Exposure to Volatile Organic Compounds and Loss of Pulmonary Function in the Elderly

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Statement of Interest
All authors declare we have no competing financial interest.
ABSTRACT

Volatile organic compounds (VOC) are reported to cause adverse effects on pulmonary function in occupationally exposed workers. However, evidence is lacking on the effect in the general population. We hypothesized that VOC impairs pulmonary function through enhancing oxidative stress, especially in the elderly population.

A longitudinal panel study of 154 elderly people was performed in South Korea. Repeated spirometric tests were performed up to 8 times on different days for each subject. We also measured urinary concentrations of metabolites of the VOC and markers of oxidative stress (malondialdehyde and 8-oxo-2’-deoxyguanosine) on the same day of spirometric tests. A mixed linear regression model was used to evaluate the association among the VOC metabolites, oxidative stress markers and spirometric tests.

We found that the urinary levels of hippuric acid and methylhippuric acid, which are metabolites of toluene and xylene, respectively, were significantly associated with reduction of FEV1, FEV1/FVC, and FEF25–75. We also found significant associations between the metabolites of VOC and the markers of oxidative stress. In addition, the oxidative stress markers were associated with pulmonary function parameters.

This study suggests that exposure to toluene and xylene exert a harmful effect on pulmonary function by exacerbating oxidative stress in elderly people.

Keywords: elderly, oxidative stress, pulmonary function, volatile organic compounds,
INTRODUCTION

There is a growing concern over health problems related to ambient air pollution. Volatile organic compounds (VOC) are one of the major ambient air pollutants emitted from diverse chemical sources in common daily use. Exposure to VOC may cause upper and lower respiratory symptoms and contribute to the worsening of asthma. The mechanisms of VOC toxicity, however, have yet to be established, even though some experimental evidence supports oxidative stress having a role (1-3).

Decline of pulmonary function has become one of the most important issues regarding health effects of air pollution because it not only reflects the individual respiratory health status, but predicts cardiovascular morbidity and mortality in the general population (4-6).

Although the diminution of pulmonary function by exposure to ozone and particulate air pollutants has been previously reported (7-9), it is not clear whether indoor or outdoor exposure to VOC in ambient air contribute to the reduction of pulmonary function. Although a recent cross-sectional study suggests an association between exposure to VOC and diminished lung function (10), evidence is still lacking to determine causality of the exposure for the effect.

Therefore, we conducted a longitudinal panel study with repeated measurements to evaluate the effects of VOC on pulmonary function and the involvement of oxidative stress in the pathogenesis in the elderly who are known to be especially susceptible to air pollution (11-13).
Methods

Study subjects
The study population consisted of 161 elderly people whose age is over 60 years at 6 day-care facilities within the three different geographic regions of Seoul (3 facilities), Cheongju (1 facility) and Gangnung (2 facilities) in Korea, which represent metropolitan area, industrialized city and rural areas, respectively. The elderly return to their home environment when they were not at the day care facilities.
Repeated spirometric tests were performed up to 8 times on different days for each subject. We also measured urinary concentrations of metabolites of the VOC and markers of oxidative stress on the same day of spirometric tests. However, 3 refused participation and 4 performed spirometric testing only once. Therefore we included 154 subjects who had performed spirometric testing twice or more in the analysis.
At the first visit, information such as demographic factors, medical history, dietary habits, smoking and alcohol consumption was obtained by a questionnaire. The first voided urines were collected for biomarker measurements at each visit day.
Written informed consent was obtained from every subject, and this study was approved by the institutional review board of the Seoul National University College of Medicine.

Spirometric Measurements
Spirometric testing was conducted 4 times in the warm season (June to September) and 4 times in the cold season (October to December) according to 2005 European Respiratory Society/American Thoracic Society recommendations. All tests were performed at the same
hour in the morning by one trained technician, and a Microlab® (Sensormedic, USA) spirometer was used.

The spirometric tests used in our analyses were forced vital capacity (FVC), forced expiratory volume in one second (FEV₁), FEV₁ as a percentage of FVC (FEV₁/FVC), and forced expiratory flow between 25 and 75% of the FVC (FEF₂₅₋₇₅).

**Urinary levels of VOC metabolites**

On the same day of spirometric tests, tt-muconic acid, hippuric acid, mandelic acid and methylhippuric acid were measured as biomarkers for exposure to benzene, toluene, ethylbenzene and xylene, respectively. To determine the tt-muconic acid levels, 200 μl of urine were mixed with 800 μl of mobile phase (1% acetic acid and methanol, 10:1, v/v) and filtered through a 0.45-μm membrane filter. Absorbance of the filtered solution was measured by HPLC-UV at 259 nm using Shiseido MF C8 SG80 5μm (4.6x150mm) column. To determine the hippuric acid, mandelic acid and methylhippuric acid levels, 200 μl of urine were mixed with 2 ml of distilled water and filtered through a 0.45-μm membrane filter. Absorbance of the filtered solution was measured by HPLC-UV at 225 nm using Waters Atlantis C18 5μm (4.6x150mm) column. The mobile phase was 0.55% n-tetrabutylammonium bromide / 0.15% KH2PO4 and MeOH (60:40, v/v).

**Urinary levels of oxidative stress biomarkers**

For evaluation of oxidative stress, urinary malondialdehyde (MDA) was determined by the measurement of MDA-thiobarbituric acid (TBA) adducts. Aliquots of the upper layer of the centrifuged urine samples were mixed with phosphoric acid and TBA reagent in an iced methanol glass tube, and were boiled for 60 minutes. After the mixture was boiled, it was cooled in ice water for 5 minutes and centrifuged after adding methanol. The absorbance was
measured by an HPLC system equipped with a SP930D solvent delivery pump and UV730 absorbance detector (Youngjin company, Korea) on a Nova-Pak C18 column (150 X 3.9 nm) at 532 nm with a mobile phase composed of 50nM KH₂PO₄ (pH 6.8)-methanol in a ratio of 58:42 (v/v) (14).

Urinary 8-oxo-2’-deoxyguanosine (8-OHDG) levels were quantitatively determined using a competitive in vitro ELISA (JICA, Fukuroi, Japan). Fifty microliters of primary monoclonal antibody and 50µl of sample or standard were added to microtitier plates, which had been pre-coated with 8-OHDG. The plates were sealed tightly and incubated at 37°C for 1 hour, and then washed with 250 µl PBS. One hundred microliters of HRP-conjugated secondary antibody were then added to each well, incubated, and washed. One hundred microliters of enzyme substrate was then added to each well. Reactions were terminated by adding 100 µl 1N phosphoric acid. Absorbance readings were taken 3 min after termination using a spectrophotometer (ELX808, Bio-Tek, Winooski, VT) at 450 nm (14).

**Ambient levels of VOC**

We measured indoor levels of benzene, toluene, ethylbenzene and xylene once at two places and outdoor levels at one place at the same time for each facility during the study period to compare ambient levels of the VOC with the levels of other reports. The sampling equipments to collect the VOC were placed on the 1.5 m above floor level indoor and ground level outdoor at the day-care facilities. Air samples were collected by passing air through adsorbent tubes (Tenax, USA). The tubes were connected with air sampler (MP-∑100H, SIBATA, Japan) for absorption of the VOC. The sampling rate was 100 ml/min for 8 hours (9 AM to 5 PM) and the samples were analyzed by GC/MSD (6890N, Agilent, USA) in a capillary column (60 m in length, 0.25 mm in inner diameter and 0.25 µm in film thickness) using Thermal Desorption System (Gerstel, Germany).
Measurements of Potential Confounders

Urinary cotinine concentrations

We measured urinary concentrations of cotinine because tobacco exposure was regarded as a confounder and cotinine is widely considered to be the best marker for monitoring tobacco exposure in either actively or passively exposed individuals. The urine samples were stored at -20 °C and analyzed in batches. Urinary cotinine level was measured using a microplate enzyme immunoassay (UMAX, USA). The microplate was coated with an anti-cotinine antibody and cotinine concentration was determined by a competitive reaction with an enzyme-bound and non-enzyme-bound antigens. Reactions were detected at 450 nm using a microplate reader (Versamax, Molecular Device, USA).

Personal exposure to PM2.5 and NO2

We measured personal exposure to PM$_{2.5}$ in participating subjects. The particles were sampled around 24 hours for each visit with a PM$_{2.5}$ cyclon (Personal Environmental Monitor, SKC company, USA) containing a pump (Mine Safety Appliances company, USA). The air flow rate (2 l/min) of the pump was adjusted before each sampling. The PM$_{2.5}$ cyclon was attached with a pin between the chest and head, and the pump was placed in a small bag that the subjects carried during measurement. The PM$_{2.5}$ cyclon and the pump were kept near the bedside during sleep. The 37 mm, 2.0 μm Teflon filters (PTFE, USA) were weighed before and after sampling using a microbalance and the concentration of ambient particulate matter was calculated considering air flow and measurement time.

Personal NO$_2$ exposures were also measured simultaneously with PM$_{2.5}$. Polyester housed badge-type NO$_2$ passive samplers (Envors Co., Korea) were used for the study. One of the investigators gave instructions on how to wear and care for the samplers. Unsealed passive
sampling badges were attached with a pin between chest and head, and were sealed the following day. Wearers were also instructed to keep the badges near the bedside when they slept. The collected samplers were sealed with a gas-tight polyester lid and kept in a zippered plastic bag before and after sampling until analysis. The ambient NO₂ concentration was calculated by using NO₂ standard solution curve assuming a given sampling rate, i.e., 16.9 mL/min, which was supplied by the manufacturer.

*Temperature and humidity*

Data for outdoor temperature and relative humidity were obtained from the Korea Meteorological Administration.

**Statistical Analysis**

We calculated the means and distribution percentiles for urinary concentrations of biomarkers. For concentrations below the limits of detection (LOD), a value equal to the LOD divided by 2 was used in the statistical analysis. We assessed associations between urinary metabolites of VOC, oxidative stress markers and pulmonary function parameters. Linear mixed-effect models were used to estimate the VOC exposure effects on the PFT parameters or the oxidative stress markers, controlling for temperature, humidity, cotinine levels, dietary habits, alcohol consumption, and individual characteristics. Personal exposures to PM₂.₅ and NO₂ were also controlled in the statistical models. Because the distributions of biomarker concentrations were skewed, we used log-transformed data for these measurements in the linear mixed models. We treated age, sex, height, weight, date, temperature, relative humidity, cotinine levels, PM₂.₅ and NO₂ exposure levels, dietary habits, alcohol consumption and biomarker concentrations as fixed effects. Each participant was
treated as a random effect in the models. Because we had similar results regardless of adjustment for urine creatinine, we report the association results with non-adjusted ones for urinary levels of VOC metabolites and oxidative stress markers.

**RESULTS**

**Participant characteristics and biochemical analyses**

The participant mean age was 73.5 years old and 63.0% were female. Of a possible total of 8 visits, mean number of visits was 6.8 visits/person. The number of persons, whose level of urinary cotinine was over 300µg/L, was 22 (14.3%). (Table 1) The indoor levels (N=12) of benzene, toluene, ethylbenzene and xylene were 5.96 (SD 4.08), 11.18 (SD 10.49), 4.55 (SD 1.78) and 5.24 (SD 1.35) µg/m³, respectively. The outdoor levels (N=6) were 4.31 (SD 3.00), 6.20 (SD 8.71), 3.10 (SD 1.56) and 2.84 (SD 1.49) µg/m³, respectively. The results of measurements for urinary markers of VOC metabolites, NO₂, PM₂.₅, outdoor temperature and relative humidity are shown in Table 2. The percentage of measurements that were lower than limit of detection (%<LOD) are also shown. Among the measured biomarkers, the %<LOD of tt-muconic acid was over 50%.

**Effects of VOC metabolites on lung function parameters**

The levels of hippuric acid were negatively associated with FEV₁ and FEF₂₅₋₇₅, and methylhippuric acid levels were negatively associated with FEV₁ and FEV₁/FVC (Table 3). However, tt-muconic acid and mandelic acid were not significantly associated with either PFT parameters.

To estimate effects of VOC metabolites on lung function, we calculated the estimated values of lung function parameters assuming average values of age, sex, height, weight, temperature,
humidity, cotinine level, PM$_{2.5}$ and NO$_2$ levels, dietary habits, and alcohol consumption. As shown in Figure 1, FEV$_1$ is decreased by 21.5ml (1.2%), FEV$_1$/FVC by 0.5% (0.6%), FEF$_{25-75}$ by 74.6ml/L (3.6%) when the value of hippuric acid is at its 90$^{th}$ percentile compared to its 10$^{th}$ percentile. FEV$_1$ decreases by 18.2ml (1.0%), FEV$_1$/FVC by 0.7% (0.7%), and FEF$_{25-75}$ by 40.5ml/L (1.9%) when methylhippuric acid is at its 90$^{th}$ compared to its 10$^{th}$ percentile.

(Figure 1)

**Associations among VOC metabolites, oxidative stress and lung function**

Except for the tt-muconic acid, all of the other VOC metabolites were significantly associated with urinary MDA levels after adjustment for age, sex, height, weight, cotinine levels, PM$_{2.5}$ and NO$_2$ levels, dietary habits, alcohol consumption, temperature, humidity. All of the measured VOC metabolites were also significantly associated with urinary 8-OHdG. (Table 4)

When we analyzed the relationships between the oxidative stress biomarkers and lung function parameters, we found that urinary MDA levels were negatively associated with FEV$_1$ and urinary 8-OHdG levels were negatively associated with FEV$_1$/FVC and FEF$_{25-75}$. However, FVC was not associated with either the MDA or 8-OHdG levels. Figure 2 shows the possible pathway for the effects of toluene and xylene exposure on lung function through oxidative stress.

**Association between indoor levels of VOCs and their metabolites**

When we analyzed the relationship between indoor VOC levels at the day-care facilities and VOC metabolites in urine, we found that urinary hippuric acid and mandelic acid levels were significantly associated with indoor toluene levels (regression coefficient $\beta$ 0.009, p-value 0.003) and ethylbenzene levels ($\beta$ 0.061, p-value <0.001) while there was no significant
association of urinary t-t-muconic acid ($\beta -0.001$, p-value 0.589) and methylhippuric acid ($\beta 0.003$, p-value 0.683) with indoor levels of benzene and xylene, respectively, after regression analysis controlling for age, sex, height, weight, date, temperature, relative humidity, cotinine levels, PM$_{2.5}$ and NO$_2$ exposure levels, dietary habits, and alcohol consumption.

**DISCUSSION**

Our study demonstrates that exposure to toluene and xylene may exert harmful effects on lung function and oxidative stress could be involved in the pathogenesis in the elderly population. Most of the reported investigations into the health effect of air pollution have been targeted to outdoor air pollutants, such as ozone and particulate matter (7, 8, 15, 16). The importance of indoor air quality, however, is progressively increasing since people spend more time indoors than outdoors. Although VOC also exists in the outdoor environment, the concentrations of VOC are two to fivefold higher indoors than outdoors, so they are generally regarded as “indoor” air pollutants (17, 18).

In this study, we found a significant association between urinary levels of hippuric acid and methylhippuric acid and decreased lung function. These are metabolites of toluene and xylene, respectively, which are widely used in our daily necessaries like paints, varnishes, rubber, and disinfectants. Although benzoic acid from dietary intake was known as one of the main sources of urinary hippuric acid, significant association between ambient levels of toluene and urinary hippuric acid was reported (19). More than 90% of xylene is known to be metabolized to methylhippuric acid, therefore urinary levels of methylhippuric acid can be used as the total body burden of xylene (20).

Muconic acid and mandelic acid, which were supposed to represent exposure to benzene and ethylbenzene, respectively, did not exhibit an association with lung function parameters.
However, this could be due to the concentration under the limit of detection (80% and 42%) rather than a true non-association. As shown in professional painters or in sick building syndrome, exposure to an unusually high concentration of VOC can result in respiratory effects (21, 22). Our panel consisted of elderly people without occupational exposure, therefore this study suggests that even ordinary exposure to VOC can affect lung function, at least in the elderly population.

Of the measured parameters of lung function, FVC did not show any association with markers of VOC exposure in contrast to FEV₁, FEV₁/FVC, and FEF₂₅-₇₅. This suggests that the primary site of the pulmonary toxicity of VOC might be airways, especially in small airways rather than the pulmonary parenchyme.

The estimated changes of FEV₁ were 21.5ml for hippuric acid and 18.2ml for methylhippuric acid, assuming the level of each metabolite changes to its 90th from the 10th percentile. Even though we assumed extreme circumstances, this is a relatively large change considering the annual loss of FEV₁ in healthy non-smokers in the general population is estimated to be approximately 30ml/year (23).

The proposed mechanism of VOC toxicity was oxidative stress (1-3), which is also thought to be the main mechanism of cigarette smoke-induced lung injury, acute exacerbation of chronic obstructive pulmonary disease, and many of the harmful effects of other air pollutants on respiratory epithelium (24-27). The results of our study support the association between exposure to VOC and increased systemic level of oxidative stress. Oxidative stress is also known to be influenced by many other factors, such as dietary intake, alcohol consumption, and cigarette smoking in addition to air pollution (19, 26-29). In our data, however, significant associations between VOC metabolites and markers of oxidative stress were found even after adjustment for dietary intake, cotinine level, alcohol consumption, PM_{2.5} and NO₂ levels.
We also found a significant association between markers of oxidative stress and parameters of lung function. This is in line with the current understanding of the relation of oxidative stress to a reduction in pulmonary function and increased cardiopulmonary mortality (20, 30). In addition, it supports the hypothesis that VOC might influence lung function by exacerbating oxidative stress.

The strengths of the present study merit discussion. Firstly, to the best of our knowledge, this is the first study to demonstrate the detrimental effects of toluene and xylene exposure on lung function in the course of ordinary environmental exposure. Secondly, the panel study design with repeated measurements of spirometric tests, urinary metabolites of VOC and oxidative stress biomarkers up to 8 times for each participant provided a good opportunity to evaluate the short-term effects of changes in VOC exposure over time. Thirdly, by using biomarkers to evaluate personal exposures and outcome, this study provided possible pathway of VOC effect which suggests involvement of oxidative stress.

However, there are also some limitations to this study. We couldn’t show associations between levels of indoor benzene and xylene and their metabolites. Because we only measured indoor levels of VOCs once at two spots in each day-care facility, the data for the levels of indoor VOCs are thought to have failed to reflect personal variation of exposure and variation along time in contrast to those of the metabolites. The other reason for discrepancy between levels of indoor benzene and xylene and their metabolites could be that they were exposed to other levels in their home. Even though we adjusted for potential sources of oxidative stress in the statistical analyses including personal exposure levels of PM$_{2.5}$ and NO$_2$ levels, concomitant exposure to other pollutants such as ozone and heavy metals could have had some impact on lung function. There is another concern whether the VOC metabolites reflect true VOC exposure in the ordinary environmental circumstances.

Although we controlled possible confounders such as dietary habit in the statistical model, we
could not prove confidently that the VOC metabolites represent exposure to the chemicals in ambient air very well when the exposure levels are not high. Finally, we didn’t adjust levels of urinary biomarkers by levels of urine creatinine. All samples used in this analysis, however, were the first morning urine to reduce the problem of dilution.

In conclusion, our findings suggest that exposure to toluene and xylene increase oxidative stress which results in a deterioration of lung function in the elderly population.
REFERENCES

Table 1. Characteristics of participants and their pulmonary function

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>No. of participants</td>
<td>57</td>
<td>97</td>
<td>154</td>
</tr>
<tr>
<td>No. of visits per person</td>
<td>6.9</td>
<td>6.8</td>
<td>6.8</td>
</tr>
<tr>
<td>Age</td>
<td>73.4 (64-88)</td>
<td>73.5 (60-91)</td>
<td>73.5 (60-91)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163.2±5.5</td>
<td>149.4±5.8</td>
<td>154.5±8.7</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>63.7±8.7</td>
<td>55.7±8.2</td>
<td>58.7±9.2</td>
</tr>
<tr>
<td>FVC (L)</td>
<td>2.33±0.55</td>
<td>1.81±0.44</td>
<td>2.01±0.55</td>
</tr>
<tr>
<td>FEV₁ (L)</td>
<td>1.95±0.52</td>
<td>1.62±0.41</td>
<td>1.75±0.49</td>
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<tr>
<td>FEV₁/FVC (%)</td>
<td>0.84±0.09</td>
<td>0.90±0.06</td>
<td>0.87±0.08</td>
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<tr>
<td>FEF₂₅-⁷₅ (L/sec)</td>
<td>2.12±0.93</td>
<td>2.02±0.79</td>
<td>2.07±0.85</td>
</tr>
<tr>
<td>Cotinine (µg/L) ≥ 300</td>
<td>18(31.6%)</td>
<td>4(4.1%)</td>
<td>22(14.3%)</td>
</tr>
</tbody>
</table>

*Plus-minus values are mean±standard deviation.

FVC: forced vital capacity,

FEV₁: forced expiratory volume in one second,

FEF₂₅-⁷₅: forced expiratory flow between 25% and 75% of forced vital capacity.
Table 2. Urinary concentrations and distributions of the VOC metabolites, NO₂, PM₂.₅, outdoor temperature and relative humidity

<table>
<thead>
<tr>
<th>VOC metabolites</th>
<th>N</th>
<th>Mean(SD)</th>
<th>LOD</th>
<th>%&lt;LOD</th>
<th>10pct</th>
<th>25pct</th>
<th>50pct</th>
<th>75pct</th>
<th>95pct</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muconic acid (mg/L)</td>
<td>988</td>
<td>0.08(0.10)</td>
<td>0.014</td>
<td>79.96</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.27</td>
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<tr>
<td>Hippuric acid (mg/ml)</td>
<td>984</td>
<td>0.53(0.65)</td>
<td>0.020</td>
<td>0.30</td>
<td>0.06</td>
<td>0.14</td>
<td>0.32</td>
<td>0.65</td>
<td>1.84</td>
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<tr>
<td>Mandelic acid (mg/ml)</td>
<td>984</td>
<td>0.08(0.11)</td>
<td>0.016</td>
<td>41.67</td>
<td>0.08</td>
<td>0.08</td>
<td>0.04</td>
<td>0.11</td>
<td>0.27</td>
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<tr>
<td>Methylhippuric acid (mg/ml)</td>
<td>984</td>
<td>0.10(0.19)</td>
<td>0.004</td>
<td>25.81</td>
<td>0.00</td>
<td>0.00</td>
<td>0.02</td>
<td>0.08</td>
<td>0.47</td>
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<tr>
<td>NO₂ (ppb)</td>
<td>1033</td>
<td>21.68(11.46)</td>
<td>0.30</td>
<td>0.0</td>
<td>8.71</td>
<td>12.36</td>
<td>19.70</td>
<td>28.80</td>
<td>43.30</td>
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<tr>
<td>PM₂.₅ (µg/m³)</td>
<td>1021</td>
<td>33.38(22.45)</td>
<td>8.87</td>
<td>17.00</td>
<td>28.81</td>
<td>43.59</td>
<td>78.31</td>
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<td>Outdoor Temperature (°C)</td>
<td>1037</td>
<td>16.79(8.91)</td>
<td>4.5</td>
<td>7.2</td>
<td>19.9</td>
<td>24.9</td>
<td>27.6</td>
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<tr>
<td>Outdoor Relative humidity (%)</td>
<td>1037</td>
<td>68.47(16.78)</td>
<td>42.3</td>
<td>58.3</td>
<td>72.6</td>
<td>80.9</td>
<td>90.8</td>
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* N denotes number of measurements,

**LOD: limit of detection,

****%<LOD: percentage of measurements whose values are under the limit of detection,

Pct: percentile.
Table 3. Estimated regression coefficients of urinary concentrations of the VOC metabolites on lung function parameters

<table>
<thead>
<tr>
<th>VOC metabolites</th>
<th>FEV₁(ml)</th>
<th>FEV₁/FVC(%)</th>
<th>FEF₂₅-₇₅(ml/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient (SE)</td>
<td>P value</td>
<td>Coefficient (SE)</td>
</tr>
<tr>
<td>Muconic acid (mg/L)</td>
<td>34.84 (44.41)</td>
<td>0.433</td>
<td>-1.40 (1.55)</td>
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<tr>
<td>Hippuric acid (mg/ml)</td>
<td>-18.23 (8.52)</td>
<td>0.033</td>
<td>-0.47 (0.29)</td>
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<tr>
<td>Methylhippuric acid (mg/ml)</td>
<td>-65.70 (30.41)</td>
<td>0.031</td>
<td>-2.44 (1.00)</td>
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<tr>
<td>Mandelic acid (mg/ml)</td>
<td>-15.10 (45.69)</td>
<td>0.741</td>
<td>-1.03 (1.58)</td>
</tr>
</tbody>
</table>

† SE: standard error

‡ P values are from mixed regression analysis adjusted for visit number, age, sex, height, weight, outdoor temperature, outdoor relative humidity, diet, cotinine, alcohol consumption, NO₂ and PM₂.₅ levels.
Table 4. Estimated regression coefficients of urinary concentrations of the VOC metabolites on markers of oxidative stress

*SE: standard error

**P values are from mixed regression analysis adjusted for visit number, age, sex, height, weight, outdoor temperature, outdoor relative humidity, diet, cotinine, alcohol consumption, NO2 and PM2.5 levels.

<table>
<thead>
<tr>
<th>VOC metabolites</th>
<th>MDA (µmol/L)</th>
<th>8-OHdG (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>coefficient</td>
<td>P value</td>
</tr>
<tr>
<td></td>
<td>(SE)</td>
<td></td>
</tr>
<tr>
<td>Muconic acid (mg/L)</td>
<td>0.54 (0.36)</td>
<td>0.132</td>
</tr>
<tr>
<td>Hippuric acid (mg/ml)</td>
<td>0.51 (0.06)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Methylhippuric acid (mg/ml)</td>
<td>0.84 (0.21)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mandelic acid (mg/ml)</td>
<td>2.20 (0.32)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Figure legend

Figure 1. Estimated effects of urinary concentrations of the hippuric acid and methylhippuric acid on lung function parameters. Each levels of three pulmonary function parameters regarding to 10, 50, 90 percentile of urinary levels of hippuric acid and methylhippuric acid were estimated. Mean values of age, sex, height, weight, outdoor temperature, outdoor relative humidity, area, cotinine, alcohol consumption were used for estimation.

Figure 2. Possible pathway for the effects of toluene and xylene exposure on lung function through oxidative stress