

# A novel approach for monitoring tobacco use in local communities by wastewater analysis

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## ABSTRACT

**Objective** We propose a novel approach for measuring tobacco use in a community through the chemical analysis of nicotine metabolites in urban wastewater. It offers frequent monitoring and ‘real-time’, ‘evidence-based’ estimates of tobacco consumption which may complement epidemiological surveillance systems normally repeated only every few years.

**Methods** Two urinary metabolites of nicotine, namely cotinine and *trans*-3'-hydroxycotinine, were selected as biomarkers of tobacco consumption in urban wastewater. During smoking, a known amount of nicotine is absorbed and after metabolism excreted as metabolites in urine, ending up in the wastewater; quantitative analysis of the metabolites in the wastewater allows back-calculation of the nicotine collectively absorbed by the population producing the sewage and, indirectly, their tobacco use. Representative samples of wastewater were collected from sewage treatment plants in eight Italian cities and analysed by mass spectrometry. Mass loads of the metabolites were used to estimate nicotine consumption.

**Results** Wastewater analysis in the cities under study was used to estimate the number of cigarettes smoked, in order to compare the results of this study with those obtained from population surveys. The number of cigarettes calculated with the two methods were closely comparable and wastewater analysis was sufficiently sensitive to confirm the differences in tobacco consumption between northern and southern Italy, previously described in population surveys.

**Conclusions** The described approach can serve as a supplementary indicator of tobacco consumption in local communities. This approach can provide objective and updated information, which are useful to assess the efficacy of tobacco-control interventions, with the aim of designing and implementing effective tobacco control plans.

## INTRODUCTION

A certain amount of the nicotine that is absorbed by the body during smoking is excreted in urine as the parent drug (nicotine) and the products of metabolism (metabolites), mainly cotinine and *trans*-3'-hydroxycotinine.<sup>1</sup> This urine then enters the wastewater in the sewerage network. The wastewater can therefore be seen as a ‘depository’ for any sufficiently stable compound excreted by the local population.<sup>2</sup> Thus, measuring the excretion products of nicotine in wastewater can serve to back-calculate the nicotine absorbed (NICabs), and indirectly assess tobacco smoking by the population producing the sewage. If regularly monitored, changing mass loads of the residues in wastewater could reflect changes in smoking giving updated

and objective information of population level nicotine consumption. This information can complement survey and cigarette sales data by providing population cigarette use estimates that are not affected by misreporting of smoking status, underestimation of cigarette use<sup>3</sup> or cigarette smuggling.<sup>4</sup>

Smoking is the first preventable cause of premature mortality worldwide<sup>5</sup> and accounts for 12.5% of total mortality in Italy,<sup>6</sup> thus an integrative method to monitor cigarette use in a certain population can also be useful to design preventive campaigns at a local level and evaluate the impact of tobacco control strategies.

As an example of this approach, the chemical analysis of wastewater has been used to estimate illicit drug consumption<sup>7</sup> and alcohol intake<sup>8</sup> in local communities. The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) has recognised the potential of this methodology to complement and extend the existing epidemiologically based illicit drug use estimation techniques.<sup>9</sup> This approach has been called ‘sewage epidemiology’<sup>7</sup> or, recently, ‘wastewater-based epidemiology’, and the analysis of a broad list of sewage biomarkers has been proposed to public health agencies for quantitative measurement of the health of a population.<sup>10</sup> To be used for this purpose, these biomarkers need to be sufficiently stable to survive the journey from toilet to sampling point (mean residence time of sewage in the sewer systems 0.5–1.5 h),<sup>11</sup> be specific for the substance used as urinary excretion products, and be excreted at sufficient concentrations to be detected. Additionally, the excretion profile of the biomarkers must be known to develop proper correction factors for back-calculating the consumption of the substance used.

This study shows that two human metabolic products of nicotine, cotinine and *trans*-3'-hydroxycotinine, can be measured in urban wastewater, and fulfil all the requirements for use as sewage biomarkers for estimating collective consumption of tobacco in communities, with the unique potential to monitor local consumption trends in real time.

## METHODS

Nicotine and its human metabolic residues were measured in urban wastewater by high-performance liquid chromatography tandem mass spectrometry. This technique was chosen because it is very sensitive and specific for quantifying traces of the selected analytes even in a complex matrix such as raw urban wastewater. The analytical method was modified from previous publications<sup>12–13</sup> and was validated according to the required good practice



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criteria in order to ensure high quality and repeatability of results.

Twenty-four hour composite samples of raw wastewater were collected daily for several consecutive days, at the entry to some sewage treatment plants (STPs), each serving a different Italian city (Milan, Como, Bologna and Turin in northern Italy, Rome in central Italy, and Naples, Bari and Palermo in southern Italy). One-week sampling was performed in October 2012 contemporaneously in all the cities investigated.

Each STP collects almost the whole amount of urban wastewater produced in a city, thus being representative of the population of that city. Sampling followed the current *best practice protocol* to obtain non-biased composite samples and was performed using a volume-proportional mode.<sup>11</sup>

Samples were filtered, enriched with labelled (deuterated) internal standards, and solid phase extracted using mixed reverse-phase cation exchange cartridges (Oasis MCX, Waters Corp, Milford, Massachusetts, USA). A triple quadrupole mass spectrometer (AB SCIEX QqQ 5500, Ontario, Canada) was used for analyses of the selected analytes. Quantification was performed using analogue labelled standards for each compound, thus enabling a higher performance of the method and the correction for matrix effects. The stability of the compounds in the sewer network and during storage and analyses was also checked experimentally and the analytes were stable in all the conditions (24 h at 4°C and 20°C, and 28 days at -20°C). Triplicate analyses have been performed for each condition and no losses of these compounds were observed during the experiment (recovery of analytes 96–104%±2–6%—relative SD). Measured concentrations of nicotine and metabolites (ng/L) were multiplied by the daily flow rate of wastewater (m<sup>3</sup>/day) at the entry of the treatment plant, to obtain a daily mass load for each substance (g/day).

Nicotine human excretion studies were analysed to develop specific correction factors to back-calculate nicotine intake by the population, starting from the metabolic residues measured in raw wastewater. From the available literature<sup>1 14 15</sup> we identified the mean percentages of excretion of cotinine and *trans*-3'-hydroxycotinine after nicotine absorption (table 1). The conjugated forms of these metabolites are completely transformed to the free form by β-glucuronidase enzymes from faecal bacteria in the raw wastewater.<sup>16 17</sup> The excretion rates of these two metabolites (% of free and conjugated forms) were summed and the mean (74%) was used to develop a correction factor. Considering this, for 1 mg of NICabs during smoking, the excretion of nicotine equivalents is 0.74 mg, hence the correction factor was calculated as follows: (1/0.74)=1.35. The mass load of the parent drug (nicotine) in the wastewater was not considered in the back-calculation, as sources other than

human excretion, such as flushing of cigarette ash or cigarettes down toilets, can contribute to its occurrence in wastewater. The NICabs (g/day) was obtained through the following equation:

$$\text{NICabs} = [(C \text{ cot} \times F) + (\text{Chy cot} \times F)] \times C_f$$

where C cot and Chy cot are, respectively, the concentration of cotinine and *trans*-3'-hydroxycotinine measured in wastewater (ng/L), F is the flow rate of wastewater (L/day), C<sub>f</sub> the correction factor (1.35).

To compare these results with those of epidemiological surveys, we used NICabs (the estimate of total nicotine absorbed by the population under study) to calculate the number of cigarettes consumed, considering that during smoking for one cigarette an average of 1.25 mg of nicotine is absorbed systemically.<sup>1</sup> Official prevalence data from one epidemiological survey conducted in 2012 by DOXA, the Italian branch of the Worldwide Independent Network/Gallup International Association (WIN/GIA) were also used to calculate the number of cigarettes smoked. This survey had a sample size of more than 3000 individuals, representative of the general population aged 15 years or over in terms of age, sex, geographic area and socioeconomic characteristics.<sup>18</sup> Prevalence data were used to calculate the number of smokers considering the population aged >14 years in each city (obtained from the Italian National Institute of Statistics),<sup>19</sup> and to estimate the number of cigarettes using the average number consumed daily by a smoker in Italy.<sup>18</sup> Finally, the number of cigarettes estimated through wastewater analysis and survey data were compared calculating the difference as number of cigarettes and percentage.

## RESULTS

Figure 1 reports an 18-day profile of excretion of nicotine, cotinine and *trans*-3'-hydroxycotinine in wastewater in Milan. Cotinine and *trans*-3'-hydroxycotinine mass loads in wastewater (about 500 and 1000 g/day, respectively) were as expected, considering the excretion in urine (table 1), and their levels were

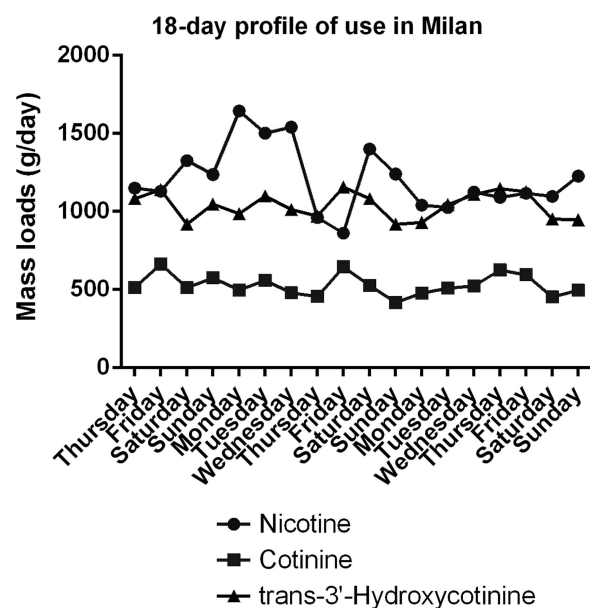


Figure 1 Eighteen-day profile of excretion of nicotine, cotinine and *trans*-3'-hydroxycotinine in wastewater in Milan. Means of two sampling campaigns, September and December 2012.

Table 1 Metabolic profile of nicotine excretion in human urine according to the literature

Investigated substances	Nicotine	Cotinine	<i>trans</i> -3'-hydroxycotinine
Human excretion (%)—free form*	10	13	35
Human excretion (%)—conjugated† form*	3	17	9
Sum free+conjugated forms (%)	13	30	44

\*Data from Ref. 14; amounts excreted were corrected to nicotine equivalents (nicotine molecular weight/metabolite molecular weight).

†Conjugated form consists of glucuronides.

**Table 2** Comparison among the number of cigarettes smoked estimated through wastewater analysis and epidemiological surveys in the population investigated

Cities investigated	Wastewater analysis			Epidemiological survey data			Difference in the number of cigarettes smoked	
	Population (>14 years) served by the STP*	Estimated NICabs (g/day; mean $\pm$ SD) <sup>†</sup>	Number of cigarettes smoked <sup>‡</sup>	Prevalence of smokers (% of population $\geq$ 15 years)	Number of smokers <sup>§</sup>	Number of cigarettes smoked <sup>¶</sup>	Number of cigarettes (survey data-wastewater analysis results)	Percentage (survey data-wastewater analysis results)
Northern Italy								
Milan	960 300	2121 $\pm$ 237	1 696 800	19.6	188 219	2 409 201	+712 401	+30
Como	79 926	241 $\pm$ 51	192 800	19.6	15 665	200 518	+7718	+4
Bologna	470 640	1360 $\pm$ 127	1 087 726	19.6	92 245	1 180 742	+93 016	+8
Turin	1 201 490	4756 $\pm$ 393	3 804 828	19.6	235 492	3 014 298	-790 530	-26
<b>Mean</b>			<b>1 695 538</b>			<b>1 701 190</b>	<b>+5651</b>	<b>+0.3</b>
Central Italy								
Rome	1 107 699	3734 $\pm$ 1779	2 987 243	19	210 463	3 114 850	+127 606	+4
Southern Italy								
Naples	549 250	2492 $\pm$ 266	1 993 948	23.3	127 975	1 714 868	-279 080	-16
Bari	296 922	1076 $\pm$ 43	860 912	23.3	69 183	927 050	+66 137	+7
Palermo	223 254	958 $\pm$ 75	766 416	23.3	52 018	697 044	-69 372	-10
<b>Mean</b>			<b>1 207 092</b>			<b>1 112 987</b>	<b>-94 105</b>	<b>-8</b>

Bold denotes the mean values.

\*The total population (served by the STP) was corrected by the percentage of people <14 years.<sup>19</sup>

<sup>†</sup>Mean and SD are calculated from the daily mass loads obtained for each city. Sampling campaigns were: 18 days in Milan and 7 days in all the other cities.

<sup>‡</sup>Calculated using the mean content of nicotine absorbed from one cigarette (1.25 mg).<sup>†</sup>

<sup>§</sup>Calculated from prevalence data considering the population served by the STPs investigated.

<sup>¶</sup>Calculated considering the average number of cigarettes smoked per day (12.8 $\pm$ 6.8 in the North of Italy, 14.8 $\pm$ 6.7 in the Center and 13.4 $\pm$ 7.7 in the South).<sup>18</sup>

NICabs, nicotine absorbed; STP, sewage treatment plant.

stable over the whole sampling period (figure 1). In contrast, parent nicotine mass loads (about 1200 g/day) were higher than expected considering only urinary excretion (table 1) and showed random variability during the sampling period. This may indicate that other sources of nicotine, such as direct disposal of ash and wash out from cigarette butts, contribute to the amount of nicotine in wastewater. This is the reason why we decided to rely on human metabolites in wastewater and not on nicotine itself to estimate the intake by the population.

Table 2 reports the comparison among the number of cigarettes smoked estimated through wastewater analysis and epidemiological surveys. Nicotine intakes (NICabs) were estimated by back-calculation, the number of cigarettes smoked was then calculated for each city and ranged between 190 000 and 3 805 000 depending on the size of the population. The number of cigarettes smoked was also calculated for each city from the prevalence of smokers estimated by epidemiological surveys in northern, central and southern Italy. The results obtained from wastewater analysis matched those from prevalence data. In the North of Italy four cities were investigated (Milan, Como, Bologna and Turin) giving a mean difference of the number of cigarettes smoked as 5651 cigarettes (0.3%). In central Italy only Rome was investigated and the mean difference of the number of cigarettes smoked was 127 606 (4%). In the South of Italy three cities were investigated (Naples, Bari and Palermo), and the mean difference of the number of cigarettes smoked was 94 105 (8%). Therefore the results obtained from wastewater analysis matched the prevalence from population surveys and confirmed epidemiological reports, indicating a higher prevalence of smoking in southern than northern and central Italy.

## DISCUSSION

Accurate monitoring of tobacco use is essential to evaluate the impact of tobacco-control policies.<sup>20</sup> However, fewer than half of European countries conduct regular tobacco use surveys annually or biennially.<sup>21</sup> The present study reports a novel approach based on the chemical analysis of nicotine excretion products in urban wastewater to evaluate tobacco use in a population. The results show good agreement between the number of cigarettes smoked calculated by wastewater analysis and from epidemiological surveys in Italy. The wastewater analysis approach was applied in several cities in different parts of Italy and the results were in good agreement with those obtained from epidemiological surveys (table 2). The differences in smoking prevalence between the northern-central and the South of Italy, already identified through epidemiological studies, could be confirmed through wastewater analysis. Similar results were obtained in Lisbon, where only cotinine was measured in three STPs to back-calculate nicotine consumption, and results were in line with a European Survey.<sup>22</sup> This confirms the reliability of the approach for monitoring tobacco use in a population. The strength of the wastewater-analysis approach is its ability to provide 'real-time' up-to-date information on smoking trends in local communities. Moreover, the information can be obtained at lower cost and in a shorter time than epidemiological surveys. However, as suggested for illicit drugs,<sup>23</sup> this approach makes no claim to replace, but only to complement epidemiological studies by providing a tool for more frequently assessing tobacco use at local levels, thus making it possible to detect and follow changing trends in populations, and to assess the efficacy of tobacco-control strategies.

As described for illicit drugs,<sup>7</sup> the wastewater approach suffers some sources of uncertainty, which can be ascribed to

variability in wastewater sampling, stability of biomarkers in sewage, accuracy of analytical measurements, uncertainty in metabolism and excretion data used for back-calculation, and in estimating population sizes.<sup>11</sup> All these factors have been recently examined in order to minimise, where possible, the uncertainty of the entire procedure and make the results more reliable. A *best practice protocol* was developed, considering the latest scientific information<sup>11 23</sup> and it was proposed to the scientific community to reduce the potential biases of this approach. In the present study, the potential uncertainty factors were carefully checked according to that proposed protocol, to ensure acceptable reliability of results. Specifically, for the adopted sampling, protocol uncertainty was lower than 10%,<sup>11</sup> the stability of nicotine excretion products in sewage was evaluated and the analytical method was fully validated keeping the specific uncertainties lower than 10%. Moreover, the pharmacokinetic data in the literature on nicotine metabolism were consistent in different papers, and the correction factor employed to back-calculate from metabolites to nicotine was therefore considered highly reliable.

Another possible confounding factor is the absorption of nicotine through routes other than smoking, such as all nicotine replacement therapies (NRTs): nicotine patches, pills and gums, and the use of electronic cigarettes. The contribution of these products to the total nicotine load was not quantified in this study but can be estimated from sales figures. In Europe, in 2010, e-cigarettes and NTRs each had a 0.4% share of the European Union (EU) tobacco and nicotine market.<sup>24</sup> Also, in a recent European Commission publication it has been estimated that among 27 EU countries, 5% of survey responders have tried e-cigarettes once and only 1% are occasional or regular user.<sup>25</sup> As mentioned before, the potential uncertainty of the protocol is below 10% and the potential estimation error due to NRTs and e-cigarettes is therefore negligible and has not been quantified in this work.

## CONCLUSIONS

To the best of our knowledge this is the first study showing that the metabolites of nicotine excreted in the urine of smokers can be measured in wastewater and that their concentrations can be used to estimate and monitor nicotine absorption and, indirectly, smoking in the communities producing the wastewater. This novel approach to estimate smoking in populations is not intended to replace, but only to complement population surveys, and will provide a tool for assessing tobacco use more frequently than by epidemiological studies, and make it possible to follow in 'real-time' changing trends in populations, and the efficacy of tobacco-control policies.

### What this paper adds?

- ▶ This paper describes a novel approach for measuring tobacco use based on wastewater analysis.
- ▶ The approach offers frequent monitoring and 'real-time', 'evidence-based' estimates of tobacco consumption.
- ▶ The number of cigarettes calculated through population surveys and wastewater analysis were closely comparable.

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