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Wastewater analysis to monitor use of caffeine and nicotine and evaluation of their metabolites as biomarkers for population size assessment

Ivan Senta [a], *Emma Gracia-Lor* [b], *Andrea Borsotti* [b], *Ettore Zuccato* [b], *Sara Castiglioni* [b,*]

[a] *Rudjer Boskovic Institute, Division for Marine and Environmental Research, Bijenicka c. 54, 10000 Zagreb, Croatia*
[b] *IRCCS – Istituto di Ricerche Farmacologiche "Mario Negri", Department of Environmental Health Sciences, Via La Masa 19, 20156 Milan, Italy*

ARTICLE INFO

Article history:
Received 10 September 2014
Received in revised form
19 December 2014
Accepted 1 February 2015
Available online 10 February 2015

Keywords:
Wastewater analysis
Caffeine
Nicotine
Urinary metabolites
Population biomarkers

ABSTRACT

The use of caffeine, nicotine and some major metabolites was investigated by wastewater analysis in 13 sewage treatment plants (STPs) across Italy, and their suitability was tested as qualitative and quantitative biomarkers for assessing population size and dynamics. A specific analytical method based on mass spectrometry was developed and validated in raw urban wastewater, and included two caffeine metabolites, 1-methylxanthine and 7-methylxanthine, never reported in wastewater before. All these compounds were found widely at the μg/L level. Mass loads, calculated by multiplying concentrations by the wastewater daily flow rate and normalized to the population served by each plant, were used to compare the profiles from different cities. Some regional differences were observed in the mass loads, especially for nicotine metabolites, which were significantly higher in the south than in the center and north of Italy, reflecting smoking prevalences from population surveys. There were no significant weekly trends, although the mean mass loads of caffeine and its metabolites were slightly lower during the weekend. Most caffeine and nicotine metabolites fulfilled the requirements for an ideal biomarker for the assessment of population size, i.e. being easily detectable in wastewater, stable in sewage and during sampling, and reflecting human metabolism. Nicotine metabolites were tested as quantitative biomarkers to estimate population size and the results agreed well with census data. Caffeine and its metabolites were confirmed as good qualitative biomarkers, but additional information is needed on the caffeine metabolism in relation to the multiple sources of its main metabolites. This exploratory study opens the way to the routine use of nicotine metabolites for estimating population size and dynamics.

1. Introduction

Caffeine and nicotine are the most widely used legal stimulants in modern societies (Garattini, 1993; WHO, 2013). Caffeine is the main stimulating ingredient in coffee, but is also found in other widely-consumed products, such as tea, soft and "energy" drinks. Nicotine is contained in cigarettes and other tobacco products and is the major addictive

* *Corresponding author*. Tel.: +39 02 39014776; fax: +39 02 39014735.
E-mail address: sara.castiglioni@marionegri.it (S. Castiglioni).
http://dx.doi.org/10.1016/j.watres.2015.02.002
0043-1354/

component of tobacco. Once consumed, these substances are extensively metabolized in the human body and excreted, mostly in the urine, as complex mixtures of parent compounds and metabolites (Garattini, 1993; Debry, 1994; Hukkanen et al., 2005), which end up in the sewage system. In fact, these substances are frequently detected in municipal wastewater at μg/L concentrations (Buerge et al., 2003, 2008; Huerta-Fontela et al., 2008; Santos et al., 2009; Rosal et al., 2010; Bueno et al., 2011) and are among the most ubiquitous waste and surface water microcontaminants (Focazio et al., 2008). However, comprehensive data on the occurrence of their metabolites are still scarce, especially for caffeine. In fact, some of caffeine's major metabolites, such as 1-methylxanthine and 7-methylxanthine, have never been analyzed in environmental samples.

In view of the widespread use of caffeine and nicotine, it has been suggested that these compounds could be used as anthropogenic markers to indicate the discharge of domestic wastewater in rivers and lakes (Buerge et al., 2003, 2008). Caffeine has also been proposed as a human biomarker for assessing population size and the dynamics of people served by a particular sewage treatment plant (STP) (Daughton, 2012).

Population size and dynamics are important parameters in many human activities, including material-flow (chemicals-flow) analysis in different environmental matrices and the per capita contributions of different pollutants (e.g. pharmaceuticals, personal care products, household chemicals, pesticides, biocides, nanomaterials) to the environment ("pollutant load per capita"; (Tsuzuki, 2006). This is particularly relevant for the "wastewater-based epidemiology" approach, which involves analysis of urban wastewater for the combined excretion products of different substances to track human habits and lifestyle (Thomas and Reid, 2011; Castiglioni et al., 2014). This approach was originally developed for the estimation of illicit drug consumption through wastewater analysis (Zuccato et al., 2005, 2008; van Nuijs et al., 2011), but can be extended to other applications, such as alcohol (Reid et al., 2011) and nicotine (Castiglioni et al., 2015). The rationale for this approach is based on the fact that almost everything we consume is excreted unchanged and/or as a mixture of metabolites in our urine and feces and ultimately ends up in the sewage network. Thus, the concentrations of metabolic residues in raw municipal wastewater can reflect the collective consumption of a substance in a community.

The accuracy of comparison of the profiles of consumption of illicit drugs and other substances in different communities relies on the estimation of the population size, i. e. the number of persons served by the STPs investigated, and the characterization of population dynamics. Current methods for population size assessment are based mainly either on public surveys, such as a census, or certain hydro-chemical parameters that are routinely determined at the STPs, including chemical oxygen demand (COD), biological oxygen demand (BOD) and total nitrogen and phosphorus (Andreottola et al., 1994; Daughton, 2012). However, since these parameters are greatly influenced by the wastewater composition (i.e. industrial, domestic or mixed), the measurement of specific substances in urban wastewater, which univocally indicates the persons served by an STP, has been proposed as an alternative for estimating population size (Daughton, 2012).

An ideal biomarker should fulfill several requirements: 1) be unique to human metabolism; 2) have no or minimal exogenous sources; 3) have a stable daily per capita excretion with minimal intra-individual and inter-individual variability; 4) be evenly distributed and stable in sewage; 5) be only minimally formed by microbial activity in sewage; 6) be determined easily, quickly and safely in environmental samples. Obviously, finding a suitable compound is a great challenge. The viability of the compounds proposed as population biomarkers, such as pharmaceuticals, coprostanol, caffeine, biocides and food additives has not been experimentally verified, but a few studies tested some of these substances in the last few years. Measuring pharmaceutical loads was first suggested to estimate the number of persons contributing to the wastewater (Lai et al., 2011). More recently, several compounds, including cotinine, have been screened using different criteria, such as quantification methods, affinity to particulate, stability in wastewater, constancy of inter-day excretion, and correlation with census population (Chen et al., 2014).

The present study tested caffeine and nicotine derivatives for the first time as quantitative human biomarkers for the assessment of population size in raw wastewater. The aims of the study were: a) to investigate the occurrence of caffeine, nicotine and some of their major metabolites in raw wastewater in Italy; b) to assess their patterns of use in different communities through wastewater analysis; c) to explore their potential as human biomarkers for population size assessment. A specific analytical method, including for the first time an almost complete set of metabolites of caffeine and nicotine, was developed and validated in raw urban wastewater. The reliability of these compounds as human biomarkers was investigated by stability tests during residence in sewage, during storage and wastewater sampling and by comparing the figures for inhabitants obtained from hydrochemical parameters and from nicotine derivatives.

2. Material and methods

2.1. *Selection of analytes*

At the beginning of the study, nine compounds were selected as possible human biomarkers for population size assessment: caffeine and its major metabolites paraxanthine (1,7-dimethylxanthine), 1-methylxanthine, 7-methylxanthine, 1,7-dimethyluric acid, 1-methyluric acid (Garattini, 1993; Baselt, 2004), and nicotine and its metabolites cotinine and *trans*-3′-hydroxycotinine (Byrd et al., 1992; Hukkanen et al., 2005). For caffeine, the first criterion for selecting metabolites was the percentage of excretion of each metabolite, which was available for a relatively low number of subjects (range 7–68) (Table S1). Generally, those accounting for more than 5% in the total human metabolism were taken into account as possible biomarkers. However, the literature suggested that some were not good candidates for biomarkers, because of instability in urine and/or in wastewater. For example, AFMU (5-acetyl-amino-6-formylamino-3-methyluracil), one of the main metabolites of caffeine, was unstable in urine (Krul and Hageman, 1998; Wong et al., 2002). For nicotine, we selected two main metabolites excreted in