We controlled for child's sex, height, child's age (months) and age squared, pets at home (at ages 5 and 7), household food insecurity score during the previous 12 months (at age 7; measured by using the US Household Food Security Instrument, Spanish version²⁴), maternal education, season of spirometry (winter/spring/summer/autumn) and technician. We also controlled for covariates previously identified in our analysis of DAPs and respiratory symptoms²⁰ (tables 3 and 4): maternal smoking during pregnancy, season of birth (mould/wet/pollen/dry), mean daily particulate matter concentrations with aerodynamic diameter ≤ 2.5 μm (PM_{2.5}) during the first 3 months of life, breast feeding duration, signs of moderate/extensive mould at home visit (6 or 12 months) and distance (≤ 150 m) from a highway (6 or 12 months). Season of birth corresponds generally to other potential exposures that might play a causal role in respiratory disease (see online supplementary methods).

Potential selection bias due to exclusion from final models of children with missing outcome data or missing covariates was addressed by comparing our results with GLM models that included stabilised inverse probability weights^{25–26} (see online supplementary material).

We analysed the data using Stata (V13.0; StataCorp, College Station, Texas, USA) for GLM models and R (V2.14.2; R Foundation for Statistical Computing, Vienna, Austria) for Super Learner models. We set statistical significance at $p < 0.05$ for all analyses. We defined borderline significance at $0.05 < p < 0.1$.

RESULTS

Most cohort participants were born to Mexican immigrant mothers who had less than a high school education. Most mothers lived in a household with at least one farm worker and almost 40% reported food insecurity when the children were 7 years old (table 1). Mean \pm SD values of FEV₁ (L/s), FVC (L), FEV₁/FVC ratio and FEF_{25–75} (L/s) were 1.70 ± 0.46 , 1.97 ± 0.51 , 0.89 ± 0.06 and 2.29 ± 0.82 , respectively (table 2). The geometric means of specific gravity-adjusted total DAP, DE and DM concentrations during pregnancy were 145, 22 and 105 nmol/L, respectively. Child creatinine-adjusted total DAP concentrations decreased with age, averaging 229, 237, 214, 161 and 128 nmol/g creatinine at 0.5, 1, 2, 3.5 and 5 years of age, respectively (see online supplementary material and table E1).

Average DAP urinary concentrations in mothers during pregnancy were not significantly associated with lung function measurements (see online supplementary table E2). Estimates from models from the first and second half of pregnancy and from models that also included children who had only one lung function measurement were also non-significant (data not shown).

We found adverse associations, between the AUC of total DAPs, DEs and DMs concentrations measured in children's urine between 0.5 and 5 years old and FEV₁, FVC and FEF_{25–75} measured at age 7 (table 3). Specifically, the highest FEV₁ measured from children who had at least two acceptable manoeuvres was significantly associated with total DAPs (adjusted β for a 10-fold increase in concentration ($a\beta$) = -0.16 , 95% CI

Table 1 Maternal and child characteristics, Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS), CA, 1999–2000 (n=279)

Characteristics	n	%
Child's sex		
Boys	129	46.2
Girls	150	53.8
Season of birth*		
Mould	105	37.6
Wet	53	19.0
Pollen	58	20.8
Dry	63	22.6
Breast feeding duration		
Never breastfed	13	4.7
≤ 6 Months	128	45.9
> 6 Months	138	49.5
Mother's country of birth		
Mexico	237	84.9
USA/other	42	15.1
Maternal education		
≤ 6 th Grade	123	44.1
7–12th Grade	97	34.8
Completed high school	59	21.1
Maternal history of asthma		
Yes	10	3.6
No	268	96.4
Mother smoked during pregnancy		
Yes	12	4.3
No	267	95.7
Home ≤ 150 m from highway 101 (6 or 12 months)		
Yes	14	5.3
No	251	94.7
Mean daily PM _{2.5} near home (0–3 months)		
< 8 $\mu\text{g}/\text{m}^3$	126	45.2
8–12 $\mu\text{g}/\text{m}^3$	121	43.4
≥ 12 $\mu\text{g}/\text{m}^3$	32	11.5
Signs of moderate/extensive mould at home visit (6 or 12 months)		
Yes	183	69.1
No	82	30.9
Pets at home at ages 5–7		
Yes	42	15.1
No	237	84.9
Agricultural workers in the household at age 7		
Yes	187	67.0
No	92	33.0
Food insecurity status at age 7		
Food secure	174	62.4
Food insecure with or without hunger	105	37.6

*Season of birth corresponds generally to other potential exposures that might play a causal role in respiratory disease. We defined the seasons as follows: pollen (mid-January to mid-May 2000), dry (mid-May to mid-August 2000), mould (mid-August to mid-January 2001), wet (mid-January to mid-March 2001), pollen (mid-March to mid-May 2001) and dry (mid-May to October 2001). Differences in the date of seasons across years are based on actual rainfall and measured pollen counts for that year.

-0.30 to -0.02 , $p=0.03$) and DEs ($a\beta$) = -0.16 , 95% CI -0.30 to -0.03 , $p=0.02$) and borderline associated with DMs ($a\beta$) = -0.12 , 95% CI -0.25 to 0.02 , $p=0.08$) (table 3). The highest FVC measured from children who had at least two acceptable manoeuvres was borderline associated with total DAPs ($a\beta$) = -0.17 , 95% CI -0.34 to 0.01 , $p=0.06$) and DEs ($a\beta$) = -0.17 ,

Table 2 Lung function measurements at age 7*†, Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) birth cohort, Salinas Valley, CA, 1999–2000 (n=279)

Variable	Mean±SD	n
FEV ₁ (L/s)	1.70±0.46	279
FVC (L)	1.97±0.51	250
FEV ₁ /FVC	0.89±0.06	250
FEF _{25–75} (L/s)	2.29±0.82	250
Height (cm)	123.56±5.66	279
Weight (kg)	29.42±7.53	279
Age (years)	7.09±0.19	279

*Average values.

†Identical dry-seal spirometers were used in the study. Routine calibration was conducted every morning. Three identical EASYONE spirometers were used by two technicians; 92% of tests were performed by the same technician. Each child performed a maximum of eight expiratory manoeuvres and up to three best acceptable tests were kept by the spirometric software.

95% CI –0.33 to 0.00, $p=0.06$) and non-significantly associated with DMs ($a\beta=-0.13$, 95% CI –0.29 to 0.04, $p=0.1$) (table 3). Additionally, the highest FEF_{25–75} measured from children that had at least two acceptable manoeuvres was borderline associated with DEs ($a\beta=-0.26$, 95% CI –0.55 to 0.03, $p=0.08$) (table 3) and was not significantly associated with total DAPs or DMs. The FEV₁/FVC ratio was not associated with any DAP measurement (table 4).

Similar results were obtained when we excluded children who had been prescribed medication for asthma, wheezing or tightness in the chest during the past 12 months and who had used any medication in the past 24 h before spirometry (see online supplementary tables E3, E6 and E7). Results were also similar when we included in the models children who had one, two or three acceptable manoeuvres and when we conducted minimally adjusted models (tables 3 and 4, see online supplementary tables E2–E7). Similar results were obtained when we did not adjust maternal DAP concentrations for specific gravity or child AUC levels for creatinine (data not shown). When we examined sex interactions in our models, we found no interaction by sex (p value for interaction: 0.3 to 0.6; data not shown). Estimates

for both inverse probability weighted and unweighted regression models yielded similar results (data not shown), suggesting that selection bias did not substantially modify our results. The attributes of families included in this analysis ($n=279$) did not differ significantly from those of families not included, including urinary DAP concentrations during childhood, maternal asthma, maternal education, marital status, poverty category and child's birth weight. However, mothers of children included in this study were slightly older (mean age 26.5 vs 25.4, $p=0.02$) and breastfed the index child for longer (9.1 months vs 4.9, $p<0.01$) than those from the initial cohort (see online supplementary methods).

DISCUSSION

Although organophosphate pesticides are still widely used in modern agriculture,^{4 5} the effects of low-level exposure on lung function of residents living in agricultural communities have not been previously well studied.³ Our study provides the first evidence for an adverse association between exposure to OPs during childhood and pulmonary function of children. We assessed this relationship using a biomarker of exposure, urinary DAP metabolites, measured during the prenatal period and multiple times during childhood, in a prospective birth cohort design. Our findings support and extend the results of previous occupational studies of OP exposure and respiratory symptoms and function,³ case studies of paediatric OP poisonings²⁷ and cross-sectional investigations of respiratory symptoms in adults and children following reported pesticide exposure.^{15–19} Our findings on pulmonary function support our previous analysis of an association between childhood DAPs and maternal report of child's respiratory symptoms,²⁰ but unlike our previous study on symptoms, we found no association between in utero exposure and pulmonary function.

It is biologically plausible that chronic exposure to OPs might result in abnormalities of the lung. In contrast to the well-known acute respiratory effects of high-dose OP exposure, which include bronchoconstriction, pulmonary oedema and respiratory muscle paralysis,²⁸ the chronic effects of low-dose OP exposure are less well understood. Recently, it has been reported that additional mechanisms, including inhibition of muscarinic receptors in the airways, may mediate effects of

Table 3 Associations*† (β (95% CI)) of lung function at age 7 with childhood concentrations of dialkyl phosphate (DAP) metabolites‡ measured at follow-up visits between 0.5 and 5 years of age

n	DEs	p Value	DMs	p Value	Total DAPs	p Value
Highest FEV ₁ for children who had one, two, or three manoeuvres						
224	–0.18 (–0.32 to –0.05)	0.009	–0.10 (–0.23 to 0.04)	0.1	–0.14 (–0.29 to 0.00)	0.05
Highest FEV ₁ for children who had at least two manoeuvres						
207	–0.16 (–0.30 to –0.03)	0.02	–0.12 (–0.25 to 0.02)	0.08	–0.16 (–0.30 to –0.02)	0.03
Highest FVC for children who had one, two, or three manoeuvres						
203	–0.18 (–0.34 to –0.02)	0.03	–0.08 (–0.24 to 0.09)	0.4	–0.12 (–0.29 to 0.05)	0.2
Highest FVC for children who had at least two manoeuvres						
175	–0.17 (–0.33 to 0.00)	0.06	–0.13 (–0.29 to 0.04)	0.1	–0.17 (–0.34 to 0.01)	0.06
Highest FEF _{25–75} for children who had one, two or three manoeuvres						
203	–0.36 (–0.64 to –0.08)	0.01	–0.09 (–0.37 to 0.20)	0.6	–0.18 (–0.49 to 0.12)	0.2
Highest FEF _{25–75} for children who had at least two manoeuvres						
175	–0.26 (–0.55 to 0.03)	0.08	–0.06 (–0.34 to 0.23)	0.7	–0.14 (–0.44 to 0.17)	0.4

*Coefficients reflect change per 10-fold increase in creatinine-adjusted (nmol/g creatinine) metabolite concentrations assessed by the area under the curve.

†Adjusted for child's sex, age, age squared, log height, maternal smoking during pregnancy, season of birth (mould/wet/pollen/dry), mean daily PM_{2.5} during the first 3 months of life, breast feeding duration, signs of moderate/extensive mould at home visit (6 or 12 months), distance (≤ 150 m) from highway (6 or 12 months), pets at home (5–7 years), household food insecurity score (7 years), maternal education, season of spirometry and technician.

‡Diethyl (DE) phosphate, dimethyl (DM) phosphate and total dialkyl phosphate (DAP) metabolites.

Table 4 Associations*† (β (95% CI)) of FEV₁/FVC ratio at age 7 with childhood concentrations of dialkyl phosphate (DAP) metabolites‡ measured at follow-up visits between 0.5 and 5 years of age

n	DEs	p Value	DMs	p Value	Total DAPs	p Value
Highest FEV ₁ /FVC ratio for children who had one, two, or three manoeuvres						
203	0.00 (−0.02 to 0.01)	0.7	0.01 (−0.01 to 0.02)	0.6	0.00 (−0.02 to 0.02)	0.8
Highest FEV ₁ /FVC ratio for children who had at least two manoeuvres						
175	−0.01 (−0.03 to 0.01)	0.5	0.01 (−0.01 to 0.03)	0.4	0.01 (−0.02 to 0.03)	0.6

*Coefficients reflect change per 10-fold increase in creatinine-adjusted (nmol/g creatinine) metabolite concentrations assessed by the area under the curve.

†Adjusted for child's sex, age, age squared, log height, maternal smoking during pregnancy, season of birth (mould/wet/pollen/dry), mean daily PM_{2.5} during first 3 months of life, breast feeding duration, signs of moderate/extensive mould at home visit (6 or 12 months), distance (≤150 m) from highway (6 or 12 months), pets at home (5–7 years), household food insecurity score (7 years), maternal education, season of spirometry and technician.

‡Diethyl (DE) phosphate, dimethyl (DM) phosphate and total dialkyl phosphate (DAP) metabolites.

chronic exposure to OPs at environmental relevant concentrations—below those needed to inhibit AChE.⁸ However, more studies are needed to clarify the underlying mechanisms between chronic OP exposure and lung impairment.

Furthermore, because both FEV₁ and FVC were associated with DAP levels, while the FEV₁/FVC ratio was not (0.3≤p≤0.9), we suggest that prolonged OP exposure may restrict the growth of lung function. Although some occupational studies have reported either a potentially obstructive²⁹ or no effect³⁰ of OP exposure, other studies suggest that a restrictive abnormality may be associated with occupational exposure to OPs.^{14 31 32} For example, Peiris-John *et al*¹⁴ reported an association of AChE levels between and during OP spraying seasons, and a reduction of FEV₁ and FVC with a normal FEV₁/FVC ratio among 25 farmers occupationally exposed to OPs. However, our results for FVC and the FEV₁/FVC ratio should be interpreted with caution since collecting high-quality spirometric data function is particularly challenging in young children.³³

Limitations of the study design include loss to follow-up and challenges in exposure assessment. Selection bias due to loss to follow-up is always a possible limitation in observational studies. However, the results of the sensitivity analysis we conducted imply that selection bias did not substantially modify our results. There are no long-term biomarkers of OP pesticide exposure. The available methods have several limitations, including a short half-life in the body, high variability in exposure and being class-specific rather than pesticide-specific.^{3 34} Owing to logistical challenges, we also used spot urine samples, which may be less representative of exposure than 24 h samples. However, we did previously find strong correlations between full-day 24 h samples and same-day spot samples.³⁴ Finally, urinary DAPs may represent exposure to preformed metabolites in food or the environment and not direct exposure to parent compounds.³⁵ Thus, our use of seven measurements collected from pregnancy to early childhood might have resulted in some exposure misclassification. Nevertheless, DAPs are commonly used in epidemiological investigations and they represent the current state-of-the-art method for assessing broad exposure to OPs,^{35 36} since there are few sensitive and specific assays for parent compounds in blood or pesticide-specific metabolites in urine. Moreover, any exposure misclassification, if it occurred, would have tended to bias our results towards the null hypothesis.

Strengths of our study include the prospective cohort design with a relatively large sample size; an objectively measured outcome, pulmonary function; and the use of a biomarker for OP exposure. Additionally, we used a biomarker that was measured twice in pregnancy and five times during early childhood, which allowed us to study the long-term effects that OPs might

have on respiratory health. Moreover, the temporal sequence between OP exposure, measured during pregnancy and at ages 0.5–5 years of age and lung function, measured at age 7, allowed us to eliminate the potential for temporal bias.

In conclusion, our findings suggest that early-childhood exposure to OP pesticides is associated with decreased pulmonary function among school-age children living in an agricultural community in California. In our study, each 10-fold increase in concentrations of OP metabolites measured through early childhood (0.5–5 years of age) was associated with a 159 mL/s decrease in FEV₁ in 7-year-old children. In comparison, 30 years ago, passive paediatric exposure to maternal cigarette smoke was found to be associated with a decrease in FEV₁ of 101 mL/s after 5 years of exposure.¹ Thus, this study adds exposure to OP pesticides to the growing list of environmental exposures, including air pollution, indoor cook stove smoke and environmental tobacco smoke,^{1 2} that may play a key role in the pathophysiology of chronic respiratory disease in children. We suggest that future studies should attempt to replicate our findings in different study populations and focus on the mechanisms by which OP exposure affects lung development and function. Given the widespread use of OP pesticides worldwide, we believe these findings deserve further attention.

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Contributors RR: carried out the scientific literature search; analysed the data; prepared the tables; drafted the manuscript; BE, KGH and AB collected the data; RR, BE, JRB and KGH interpreted the data; BE, JRB, KGH, RBG, SM and AB provided critical revisions. All authors provided substantial contribution to the conception/design of work; approved the current version and state that it is ready to be published; and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Competing interests AB has served as a consultant on cases unrelated to the topics covered in this paper and has participated as a member of the Board for The Organic Center, a non-profit organisation that provides information for scientific research about organic food and farming. JRB is the physician member of the California Air Resources Board, a paid position.

Patient consent Obtained.

Ethics approval Research protocols were approved by the University of California, Berkeley, Committee for the Protection of Human Subjects.

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REFERENCES

- 1 Tager IB, Weiss ST, Muñoz A, *et al.* Longitudinal study of the effects of maternal smoking on pulmonary function in children. *N Engl J Med* 1983;309:699–703.
- 2 Franklin P, Kusel M. Environmental contributions to childhood asthma. *J Environ Immunol Toxicol* 2013;1:53–7.
- 3 Amaral AF. Pesticides and asthma: challenges for epidemiology. *Front Public Health* 2014;2:6.
- 4 Grube A, Donaldson D, Kiely T, *et al.* Pesticides industry sales and usage: 2006 and 2007 market estimates. Environmental Protection Agency, US, Office of Chemical Safety and Pollution Prevention, 2011. http://www.epa.gov/opp00001/pestsales/07pestsales/market_estimates2007.pdf (accessed 4 Nov 2014).
- 5 Pesticide registration status for Organophosphates, U.S. EPA (U.S. Environmental Protection Agency), 2013. http://www.epa.gov/opsrrd1/reregistration/status_op.htm. (accessed 4 Nov 2014).
- 6 CDC (Centers for Disease Control and Prevention). Fourth Report on Human Exposure to Environmental Chemicals, Updated Tables, (July, 2014). Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, 2014. <http://www.cdc.gov/exposurereport/> (accessed 4 Nov 2014).
- 7 Barnes PJ. Neural control of human airways in health and disease. *Am Rev Respir Dis* 1986;134:1289–314.
- 8 Fryer AD, Lein PJ, Howard AS, *et al.* Mechanisms of organophosphate insecticide-induced airway hyperreactivity. *Am J Physiol Lung Cell Mol Physiol* 2004;286:963–9.
- 9 Mamane A, Baldi I, Tessier JF, *et al.* Occupational exposure to pesticides and respiratory health. *Eur Respir Rev* 2015;24:306–19.
- 10 Hoppin JA, Umbach DM, London SJ, *et al.* Pesticides and adult respiratory outcomes in the agricultural health study. *Ann N Y Acad Sci* 2006;1076:343–54.
- 11 Hernández AF, Parrón T, Alarcón R. Pesticides and asthma. *Curr Opin Allergy Clin Immunol* 2011;11:90–6.
- 12 Zock JP, Sunyer J, Kogevinas M, *et al.* Occupation, chronic bronchitis and lung function in young adults: an international study. *Am J Respir Crit Care Med* 2001;163:1572–7.
- 13 de Jong K, Boezen HM, Kromhout H, *et al.* Pesticides and other occupational exposures are associated with airway obstruction: the LifeLines cohort study. *Occup Environ Med* 2014;71:88–96.
- 14 Peiris-John RJ, Ruberu DK, Wickremasinghe AR, *et al.* Low-level exposure to organophosphate pesticides leads to restrictive lung dysfunction. *Respir Med* 2005;99:1319–24.
- 15 Nriagu J, Robins T, Gary L, *et al.* Prevalence of asthma and respiratory symptoms in south-central Durban, South Africa. *Eur J Epidemiol* 1999;15:747–55.
- 16 Yemaneberhan H, Bekele Z, Venn A, *et al.* Prevalence of wheeze and asthma and relation to atopy in urban and rural Ethiopia. *Lancet* 1997;350:85–90.
- 17 Salameh P, Baldi I, Brochard P, *et al.* Respiratory symptoms in children and exposure to pesticides. *Eur Respir J* 2003;22:507–12.
- 18 Salam MT, Li YF, Langholz B, *et al.* Early-life environmental risk factors for asthma: findings from the Children's Health Study. *Environ Health Perspect* 2004;112:760–5.
- 19 Xu X, Nembhard WN, Kan H, *et al.* Residential pesticide use is associated with children's respiratory symptoms. *J Occup Environ Med* 2012;54:1281–7.
- 20 Raanan R, Harley KG, Balmes JR, *et al.* Early-life exposure to organophosphate pesticides and pediatric respiratory symptoms in the CHAMACOS cohort. *Environ Health Perspect* 2015;123:179–85.
- 21 Eskenazi B, Marks AR, Bradman A, *et al.* In utero exposure to dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethylene (DDE) and neurodevelopment among young Mexican American children. *Pediatrics* 2006;118:233–41.
- 22 Bradman A, Eskenazi B, Barr DB, *et al.* Organophosphate urinary metabolite levels during pregnancy and after delivery in women living in an agricultural community. *Environ Health Perspect* 2005;113:1802–7.
- 23 Bravo R, Driskell WJ, Whitehead RD, *et al.* Quantitation of dialkyl phosphate metabolites of organophosphate pesticides in human urine using GC-MS-MS with isotopic internal standards. *J Anal Toxicol* 2002;26:245–52.
- 24 Harrison GG, Stormer A, Herman DR, *et al.* Development of a spanish-language version of the U.S. household food security survey module. *J Nutr* 2003;133:1192–7.
- 25 Hernan MA, Hernandez-Diaz S, Robins JM. A structural approach to selection bias. *Epidemiology* 2004;15:615–25.
- 26 van der Laan MJ, Polley EC, Hubbard AE. *Super Learner*. UC Berkeley Division of Biostatistics Working Paper Series 2007. Working Paper 222. <http://biostats.bepress.com/ucbbiostat/paper22>
- 27 Cavari Y, Lifshitz M, Leibson T, *et al.* Severe and uncommon complications of anticholinesterase intoxication in children. *Harefuah* 2013;152:391–4.
- 28 Carey JL, Dunn C, Gaspari RJ. Central respiratory failure during acute organophosphate poisoning. *Respir Physiol Neurobiol* 2013;189:403–10.
- 29 Salameh P, Waked M, Baldi I, *et al.* Spirometric changes following the use of pesticides. *East Mediterr Health J* 2005;11:126.
- 30 Fieten KB, Kromhout H, Heederik D, *et al.* Pesticide exposure and respiratory health of indigenous women in Costa Rica. *Am J Epidemiol* 2009;169:1500–6.
- 31 Rastogi SK, Gupta BN, Husain T, *et al.* Study of respiratory impairment among pesticide sprayers in Mango plantations. *Am J Ind Med* 1989;16:529–38.
- 32 Chakraborty S, Mukherjee S, Roychoudhury S, *et al.* Chronic exposures to cholinesterase-inhibiting pesticides adversely affect respiratory health of agricultural workers in India. *J Occup Health* 2009;51:488–97.
- 33 Hall GL, Brookes IM. Techniques for the measurement of lung function in toddlers and preschool children. In: Hammer J, Eber E, eds. *Paediatric Pulmonary Function Testing*. Basel: Karger Publishers. 2005:66–77.
- 34 Bradman A, Kogut K, Eisen EA, *et al.* Variability of organophosphorous pesticide metabolite levels in spot and 24-hr urine samples collected from young children during 1 week. *Environ Health Perspect* 2013;121:118–24.
- 35 Sudakin DL, Stone DL. Dialkyl phosphates as biomarkers of organophosphates: the current divide between epidemiology and clinical toxicology. *Clin Toxicol (Phila)* 2011;49:771–81.
- 36 Chen L, Zhao T, Pan C, *et al.* Preformed biomarkers including dialkylphosphates (DAPs) in produce may confound biomonitoring in pesticide exposure and risk assessment. *J Agric Food Chem* 2012;60:9342–51.

Supplemental Material:

1. Title: Decreased Lung Function in 7-year-old Children with Early-Life Organophosphate Exposure

2. Authors: Rachel Raanan, PhD, John R. Balmes, MD, Kim G. Harley, PhD, Robert B. Gunier, PhD, Sheryl Magzamen, PhD, Asa Bradman, PhD, Brenda Eskenazi, PhD

Supplemental Methods:

Study setting and design. Pregnant mothers were recruited to the CHAMACOS study during October 1999-October 2000. Women were eligible for the study if they were ≥ 18 years of age, <20 weeks gestation who were planning to deliver at the county hospital, English or Spanish speaking, and Medi-Cal eligible. Medi-Cal is California's Medicaid health care program, and eligibility is based on economic need.

Spirometry. The children practiced blowing with a whistle before they practiced with the spirometer. They were instructed to blow into the whistle as loud and long as they can. Then, the technician would explain the purpose of the test and demonstrate the maneuver by blowing into the spirometer, and the children practiced blowing into the spirometer. Children were instructed to sit up straight on a stool with their feet flat on the floor, and take a deep breath and fill up their lungs with as much air as they can – till they feel like they cannot get any more in. The children were asked to place the mouthpiece in their mouth and seal it with their lips, and not to bite or stick their tongue in the hole, and then were coached to blast the air out as fast and as hard as they can, and not to stop until they are told. The technician kept encouraging the children to blow

until the test was completed. Children were instructed by demonstration not to lean forward when they blast out and to try to scrunch down instead.

After the practice, each child performed a maximum of eight expiratory maneuvers and up to three best acceptable tests were kept by the spirometric software. Each acceptable blow lasted at least three seconds. The children were coached to blow until the EasyOne signaled that the test has ended. All attempts were coached. Quality control was also achieved by verifying that the child did not lean forward; air did not leak out of the side; the child blew enough air in and took a deep breath right from the beginning; blew enough air out; did not stop blowing too soon; and took a big smooth breath. All maneuvers were reviewed again and verified by the two physicians to assure quality control.

Data analysis. Season of birth corresponds generally to other potential exposures that might play a causal role in respiratory disease. We defined the seasons as follows: pollen (mid-January to mid-May 2000), dry (mid-May to mid-August 2000), mold (mid-August to mid-January 2001), wet (mid-January to mid-March 2001), pollen (mid-March to mid-May 2001) and dry (mid-May to October 2001). Discrete seasons of high spore and pollen concentrations were determined by ambient aeroallergen concentrations that were measured throughout the birth periods of the participants. Differences in the date of seasons across years are based on actual rainfall and measured pollen counts for that year. Detailed methods for the differentiation of the four seasons have been described elsewhere¹. Data from the air monitoring station the Monterey Unified Air Pollution Control District (MBAPCD) was used to calculate the mean PM_{2.5} concentration during the first three months of life. Measurements have been conducted every sixth day with high-volume Sierra-Andersen gravimetric samplers for 24 hours (Thermo

Scientific, Waltham, MA). The covariate pets at home (at ages 5 and 7) included indoor dogs, cats and other furry pets.

Supplemental Results:

A total of 601 pregnant women were enrolled in the CHAMACOS cohort, of whom 347 children were followed when the children were approximately seven years of age². 306 children attempted to complete the spirometry test. Of these, 27 children performed unacceptable maneuvers (either all spirometric measurements were unacceptable or only PEF measurements were acceptable). A total of 279 children (80.4% of the total number of children who were followed at seven years of age) completed the spirometry test. Families included in this analysis (n=279) did not differ significantly from families not included on most attributes, including urinary DAP concentrations during childhood (2,598 versus 2,337 (0.5-5 years; nmol/year/g crt)), maternal asthma (3.6 versus 6.5 percent), maternal education (78.9% versus 80.1% of mothers completed less than a high school education), marital status (marriage percentage—82% versus 80%), poverty category (63.1% versus 60.1%), and child's birth weight (3,437 versus 3,440 g). However, mothers of children included in the present study were slightly older (mean age 26.5 versus 25.4, p=0.02), and breastfed the index child longer (9.1 months versus 4.9, p<0.01) than those from the initial cohort.

Families included in the analysis of participants who had at least two acceptable measurements (n=212) did not differ significantly from families included in the analysis of participants who had only one acceptable measurement (n=19) on most attributes, including urinary DAP concentrations during childhood, maternal age, maternal asthma, maternal

education, marital status, and poverty category. However, children included in the analysis of participants who had at least two acceptable measurements had a higher birth weight (mean birth weight 3,474 kg versus 3,173 kg, $p < 0.01$) than those included in the analysis of children who had only one acceptable measurement.

When BMI was considered as a covariate in sensitivity analyses, it was not found to alter the associations reported.

References:

1. Harley KG, Macher JM, Lipsett M, et al. Fungi and Pollen Exposure in the First Months of Life and Risk of Early Childhood Wheezing. *Thorax* 2009;64(4):353–58. DOI: 10.1136/thx.2007.090241.
2. Raanan R, Harley KG, Balmes JR, et al. Early-life exposure to organophosphate pesticides and pediatric respiratory symptoms in the CHAMACOS cohort. *Environ Health Perspect* 2015;123(2):179–85. DOI: 10.1289/ehp.1408235. Epub 2014 Nov 4.

Supplemental Table E1. DAP metabolite concentrations^{*†}, measured in maternal urine during pregnancy (nmol/L) and in children's urine at follow-up visits (nmol/g creatinine) between ages 0.5-5 years), CHAMACOS.

	n	DF (%)	GM (95% CI)	min	25 th	50 th	75 th	90 th	max
Measurements of pregnancy (nmol/L)									
First Half of Pregnancy									
Total DAPs	207	(89.4)	108 (89,131)	4	39	105	275	826	5026
DE	207	(74.5)	14 (12,18)	0.2	5	13	33	94	2436
DM	207	(83.6)	73 (59,90)	2	21	70	213	622	5019
Second Half of Pregnancy									
Total DAPs	258	(99.2)	123 (109,140)	6	64	120	229	515	2366
DE	258	(97.7)	19 (16,23)	0.7	6	22	51	120	630
DM	259	(99.2)	85	2	41	84	171	403	3175

(74,98)

Pregnancy Average

Total DAPs	275	(100)	145	10	72	132	285	585	2554
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(128,163)

DE	275	(100)	22	0.4	9	22	50	94	1245
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(19,25)

DM	276	(100)	105	6	51	97	225	530	3175
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(92,121)

Measurements of childhood (nmol/g crt)

At 6 Months

Total DAPs	251	(99.2)	229	2	88	190	674	1893	78235
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(188,280)

DE	251	(90.1)	43	0.2	14	67	147	307	78010
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(34,54)

DM	251	(88.1)	108	0.7	29	93	398	1679	10073
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(85,137)

At 1 Year

Total DAPs	262	(95.4)	237	4	86	229	647	1638	10552
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			(197,285)						
DE	262	(93.1)	61	0.8	31	69	148	258	1626
			(52,72)						
DM	262	(79.8)	115	0.8	28	121	484	1408	10298
			(91,147)						
At 2 Years									
Total DAPs	263	(96.6)	214	3	101	216	587	1324	5943
			(179,256)						
DE	263	(71.5)	22	0.0	3	47	117	302	1325
			(17,29)						
DM	263	(96.2)	149	2	56	164	423	1025	5843
			(124,179)						
At 3.5 Years									
Total DAPs	217	(94.5)	161	2	55	179	520	948	9240
			(130,198)						
DE	217	(63.1)	6	0.0	0.6	14	54	162	546
			(4,8)						

DM	217	(93.1)	132	2	44	143	361	929	8694
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(107,164)

At 5 Years

Total DAPs	259	(91.2)	128	0.9	45	144	334	930	10085
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(105,155)

DE	259	(50.1)	3	0.0	0.2	5	42	113	519
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(2,4)

DM	259	(88)	101	0.8	39	97	288	840	10052
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(82,124)

AUC (0.5-5 years; nmol/year/g crt)

Total DAPs	231	(90.9-	1687	118	861	1702	3095	6335	18927
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99.1) (1493,1905)

DE	231	(51.5-	258	14	137	255	525	812	16580
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92.5) (227,293)

DM	231	(78.3-	1311	79	608	1350	2598	5636	15460
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96.4) (1150,1495)

Abbreviations: DF= detection frequency; GM= geometric mean; CI= Confidence Interval;
crt=creatinine; diethyl (DE) phosphate, dimethyl (DM) phosphate and total dialkyl phosphate
(DAP) metabolites.

*Limits of detection for all DE analytes ranged from 0.05-0.2 ug/L, and for all DM analytes 0.08-0.58 ug/L.

†Pregnancy measurements were specific gravity adjusted and childhood measurements were creatinine adjusted.

Supplemental Table E2. Associations^{*†} [β (95% CI)] of lung function[‡] at age 7 with average[§] prenatal concentrations of dialkyl phosphate (DAP) metabolites^{||} measured in maternal urine collected twice during pregnancy.

	n	DEs	p- Value	DMs	p- Value	Total DAPs	p- Value
FEV ₁ (L/s)	242	-0.02 (-0.12,0.08)	0.7	0.00 (-0.12,0.11)	1	-0.01 (0.14,0.11)	0.8
FVC (L)	205	-0.03 (-0.16,0.09)	0.6	-0.01 (-0.15,0.13)	0.9	-0.05 (-0.21,0.12)	0.6
FEF ₂₅₇₅ (L/s)	205	-0.06 (-0.27,0.15)	0.6	0.02 (-0.23,0.27)	0.9	-0.03 (-0.30,0.25)	0.9
FEV ₁ /FVC	205	0.00 (-0.01,0.02)	0.7	0.01 (-0.01,0.02)	0.6	0.01 (-0.01,0.03)	0.5

^{*}Coefficients reflect change per 10-fold increase in specific-gravity adjusted metabolite concentrations.

[†]Adjusted for child's sex, age and age squared, log height, maternal smoking during pregnancy, season of birth (mold/wet/pollen/dry), mean daily PM_{2.5} during first 3 months of life, breast feeding duration, signs of moderate/extensive mold at home visit (6 or 12 months), distance (≤ 150 m) from highway (6 or 12 months), pets at home (5-7 years), household food insecurity score (7 years), maternal education, season of

spirometry and technician. [‡]Highest lung function measurements of children that had at least two blows.

[§]Comprised from the average of the two measurements taken during pregnancy; for mothers who did not have both measurements, the average reflects the single measurement available. ^{||}Diethyl (DE) phosphate, dimethyl (DM) phosphate and total dialkyl phosphate (DAP) metabolites.

Supplemental Table E3. Associations^{*†} [β (95% CI)] of lung function[‡] at age 7, excluding children that took any medication 24 hours before the test who were prescribed respiratory medication during the last 12 months, with average[§] prenatal concentrations of dialkyl phosphate (DAP) metabolites^{||} measured in maternal urine collected twice during pregnancy.

			p-		p-		p-
	n	DEs	Value	DMs	Value	Total DAPs	Value
FEV ₁ (L/s)	236	0.00 (0.10,0.10)	1	0.03 (-0.09,0.14)	0.6	0.02 (-0.11,0.15)	0.8
FVC (L)	203	-0.03 (0.15,0.01)	0.7	0.01 (-0.14,0.15)	0.9	-0.03 (-0.19,0.14)	0.7
FEF ₂₅₇₅ (L/s)	203	-0.06 (-0.27,0.16)	0.6	0.03 (-0.22,0.28)	0.8	-0.02 (-0.30,0.27)	0.9
FEV ₁ /FVC	203	0.00 (-0.01,0.02)	0.7	0.00 (-0.01,0.02)	0.7	0.01 (-0.01,0.03)	0.6

^{*}Coefficients reflect change per 10-fold increase in specific-gravity adjusted metabolite concentrations.

[†]Adjusted for child's sex, age and age squared, log height, maternal smoking during pregnancy, season of birth (mold/wet/pollen/dry), mean daily PM_{2.5} during first 3 months of life, breast feeding duration, signs of moderate/extensive mold at home visit (6 or 12 months), distance (≤ 150 m) from highway (6 or 12 months), pets at home (5-7 years), household food insecurity score (7 years), maternal education, season of spirometry and technician. [‡]Highest lung function measurements of children that had at least two blows.

[§]Comprised from the average of the two measurements taken during pregnancy; for mothers who did not have both measurements, the average reflects the single measurement available. ^{||}Diethyl (DE) phosphate, dimethyl (DM) phosphate and total dialkyl phosphate (DAP) metabolites.

Supplemental Table E4. Associations^{*†} [β (95% CI)] of lung function at age 7 with childhood concentrations of dialkyl phosphate (DAP) metabolites[‡] measured at follow-up visits between 0.5-5 years of age.

			p-		p-		p-
	n	DEs	Value	DMs	Value	Total DAPs	Value
Highest FEV ₁ for children who had one, two, or three maneuvers							
Minimally adjusted	231	-0.14 (-0.29,0.00)	0.06	-0.14 (0.28,0.01)	0.06	-0.16 (-0.31,0.01)	0.04
Adjusted	224	-0.18 (-0.32,-0.05)	0.009	-0.10 (-0.23,0.04)	0.1	-0.14 (-0.29, 0.00)	0.05
Highest FEV ₁ for children who had at least two maneuvers							
Minimally adjusted	212	-0.12 (-0.27,0.02)	0.09	-0.15 (-0.29,0.01)	0.04	-0.17 (-0.32,0.03)	0.02
Adjusted	207	-0.16 (-0.30,-0.03)	0.02	-0.12 (-0.25,0.02)	0.08	-0.16 (-0.30,0.02)	0.03
Highest FVC for children who had one, two, or three maneuvers							
Minimally adjusted	206	-0.16 (-0.32,0.02)	0.07	-0.12 (-0.29,0.05)	0.2	-0.15 (-0.33,0.03)	0.1

Adjusted	203	-0.18 (-0.34,-0.02)	0.03	-0.08 (-0.24,0.09)	0.4	-0.12 (-0.29,0.05)	0.2
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Highest FVC for children who had at least two maneuvers

Minimally adjusted	178	-0.14 (-0.32,0.05)	0.1	-0.15 (-0.33,0.03)	0.1	-0.17 (-0.36,0.02)	0.07
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Adjusted	175	-0.17 (-0.33,0.00)	0.06	-0.13 (-0.29,0.04)	0.1	-0.17 (-0.34,0.01)	0.06
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Highest FEF₂₅₇₅ for children who had one, two, or three maneuvers

Minimally adjusted	206	-0.30 (-0.80,-0.01)	0.04	-0.10 (-0.39,0.18)	0.5	-0.17 (-0.47,0.13)	0.3
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Adjusted	203	-0.36 (-0.64,-0.08)	0.01	-0.09 (-0.37,0.20)	0.6	-0.18 (-0.49,0.12)	0.2
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Highest FEF₂₅₇₅ for children who had at least two maneuvers

Minimally adjusted	178	-0.22 (-0.51,0.07)	0.1	-0.07 (-0.35,0.21)	0.6	-0.12 (-0.42,0.18)	0.4
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Adjusted	175	-0.26 (-0.55,0.03)	0.08	-0.06 (-0.34,0.23)	0.7	-0.14 (-0.44,0.17)	0.4
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*Coefficients reflect change per 10-fold increase in creatinine-adjusted (nmol/g creatinine) metabolite concentrations assessed by the Area Under Curve. †Minimally adjusted for child's sex, age, age squared, and log height. Fully adjusted models also include maternal smoking during pregnancy, season of birth

(mold/wet/pollen/dry), mean daily PM_{2.5} during first 3 months of life, breast feeding duration, signs of moderate/extensive mold at home visit (6 or 12 months), distance ($\leq 150\text{m}$) from highway (6 or 12 months), pets at home (5-7 years), household food insecurity score (7 years), maternal education, season of spirometry and technician. [‡]Diethyl (DE) phosphate, dimethyl (DM) phosphate and total dialkyl phosphate (DAP) metabolites.

Supplemental Table E5. Associations^{*†} [β (95% CI)] of FEV₁/FVC ratio at age 7 with childhood concentrations of dialkyl phosphate (DAP) metabolites[‡] measured at follow-up visits between 0.5-5 years of age.

			p-		p-		p-
	n	DEs	Value	DMs	Value	Total DAPs	Value
Highest FEV ₁ /FVC ratio for children who had one, two, or three maneuvers							
Minimally adjusted	206	0.00 (-0.02,0.02)	0.9	0.01 (-0.01,0.03)	0.5	0.01 (-0.02,0.03)	0.6
Adjusted	203	0.00 (-0.02,0.01)	0.7	0.01 (-0.01,0.02)	0.6	0.00 (-0.02,0.02)	0.8
Highest FEV ₁ /FVC ratio for children who had at least two maneuvers							
Minimally adjusted	178	-0.01 (-0.03,0.01)	0.6	0.01 (-0.01,0.03)	0.3	0.01 (-0.01,0.03)	0.5
Adjusted	175	-0.01 (-0.03,0.01)	0.5	0.01 (-0.01,0.03)	0.4	0.01 (-0.02,0.03)	0.6

^{*}Coefficients reflect change per 10-fold increase in creatinine-adjusted (nmol/g creatinine) metabolite

concentrations assessed by the Area Under Curve. [†]Minimally adjusted for child's sex, age, age squared, and log height. Fully adjusted models also include maternal smoking during pregnancy, season of birth (mold/wet/pollen/dry), mean daily PM_{2.5} during first 3 months of life, breast feeding duration, signs of moderate/extensive mold at home visit (6 or 12 months), distance ($\leq 150\text{m}$) from highway (6 or 12 months), pets at home (5-7 years), household food insecurity score (7 years), maternal education, season of spirometry and technician. [‡]Diethyl (DE) phosphate, dimethyl (DM) phosphate and total dialkyl phosphate (DAP) metabolites.

Supplemental Table E6. Associations^{*†} [β (95% CI)] of lung function at age 7, excluding children that took any medication 24 hours before the test who were prescribed respiratory medication during the last 12 months, with childhood concentrations of dialkyl phosphate (DAP) metabolites[‡] measured at follow-up visits between 0.5-5 years.

			p-		p-		p-
	n	DEs	Value	DMs	Value	Total DAPs	Value
Highest FEV ₁ for children who had one, two, or three maneuvers							
Minimally adjusted	226	-0.13 (-0.28,0.02)	0.09	-0.13 (-0.28,0.02)	0.08	-0.16 (-0.31,0.00)	0.05
Adjusted	219	-0.17 (-0.31,-0.03)	0.02	-0.09 (-0.23,0.05)	0.2	-0.14 (-0.28,0.01)	0.07
Highest FEV ₁ for children who had at least two maneuvers							
Minimally adjusted	207	-0.11 (-0.25,0.04)	0.2	-0.14 (-0.29,0.00)	0.05	-0.17 (-0.32,0.01)	0.04
Adjusted	202	-0.15 (-0.29,-0.01)	0.03	-0.11 (-0.25,0.03)	0.1	-0.15 (-0.30,0.01)	0.04
Highest FVC for children who had one, two, or three maneuvers							

Minimally adjusted	203	-0.14 (-0.32,0.03)	0.1	-0.11 (-0.29,0.06)	0.2	-0.14 (-0.33,0.05)	0.1
Adjusted	200	-0.18 (-0.34,-0.02)	0.03	-0.077 (- 0.24,0.09)	0.4	-0.13 (-0.30,0.05)	0.2
Highest FVC for children who had at least two maneuvers							
Minimally adjusted	176	-0.12 (-0.31,0.06)	0.2	-0.14 (-0.32,0.05)	0.1	-0.16 (-0.35,0.03)	0.1
Adjusted	173	-0.16 (-0.33,0.01)	0.07	-0.12 (-0.29,0.05)	0.2	-0.16 (-0.34,0.02)	0.08
Highest FEF ₂₅₇₅ for children who had one, two, or three maneuvers							
Minimally adjusted	203	-0.29 (-0.57,-0.01)	0.05	-0.10 (-0.39,0.19)	0.5	-0.17 (-0.48,0.14)	0.3
Adjusted	200	-0.36 (-0.65,-0.08)	0.01	-0.10 (-0.39,0.19)	0.5	-0.20 (-0.51,0.11)	0.2
Highest FEF ₂₅₇₅ for children who had at least two maneuvers							
Minimally adjusted	176	-0.21 (-0.50,0.08)	0.2	-0.05 (-0.34,0.24)	0.7	-0.10 (-0.41,0.21)	0.5

Adjusted	173	-0.26 (-0.55,0.04)	0.09	-0.05 (-0.34,0.25)	0.8	-0.13 (-0.44,0.18)	0.4
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*Coefficients reflect change per 10-fold increase in creatinine-adjusted (nmol/g creatinine) metabolite concentrations assessed by the Area Under Curve. †Minimally adjusted for child's sex, age, age squared, and log height. Fully adjusted models also include maternal smoking during pregnancy, season of birth (mold/wet/pollen/dry), mean daily PM2.5 during first 3 months of life, breast feeding duration, signs of moderate/extensive mold at home visit (6 or 12 months), distance (≤ 150 m) from highway (6 or 12 months), pets at home (5-7 years), household food insecurity score (7 years), maternal education, season of spirometry and technician. ‡Diethyl (DE) phosphate, dimethyl (DM) phosphate and total dialkyl phosphate (DAP) metabolites.

Supplemental Table E7. Associations^{*†} [β (95% CI)] of FEV₁/FVC ratio at age 7, excluding children that took any medication 24 hours before the test who were prescribed respiratory medication during the last 12 months, with childhood concentrations of dialkyl phosphate (DAP) metabolites[‡] measured at follow-up visits between 0.5-5 years.

				p-			p-			p-
	n	DEs	Value	DMs	Value	Total DAPs	Value			
Highest FEV ₁ /FVC ratio for children who had one, two, or three maneuvers										
Minimally adjusted	203	0.00 (-0.02,0.02)	0.7	0.01 (-0.01,0.02)	0.6	0.00 (-0.02,0.02)	0.8			
Adjusted	200	-0.01 (-0.03,0.01)	0.5	0.00 (-0.02,0.02)	0.8	0.00 (-0.02,0.02)	1			
Highest FEV ₁ /FVC ratio for children who had at least two maneuvers										
Minimally adjusted	176	-0.01 (-0.03,0.01)	0.5	0.01 (-0.01,0.03)	0.4	0.01 (-0.02,0.03)	0.6			
Adjusted	173	-0.01 (-0.03,0.01)	0.4	0.01 (-0.01,0.03)	0.5	0.00 (-0.02,0.03)	0.7			

*Coefficients reflect change per 10-fold increase in creatinine-adjusted (nmol/g creatinine) metabolite

concentrations assessed by the Area Under Curve. [†]Minimally adjusted for child's sex, age, age squared, and log height. Fully adjusted models also include maternal smoking during pregnancy, season of birth (mold/wet/pollen/dry), mean daily PM2.5 during first 3 months of life, breast feeding duration, signs of moderate/extensive mold at home visit (6 or 12 months), distance ($\leq 150\text{m}$) from highway (6 or 12 months), pets at home (5-7 years), household food insecurity score (7 years), maternal education, season of spirometry and technician. [‡]Diethyl (DE) phosphate, dimethyl (DM) phosphate and total dialkyl phosphate (DAP) metabolites.