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Urinary Dialkyl Phosphate Concentrations and Lung Function Parameters in Adolescents and Adults: Results from the Canadian Health Measures Survey

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ABSTRACT

Background: Epidemiological studies have reported associations between lung function parameters and organophosphate (OP) pesticide exposures in agricultural occupations, but to our knowledge, associations have not been evaluated in general populations.

Objectives: To examine associations between OP metabolite dialkyl phosphates (DAPs) and lung function using data from the Canadian Health Measures Survey (CHMS) Cycle 1.

Methods: Forced vital capacity (FVC), forced expiratory volume in one second (FEV₁), FEV₁/FVC ratio and forced expiratory flow between 25% and 75% of FVC (FEF_{25%-75%}) were measured for 4,446 CHMS participants. Urinary concentrations of six DAP metabolites (DMP, DMTP, DMDTP, DEP, DETP and DEDTP), smoking status, and other predictors of lung function, were also measured in the CHMS-Cycle 1. Multiple linear regression analyses were used to examine the relationship between total DAP concentrations (Σ DAPs) and lung function in adolescents (12-19 years) and adults (20-79 years).

Results: In adults, estimates from multiple regression analyses suggested that one unit increase on natural logarithmic scale (171% increase on the original scale) in the creatinine corrected urinary concentration (nmol/g creatinine) of Σ DAP was associated with a 32.6 (-57.2, -8.1) mL reduction in FVC, 32.6 (-59.0, -6.3) mL reduction in FEV₁, 0.2% (-0.6, 0.2) reduction in FEV₁/FVC ratio and 53.1 (-113.9, 7.7) mL/s reduction in FEF_{25%-75%}. In adolescents, associations between Σ DAP and FEV₁ were closer to the null and positive for FVC, while associations with FEV₁/FVC and FEF_{25%-75%} were negative, as in adults. However, none of the associations were significant in adolescents.

Conclusions: The negative association between Σ DAP and lung function in adult participants suggests a detrimental effect of OP pesticides on lung function in the adult general population. Further studies using prospective designs are warranted to confirm the findings reported in this study.

INTRODUCTION

Organophosphate (OP) pesticides have been extensively used in agricultural and residential applications. Humans may be exposed occupationally, environmentally and through household exposure. Occupational exposures are mainly from agricultural occupations (EPA, 2011) and environmental exposures can be from land run-off from the OP-treated areas (Kolpin et al., 1998). Mixing OP insecticides without personal protection can cause dermal absorption due to the high lipophilicity of OP insecticides (Reigart et al., 1999). Ingestion of food and water contaminated with OPs is also a major route of exposure for general populations (Reigart et al., 1999; Ye et al., 2015).

After entering the body, OP and/or its activated desulfurated 'oxon' form (EPA, 2006a), are rapidly hydrolyzed by phosphotriesterase paraoxonase 1 (PON1) to form dialkyl phosphate (DAP) metabolites that are subsequently excreted in the urine (Jansen et al., 2009). In the environment, generation of DAPs also occurs naturally when OP pesticides are degraded in soil, sediment and surface water (Walker, 2001).

There are six dialkyl phosphate metabolites: dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), dimethyl dithiophosphate (DMDTP), diethyl phosphate (DEP), diethyl thiophosphate (DETP) and diethyl dithiophosphate (DEDTP). These dialkyl phosphates are common metabolites of OPs, but are not pesticide specific (Kapka-Skrzypczak et al., 2011). Blood or urinary levels of DAPs are often considered as biomarkers of exposures to parental OP pesticides or their metabolites in the environment (Kapka-Skrzypczak et al., 2011). The detection of DAPs in urine samples is generally believed to reflect recent exposures to OPs over the past few days (Kapka-Skrzypczak et al., 2011).

OP pesticides function as cholinesterase-inhibitors and thus interfere with neural transmission in the nervous system (Androutsopoulos et al., 2012). In humans, health concerns related to OP exposures mainly focus on their high acute neurotoxicity (Keifer and Firestone, 2007). Exposures to high doses of OP can cause death due to respiratory paralysis and bradycardia (Keifer and Firestone, 2007). Other adverse health effects associated with OP exposures include chronic neurological (Abou-Donia, 2003), neurodevelopmental (Eskenazi et al., 2007), immunological (Corsini et al., 2008), endocrine disruptive (De Coster and van Larebeke, 2012) and respiratory effects (Ye et al., 2013). Because of these health concerns, OP pesticides have been largely restricted to use in agricultural applications in many countries. For example, chlorpyrifos, has been banned for residential use in the US since 2001 (EPA, 2006b).

Exposures to OP insecticides in agricultural occupations have been associated with both respiratory symptoms and diseases. In a matched case-control study of 376 agricultural workers with 348 age- and sex- matched control subjects in Eastern India, exposures to OP insecticides were significantly associated with runny or stuffy nose, sore throat, dry cough, wheezing, breathlessness, chest tightness and dyspnea (Chakraborty et al., 2009). The Agricultural Health Study conducted in the United States (US) reported that exposures to OP insecticides chlorpyrifos and parathion were associated with wheezing and adult-onset asthma (Hoppin et al., 2002; Hoppin et al., 2006). In addition, the prevalence of chronic bronchitis has been associated with occupational exposures to OP insecticides among agricultural workers in India (Chakraborty et al., 2009) and in the US (Hoppin et al., 2007; Valcin et al., 2007).

Few studies on OP exposures in agriculture have examined the association of OP pesticides on lung function. Peiris-John et al. reported that reductions in forced expiratory volume in one second (FEV₁) and forced vital capacity (FVC) were significantly associated with

an OP insecticide application period among farm workers (N=25, $p<0.05$) in Sri Lanka (Peiris-John et al., 2005). In a study from India, exposures to OP insecticides among agricultural sprayers were significantly associated with lower mean FVC (sprayers vs control: 2.23 L vs 2.58 L), FEV₁ (sprayers vs control: 1.94 L vs 2.36 L), FEV₁/FVC ratio (sprayers vs control: 87.0% vs 91.5%), forced expiratory flow between 25% and 75% of FVC (FEF_{25%-75%}) (sprayers vs control: 2.25 L/s vs 2.81 L/s) and peak expiratory flow rate (PEFR) (sprayers vs control: 2.04 L/s vs 2.73 L/s) among agricultural workers (N=724, $p<0.0001$) (Chakraborty et al., 2009). In addition, impaired lung function has been found to be associated with OP-induced cholinesterase inhibition (Fareed et al., 2013). A cross-sectional study of pesticide sprayers (N=166) in India showed that impaired lung function was significantly correlated with lower activities of acetylcholinesterase ($p<0.01$) and butylcholinesterase ($p<0.05$), suggesting an adverse effect of OPs on lung function (Fareed et al., 2013).

While there have been a few studies on the association between OP exposures and lung function in farm workers, to our knowledge, none have reported its impact on lung function in the general population. In the current study, urinary concentrations of dialkyl phosphate metabolites and their association with lung function were characterized among a Canadian general population.

METHODS

In this study, data from the first Canadian Health Measures Survey (CHMS-Cycle 1), a nation-wide cross-sectional survey conducted by the Statistics Canada in 2007-2009 were utilised (Statistics Canada, 2011). Statistics Canada considered that the CHMS participants were a representative sample of the Canadian general population (Statistics Canada, 2011). The study participants comprised 4,446 CHMS participants, including 980 adolescents aged 12 to 19 years

and 3,466 adults aged 20 to 79 years, who had data available on urinary concentrations of DAPs (Health Canada, 2010), spirometric measurements of lung function, smoking status, and other predictors of lung function.

The CHMS-Cycle 1 participants were chosen using a multi-stage sampling strategy. Collection sites were stratified by geographic region and by the Census Metropolitan Area (CMA), and then selected according to the population size. Inhabitants of dwellings gave the household composition information of each dwelling. Dwellings in the collection sites were stratified by age groups according to the probability of having inhabitants with desired age in each dwelling and then equal number of dwellings was sampled within each age stratum. Participants were then sampled from chosen dwellings in each age stratum (Statistics Canada, 2011). People living on reserves and Aboriginal settlements, residents of institutions, members of the Canadian Forces and those living in remote areas with too low population density, were excluded (Statistics Canada, 2011). The overall response rate was 51.7% for the CHMS-Cycle 1 (Statistics Canada, 2011). A detailed description of the CHMS-Cycle 1 can be obtained from Statistics Canada (Statistics Canada, 2011).

Participation in the CHMS was voluntary and all 4,446 subjects provided informed consent to store and use their urine samples (Statistics Canada, 2011). This study was approved by the Health Research Ethics Board of the University of Alberta.

Urinary concentrations of dialkyl phosphates

Approximately 60 mL of mid-stream urine was collected from each CHMS participant using a urine specimen container. After collection, urine samples were refrigerated immediately and transported as soon as possible using refrigerated shipment to an analytical laboratory at the National Public Health Institute of Quebec (INSPQ) in Quebec City for analyzing dialkyl

phosphate (DAP) metabolites (Statistics Canada, 2011). Concentrations of six DAP metabolites (DMP, DMTP, DMDTP, DEP, DETP and DEDTP) were measured using gas chromatography–mass spectrometry (GC-MS) (Health Canada, 2010; Statistics Canada, 2011). Limits of detection (LOD) for measuring DMP, DMTP, DMDTP, DEP, DETP and DEDTP were 7.9 nmol/L, 4.2 nmol/L, 1.9 nmol/L, 6.5 nmol/L, 3.5 nmol/L and 1.6 nmol/L, respectively (Health Canada, 2010). In addition, urinary creatinine concentration was measured using the colorimetric Jaffe method and concentrations of DAPs were normalized to allow for urine dilution (Barr et al., 2005).

The total concentration of all six DAP metabolites (Σ DAP), the three dimethyl alkylphosphate metabolites (Σ DMAP) and the three diethyl alkylphosphate metabolites (Σ DEAP), to was used to estimate the overall exposures to all OPs, OPs with DMAP metabolites such as malathion, and OPs with DEAP metabolites such as chlorpyrifos, respectively (Oulhote and Bouchard, 2013). To calculate Σ DAP, Σ DMAP, and Σ DEAP, mass concentrations of DAPs in urine ($\mu\text{g/g}$ creatinine) were converted to molar concentrations (nmol/g creatinine) using respective molecular weights of DAPs. Samples with DAP concentrations less than LOD were assigned as $0.5 \times \text{LOD}$ (Hornung and Reed, 1990).

Lung function measurements

Lung function parameters FVC, FEV_1 , FEV_1/FVC ratio and $\text{FEF}_{25\%-75\%}$ were considered in the current study. Lung function tests were performed at the Mobile Examination Center (MEC) on the same day when each participant's urine sample was collected.

Trained technologists measured the lung function of participants using a portable flow-based spirometer (Koko[®], PDS Instrumentation Inc., Louisville, Colorado US). American Thoracic Society (ATS) recommendations for performance of spirometry were followed,

including calibrating spirometers with a 3 liter syringe and obtaining a minimum of 3 acceptable trials from a maximum of 8 maneuvers based on the ATS definition of within- and between-manoeuvre criteria for usable and acceptable trials (Statistics Canada, 2011; Miller et al., 2005). Lung function measures were standardized to body temperature, barometric pressure and water saturation (BTPS) measured at the MEC on the same day (Statistics Canada, 2011).

For FVC and FEV₁ measurements, the largest value of acceptable trials was used, and for FEF_{25%-75%} measurements, the mean flow rate (L/s) of the acceptable trial with the largest sum of FVC and FEV₁ was used (Statistics Canada, 2011). Subjects with difficulty in breathing at rest, acute (e.g., cold, bronchitis and flu) or chronic respiratory condition (e.g. persistent cough), taking medication for tuberculosis, recent eye (within 6 weeks), chest or abdominal (within 3 months) surgery, pregnancy (> 27 weeks), or with an important language barrier, were excluded from lung function testing (Statistics Canada, 2011).

Factors related to lung function

Information on age, sex and ethnicity were collected using the CHMS-Cycle 1 household questionnaire (Statistics Canada, 2011). Standing height and weight were objectively measured by a fixed stadiometer with scales using a standard procedure based on the Canadian Physical Activity, Fitness and Lifestyle Approach (CPAFLA) (Statistics Canada, 2011).

Information on tobacco smoking was obtained for participants aged 12 years and above using the CHMS-Cycle 1 household questionnaire regarding the frequency (daily, occasionally, or not at all) and the duration of cigarette smoking (ages when started smoking at least 1 cigarette/month and ages when stopped smoking completely) (Statistics Canada, 2011). Based on the responses to the questionnaire, a variable with three categories was defined by CHMS to indicate never, former smoker (including former daily and former occasional smoker) and

current smoker, respectively. Pack-years, defined as number of packs of cigarettes smoked per day multiplied by number of years of smoking, were also calculated using detailed information collected on smoking in the CHMS-Cycle 1 (Statistics Canada, 2011). In the pack-years calculation, never smokers and former occasional smokers (< 1 cigarettes smoked / day in the past) were assigned a value of 0 pack-years.

Other lung function related factors, including environmental tobacco exposure (exposed to second-hand smoke inside their home, in their private vehicle, in public places, such as bars, restaurants, shopping malls, or at their place of work), types of heating source used at home (gas furnace/fireplace, oil furnace, electric heat, or wood burning fireplace/stove), and air quality (ambient concentrations of PM_{2.5}, NO₂ and O₃ that were recorded hourly by the National Air Pollution Surveillance Program on the same day when the spirometry tests were performed) , were also measured as part of CHMS-Cycle 1 (Statistics Canada, 2011).

Statistical analyses

Lung function parameters FVC, FEV₁, FEV₁/FVC and FEF_{25%-75%} were considered as continuous outcome variables. Natural log-transformed total concentrations of dialkyl phosphates (Σ DAPs, Σ DMAPs, and Σ DEAPs) were considered as continuous exposure variables in the analyses to reduce the skewness of the distribution, which leads to a one unit increase in log transformed DAP concentrations equivalent to 171% increase in the actual concentrations.

In the descriptive analyses, geometric means, medians, interquartile ranges (IQRs) of urinary concentrations (nmol/g creatinine), and proportions of subjects with detectable urinary concentrations (\geq LOD) of DAPs, were calculated for each DAP metabolite, as well as for Σ DAP, Σ DMAP, and Σ DEAP. Descriptive statistics were not calculated for individual metabolites that were below LOD in > 40% of samples (Health Canada, 2010). Demographic and

anthropomorphic characteristics and smoking status of participants were described by means with standard errors (SE) or proportions.

We incorporated sampling design weights provided by Statistics Canada in our statistical analyses to adjust for post-stratification in the multistage sampling, units with no responses and out of scope responses (Statistics Canada, 2011). In order to allow for the complex sampling design, 500 bootstrap weights, provided by Statistics Canada, were applied in variance estimation for descriptive statistics, regression coefficients and 95% confidence intervals (Statistics Canada, 2011).

Bivariate analyses were initially conducted to examine the relationship between risk factors, including urinary concentrations of DAPs, and lung function. Factors that were significant at $p \leq 0.1$ were considered in the multiple regression model for each individual lung function parameter. In multiple regression models, a purposeful selection method along with backward step-wise model building was used to determine the final models, i.e. the variables that were consistently shown as predictors of lung function in the literature, including age, sex, ethnicity, height and smoking, were forced into the final models. Other variables that were non-significant at $p = 0.05$ in the step-wise model building, including environmental tobacco exposure (Yes/No), types of heating source (categorical variable) used at home and ambient air pollutants (continuous concentrations of $PM_{2.5}$, NO_2 and O_3), were excluded from the final models. Associations between urinary concentrations of DAPs (ΣDAP , $\Sigma DMAP$ or $\Sigma DEAP$) and lung function were determined by the final multiple linear regression models with lung function parameters as dependent variables and log (natural) transformed creatinine-corrected urinary DAP concentrations as independent variables, adjusting for age (continuous), sex, ethnicity (Caucasian or other), height (continuous), smoking status (never, former, current) and weight

(continuous). In addition, product interaction terms between urinary DAP concentrations and age (continuous), sex (male/female), ethnicity (Caucasian or other), smoking status (never, former, current) on the association with lung function outcomes were also examined, with $p \leq 0.05$ being considered in the final models. Separate regression models were used to examine the association among adolescent (12-19 years) and adult (20-79 years) participants, respectively.

Sensitivity analyses were performed using mass volume concentrations (nmol/L) of Σ DAP as an exposure variable and adjusting for urinary creatinine concentration (g/L) as a separate independent covariate, instead of modeling creatinine-corrected Σ DAP (Barr et al., 2005).

Statistical analyses were performed using STATA (StataCorp LP. 2007, Release 12) and SAS (SAS Institute Inc. 2011, SAS® 9.3) software with procedures for the complex survey data analysis. In this study, we used default alpha level in STATA, i.e. $p \leq 0.05$ was considered as statistical significance.

RESULTS

Characteristics of the study participants

Demographic and anthropometric characteristics, and smoking status of participants, are summarized in Table 1. Among 4,446 participants in the CHMS Cycle 1 (2007-2009), 980 (22.0%) were adolescents (12-19 years) with mean age of 15.5 years and 3,466 (78.0%) were adults (20-79 years) with mean age of 45.5 years. Overall, males and females were almost equally represented (Table 1). Approximately 62.7% of the participants aged 12-19 years and 71.7% of the participants aged 20-79 years self-identified as of Caucasian ethnicity. While the majority of adolescent participants (85.6%) had never smoked, approximately half of the adult participants ever smoked at some time during their lifetime (Table 1).

Lung function among the study participants

Lung function parameters across demographic groups and smoking status are summarized in Table 2. In adults, lung function was larger in young adults (age 20-29 years) than older adults (30–79 years) (Table 2). Although there was no significant difference in the mean lung function measures between smoking categories in the univariate analyses, after adjusting for age, sex, ethnicity, weight and height, for both adolescent and adult participants, former and current smokers had statistically lower mean values of FEV₁, FEV₁/FVC ratio and FEF_{25%-75%} compared to non-smokers ($p < 0.01$, data not shown). After controlling for age, sex, ethnicity, weight, height and smoking status, lung function parameters followed a reasonably normal distribution for the CHMS participants (data not shown). In addition, there were no significant associations in adolescents or adults between lung function parameters and exposure to environmental tobacco smoking, types of heating source used at home, or ambient concentration of PM_{2.5}, NO₂, and O₃ (data not shown).

Urinary concentrations of dialkyl phosphates in the study participants

Among the total study participants, 91.3% had at least one of the six dialkyl phosphate metabolites detectable in their urine samples. The mean (both arithmetic and geometric means) concentrations (creatinine-adjusted) of DAP metabolites were higher among adults than adolescents (Table 3).

DMP and DEP were the most prevalent DAP metabolites (detected in approximately 80.0% of both adolescent and adult participants) while DEDTP was the least prevalent metabolite (detected in less than 5.0% of adolescent and adult participants) (Table 3). In addition, the mean concentrations (creatinine-adjusted) of total methyl DAPs (Σ DMAP) was significantly higher than the total ethyl DAPs (Σ DEAP) in both adolescent and adult participants (Table 3).

The geometric mean concentrations of total DAP (Σ DAP) were 83.8 ± 5.5 (nmol/g creatinine) and 90.5 ± 4.5 (nmol/g creatinine) for adolescent participants and adult participants, respectively (Table 3). In both adolescent and adult participants, females had significantly higher mean concentrations of DAP metabolites, including Σ DAP, Σ DMAP and Σ DEAP, than male participants ($p < 0.05$) (Table 4). In addition, among adult participants, current smokers had statistically significant lower mean concentrations of DAP metabolites (Σ DAP, Σ DMAP and Σ DEAP) than former smokers and the participants who never smoked (for current vs. never and current vs. former smokers, both p -values < 0.01 , Table 4). In both adolescents and adults, no significant difference in the mean concentrations of DAP metabolites was observed between Caucasians and participants in other ethnic groups (Table 4).

Relationships between DAP concentrations and lung function

In the multiple regression analyses of adult participants aged 20-79 years, one unit increase on natural logarithmic scale (171% increase on the original scale) in the creatinine corrected urinary concentration of Σ DAP was associated with a 32.63 (95% CI: -57.21, -8.05) mL reduction in FVC, a 32.66 (95% CI: -59.02, -6.28) mL reduction in FEV₁, 0.18% (95% CI: -0.61%, 0.24%) reduction in FEV₁/FVC ratio and 53.11 (95% CI: -113.90, 7.68) mL/s reduction in FEF_{25%-75%} after adjusting for age, sex, ethnicity, height, weight and smoking status (Table 5). In addition, urinary concentration of Σ DMAP and Σ DEAP were also negatively associated with FVC and FEV₁ in adults, but associations were statistically significant for Σ DMAP only (Table 5). No interactions between DAP concentrations and age, sex, ethnicity, smoking status were significant at $p \leq 0.05$ (data not shown). In addition, model estimates were similar when adjusting for pack-years as an untransformed continuous variable instead of smoking status (data not shown).

In adolescents, associations between urinary DAP concentrations (including Σ DAP, Σ DMAP and Σ DEAP) and lung function parameters were positive for FVC, close to null for FEV₁, and negative for FEV₁/FVC and FEF_{25%-75%} after adjusting for age, sex, ethnicity, height, weight and smoking status. However, none of the associations were statistically significant in adolescents (Table 5).

Associations based on models of Σ DAP (nmol/L urine), as well as Σ DMAP (nmol/L urine) and Σ DEAP (nmol/L urine) that included creatinine as a covariate were consistent with findings from models of creatinine-adjusted DAP concentrations (nmol/g creatinine) for adolescent and adult participants (data not shown).

DISCUSSION

Our results also showed that over 90% of the CHMS Cycle 1 participants aged 12-79 years had at least one species of DAP metabolite detectable in their urine. The prevalence and geometric mean concentration of most of the DAP metabolites reported in the current study (CHMS 2007-2009) were higher than those reported in a similar year for the US population (NHANES 2007-2008) (CDC, 2013). For example, the geometric means of urinary concentrations of DMP and DEP were 51.5 nmol/g and 28.1 nmol/g for participants of this study, but lower than LOD in the US population (NHANES 2007-2008) (CDC, 2013), which may be resulted from a higher extent of OP pesticide exposures in Canada than the US when assuming high correlation between the biomonitoring concentrations and exposures to OP pesticides. Given the short half-life of most OP pesticides in the environment (Pehkonen S, 2002) and the short elimination half-life in humans (Kapka-Skrzypczak et al., 2011), the detection of DAP metabolites among the majority of the participants suggests that exposures to OP pesticides are common and ongoing in the Canadian general population.

In the current study, we estimated associations between urinary concentration of DAPs and lung function parameters among the Canadian Health Measures Survey-Cycle 1 participants aged 12-79 years, a representative sample of the Canadian adolescents and adults (Statistics Canada, 2011). To the best of our knowledge, the current study is the first nation-wide population-based investigation on the relationships between DAP metabolites and lung function among the Canadian general population.

Among the adult participants, urinary concentrations of total DAPs (Σ DAP) were significantly associated with the reduction in FVC and FEV₁. The differences in FVC and FEV₁ between adult participants at the 25th (43.2 nmol/g) and 75th (175.1 nmol/g) percentile of urinary concentrations of Σ DAP, calculated by the product of beta coefficient for FVC or FEV₁ in Table 5 with $\log(25^{\text{th}} \text{ percentile of concentration} / 75^{\text{th}} \text{ percentile of concentration})$, would be 45.67 mL and 45.70 mL, respectively, which is around 1-2% of typical lung function. The Σ DAP associated 45.67 mL and 45.70 mL reduction in FVC and FEV₁ respectively after adjusting for age, sex, ethnicity, height, weight and smoking status in this cross-sectional study was similar in size to the natural age-related decline of lung function per year for healthy non-smoking adults (approximately 30mL/year in FVC and 20-30 mL/year in FEV₁) (Burrows et al., 1983; Peat et al., 1990). Nevertheless, the magnitude of any DAP concentration associated lung function reduction would be better characterized in studies with longitudinal designs.

Results from the multiple regression analyses showed significant association between DAP concentrations and lung function in adult participants (20-79 years) but not in adolescent participants (12-19 years). The reasons for this difference are unclear but may be due to the rapid growth of the lungs during the growth spurt in adolescents increasing variance in lung function parameters (Pellegrino et al., 2005), which will result in larger uncertainties in characterizing the

association between insecticide exposures and lung function. This difference could be also due to differences in the magnitude, including duration and/or timing, of OP exposures, uncontrolled confounding effect, the smaller sample size of adolescent versus adult participants, and/or any other sources of bias that lead to differences between adults and adolescents.

Although both methyl DAPs and ethyl DAPs were negatively associated with lung function among adults, they were only statistically significant for Σ DMAP. This result could be due to less variation (smaller IQR) in Σ DEAP concentrations. In addition, a higher mean concentration of Σ DMAP than Σ DEAP might suggest that the potential OP related lung function changes identified could be mainly from the OP insecticides with methyl DAP metabolites, although future work is required to confirm this.

Several studies in the literature have suggested that exposures to OP pesticides in agricultural occupations were associated with a reduction in lung function parameters, including FEV₁ (Chakraborty et al., 2009; Fareed et al., 2013; Peiris-John et al., 2005), FVC (Chakraborty et al., 2009; Peiris-John et al., 2005), FEV₁/FVC ratio (Chakraborty et al., 2009; Fareed et al., 2013), FEF_{25%-75%} (Chakraborty et al., 2009) and peak expiratory flow rate (Chakraborty et al., 2009; Fareed et al., 2013). In this study, the urinary Σ DAP level was associated with reductions in both FVC and FEV₁ among the adult general population, which is consistent with the findings in agricultural occupations (Chakraborty et al., 2009; Fareed et al., 2013; Peiris-John et al., 2005).

OP pesticides are neurotoxicants that bind to the serine residue in acetylcholine esterase (AChE), resulting in an accumulation of acetylcholine (ACh) and overstimulation of postsynaptic cholinergic nerves (Androutsopoulos et al., 2012). This suggests a number of possible mechanisms by which OP exposure could affect lung function. Muscarinic 3 (M3) receptors, a stimulatory type of muscarinic ACh receptors, are expressed on both pulmonary nerves and

smooth muscles (Racke and Matthiesen, 2004). Stimulation of M3 receptors by Ach would potentially lead to the contraction of airway smooth muscles *ex vivo* (Fryer and Jacoby, 1998). Another muscarinic receptor, the M2 receptor located on the pulmonary prejunctional nerves and smooth muscles can inhibit further release of Ach from prejunctional nerve ends (Costello et al., 1998). Based on studies of guinea pigs, at a low-dose level, which may be particularly relevant among general populations, OP pesticides do not seem to inhibit AchE but have the potential to disrupt the auto-inhibitory function of pulmonary prejunctional M2 receptors (Lein and Fryer, 2005; Proskocil et al., 2010), so leading to an unopposed release of ACh from prejunctional parasympathetic nerves which again might cause excessive bronchoconstriction (Minette and Barnes, 1988). Nevertheless, mechanisms of OP-associated lung function reduction in humans may not be the same as these experimental findings.

Inhalation of OP-containing gases, vapors or aerosols into airways can also lead to production of reactive oxygen species (ROS) and subsequent activation of a series of stress-responsive signalling pathways, including ERK(extracellular-signal-regulated kinase)-MAPK (mitogen-activated protein kinase), JNK (c-Jun N-terminal kinases) and NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells) signalling pathways (Terry, 2012). This may in turn cause contraction of airway smooth muscles and airway narrowing (Abdollahi et al., 2004; Tomasic et al., 1992).

Notwithstanding our consideration of these actions of OPs, the biological mechanisms underlying any OP-related reduction of lung function remain unclear. The proposed biological mechanisms are not exclusive for any specific type of pulmonary diseases. Further study to characterize the biological plausibility of the OP-associated type of lung function impairment is necessary.

For the general population, dietary intake of trace amounts of OPs from pesticide-sprayed or treated fruits and vegetables is a major source of OP exposures (Reigart et al, 1999). However, as suggested by Zhang et al, ingestion of environmentally preformed DAPs can also lead to the detection of DAP metabolites in urine samples (Zhang et al., 2008). Therefore, in addition to OP pesticides, DAP metabolites detected in urine samples in the current study may have resulted from the direct exposure to environmental DAPs, which are currently not known to be toxic to human health.

There are several limitations in our data. Firstly, data from the CHMS did not cover the entire Canadian population. Aboriginal people living on reserves and Aboriginal settlements, people living in remote areas, institutional residents and full-time members of the Canadian Force were excluded from the CHMS-Cycle 1 (Statistics Canada, 2011). However, it is unlikely that the exclusion of these groups would change the relationships reported in this study, since the excluded populations in the CHMS-Cycle 1 represent less than 4% of the total Canadian population (Statistics Canada, 2011). Secondly, subjects with chronic or acute respiratory conditions and taking medication for tuberculosis (Statistics Canada, 2011), were excluded from the regression analyses, which will limit our ability to generalize our results to a broader population. Thirdly, while urinary levels of DAP metabolites can be considered as an objective measure of actual body burden arising from OP pesticide exposures (Kapka-Skrzypczak et al., 2011), they lack specificity in identifying corresponding pesticides, and therefore the current study was not able to provide information on specific OP pesticides that the participants were exposed to. Moreover, this study cannot distinguish between environmentally preformed DAPs and metabolite DAPs resulting from exposure to the parent compounds. Lastly, due to the cross-sectional nature of the CHMS, our data provide only a snapshot of urinary DAP concentrations

and repeated DAP measurements were not conducted longitudinally during the year, which may not be directly related to peak or cumulative OP exposures (Kapka-Skrzypczak et al., 2011) and it was also not possible to examine the potential effect of measurement variability on the results. Moreover, the temporal sequence between changes in lung function and exposures to OPs cannot be determined in the current study.

CONCLUSIONS

Our results showed that urinary concentrations of total DAPs were significantly associated with reductions in FVC and FEV₁ among the adult participants aged 20-79 years, a representative sample of the Canadian general population.

Although many organophosphate (OP) pesticides have been restricted for agricultural uses only, exposures remain common and may still pose risks to public health (EPA, 2006b). Further research using prospective designs is warranted to confirm the associations reported in this study.

REFERENCES

- Abdollahi, M., et al., 2004. Pesticides and oxidative stress: a review. *Med Sci Monit.* 10: RA141-147.
- Abou-Donia, M. B., 2003. Organophosphorus ester-induced chronic neurotoxicity. *Arch Environ Health.* 58: 484-497.
- Androutsopoulos, V. P., et al., 2013. A mechanistic overview of health associated effects of low levels of organochlorine and organophosphorous pesticides. *Toxicology.* 307: 89-94.
- Barr, D. B., et al., 2005. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. *Environ Health Perspect.* 113: 192-200.
- Burrows, B., et al., 1983. A descriptive analysis of the growth and decline of the FVC and FEV1. *Chest.* 83: 717-724.
- CDC, 2013. Fourth national report on human exposure to environmental chemicals-updated tables. Centers for disease control and prevention. Available: <http://www.cdc.gov/exposurereport/> [accessed March 17 2015].
- Chakraborty, S., et al., 2009. Chronic exposures to cholinesterase-inhibiting pesticides adversely affect respiratory health of agricultural workers in India. *J Occup Health.* 51: 488-497.
- Corsini, E., et al., 2008. Effects of pesticide exposure on the human immune system. *Hum Exp Toxicol.* 27: 671-680.
- Costello, R. W., et al., 1998. Pulmonary neuronal M2 muscarinic receptor function in asthma and animal models of hyperreactivity. *Thorax.* 53: 613-616.
- De Coster, S., van Larebeke, N., 2012. Endocrine-disrupting chemicals: associated disorders and mechanisms of action. *J Environ Public Health.* 2012, 713696.
- EPA. 2006a. Reregistration Eligibility Decision(RED) for Malathion. Vol. 2012. US-Environmental Protection Agency, 2006b.
- EPA. 2006b. Reregistration Eligibility Decision for Chlorpyrifos. US-Environmental Protection Agency.
- EPA. 2011. Pesticide market estimates: 2006-2007. . Available: http://www.epa.gov/pesticides/pestsales/07pestsales/table_of_contents2007.htm [accessed Mar 14 2015].
- Eskenazi, B., et al., 2007. Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children. *Environ Health Perspect.* 115: 792-798.

- Fareed, M., et al., 2013. Adverse respiratory health and hematological alterations among agricultural workers occupationally exposed to organophosphate pesticides: a cross-sectional study in North India. *PLoS One*. 8, e69755.
- Fryer, A. D., Jacoby, D. B., 1998. Muscarinic receptors and control of airway smooth muscle. *Am J Respir Crit Care Med*. 158: S154-160.
- Health Canada. 2010. Report on Human Biomonitoring of Environmental Chemicals in Canada- Results of the Canadian Health Measures Survey Cycle 1 (2007-2009). Vol. 2012. Health Canada.
- Hoppin, J. A., et al., 2002. Chemical predictors of wheeze among farmer pesticide applicators in the Agricultural Health Study. *Am J Respir Crit Care Med*. 165, 683-9.
- Hoppin, J. A., et al., 2006. Pesticides and adult respiratory outcomes in the agricultural health study. *Ann N Y Acad Sci*. 1076, 343-54.
- Hoppin, J. A., et al., 2007. Pesticide use and chronic bronchitis among farmers in the Agricultural Health Study. *Am J Ind Med*. 50, 969-79.
- Hornung, R. W., Reed, L. D., 1990. Estimation of average concentration in the presence of nondetectable values. *Applied Occupational and Environmental Hygiene*. 5: 46-51.
- Jansen, K. L., et al., 2009. Paraoxonase 1 (PON1) modulates the toxicity of mixed organophosphorus compounds. *Toxicol Appl Pharmacol*. 236: 142-153.
- Kapka-Skrzypczak, L., et al., 2011. Biomonitoring and biomarkers of organophosphate pesticides exposure - state of the art. *Ann Agric Environ Med*. 18: 294-303.
- Keifer, M. C., Firestone, J., 2007. Neurotoxicity of pesticides. *J Agromedicine*. 12: 17-25.
- Kolpin, D., et al., 1998. Occurrence of pesticides in shallow groundwater of the United States: initial results from the National Water-Quality Assessment program. *Environ Sci Technol*. 32: 558-566.
- Lein, P. J., Fryer, A. D., 2005. Organophosphorus insecticides induce airway hyperreactivity by decreasing neuronal M2 muscarinic receptor function independent of acetylcholinesterase inhibition. *Toxicol Sci*. 83: 166-176.
- Miller, M. R., et al., 2005. Standardisation of spirometry. *Eur Respir J*. 26: 319-338.
- Minette, P. A., Barnes, P. J., 1988. Prejunctional inhibitory muscarinic receptors on cholinergic nerves in human and guinea pig airways. *J Appl Physiol*. 64: 2532-2537.

- Oulhote, Y., Bouchard, M. F., 2013. Urinary metabolites of organophosphate and pyrethroid pesticides and behavioral problems in canadian children. *Environ Health Perspect.* 121: 1378-1384.
- Peat, J. K., et al., 1990. Decline of lung function and development of chronic airflow limitation: a longitudinal study of non-smokers and smokers in Busselton, Western Australia. *Thorax.* 45: 32-37.
- Pehkonen S, Z. Q., 2002. The Degradation of Organophosphorus Pesticides in Natural Waters: A Critical Review. *Critical Reviews in Environmental Science and Technology.* 32: 17-72.
- Peiris-John, R. J., et al., 2005. Low-level exposure to organophosphate pesticides leads to restrictive lung dysfunction. *Respir Med.* 99: 1319-1324.
- Pellegrino, R., et al., 2005. Interpretative strategies for lung function tests. *Eur Respir J.* 26, 948-968.
- Proskocil, B. J., et al., 2010. Organophosphorus pesticides decrease M2 muscarinic receptor function in guinea pig airway nerves via indirect mechanisms. *PLoS One.* 5, e10562.
- Racke, K., Matthiesen, S., 2004. The airway cholinergic system: physiology and pharmacology. *Pulm Pharmacol Ther.* 17: 181-198.
- Reigart JR, Robert JR, Recognition and Management of Pesticide Poisonings. Vol. 2012. National Pesticide Information Center, 1999.
- Statistics Canada. 2010. Canadian Health Measures Survey (Cycle 1) - Clinic Questionnaire Vol. 2012. Statistics Canada.
- Statistics Canada. 2011. Canadian Health Measures Survey (CHMS) Data User Guide: Cycle 1. Vol. 2012. Statistics Canada.
- Terry, A. V., Jr., 2012. Functional consequences of repeated organophosphate exposure: potential non-cholinergic mechanisms. *Pharmacol Ther.* 134: 355-365.
- Tomasic, M., et al., 1992. Contractile agonists activate voltage-dependent calcium channels in airway smooth muscle cells. *Am J Physiol.* 263: C106-113.
- Valcin, M., et al., 2007. Chronic bronchitis among nonsmoking farm women in the agricultural health study. *J Occup Environ Med.* 49, 574-83.
- Walker, C., 2001. Organic pollutants, an ecotoxicological perspectives. Taylor and Francis, London.

- Ye, M., et al., 2013. Occupational pesticide exposures and respiratory health. *Int J Environ Res Public Health*. 10: 6442-6471.
- Ye M, Beach J, Martin JW, Senthilselvan A. 2015. Associations between dietary factors and urinary concentrations of organophosphate and pyrethroid metabolites in a canadian general population. *Int J Hyg Environ Health*. doi: 10.1016/j.ijheh.2015.06.006
- Zhang, X., et al., 2008. Dialkylphosphates (DAPs) in fruits and vegetables may confound biomonitoring in organophosphorus insecticide exposure and risk assessment. *J Agric Food Chem*. 56: 10638-10645.

Table 1. Characteristics of the study population by age group[†]

Characteristics	Age groups (Total N=4,446)	
	12-19 years (N=980) [‡]	20-79 years (N=3,466) [‡]
Sex (%)		
Female	48.8	50.5
Male	51.2	49.5
Height (cm)	166.70 (0.24)	168.58 (0.28)
Weight (kg)	63.16 (0.98)	77.65 (0.76)
Ethnicity (%)		
Caucasian	62.7	71.7
Other	37.3	28.3
Province of residence (%)		
New Brunswick	7.1	7.2
Quebec	23.3	23.7
Ontario	39.2	38.7
Alberta	16.9	16.8
British Columbia	13.5	13.6
Smoking status (%)		
Never	85.6	47.9
Former smoker	2.4	30.6
Current smoker	12.0	21.5
Pack-years for smokers (pack-years)	1.00 (0.20)	9.90 (0.42)

[†] Survey design weights and 500 bootstrap weights were used in calculating percentages, mean values and standard errors (S.E.)

[‡] Among 4,446 participants, 22.0% were adolescents aged 12-19 years, and 78.0% were adults aged 20-79 years.

Table 2. Distribution of the means of lung function parameters by demographic factors and smoking status by age group

Characteristics	FVC (L) Mean (SE) †	FEV₁ (L) Mean (SE) †	FEV₁/FVC (%) Mean (SE) †	FEF_{25%-75%} (L/s) Mean (SE) †
12-19 years (N=980)				
Total sample	4.14 (0.04)	3.46 (0.03)	84.0 (0.3)	3.55 (0.05)
Sex				
Female	3.71 (0.03)	3.16 (0.03)	85.5 (0.3)	3.39 (0.06)
Male	4.54 (0.07) *	3.74 (0.05) *	82.6 (0.5) *	3.69 (0.07) *
Ethnicity				
Caucasian	4.28 (0.03)	3.55 (0.02)	83.4 (0.3)	3.60 (0.06)
Others	3.88 (0.07) *	3.29 (0.05) *	85.2 (0.5) *	3.45 (0.06) *
Smoking status				
Never	4.06 (0.03)	3.41 (0.03)	84.3 (0.3)	3.52 (0.05)
Former smoker	4.36 (0.13) *	3.58 (0.07) *	82.5 (2.0) *	3.65 (0.17) *
Current smoker	4.61 (0.10) *	3.77 (0.09) *	82.3 (1.0) *	3.71 (0.20) *
20-79 years (N=3,466)				
Total sample	4.13 (0.04)	3.19 (0.03)	77.1 (0.3)	2.88 (0.04)
Age group				
20-29 years	4.71 (0.10)	3.81 (0.08)	81.4 (0.5)	3.69 (0.12)
30-79 years	4.00 (0.03) *	3.05 (0.02) *	76.2 (0.3) *	2.70 (0.03) *
Sex *				
Female	3.45 (0.02)	2.68 (0.02)	77.6 (0.3)	2.48 (0.04)
Male	4.81 (0.05) *	3.69 (0.04) *	76.6 (0.4) *	3.28 (0.06) *
Ethnicity				
Caucasian	4.19 (0.03)	3.20 (0.03)	76.3 (0.3)	2.84 (0.04)
Others	3.98 (0.06) *	3.14 (0.04) *	79.1 (0.5) *	2.97 (0.04) *
Smoking status				
Never	4.12 (0.07)	3.26 (0.05)	79.2 (0.3)	3.09 (0.06)
Former smoker	4.02 (0.05) *	3.06 (0.04) *	75.9 (0.3) *	2.67 (0.06) *
Current smoker	4.30 (0.05) *	3.21 (0.04) *	74.4 (0.4) *	2.72 (0.05) *

†Survey design weights and 500 bootstrap weights were used in calculating arithmetic mean values and standard errors (S.E.)

* Statistically significant differences in lung function from the reference group (e.g. “Female” for sex) with $p < 0.01$.

Table 3. Distribution of creatinine-corrected urinary concentrations of organophosphate metabolites in the study population by age group

Organophosphate pesticide metabolites	Detection limit (nmol/L)	Percentage \geq LOD [†] (%)	A.mean [‡] (nmol/g, S.E. [§])	G.mean [‡] (nmol/g, S.E. [§])	Median (nmol/g, S.E. [§])	IQR (nmol/g)
12-19 years (N=980)						
DMP	7.9	82.3	51.5 (3.0)	27.1 (2.1)	29.0 (2.7)	13.2-63.0
DMTP	4.2	68.6	55.0 (6.3)	14.0 (1.3)	14.1 (1.5)	<LOD-42.9
DMDT	1.9	35.4	–	–	<LOD	<LOD-3.6
DEP	6.5	82.0	28.1 (2.0)	16.8 (1.3)	16.9 (1.3)	9.0-33.7
DETP	3.5	44.6	–	–	<LOD	<LOD-<LOD
DEDT	1.6	4.4	–	–	<LOD	<LOD-<LOD
ΣDAP	n/a	93.8	151.8 (12.0)	83.8 (5.5)	78.2 (7.8)	39.9-167.5
ΣDMAP	n/a	88.4	116.0 (9.9)	51.6 (3.6)	48.4 (4.6)	22.3-119.5
ΣDEAP	n/a	83.1	35.7 (2.5)	22.6 (1.5)	21.8 (1.8)	12.4-41.9
20-79 years (N=3,466)						
DMP	7.9	76.4	53.3 (3.5)	27.5 (2.0)	27.5 (2.0)	13.1-56.9
DMTP	4.2	66.7	68.8 (5.0)	17.2 (1.3)	14.5 (1.6)	<LOD-55.4
DMDT	1.9	36.4	–	–	<LOD	<LOD-5.5
DEP	6.5	77.9	27.6 (1.0)	17.5 (0.8)	17.9 (1.0)	9.8-33.4
DETP	3.5	36.1	–	–	<LOD	<LOD-<LOD
DEDT	1.6	2.3	–	–	<LOD	<LOD-<LOD
ΣDAP	n/a	91.0	169.7 (9.5)	90.5 (4.5)	84.8 (5.1)	43.2-175.1
ΣDMAP	n/a	82.4	132.7 (8.9)	55.5 (3.7)	50.7 (3.7)	23.2-125.0
ΣDEAP	n/a	78.6	36.9 (1.1)	24.3 (0.9)	24.3 (1.0)	13.6-43.4

[†] Percentage of participants with at least one of the organophosphate metabolites in the sum \geq LOD

[‡] If <60% of samples had detectable organophosphate metabolites, means were not calculated. A.mean: Arithmetic means. G.mean: Geometric means

[§] Survey design weights and 500 bootstrap weights were used in calculating percentages, mean values, standard errors (S.E.) and 95% confidence intervals.

Table 4. Distribution of creatinine-corrected urinary concentrations of organophosphate metabolites by demographic factors and smoking status by age group

Geometric means (nmol/g creatinine, S.E.) [†]						
Characteristics	12-19 years (N=980)			20-79 years (N=3,466)		
	ΣDAP	ΣDMAP	ΣDEAP	ΣDAP	ΣDMAP	ΣDEAP
Average	83.8 (5.5)	51.6 (3.6)	22.6 (1.5)	90.5 (4.5)	55.5 (3.7)	24.3 (0.9)
Sex						
Female	95.3 (7.4)	59.9 (5.0)	24.3 (2.1)	112.2 (6.6)	68.5 (5.8)	29.8 (1.3)
Male	74.4 (5.3) [*]	44.9 (3.7) [*]	21.2 (1.2) [*]	72.6 (3.7) [*]	44.7 (2.9) [*]	19.7 (0.7) [*]
Ethnicity						
Caucasian	84.5 (7.3)	51.2 (4.7)	23.5 (2.0)	90.4 (4.4)	55.5 (3.6)	24.7 (1.0)
Others	83.6 (5.9)	53.2 (4.1)	21.2 (1.4)	91.4 (6.8)	56.1 (5.4)	23.4 (1.5)
Smoking status						
Never	87.8 (6.3)	54.3 (4.2)	23.4 (1.7)	96.7 (6.1)	60.2 (4.7)	25.3 (1.3)
Former smoker	70.8 (33.7)	46.7 (27.6)	16.7 (3.3)	104.0 (7.5)	64.5 (5.5)	26.0 (1.5)
Current smoker	61.8 (9.5)	36.3 (9.3)	18.7 (2.4)	63.8 (4.8)	37.3 (3.4)	20.2 (1.1)

[†]Survey design weights and 500 bootstrap weights were used in calculating geometric mean values and standard errors (S.E.)

^{*}Statistically significant differences in DAP concentrations from the reference group (e.g. “Female” for sex) with $p < 0.05$.

Table 5. Association between natural log transformed creatinine-corrected urinary concentrations of Σ DAP, Σ DMAP and Σ DEAP and lung function parameters by age group[†]

per natural log transformed [nmol /g creatinine]	FVC (mL)		FEV ₁ (mL)		FEV ₁ /FVC (%)		FEF _{25-75%} (mL/s)	
	Beta (95% CI) [‡]	<i>p</i> -value	Beta (95% CI) [‡]	<i>p</i> -value	Beta (95% CI) [‡]	<i>p</i> -value	Beta (95% CI) [‡]	<i>p</i> -value
12-19 years (N=980)								
Σ DAP	13.93 (-24.37, 52.24)	0.44	-2.36 (-35.74, 31.02)	0.88	-0.33 (-0.86, 0.20)	0.20	-27.05 (-114.95, 60.85)	0.51
Σ DMAP	12.34 (-18.19, 42.87)	0.39	1.12 (-23.00, 25.24)	0.92	-0.22 (-0.68, 0.24)	0.31	-17.88 (-87.58, 51.82)	0.58
Σ DEAP	21.58 (-46.07, 89.22)	0.50	2.75 (-49.32, 54.83)	0.91	-0.34 (-0.86, 0.18)	0.18	-6.26 (-93.57, 81.05)	0.88
20-79 years (N=3,466)								
Σ DAP	-32.63 (-57.21, -8.05)	0.014	-32.65 (-59.02, -6.28)	0.02	-0.18 (-0.61, 0.24)	0.36	-53.11 (-113.90, 7.68)	0.081
Σ DMAP	-24.29 (-45.39, -3.18)	0.028	-24.18 (-45.52, -2.85)	0.03	-0.15 (-0.51, 0.21)	0.39	-38.73 (-90.26, 12.80)	0.13
Σ DEAP	-20.38 (-57.16, 16.39)	0.25	-26.34 (-61.08, 8.39)	0.12	-0.20 (-0.55, 0.14)	0.22	-62.17 (-113.57, -10.76)	0.022

[†]Associations were characterized by the multiple linear regression analyses with controlling for age, sex, ethnicity, height, weight and smoking status.

[‡]Survey design weights and 500 bootstrap weights were included in calculating β coefficients and 95% confidence interval (95% CI). Beta= β coefficients