



Wastewater-based epidemiological evaluation of the effect of air pollution on short-acting beta-agonist consumption for acute asthma treatment

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ABSTRACT

Asthma, one of the most common chronic diseases in the world and a leading cause of hospitalization among children, has been associated with outdoor air pollution.

We applied the wastewater-based epidemiology (WBE) approach to study the association between the use of salbutamol, a short-acting beta-agonist used to treat acute bronchospasm, and air pollution in the population of Milan, Italy.

Composite 24-h samples of untreated wastewater were collected daily and analyzed for human metabolic residues of salbutamol by liquid chromatography tandem mass spectrometry. Corresponding daily outdoor concentrations of particulate matter up to 10 µm (PM₁₀) and 2.5 µm (PM_{2.5}) in aerodynamic diameter, nitrogen dioxide, ozone, sulfur dioxide, and benzene were collected from the public air monitoring network. Associations at different lag times (0–10 days) were assessed by a log-linear Poisson regression model.

We found significant direct associations between defined daily doses (DDD) of salbutamol and mean daily concentrations of PM₁₀ and PM_{2.5} up to nine days of lag time. The highest rate ratio, and 95% confidence interval (CI), of DDD of salbutamol was 1.06 (95% CI: 1.02–1.10) and 1.07 (95% CI: 1.02–1.12) at seven days of lag time and for an increase of 10 µg/m³ of PM₁₀ and PM_{2.5}, respectively. Reducing the mean daily PM₁₀ concentration in Milan from 50 to 30 µg/m³ means that 852 (95% CI: 483–1504) daily doses of salbutamol per day would not be used. These results confirm the association between asthma and outdoor PM₁₀ and PM_{2.5} and prove the potential of the WBE approach to quantitatively estimate the relation between environmental exposures and diseases.

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Abbreviations: ATC, anatomic therapeutic chemical; ARPA, Regional Agency for Environmental Protection; CI, confidence interval; DDD, defined daily dose; IS, internal standard; LABAs, long-acting beta-agonists; PM_{2.5}, particulate matter with aerodynamic diameter ≤ 2.5 µm; PM₁₀, particulate matter with aerodynamic diameter ≤ 10 µm; RR, rate ratio; SABAs, short-acting beta-agonists; WBE, wastewater-based epidemiology; WHO, World Health Organization

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1. Introduction

Asthma is one of the most common chronic diseases in the world and one of the leading causes of hospitalization among children, its prevalence has increased during the second half of the 20th century and is expected to rise further in the next 15–20 years (Eder et al., 2006; Masoli et al., 2004). It is a complex immunologic and inflammatory disease comprising different overlapping phenotypes with defined clinical and physiological characteristics (Wenzel, 2006). Current pharmacological management includes the treatment of acute episodes and the control of chronic symptoms. The acute episodes are mainly treated using short-acting beta-agonists (SABAs), which cause reactive relief of bronchospasm, whereas, for the control of the chronic symptoms, long-acting beta-agonists (LABAs) are used regularly on a daily basis. Nowadays the combinations of inhaled corticosteroids and

LABAs have become established as the most efficacious treatment (Chung, 2015). However, asthma is not cured by any therapeutic regimen and not all patients respond well to current treatments (Adcock et al., 2008). Thus, primary prevention strategies to avoid the determinants of asthma are fundamental to reduce the morbidity and mortality of this disease (Beasley et al., 2015).

In the last decade scientific evidence has emerged about an association between air pollution and exacerbations of existing asthma and new-onset asthma attacks in children and adults (Kunzli et al., 2009; McConnell et al., 2010; Weinmayr et al., 2010). The mechanisms by which the pollutants induce these effects are not completely understood, although four main mechanisms have been proposed: 1) oxidative stress and damage, 2) airway remodelling, 3) inflammatory pathway and immunological responses, and 4) enhanced respiratory sensitization to aeroallergens (Guarnieri and Balmes, 2014).

Most studies on the association of air pollution with asthma assessed asthma as self-reported, emergency department visits and hospitalizations, and fewer have examined surrogate markers for asthma morbidity, such as medicine sales or prescriptions. SABA prescriptions have been seen to be closely associated with emergency department visits, and hospital admissions for asthma (Naureckas et al., 2005), and ecological studies based on respiratory drug sales may have higher statistical power than studies on mortality or hospital admissions (Pitard et al., 2004). However, the association between anti-asthmatic sales or prescriptions and air pollution may underestimate the effect of air pollution, since patients suffering from asthma are generally using self medications by using a SABA drug always available in case of incipient asthma attacks (Urbano, 2008).

The “wastewater-based epidemiology” (WBE) approach (Daughton, 2001) involves chemical analysis of human metabolic residues in the raw wastewaters produced by a population, in order to study the collective use of a wide range of substances. This approach has been successfully applied to drugs of abuse (Castiglioni et al., 2006; van Nuijs et al., 2011; Zuccato et al., 2008), alcohol consumption (Reid et al., 2011; Rodriguez-Alvarez et al., 2015) and tobacco (Castiglioni et al., 2015), and is potentially applicable to other classes of chemicals, including therapeutic drugs, once the drug environmental sources, urinary excretion, metabolism and stability have been carefully assessed (Castiglioni et al., 2013).

In this study, we applied the WBE approach to study the association between the use of SABAs and air pollution. Salbutamol, one of the most popular SABAs, was the chemical measured in wastewaters. We report the results of a pilot phase of data collection for three months.

2. Methods

2.1. Sampling and data collection

The study area was Milan, the second largest city in Italy, where, on a large number of days during the year, air quality limits are exceeded with some very strong pollution episodes (Masiol et al., 2014).

Composite 24-hour samples of untreated wastewater were collected daily, from September 30 to December 22, 2013, from the inlet of the wastewater treatment plant at Nosedo, the largest in Milan, with a treatment capacity of about 1,100,000 inhabitant equivalents. Wastewater samples were collected every 15–30 min for 24 h with an automatic computer-controlled sampling device and were stored frozen at -20°C until analysis.

In the same period, daily concentrations of particulate matter up to $10\text{ }\mu\text{m}$ (PM_{10}) and $2.5\text{ }\mu\text{m}$ ($\text{PM}_{2.5}$) in aerodynamic diameter,

nitrogen dioxide, ozone, sulfur dioxide, and benzene were collected from the monitoring network of the Lombardy Regional Environmental Protection Agency (ARPA, 2016); in addition, meteorological variables (temperature and relative humidity) were recorded from ARPA. Data from three monitoring stations in three different areas in Milan were used and averaged to cover the metropolitan area served by the wastewater treatment plant.

2.2. Analytical method

Wastewater samples were analyzed for salbutamol within few days from their arrival to the laboratory using solid phase extraction and liquid chromatography tandem mass spectrometry. The method was adapted from that of previous studies (Castiglioni et al., 2005; Zuccato et al., 2000). In brief, wastewater samples (100 mL) were filtered, acidified to pH 2.0 with 37% HCl, spiked with labelled internal standard (IS) and solid-phase extracted using mixed reverse-phase cation exchange cartridges (Oasis-MCX, Waters Corp., Milford, MA). Cartridges were then vacuum-dried for 10 min and eluted with 2 mL of methanol and 2 mL of a 2% ammonia solution in methanol. The eluates were pooled, dried under a nitrogen stream and re-dissolved in ultrapure water for instrumental analysis.

A high-performance liquid chromatograph 1200 Series (Agilent Technologies, Santa Clara, CA, USA) coupled to a 6410 QqQ mass spectrometer equipped with an electrospray ionization ion source (Agilent Technologies, Santa Clara, CA, USA) was used. The analysis was run in positive ionization mode using the selected reaction monitoring mode. Quantification was done by isotope dilution using the most abundant precursor/product ion transition (quantifier ion) and comparing the area with its corresponding IS. The conditions used for analysis and quantification and the validation parameters of the method are detailed elsewhere (Re Depaolini et al., 2016). A stability tests showed that salbutamol was stable during sampling and for up to eight weeks during storage at -20°C (Re Depaolini et al., 2016).

2.3. Data analysis

We transformed salbutamol wastewater concentrations to the number of daily doses consumed, by using the defined daily dose (DDD) of the WHO (WHO, 2016) and the metabolic excretion rate for salbutamol (Dollery, 1991) by the following formula:

$$\text{No DDD} = \frac{C \times F}{E \times \text{DDD}}$$

Where: No DDD=number of DDD excreted daily; C=concentration of salbutamol in wastewaters (mg/l); F=flow rate of wastewaters entering into the treatment plant (L/day); E=metabolic extraction rate of salbutamol (0.3); DDD=0.8 mg.

We also calculated the average number of salbutamol DDD prescribed daily for the Milan population, using the pharmacy prescription database of the Lombardy Region. Prescription data for 2012 were used because data were not available for the subsequent years. The prescription database contains the medication name and anatomic therapeutic chemical (ATC) classification code, quantity and dispensation date. Data were analyzed for the Milan Local Health Authority, in order to identify prescriptions of salbutamol by its ATC code (R03AC02) and determine the numbers of boxes dispensed for each salbutamol pharmaceutical specialty.

We provided descriptive statistics for all the variables of interest (number of doses of salbutamol, air pollutants, and meteorological variables), calculated the correlation between them (Pearson's correlation coefficient), and plotted salbutamol versus each air pollutant by a linear regression model.

To assess the association between salbutamol and air

pollutants, we modelled data by a log-linear Poisson regression (Schwartz et al., 1996), adjusted for temperature and relative humidity, as continuous variables, as in the following formula:

$$\log(E(Y)) = \text{Intercept} + \beta_1 \times \text{Alag}_x + \beta_2 \times T + \beta_3 \times RH$$

Where: Y=DDD for salbutamol, Alag_x =moving average of the air pollutant "A" over the previous x days; T=temperature; RH=relative humidity. We also used alternative models adjusted for month and weekday. The resulting estimate of the effect was the Rate Ratio (RR) of salbutamol DDD associated with a $10 \mu\text{g}/\text{m}^3$ increase in each pollutant. This means the increase of salbutamol DDD consumed as a function of an increase of $10 \mu\text{g}/\text{m}^3$ for a specific pollutant. For each pollutant, different lag times (time between air pollution measurement and the resulting drug use), from 0 to 10 days, as moving average of the daily mean concentrations over the days t and $t-1$, were considered and each lag time was fitted in the model one at a time, to calculate the corresponding RR. Days with missing values for one of the variables of interest were dropped from the analyses. A sensitivity analysis where we inputted missing data by linear interpolation was also conducted. Results were considered significant at $p < 0.05$. Statistical analyses were done using SAS software (version 9.2; SAS Institute Inc., Cary, NC).

3. Results

Of the 84 study days, between September 29 and December 22, 57 (69%) had the complete measures for all the variables (daily-specific measurements are provided in the [Supplementary material](#)). Wastewater analysis showed concentrations of salbutamol ranging 2.95–9.97 ng/L, corresponding to a daily load entering the treatment plant of 1.26–4.53 g/day (Table 1). This load corresponded to 5249–14,845 daily doses of salbutamol consumed by the population of Milan. The count of pharmaceutical prescriptions showed 5,848,350 salbutamol DDD prescribed in 2012 for the population of Milan, corresponding to an average of 16,023 DDD of salbutamol daily.

Concentrations of air pollutants are shown in Table 1. As expected, daily concentrations of PM_{10} and $\text{PM}_{2.5}$ exceeded the WHO air quality guidelines (Krzyzanowski, 2008), of 50 and $25 \mu\text{g}/\text{m}^3$ respectively, on 28 and 45 days, out of 71 (data not shown). The average concentration for nitrogen dioxide was above the air guideline of $40 \mu\text{g}/\text{m}^3$ for the annual mean, while concentrations of ozone and sulfur dioxide were below. For benzene no guidelines are recommended by the WHO, but measured concentrations were below the air quality limit of $5 \mu\text{g}/\text{m}^3$ as annual average, accepted by Italian legislation (D.Lgs., 2010).

The correlation matrix at lag time 0 (Table 2) showed that the

daily doses of salbutamol were significantly directly correlated to PM_{10} , while the correlation with $\text{PM}_{2.5}$ was of borderline significance. As expected, PM_{10} , $\text{PM}_{2.5}$, nitrogen dioxide, sulfur dioxide and benzene, were significantly directly associated with each other and inversely associated with ozone. Temperature was significantly associated to all air pollutants, but relative humidity only to ozone.

The daily trend and simple regression analysis for salbutamol and PM_{10} and $\text{PM}_{2.5}$ are shown in Fig. 1. Results of the Poisson analysis for different lag times (0–10 days), adjusted for temperature and relative humidity (Table 3), showed a significant direct association between number of DDD of salbutamol and concentrations of PM_{10} and $\text{PM}_{2.5}$, but no associations for other air pollutants. Further adjustment for month and weekday did not meaningfully modify the associations. The strength of the association increased up to seven days of lag time, then decreased, losing significance at ten days. The association was very similar for PM_{10} and $\text{PM}_{2.5}$ though slightly stronger for $\text{PM}_{2.5}$. The strongest associations at lag-time of seven days resulted in RR of 1.06 (95% confidence interval, CI: 1.02–1.10) and 1.07 (95% CI: 1.02–1.12), for an increase of $10 \mu\text{g}/\text{m}^3$ of PM_{10} and $\text{PM}_{2.5}$, respectively. No significant association was observed for salbutamol and any other air pollutant. Results were consistent adjusting RR estimates by temperature and relative humidity in categories, and when we input missing data by linear interpolation.

4. Discussion

To our knowledge, this is the first study to investigate the association between consumption of salbutamol, assessed by wastewater analysis, and outdoor air pollution. We found a direct and significant association between the number of daily doses of salbutamol and the levels of PM_{10} and $\text{PM}_{2.5}$ to which the population was exposed. This provides direct evidence of the relation between ambient air pollution and asthma attacks, since salbutamol is widely used to treat acute exacerbations of obstructive lung diseases, is excreted with urine in wastewaters after consumption, and no other important sources of salbutamol are expected in the city wastewaters. The association between salbutamol and $\text{PM}_{2.5}$ and PM_{10} was stronger for time-weighted longer periods (lag time 7–8 days) than for short-term exposures (lag time 1 d), suggesting a cumulative effect of PM_{10} and $\text{PM}_{2.5}$ on asthma outcomes.

We saw no significant associations between salbutamol and nitrogen dioxide, sulfur dioxide, ozone and benzene. These pollutants have been associated with asthma in several studies (Jassal, 2015). However, for nitrogen dioxide the association with asthma attacks was not robust and to date there is no firm toxicological evidence of such a relation at ambient concentrations (Weinmayr

Table 1
Descriptive statistics for wastewater concentrations, daily loads and defined daily doses (DDD) of salbutamol, and for air pollutants and meteorological variables.

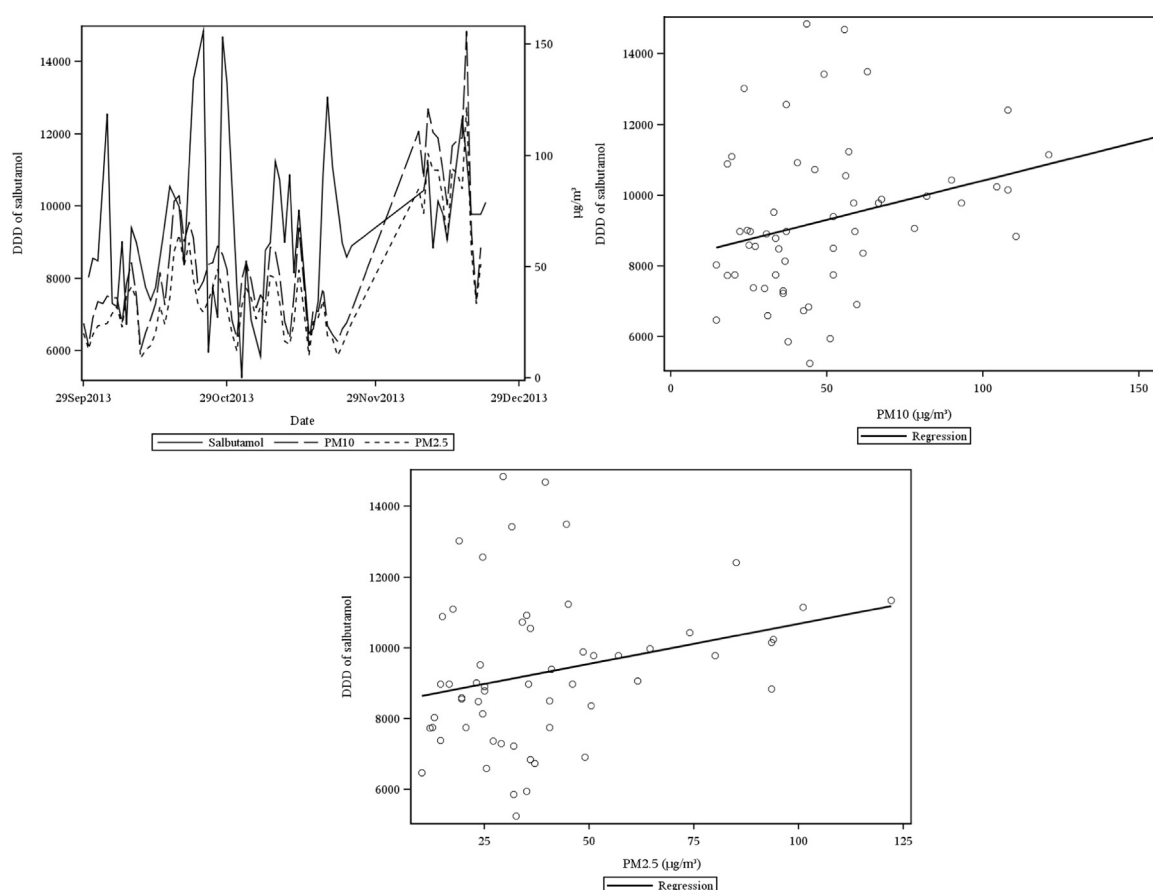
Variable	N	Mean	SD	Median	Min	Max
Salbutamol						
Wastewater concentrations (ng/L)	58	5.80	1.48	5.85	2.95	9.97
Daily loads (g/day)	58	2.35	0.66	2.22	1.26	4.53
Daily doses	58	9340	2145	8988	5249	14,845
PM_{10} ($\mu\text{g}/\text{m}^3$)	71	50.3	30.1	42.5	12.5	156
$\text{PM}_{2.5}$ ($\mu\text{g}/\text{m}^3$)	71	39.7	25.3	32.5	9.0	122
Nitrogen dioxide ($\mu\text{g}/\text{m}^3$)	71	59.2	22.3	52.5	29.3	158
Ozone ($\mu\text{g}/\text{m}^3$)	71	12.5	8.96	9.49	4.29	41.4
Sulfur dioxide ($\mu\text{g}/\text{m}^3$)	57	5.82	3.53	4.84	0.76	15.6
Benzene ($\mu\text{g}/\text{m}^3$)	58	2.05	1.73	1.50	0.36	6.54
Relative humidity (%)	58	81.2	11.8	85.0	26.0	95.0
Temperature ($^{\circ}\text{C}$)	59	10.8	5.6	12.5	0.2	17.6

PM_{10} =particulate matter of aerodynamic diameter $\leq 10 \mu\text{m}$; $\text{PM}_{2.5}$ =particulate matter of aerodynamic diameter $\leq 2.5 \mu\text{m}$

Table 2

Correlations between defined daily doses (DDD) of salbutamol, air pollutants, relative humidity and temperature.

	Pearson's coefficient (p-value)								
	Salbutamol	PM ₁₀	PM _{2.5}	Nitrogen dioxide	Ozone	Sulfur dioxide	Benzene	Relative humidity	Temperature
Salbutamol	1.00								
PM ₁₀	0.31 (0.033)	1.00							
PM _{2.5}	0.29 (0.054)	0.99	1.00						
		(< 0.0001)							
Nitrogen dioxide	0.17 (0.278)	0.85	0.84	1.00					
		(< 0.0001)	(< 0.0001)						
Ozone	−0.11 (0.463)	−0.47 (0.001)	−0.47 (0.001)	−0.43 (0.004)	1.00				
Sulfur dioxide	0.18 (0.248)	0.65	0.66	0.84	−0.28	1.00			
		(< 0.0001)	(< 0.0001)	(< 0.0001)	(0.061)				
Benzene	0.26 (0.079)	0.94	0.94	0.83	−0.46	0.71	1.00		
		(< 0.0001)	(< 0.0001)	(< 0.0001)	(0.002)	(< 0.0001)			
Relative humidity	−0.01 (0.946)	0.18 (0.239)	0.21 (0.161)	−0.05 (0.756)	−0.55	−0.12 (0.442)	0.20 (0.199)	1.00	
					(0.0001)				
Temperature	−0.08 (0.605)	−0.63	−0.67	−0.73	0.43 (0.006)	−0.78	−0.65	−0.13 (0.388)	1.00
		(< 0.0001)	(< 0.0001)	(< 0.0001)		(< 0.0001)	(< 0.0001)		

PM₁₀=particulate matter of aerodynamic diameter $\leq 10 \mu\text{m}$; PM_{2.5}=particulate matter of aerodynamic diameter $\leq 2.5 \mu\text{m}$ **Fig. 1.** (a) Time course of defined daily doses (DDD) of salbutamol, particulate matter 10 μm aerodynamic diameter (PM₁₀), and particulate matter 2.5 μm aerodynamic diameter (PM_{2.5}); (b) regression line for DDD of salbutamol and PM₁₀; (c) regression line for DDD of salbutamol and PM_{2.5}, in Milan from 29 September to 22 December 2013.

et al., 2010). In contrast, for ozone and sulfur dioxide the relation with asthma is firmer, but the environmental concentrations of these pollutants measured in the present investigation were probably too low to allow to show any effect. Tropospheric ozone is a secondary pollutant requiring UV radiation to be formed so that only low concentrations are expected in winter, when this study was conducted. The low concentrations of sulfur dioxide, instead, are quite likely due to the dramatic reduction of atmospheric emissions as a consequence of the efficient abatement of

sulfur in fossil fuel.

Finally, the association between benzene and asthma has been reviewed recently (Bolden et al., 2015), and some studies found a significant association with environmental outdoor concentrations. However, in the present study, the length of the sampling period was probably too short to detect any association at the measured concentrations of benzene.

The findings of this study agree with the associations found by other investigations between particulate matter and medical

Table 3

Rate ratio (RR)^a, and corresponding 95% confidence intervals (CI), for the association of defined daily doses (DDD) of salbutamol with PM₁₀ and PM_{2.5}, for different lag times.

Lag time (days)	RR (95% CI)	
	PM10	PM2.5
0	1.03 (1.01, 1.06)	1.04(1.01, 1.07)
1	1.04 (1.01, 1.07)	1.05(1.01, 1.08)
2	1.04 (1.01, 1.07)	1.05(1.02, 1.09)
3	1.04 (1.01, 1.08)	1.05(1.01, 1.09)
4	1.04 (1.01, 1.08)	1.05(1.01, 1.10)
5	1.05 (1.01, 1.08)	1.06(1.02, 1.10)
6	1.05 (1.02, 1.09)	1.06(1.02, 1.11)
7	1.06 (1.02, 1.10)	1.07(1.02, 1.12)
8	1.05 (1.01, 1.10)	1.07(1.02, 1.12)
9	1.05 (1.01, 1.10)	1.06(1.01, 1.12)
10	1.02 (0.98, 1.06)	1.05(1.00, 1.11)

PM₁₀=particulate matter of aerodynamic diameter $\leq 10 \mu\text{m}$; PM_{2.5}=particulate matter of aerodynamic diameter $\leq 2.5 \mu\text{m}$

^a Results from Poisson regression models, adjusted for relative humidity and temperature.

dispensations or prescriptions of anti-asthma drugs. Slaughter and co-workers (Slaughter et al., 2003) found odd ratios for medication use in asthmatic children of 1.08 (95% CI: 1.01–1.15) and 1.05 (95% CI: 1.00–1.09) for increases of $10 \mu\text{g}/\text{m}^3$ of PM_{2.5} and PM₁₀, respectively. Salbutamol prescriptions were clearly associated with fire-related PM_{2.5}, in a population of British Columbia, Canada, with a RR of 1.06 (95% CI: 1.04–1.06) for a $10 \mu\text{g}/\text{m}^3$ increase of PM_{2.5}. In a population-based ecological study in UK, an increase of $10 \mu\text{g}/\text{m}^3$ of PM₁₀ was associated with a 1% (95% CI: 0.1–2) increase in salbutamol prescriptions. Finally, Carlsen and co-workers (Carlsen et al., 2012) found a 0.9% (95% CI: 0.1–1.8) increase of dispensations of anti-asthma drugs for a $10 \mu\text{g}/\text{m}^3$ increase of PM₁₀ in the Icelandic capital.

From the log-linear Poisson regression in this study, the daily doses consumed by the population of Milan would be 6537 (95% CI: 3708–11,563) at a hypothetical PM₁₀ concentration of $7.5 \mu\text{g}/\text{m}^3$, which is considered a natural background level, and would increase up to 8241 (95% CI: 4675–14,544) for a PM₁₀ concentration of $50 \mu\text{g}/\text{m}^3$, which is the average concentration measured in Milan in this study. This result means that reducing the mean daily PM₁₀ concentration in Milan by $20 \mu\text{g}/\text{m}^3$, from 50 to $30 \mu\text{g}/\text{m}^3$, would reduce salbutamol consumption by 852 (95% CI: 483–1504) doses per day, presumably corresponding to the same number of people not suffering new attacks or exacerbations of asthma every day and, therefore, confirming that policies aimed at reduction or abatement of fine particulate matter in urban areas would give important individual health benefits, especially for children.

The present investigation also shows the potential of the WBE approach in the study of the environmental diseases. First of all this method serves to investigate the relations between environmental exposures and diseases by direct comparison of pharmaceutical consumption and pollution levels on the same day. This would be difficult from prescriptions alone, since patients can use their personal store, or from hospital records, which would only collect information on the most severe cases. In addition, the method is suitable for real-time analysis, as a tool to check whether strategies for risk reduction or smog alerts are really effective in terms of health benefit, or, else to detect excessive use of drugs which can be a consequence of unknown environmental exposures. Finally, the small-scale application of this approach can be used to estimate specific effects of air pollution on sensitive subpopulations, such children or patients. This would be possible by sampling the wastewaters of the outgoing collectors from hospitals or schools before they reach the city treatment plant.

The study suffers by some limitations and uncertainties. One limitation is the short sampling period, which covered less than three months during the cold season so we could not assess the effect of ozone and possibly of other pollutants. However, it was long enough to obtain significant results both in the simple correlation analysis and in Poisson regression analysis for PM₁₀ and PM_{2.5}, indicating the soundness of the association.

Another limitation is that we could not compare the DDD results obtained by the WBE approach with those based on drug prescription or dispensation data for the corresponding period, because no such data was available for 2013. However, a rough comparison with the data for 2012, which are probably not markedly different from 2013, gave consistent results, since the prescribed or dispensed DDD exceed not more than the double the DDD measured in wastewaters. Indeed the wastewater treatment plant covers most but not all the Milan population, whereas the prescribed or dispensed DDD are related to all the residents. In addition, since this drug cannot be sold without a medical prescription, the DDD excreted should be less than those prescribed because it is likely that not all the doses prescribed and dispensed from the National Health System are actually used.

In conclusion, this study confirmed the association between asthma and outdoor PM₁₀ and PM_{2.5} levels by a new application of the WBE approach, providing direct evidence of the effect of outdoor ambient air pollution on asthma. The study also shows the potential of the WBE approach to quantitatively estimate the relation between environmental exposures and diseases, which includes real-time analyses to check the health benefits of strategies for the reduction of environmental exposures and small-scale applications to identify or investigate sensitive subpopulations.

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Authors' contribution

Elena Fattore and Enrico Davoli equally contributed to this work.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.envres.2016.05.051>.

References

- Adcock, I.M., Caramori, G., Chung, K.F., 2008. New targets for drug development in asthma. *Lancet* 372, 1073–1087.
- ARPA 2016. Lombardy Regional Environmental Protection Agency. (http://www2.arpalombardia.it/sites/QAria/_layouts/15/QAria/IDati.aspx?v=2) (accessed 14.01.16).
- Beasley, R., Semprini, A., Mitchell, E.A., 2015. Risk factors for asthma: is prevention possible? *Lancet* 386, 1075–1085.

- Bolden, A.L., Kwiatkowski, C.F., Colborn, T., 2015. Correction to New Look at BTEX: Are Ambient Levels a Problem? *Environ. Sci. Technol.* 49, 11984–11989.
- Carlsen, H.K., Zoega, H., Valdimarsdottir, U., Gislason, T., Hrafnkelsson, B., 2012. Hydrogen sulfide and particle matter levels associated with increased dispensing of anti-asthma drugs in Iceland's capital. *Environ. Res.* 113, 33–39.
- Castiglioni, S., Bagnati, R., Calamari, D., Fanelli, R., Zuccato, E., 2005. A multiresidue analytical method using solid-phase extraction and high-pressure liquid chromatography tandem mass spectrometry to measure pharmaceuticals of different therapeutic classes in urban wastewaters. *J. Chromatogr. A* 1092, 206–215.
- Castiglioni, S., Bijlsma, L., Covaci, A., Emke, E., Hernandez, F., Reid, M., Ort, C., Thomas, K.V., van Nuijs, A.L., de Voigt, P., Zuccato, E., 2013. Evaluation of uncertainties associated with the determination of community drug use through the measurement of sewage drug biomarkers. *Environ. Sci. Technol.* 47, 1452–1460.
- Castiglioni, S., Senta, I., Borsotti, A., Davoli, E., Zuccato, E., 2015. A novel approach for monitoring tobacco use in local communities by wastewater analysis. *Tob. Control* 24, 38–42.
- Castiglioni, S., Zuccato, E., Crisci, E., Chiabrando, C., Fanelli, R., Bagnati, R., 2006. Identification and measurement of illicit drugs and their metabolites in urban wastewater by liquid chromatography-tandem mass spectrometry. *Anal. Chem.* 78, 8421–8429.
- Chung, K.F., 2015. Targeting the interleukin pathway in the treatment of asthma. *Lancet* 386, 1086–1096.
- D.Lgs. 2010. (<http://www.minambiente.it/normative/dlgs-13-agosto-2010-n-155-at-tuazione-della-direttiva-200850ce-relativa-alla-qualita>) (accessed on 28.04.16).
- Daughton, C.G., 2001. Illicit drugs in municipal sewage: proposed new non-intrusive tool to heighten public awareness of societal use of illicit/abused drugs and their potential for ecological consequences. In: Daughton, C.G., Jones-Lepp, T.L. (Eds.), *Pharmaceuticals and Personal Care products in the Environment*, Scientific and regulatory Issue. American Chemical Society, Washington, DC, pp. 348–364.
- Dollery, C., 1991. *Therapeutic Drugs*. In: C. Livingstone (Ed.). Edinburg.
- Eder, W., Ege, M.J., von Mutius, E., 2006. The asthma epidemic. *N. Engl. J. Med.* 355, 2226–2235.
- Guarnieri, M., Balmes, J.R., 2014. Outdoor air pollution and asthma. *Lancet* 383, 1581–1592.
- Jassal, M.S., 2015. Pediatric asthma and ambient pollutant levels in industrializing nations. *Int. Health* 7, 7–15.
- Krzyzanowski, M., 2008. WHO air quality guidelines for Europe. *J. Toxicol. Environ. Health A* 71, 47–50.
- Kunzli, N., Bridevaux, P.O., Liu, L.J., Garcia-Esteban, R., Schindler, C., Gerbase, M.W., Sunyer, J., Keidel, D., Rochat, T., 2009. Traffic-related air pollution correlates with adult-onset asthma among never-smokers. *Thorax* 64, 664–670.
- Masiol, M., Agostinelli, C., Formenton, G., Tarabotti, E., Pavoni, B., 2014. Thirteen years of air pollution hourly monitoring in a large city: potential sources, trends, cycles and effects of car-free days. *Sci. Total Environ.* 494–495C, 84–96.
- Masoli, M., Fabian, D., Holt, S., Beasley, R., 2004. The global burden of asthma: executive summary of the GINA dissemination committee report. *Allergy* 59, 469–478.
- McConnell, R., Islam, T., Shankardass, K., Jerrett, M., Lurmann, F., Gilliland, F., Gauderman, J., Avol, E., Kunzli, N., Yao, L., Peters, J., Berhane, K., 2010. Childhood incident asthma and traffic-related air pollution at home and school. *Environ. Health Perspect.* 118, 1021–1026.
- Naureckas, E.T., Dukic, V., Bao, X., Rathouz, P., 2005. Short-acting beta-agonist prescription fills as a marker for asthma morbidity. *Chest* 128, 602–608.
- van Nuijs, A.L., Castiglioni, S., Tarcomnicu, I., Postigo, C., Lopez de Alda, M., Neels, H., Zuccato, E., Barcelo, D., Covaci, A., 2011. Illicit drug consumption estimations derived from wastewater analysis: a critical review. *Sci. Total Environ.* 409, 3564–3577.
- Pitard, A., Zeghnoun, A., Courseaux, A., Lambert, J., Delmas, V., Fossard, J.L., Villet, H., 2004. Short-term associations between air pollution and respiratory drug sales. *Environ. Res.* 95, 43–52.
- Re Depaolini, A., Fattore, E., Cappelli, F., Pellegrino, R., Castiglioni, S., Zuccato, E., Fanelli, R., Davoli, E., 2016. Source discrimination of drug residues in wastewater: the case of salbutamol. *J. Chromatogr. B* 1023, 62–67.
- Reid, M.J., Langford, K.H., Morland, J., Thomas, K.V., 2011. Analysis and interpretation of specific ethanol metabolites, ethyl sulfate, and ethyl glucuronide in sewage effluent for the quantitative measurement of regional alcohol consumption. *Alcohol Clin. Exp. Res.* 35, 1593–1599.
- Rodriguez-Alvarez, T., Racamonde, I., Gonzalez-Marino, I., Borsotti, A., Rodil, R., Rodriguez, I., Zuccato, E., Quintana, J.B., Castiglioni, S., 2015. Alcohol and cocaine co-consumption in two European cities assessed by wastewater analysis. *Sci. Total Environ.* 536, 91–98.
- Schwartz, J., Spix, C., Touloumi, G., Bacharova, L., Barumamdzadeh, T., le Tertre, A., Piekarski, T., Ponce de Leon, A., Ponka, A., Rossi, G., Saez, M., Schouten, J.P., 1996. Methodological issues in studies of air pollution and daily counts of deaths or hospital admissions. *J. Epidemiol. Community Health* 50 (Suppl. 1), S3–S11.
- Slaughter, J.C., Lumley, T., Sheppard, L., Koenig, J.Q., Shapiro, G.G., 2003. Effects of ambient air pollution on symptom severity and medication use in children with asthma. *Ann. Allergy Asthma Immunol.* 91, 346–353.
- Urbano, F.L., 2008. Review of the NAEPP 2007 expert panel report (EPR-3) on asthma diagnosis and treatment guidelines. *J. Manag. Care Pharm.* 14, 41–49.
- Weinmayr, G., Romeo, E., De Sario, M., Weiland, S.K., Forastiere, F., 2010. Short-term effects of PM10 and NO2 on respiratory health among children with asthma or asthma-like symptoms: a systematic review and meta-analysis. *Environ. Health Perspect.* 118, 449–457.
- Wenzel, S.E., 2006. Asthma: defining of the persistent adult phenotypes. *Lancet* 368, 804–813.
- WHO 2016. World Health Organization. Collaborating centre for drugs statistics and methodology. ATC/DDD Index 2016. (accessed 14.01.16).
- Zuccato, E., Calamari, D., Natangelo, M., Fanelli, R., 2000. Presence of therapeutic drugs in the environment. *Lancet* 355, 1789–1790.
- Zuccato, E., Chiabrando, C., Castiglioni, S., Bagnati, R., Fanelli, R., 2008. Estimating community drug abuse by wastewater analysis. *Environ. Health Perspect.* 116, 1027–1032.