This item is the archived peer-reviewed author-version of:

Can wastewater-based epidemiology be used to evaluate the health impact of temperature? An exploratory study in an Australian population

Reference:
Phung Dung, Mueller Jochen, Lai Foon Yin, O'Brien Jake, Dang Nhun, Morawska Lidia, Thai Phong K.- Can wastewater-based epidemiology be used to evaluate the health impact of temperature? An exploratory study in an Australian population
ENVIRONMENTAL RESEARCH - ISSN 0013-9351 - 156(2017), p. 113-119
Full text (Publisher's DOI): https://doi.org/10.1016/J.ENVRES.2017.03.023
To cite this reference: http://hdl.handle.net/10067/144159151162165141
Can wastewater-based epidemiology be used to evaluate the health impact of temperature? –

an exploratory study in an Australian population

Dung Phung\textsuperscript{a}, Jochen Mueller\textsuperscript{b}, Foon Yin Lai\textsuperscript{b}, Jake O’Brien\textsuperscript{b}, Nhung Dang\textsuperscript{c}, Lidia Morawska\textsuperscript{d}, Phong K. Thai\textsuperscript{d,*}

\textsuperscript{a}Centre for Environment and Population Health, Griffith University, Brisbane, QLD, Australia
\textsuperscript{b}Queensland Alliance for Environmental Health Sciences, University of Queensland, Brisbane, QLD, Australia;
\textsuperscript{c}Dermatology Research Centre, University of Queensland, Brisbane, QLD, Australia;
\textsuperscript{d}International Laboratory for Air Quality and Health, Queensland University of Technology, Brisbane, QLD, Australia;

*Corresponding author:

Phong Thai, Queensland University of Technology

(e) phong.thai@qut.edu.au;
Abstract

Ambient temperature is known to have impact on population health but assessing its impact by the traditional cohort approach is resource intensive. Wastewater-based epidemiology (WBE) could be an alternative for the traditional approach. This study was to provide the first evaluation to see if WBE can be used to assess the impact of temperature exposure to a population in South East Queensland, Australia using selected pharmaceuticals and personal care products (PPCPs) as biomarkers. Daily loads of eight PPCPs in wastewater collected from a wastewater treatment plant were measured from February 2011 to June 2012. Corresponding daily weather data were obtained from the closest weather station. Missing data of PPCPs were handled using the multiple imputation (MI) method, then we used a one-way between-groups analysis of variance to examine the seasonal effect on daily variation of PPCPs by seasons. Finally, an MI estimate was performed to evaluate the continuous relationship between daily average temperature and each multiply-imputed PPCP using time-series regression analysis. The results indicated that an increase of 1°C in average temperature associated with decrease at 1.3 g/d (95% CI: -2.2--(4.4), p<0.05) for atenolol, increase at 36.5 g/d (95% CI: 25.2-47.8, p<0.01) for acesulfame, and increase at 0.8 g/d (95% CI: 0.02-1.55, p=0.05) for naproxen. No significant association was observed between temperature and the remaining PPCPs, comprising: caffeine, carbamazepine, codeine, hydrochlorothiazide, and salicylic acid. The findings suggested that consumption of sweetened drinks, risk of worsening cardiovascular conditions and pains are associated with variation in ambient temperature. WBE can thus be used as a complementary method to traditional cohort studies in epidemiological evaluation of the association between environmental factors and health outcomes provided that specific biomarkers of such health outcomes can be identified.

Keywords: Wastewater analysis; ambient temperature; atenolol; sweetened drinks; cardiovascular diseases.
1. Introduction

Sampling and analysis of wastewater influent from wastewater treatment plants (WWTPs) has become a useful tool for measuring the consumption of chemicals in the population. Initially proposed in 2001 (Daughton, 2001), with the first study published in 2005 (Zuccato et al., 2005), the approach has been validated and successfully applied to monitor substances of abuse in large scale studies across the globe (e.g. Ort et al. (2014) for illicit drugs, Castiglioni et al. (2015) for tobacco, and Ryu et al. (2016) for alcohol consumption). Wastewater-based epidemiology (WBE), as it is commonly referred to, can also be used for other chemicals that are excreted to the sewer system after consumption and reach the WWTPs. In fact WBE has been applied to measure the concentrations of several pharmaceutical and personal care products (PPCPs) in order to estimate the population residing in the monitored catchment (Gao et al., 2016; Lai et al., 2015; O’Brien et al., 2014; Rico et al., 2017).

Until now, few WBE studies have interrogated the association of the levels of chemical consumption measured in a population with any health impacts or environmental health factors. To our knowledge, only one pilot study by Fattore et al. (2016) has reported the association between asthma and outdoor PM$_{10}$ and PM$_{2.5}$ levels by using the levels of salbutamol in wastewater as an indicator of the occurrence of asthma. Such findings provided direct evidence of the effect of outdoor ambient air pollution on asthma which is usually difficult to obtain by other methods.

Ambient temperature is an important environmental risk factor. A recent study involving multiple countries has indicated that even mild temperature changes can increase the mortality risk to people (Gasparrini et al., 2015). The effect of ambient temperature on morbidity is also considered a significant public health issue with a large number of hospitalizations associated with exposure to extreme ambient temperatures such as heat waves and cold spells (Phung et al., 2016). Assessing the impact of ambient temperature on morbidity is difficult because the use of emergency department visits and hospitalizations, and medicine sales or prescriptions as markers for morbidity
may underestimate the effect of mild temperature change since people can also use medications at home when they know the symptoms of these chronic diseases.

In this study, we utilised a unique WBE data set to investigate the association between the ambient temperature and the levels of eight PPCPs measured in wastewater from a South East Queensland catchment in Australia during a period of more than a year (Lai et al., 2015). The outcome was consequently used to select good candidate biomarkers for health impact of ambient temperature for future WBE studies.

2. Materials and Methods

2.1 WBE data on the daily loads of PPCPs

The dataset of eight PPCPs (Table 1) in wastewater used in this study had been used previously to estimate the daily de facto population in the catchment of 230,000 (Census 2011 data) through Bayesian modelling (Lai et al., 2015). They are the only PPCPs that could be detected and measured in all daily samples at low cost (i.e. by direct injection LCMS as described below). The sample collection and chemical analysis were described in details in Lai et al. (2015). Briefly, daily composite wastewater samples were collected between February 2011 and June 2012 (with several days missing due to logistical or technical reasons) using a continuous flow-proportional sampling technique. The samples were refrigerated at 4 °C during collection, acidified on site to pH 2 using 2 M hydrochloric acid, and then frozen until analysis. Data on daily wastewater volumes were recorded and provided by the WWTP.

The concentrations of the PPCPs were measured in wastewater samples by direct injection liquid chromatography (Shimadzu, Nexera UHPLC system, Kyoto, Japan) coupled with tandem mass spectrometry (AB SCIEX QTRAP5500, Ontario, Canada) (LC-MS/MS). Separation of the targeted PPCPs was performed on a Kinetics C18 column (100 x 3 mm, 2.6 μm) using gradient mobile phases. Together with the calibration standards, concentrations of the PPCPs were measured using mass spectrometry with a multireaction monitoring (MRM) scheme. Two MRM transitions were
used for identification and quantification of each analyte. Concentrations of the analytes were quantified using the isotope dilution method. Quality assurance and quality control (QA/QC) measures were performed for every batch of analysis. All results were within the acceptable range with detailed QA/QC information (Lai et al., 2015). The concentrations of PPCPs were then multiplied with the daily flow data to produce the daily loads which were subsequently used in the statistical analysis.

2.2 Temperature data retrieval and statistical analysis

Daily weather data were obtained from the closest airport weather station to the WWTP catchment for the corresponding time-span of PPCPs monitoring time (475 days). The data comprise of daily minimum, maximum, and average temperature (°C); relative humidity (%) and cumulative rainfall (mm). Three steps were involved in the data analysis. Firstly, the missing data of PPCPs was handled using the multiple imputation (MI) method which is a simulation-based approach for analysing incomplete data and is described elsewhere (Marchenko, 2010; Soley-Bori, 2013; Yang, 2017). Multiple imputation provides a useful strategy for dealing with data sets with missing values, in which each missing value with a set of plausible values is replaced by Rubin’s (1987) multiple imputation procedure instead of filling single value for each missing value, then these multiple imputed datasets involved analysis by using standard procedures for complete data and combining the results from these analyses. Regardless of which complete-date analysis is used, the process of combining results from different imputed data sets is essentially the same. In this study, we used multiple imputation procedure in Stata statistical software (Stata Corporation, College Station, TX, USA) to impute missing data. Variables comprising the weather variables (average temperature, humidity, and cumulative rainfall), which were strongly correlated with incomplete PPCPs values were investigated using Pearson product-moment correlation coefficient, were fitted with the regression model where each PCPP was the dependent variable consisting of missing values, then 10 complete datasets for each PPCP were created using these selected multiple weather variables. A
total run length of Monte Carlo simulations used with imputations was 10,000. The 10 imputed data sets were then used for regression analysis using the command “mi estimate: regress (vars)” created for mi package in Stata software 11.

We tested normality of PCPPs data by plotting probability density for visual check (Supplement 1) and used the Shapiro-Wilk and Kolmogorov-Smirnov tests. Secondly, we used a one-way between-groups analysis of variance to examine the seasonal effect on daily variation of PPCPs by seasons comprising: summer (December-February), autumn (March-May), winter (June-August), and spring (September-November). The statistical significance was set up at the level of p<=0.05. Finally, we performed MI estimate using standard linear regression (Equation 1) to evaluate the continuous relationship between daily average temperature and each multiply-imputed PPCP using time-series linear regression functions. This step determined how much the daily load of each PPCP changed in relation to 1°C increase in temperature. We used Augmented Dickey-Fuller test (Mushtaq, 2011) to check stability of the data before running time-series regression analysis. We also conducted sensitivity analysis using different temperature (minimum and maximum) for the PPCPs which were statistically associated with average temperature. We controlled potential confounding factors that can influence on daily relationship between temperatures and PCPPs comprising: relative humidity (%), cumulative rainfall (mm), day of week, and month of year to control for seasonal effect.

\[ Y_j = \alpha + \beta_1 T_j + \beta_2 H_j + \beta_3 R_j + \beta_4 DOW + \beta_5 MOY \]

where \( Y_j \) is mass load of each PCPP on day \( j \); \( T_j \) is daily average temperature on day \( j \); \( H_j \) is daily average relative humidity on day \( j \); \( R_j \) is cumulative rainfall on day \( j \)

3. Results

The descriptive statistics of PPCPs and weather factors are presented in Table 2. The average mass load (g/d) for those PPCPs varied from as low as 27 for carbamazepine to 2600 for salicylic acid. The widest range of values was found with caffeine, of which the maximum value was about 340
times higher than the minimum value. The distributional plots are likely to present normal
distributions for most of the PPCPs (Supplement 1). The average values of PPCPs calculated from
10 imputations using the multivariate imputation method were not significantly different (p>0.05)
from the observed values with missing data, with the values of differences ranging from 0.2 to 15
g/d across the PPCPs (Table 2). The temporal plots of daily load of eight PPCPs including their
imputed values are shown in Figure 1.

The average daily temperatures at the research location ranged from 10 to 28°C (mean of 20%), and
the average relative humidity ranged from 48 to 94% (mean of 74%) during the study period. The
sum of cumulative rainfall was observed at 1020 mm with 147 rainy days for the whole study
period.

The seasonal effects varied among PPCPs (Figure 2). While there is no seasonal effect observed
with codeine and hydrochlorothiazide, the results of one-way ANOVA test indicated some effects
with the rest of PPCPs. Atenolol (winter, 120 vs autumn, 111; summer, 107; spring, 109) and
carbamazepine (winter, 29 vs autumn, 26; summer, 26; spring, 27) were highest in winter in
comparison with other seasons (p<0.05). On the contrary, acesulfame (summer, 2033 vs autumn,
1868; winter, 1748; spring, 2029) and naproxen (summer, 120 vs autumn, 103; winter, 111; spring,
111) were observed with the highest level in summer compared with the rest of the year (p<0.05).
Caffeine (autumn, 2234 vs summer, 2526; winter, 2547; spring, 2532) and salicylic acid (autumn,
2342 vs summer, 2836; winter, 2649; spring, 2891) were lowest in autumn in comparison with the
rest of seasons which have similar masses of these PPCPs (Figure 2).

The results of MI estimate using linear regression indicated significant association between ambient
temperatures and atenolol, acesulfame, and naproxen. An increase of 1°C in average temperature
associated with decrease at 1.3 g/d (95% CI: -2.2 to -0.4, p<0.05) for atenolol while an associated
increase for acesulfame was observed at 36.5 g/d (95% CI: 25.2 to 47.8, p<0.01) and 0.8 g/d (95% CI:
0.02 to 1.55, p=0.05) for naproxen. No significant association was observed between temperature and
the remaining PPCPs. Quantitatively, an increase of 1°C in average temperature associated with
increase of 0.05 g/d (95% CI: -0.6-0.7, p=0.8) for codeine and 6.8 g/d (95% CI: -20.5-34.1, p=0.6) for salicylic acid; whereas, the inverse relationship was found for caffeine with a decrease of 20.4 g/d (95% CI: -42.9-2.17, p=0.08), for hydrochlorothiazide with a decrease of 0.3 g/d (95% CI: -0.8-0.3, p=0.3), and for carbamazepine with a decrease of 0.02 g/d (95% CI: -0.5-0.5, p=0.9) as presented in Figure 3. The results of sensitivity analysis, which replaces average temperatures with maximum and minimum temperatures in regression analyses for 3 PPCPs with significant associations were reported in Supplement 2. A decrease of 1.2 g/d (95% CI: -1.83-(-.55) and 1 g/d (95% CI: -1.43-(-.58) of atenolol daily load were associated with 1°C increase in maximum and minimum temperatures, respectively. In contrast, an increase of 44 g/d (95% CI: 36.7-51.2) and 24.8 g/d (95% CI: 19.8-29.9) of acesulfame daily load were associated with 1°C increase in maximum and minimum temperatures. Likewise, an increase of naproxen of 0.7 g/d (95% CI: 0.27-1.16) was associated with 1°C increase in minimum temperatures but not significant with increase in maximum temperatures.

4. Discussion

This is the first study to investigate the association between the consumption of several PPCPs and ambient outdoor temperature using wastewater analysis. Significant association was found between the daily loads of three PPCPs, namely atenolol, acesulfame and naproxen, and the daily average temperature recorded in the same catchment while daily loads of caffeine, codeine, carbamazepine, hydrochlorothiazide, and salicylic acid showed no association with daily ambient temperature.

4.1. Compounds having significant association with ambient temperature

+ Acesulfame

Acesulfame is an intense sweetener which can be used as a flavour enhancer or a substitute for sugars. It is used in a variety of products, many of them beverage and juices that are consumed during warm temperature such as Coke and Pepsi (acesulfame is used in combination with another
intense sweetener, aspartame). Rossato et al. (2015) has indeed reported the higher consumption of sweetened beverage in summer than in winter in southern Brazil where the climate is similar to the studied catchment. Therefore, it is reasonable to expect that the consumption of acesulfame will increase during warmer months of the year. The strong association between ambient temperature and the consumption of soft drinks as represented by the level of acesulfame in wastewater found in this study (p<0.001) proved that wastewater analysis can be used to investigate associations in epidemiological studies.

+ Atenolol

Atenolol is a drug belonging to the group of beta blockers, a class of drugs used primarily to treat cardiovascular diseases. Atenolol is used to treat high blood pressure (hypertension), chest pain (angina) and to improve survival after a heart attack.

The strong invert association of atenolol consumption and ambient temperature observed in this study (p=0.007) is in agreement with evidence from traditional epidemiological studies about the seasonal variability of cardiovascular risks (Lichtman et al., 2016; Marti-Soler et al., 2014). The highest risk was observed during winter when atenolol was consumed at the highest level. But it is noted that although average consumption of atenolol was lowest during summer, the variation in this season was also highest because the occurrence of very high temperature could increase the risk of cardiovascular disease as well (Phung et al, 2016).

+ Naproxen

Naproxen is a non-steroidal anti-inflammatory drug (NSAID) with analgesic, anti-inflammatory and antipyretic properties. In Australia, it is a prescription drug mainly for arthritis but it can also be purchased over-the-counter for dysmenorrhoea and other general pains.

Since naproxen is used to treat a wide range of symptoms, it is difficult to attribute the positive association of its daily load with ambient temperature (p=0.045) to a specific disease. Regarding
arthritis, since there are two opposite impacts of seasonal change to its prevalence (high temperature in summer reduce the occurrence of arthritis but at the same time, higher relative humidity in summer can cause more arthritis cases - (Patberg and Rasker 2004), the overall seasonal variation of arthritis incidents (and arthritis-related naproxen consumption) would be insignificant. It would be very useful for the investigation of weather effects on arthritis if an arthritis-specific chemical can be identified in wastewater.

4.2. Compounds having non-significant association with ambient temperature

+ Caffeine

Caffeine occurs naturally in certain food and drinks such as coffee, tea and cocoa and has a long history of safe use as a mild stimulant. Caffeine can also be added into other products including cola-type soft drinks, formulated caffeinated beverages (energy drinks) and energy shots (FSANZ 2016). Such a wide range of both hot and cool products containing caffeine may be the reason why there was no significant association between caffeine consumption and ambient temperature. Although not significant (p=0.076), there was an approximately 1% decrease in caffeine consumption for each 1°C increase in ambient temperature. As coffee and tea are likely the main caffeine containing products consumed by the general population, such an observation seems logical. The increase in consumption of caffeinated drinks (cola-type soft drink) as indicated by the increase of acesulfame possibly contribute to make the decrease insignificant.

It is noted that caffeine is not completely stable in the sewer system (O’Brien et al., 2017) although it has been reported that it is stable in wastewater only (Senta et al., 2015). Therefore, there may have been more degradation of caffeine in the sewer system in the summer than in the winter, which also leads to a reduction in the daily load of caffeine measured during summer time. In our opinion, caffeine is not a good biomarker since there are too many potential contributors to its load in wastewater and its degradation in the sewer system would add more uncertainty to the temporal variation of daily loads.
Codeine is a pain killer belonging to the opioid group. Codeine is used to provide relief from a number of conditions including pain (sometimes in combination with aspirin or paracetamol), dry irritating cough, diarrhoea, cold and flu. Codeine is available in Australia in both prescription and over-the-counter forms (Gisev et al., 2016).

There was no association between codeine consumption and ambient temperature with a near zero coefficient. It means in average, consumption of codeine is flat all year round. This is probably the combined effect of decrease consumption for cold and flu and increase consumption for other pains (similar to the case of naproxen) when the temperature increases. O’Brien et al. (2017) reported the potential degradation of codeine in the sewer system, which is different from naproxen, which may have attenuated the increase use of codeine in warmer seasons like in the case of naproxen.

Carbamazepine is an antiepileptic drug which is only available by prescription. It is used to treat seizures and nerve pain. We saw no significant association between carbamazepine daily loads and ambient temperatures (p=0.948) and a near zero coefficient. This finding indicated that the occurrence of epilepsy/seizures is not influenced by ambient temperature (or seasons). Such observation is in agreement with previous epidemiological study in epilepsy which reported no seasonality in epilepsy-related deaths (Bell et al., 2010). The consumption of carbamazepine as preventative/maintenance treatment could also result in the stable level of this drug in wastewater.

Hydrochlorothiazide is a diuretic drug which is used to treat high blood pressure and swelling. In Australia, it is a prescription drug mainly used for high blood pressure. No significant association between consumption of hydrochlorothiazide with ambient temperature was observed in this study although there was a slight (nonsignificant) decrease in the daily load of hydrochlorothiazide in
wastewater when ambient temperature increased. In the literature, blood pressure is reported to be higher in colder months with physiological cause of reflex coronary and systemic vasoconstriction (Cuspidi et al., 2012). It seems plausible that physical activity increases in warmer months thus reducing blood pressure (Rossi et al., 2012). That means an increase in temperature is likely to lead to a reduction in the use of antihypertensive drugs including hydrochlorothiazide (Cuspidi et al., 2012). The finding of this study could not confirm this hypothesis, which probably indicates that hydrochlorothiazide is mainly used for preventative/maintenance treatment of hypertension in the monitored population.

+ Salicylic acid

Salicylic acid is a topical drug, which is used to treat some skin conditions and certain fungal infections. It is also the metabolite of aspirin, a drug used to treat pain, fever, and inflammation. The topical administration of this drug means that almost all of the applied drug will be released into the sewer, not through the toilet like most other drugs, but through the bathing and showering. The wide range of diseases that relate to the release of salicylic acid into the sewer make it difficult to distinguish the effect of each disease to the overall daily load of this chemical. Salicylic acid is also degradable during passage in the sewer system which complicates the interpretation of the results. In summary, salicylic acid is not a good candidate to use as biomarker for epidemiological evaluation in wastewater analysis.

4.3 Implication of the findings

This study shows that wastewater analysis is novel, objective approach to conduct epidemiological evaluation of the association between disease/behaviour and environmental factors such as ambient temperature provided that the biomarkers used in wastewater analysis are sufficiently disease/behaviour specific and relatively stable in the sewer. In the case where the unique biomarker is prone to degradation in the sewer then it can still be used as long as the level of
degradation is quantifiable but more research is needed to quantify the degradation in different sewer conditions.

The outcome of this study also indicated the significant seasonal variation of some chemicals such as acesulfame that could affect its use as biomarker for population size estimation (O’Brien et al., 2014, Lai et al., 2015), especially when it is used to estimate the population in a narrow period of time (Gao et al., 2016). The knowledge of seasonal variability obtained from this study should be utilised and incorporated into the model in the future to improve the accuracy of the model prediction.

4.4 Limitations & recommendations

+ Representative biomarkers of temperature-adverse health effects

In this study, the PPCPs were monitored based on the analytical capability, so they might not be the most appropriate biomarkers of temperature-health adverse effect. Future studies should consider developing sensitive methods for biomarkers that can represent most of adverse health effects of climatic conditions (e.g. residues of pharmaceuticals related to cold and flu).

+ Stability of the PPCPs in the sewer:

Amongst the eight PPCPs investigated in this study, five are considered stable at relative high temperature (20°C) while three could be degraded under sewer conditions, namely codeine, caffeine, and salicylic acid (O’Brien et al., 2017). For those unstable chemicals, it is difficult to distinguish between the impact of temperature to disease and drug consumption with the impact of degradation in the sewer. Therefore, future research into the impact of temperature should focus/select biomarkers that are stable in the sewer system to avoid the confounding factor of different degradation due to different temperature of the wastewater.

+ Variation in daily (de facto) population:
Change in daily load of PPCPs in wastewater could be due to change in per capita consumption, as assumed in this study. But it could also be the result of change in population in the catchment where larger sewage catchment produces higher daily load of PPCPs (O’Brien et al., 2014). At the moment, there is no data of daily population available for the studied catchment to test the impact of population variation to the associations found in this study (i.e. to use daily population as a confounding factor) although it is possible that summer would attract more people to visit and stay in the studied catchment. Nevertheless, the significant negative association of atenolol with temperature indicated that temperature did have impact on the consumption of this drug independent from the influence of the possible population increase during the period of high temperature.

+ Imputation

There were missing values of for the PPCPs monitored during April and May, so the replaced values of the PPCPs which were obtained using multiple imputation techniques, were used for data analysis. The imputed values might not reflect the outliers that were correspondent to outbreaks of medication usage if they happened during the missing-data period. However, the imputed values made the relatively similar statistical mean to values actually observed under the normal context.

+ Lacking of individual data:

The study had no access to individual-level data on substance usage, socio-economic, and adaptive factors (e.g. air conditioning) which might reflect the actual relationship between high ambient temperatures and health conditions, resulting to substance use (i.e. medication). It would be more comprehensive if these factors are investigated and considered in analysis in future study. There are also lack of data on sweetened drinks and drug sales to validate the measured loads from the sewage system, which somewhat limited the validity of the study outcomes.
5. Conclusions

This study proved the potential of the WBE approach to quantitatively estimate the relationship between environmental exposures and diseases using long term daily loads of selected PPCPs in a relatively large Australian population. Based on the findings, we suggested that increase in ambient temperature may result to increased consumption of sweetened drinks as well as increased risk of cardiovascular diseases and pains. This study also suggested the importance of finding specific biomarkers for health outcome in order to expand the applicability of WBE.

Acknowledgments

The Queensland Alliance for Environmental Health Sciences, The University of Queensland gratefully acknowledges the financial support of the Queensland Department of Health. DP is funded by a Griffith Postdoctoral Research Fellowship. JFM is funded by an ARC Future Fellowship. PT is funded by a QUT VC Research Fellowship.

6. References


Daughton, C.G. 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. Pharmaceuticals and Personal Care products in the Environment, Scientific and regulatory Issue 2001:348-364


FSANZ. Caffeine. FSANZ; 2016


Kim, H.; Armstrong, B. Mortality risk attributable to high and low ambient temperature: A multicountry observational study. The Lancet 2015;386:369-375


Marchenko, Y. Multiple-imputation analysis using Stata's mi command. Stata Conference, Boston 2010. 2010


Senta, I.; Gracia-Lor, E.; Borsotti, A.; Zuccato, E.; Castiglioni, S. Wastewater analysis to monitor use of caffeine and nicotine and evaluation of their metabolites as biomarkers for population size assessment. Water Research 2015;74:23-33

