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

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

^a Centre for Environment and Population Health, Griffith University, Brisbane, QLD, Australia

^b Queensland Alliance for Environmental Health Sciences, University of Queensland, Brisbane,
QLD, Australia;

^c Dermatology Research Centre, University of Queensland, Brisbane, QLD, Australia;

^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;

17 **Abstract**

18 Ambient temperature is known to have impact on population health but assessing its impact by the
19 traditional cohort approach is resource intensive. Wastewater-based epidemiology (WBE) could be
20 an alternative for the traditional approach. This study was to provide the first evaluation to see if
21 WBE can be used to assess the impact of temperature exposure to a population in South East
22 Queensland, Australia using selected pharmaceuticals and personal care products (PPCPs) as
23 biomarkers. Daily loads of eight PPCPs in wastewater collected from a wastewater treatment plant
24 were measured from February 2011 to June 2012. Corresponding daily weather data were obtained
25 from the closest weather station. Missing data of PPCPs were handled using the multiple imputation
26 (MI) method, then we used a one-way between-groups analysis of variance to examine the seasonal
27 effect on daily variation of PPCPs by seasons. Finally, an MI estimate was performed to evaluate
28 the continuous relationship between daily average temperature and each multiply-imputed PPCP
29 using time-series regression analysis. The results indicated that an increase of 1°C in average
30 temperature associated with decrease at 1.3 g/d (95% CI: -2.2-(-.4), $p<0.05$) for atenolol, increase at
31 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,
32 $p=0.05$) for naproxen. No significant association was observed between temperature and the
33 remaining PPCPs, comprising: caffeine, carbamazepine, codeine, hydrochlorothiazide, and salicylic
34 acid. The findings suggested that consumption of sweetened drinks, risk of worsening
35 cardiovascular conditions and pains are associated with variation in ambient temperature. WBE can
36 thus be used as a complementary method to traditional cohort studies in epidemiological evaluation
37 of the association between environmental factors and health outcomes provided that specific
38 biomarkers of such health outcomes can be identified.

39

40 **Keywords:** Wastewater analysis; ambient temperature; atenolol; sweetened drinks; cardiovascular
41 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 PM₁₀ 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

67 may underestimate the effect of mild temperature change since people can also use medications at
68 home when they know the symptoms of these chronic diseases.

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

74

75 **2. Materials and Methods**

76 **2.1 WBE data on the daily loads of PPCPs**

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

87 The concentrations of the PPCPs were measured in wastewater samples by direct injection liquid
88 chromatography (Shimadzu, Nexera UHPLC system, Kyoto, Japan) coupled with tandem mass
89 spectrometry (AB SCIEX QTRAP5500, Ontario, Canada) (LC-MS/MS). Separation of the targeted
90 PPCPs was performed on a Kinetics C18 column (100 x 3 mm, 2.6 µm) using gradient mobile
91 phases. Together with the calibration standards, concentrations of the PPCPs were measured using
92 mass spectrometry with a multireaction monitoring (MRM) scheme. Two MRM transitions were

93 used for identification and quantification of each analyte. Concentrations of the analytes were
94 quantified using the isotope dilution method. Quality assurance and quality control (QA/QC)
95 measures were performed for every batch of analysis. All results were within the acceptable range
96 with detailed QA/QC information (Lai et al., 2015). The concentrations of PPCPs were then
97 multiplied with the daily flow data to produce the daily loads which were subsequently used in the
98 statistical analysis.

99

100 2.2 Temperature data retrieval and statistical analysis

101 Daily weather data were obtained from the closest airport weather station to the WWTP catchment
102 for the corresponding time-span of PPCPs monitoring time (475 days). The data comprise of daily
103 minimum, maximum, and average temperature (°C); relative humidity (%) and cumulative rainfall
104 (mm). Three steps were involved in the data analysis. Firstly, the missing data of PPCPs was
105 handled using the multiple imputation (MI) method which is a simulation-based approach for
106 analysing incomplete data and is described elsewhere (Marchenko, 2010; Soley-Bori, 2013; Yang,
107 2017). Multiple imputation provides a useful strategy for dealing with data sets with missing values,
108 in which each missing value with a set of plausible values is replaced by Rubin's (1987) multiple
109 imputation procedure instead of filling single value for each missing value, then these multiple
110 imputed datasets involved analysis by using standard procedures for complete data and combining
111 the results from these analyses. Regardless of which complete-date analysis is used, the process of
112 combining results from different imputed data sets is essentially the same. In this study, we used
113 multiple imputation procedure in Stata statistical software (Stata Corporation, College Station, TX,
114 USA) to impute missing data. Variables comprising the weather variables (average temperature,
115 humidity, and cumulative rainfall), which were strongly correlated with incomplete PPCPs values
116 were investigated using Pearson product-moment correlation coefficient, were fitted with the
117 regression model where each PCPP was the dependent variable consisting of missing values, then
118 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 $p \leq 0.05$. 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

145 times higher than the minimum value. The distributional plots are likely to present normal
146 distributions for most of the PPCPs (Supplement 1). The average values of PPCPs calculated from
147 10 imputations using the multivariate imputation method were not significantly different ($p>0.05$)
148 from the observed values with missing data, with the values of differences ranging from 0.2 to 15
149 g/d across the PPCPs (Table 2). The temporal plots of daily load of eight PPCPs including their
150 imputed values are shown in Figure 1.

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

155 The seasonal effects varied among PPCPs (Figure 2). While there is no seasonal effect observed
156 with codeine and hydrochlorthiazide, the results of one-way ANOVA test indicated some effects
157 with the rest of PPCPs. Atenolol (winter, 120 vs autumn, 111; summer, 107; spring, 109) and
158 carbamazepine (winter, 29 vs autumn, 26; summer, 26; spring, 27) were highest in winter in
159 comparison with other seasons ($p<0.05$). On the contrary, acesulfame (summer, 2033 vs autumn,
160 1868; winter, 1748; spring, 2029) and naproxen (summer, 120 vs autumn, 103; winter, 111; spring,
161 111) were observed with the highest level in summer compared with the rest of the year ($p<0.05$).
162 Caffeine (autumn, 2234 vs summer, 2526; winter, 2547; spring, 2532) and salicylic acid (autumn,
163 2342 vs summer, 2836; winter, 2649; spring, 2891) were lowest in autumn in comparison with the
164 rest of seasons which have similar masses of these PPCPs (Figure 2).

165 The results of MI estimate using linear regression indicated significant association between ambient
166 temperatures and atenolol, acesulfame, and naproxen. An increase of 1°C in average temperature
167 associated with decrease at 1.3 g/d (95% CI: -2.2-(-.4), $p<0.05$) for atenolol while an associated
168 increase for acesulfame was observed at 36.5 g/d (95% CI: 25.2-47.8, $p<0.01$) and 0.8 g/d (95% CI:
169 0.02-1.55, $p=0.05$) for naproxen. No significant association was observed between temperature and
170 the remaining PPCPs. Quantitatively, an increase of 1°C in average temperature associated with

171 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)
172 for salicylic acid; whereas, the inverse relationship was found for caffeine with a decrease of 20.4
173 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-
174 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
175 presented in Figure 3. The results of sensitivity analysis, which replaces average temperatures with
176 maximum and minimum temperatures in regression analyses for 3 PPCPs with significant
177 associations were reported in Supplement 2. A decrease of 1.2 g/d (95%CI: -1.83-(-.55) and 1 g/d
178 (95%CI: -1.43-(-.58) of atenolol daily load were associated with 1°C increase in maximum and
179 minimum temperatures, respectively. In contrast, an increase of 44 g/d (95%CI: 36.7-51.2) and 24.8
180 (95%CI: 19.8-29.9) of acesulfame daily load were associated with 1°C increase in maximum and
181 minimum temperatures. Likewise, an increase of naproxen of 0.7 g/d (95%CI: 0.27-1.16) was
182 associated with 1°C increase in minimum temperatures but not significant with increase in
183 maximum temperatures.

184

185 **4. Discussion**

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

191

192 4.1. Compounds having significant association with ambient temperature

193 + Acesulfame

194 Acesulfame is an intense sweetener which can be used as a flavour enhancer or a substitute for
195 sugars. It is used in a variety of products, many of them beverage and juices that are consumed
196 during warm temperature such as Coke and Pepsi (acesulfame is used in combination with another

197 intense sweetener, aspartame). Rossato et al. (2015) has indeed reported the higher consumption of
198 sweetened beverage in summer than in winter in southern Brazil where the climate is similar to the
199 studied catchment. Therefore, it is reasonable to expect that the consumption of acesulfame will
200 increase during warmer months of the year. The strong association between ambient temperature
201 and the consumption of soft drinks as represented by the level of acesulfame in wastewater found in
202 this study ($p<0.001$) proved that wastewater analysis can be used to investigate associations in
203 epidemiological studies.

204

205 + Atenolol

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

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

216

217 + Naproxen

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

221 Since naproxen is used to treat a wide range of symptoms, it is difficult to attribute the positive
222 association of its daily load with ambient temperature ($p=0.045$) to a specific disease. Regarding

223 arthritis, since there are two opposite impacts of seasonal change to its prevalence (high temperature
224 in summer reduce the occurrence of arthritis but at the same time, higher relative humidity in
225 summer can cause more arthritis cases - (Patberg and Rasker 2004), the overall seasonal variation of
226 arthritis incidents (and arthritis-related naproxen consumption) would be insignificant. It would be
227 very useful for the investigation of weather effects on arthritis if an arthritis-specific chemical can
228 be identified in wastewater.

229

230 4.2. Compounds having non-significant association with ambient temperature

231 + Caffeine

232 Caffeine occurs naturally in certain food and drinks such as coffee, tea and cocoa and has a long
233 history of safe use as a mild stimulant. Caffeine can also be added into other products including
234 cola-type soft drinks, formulated caffeinated beverages (energy drinks) and energy shots (FSANZ
235 2016). Such a wide range of both hot and cool products containing caffeine may be the reason why
236 there was no significant association between caffeine consumption and ambient temperature.

237 Although not significant ($p=0.076$), there was an approximately 1% decrease in caffeine
238 consumption for each 1°C increase in ambient temperature. As coffee and tea are likely the main
239 caffeine containing products consumed by the general population, such an observation seems
240 logical. The increase in consumption of caffeinated drinks (cola-type soft drink) as indicated by the
241 increase of acesulfame possibly contribute to make the decrease insignificant.

242 It is noted that caffeine is not completely stable in the sewer system (O'Brien et al., 2017) although
243 it has been reported that it is stable in wastewater only (Senta et al., 2015). Therefore, there may
244 have been more degradation of caffeine in the sewer system in the summer than in the winter, which
245 also leads to a reduction in the daily load of caffeine measured during summer time. In our opinion,
246 caffeine is not a good biomarker since there are too many potential contributors to its load in
247 wastewater and its degradation in the sewer system would add more uncertainty to the temporal
248 variation of daily loads.

249 + Codeine

250 Codeine is a pain killer belonging to the opioid group. Codeine is used to provide relief from a
251 number of conditions including pain (sometimes in combination with aspirin or paracetamol), dry
252 irritating cough, diarrhoea, cold and flu. Codeine is available in Australia in both prescription and
253 over-the-counter forms (Gisev et al., 2016).

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

260

261 + Carbamazepine

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

269

270 + Hydrochlorothiazide

271 Hydrochlorothiazide is a diuretic drug which is used to treat high blood pressure and swelling. In
272 Australia, it is a prescription drug mainly used for high blood pressure. No significant association
273 between consumption of hydrochlorothiazide with ambient temperature was observed in this study
274 although there was a slight (nonsignificant) decrease in the daily load of hydrochlorothiazide in

275 wastewater when ambient temperature increased. In the literature, blood pressure is reported to be
276 higher in colder months with physiological cause of reflex coronary and systemic vasoconstriction
277 (Cuspidi et al., 2012). It seems plausible that physical activity increases in warmer months thus
278 reducing blood pressure (Rossi et al., 2012). That means an increase in temperature is likely to lead
279 to a reduction in the use of antihypertensive drugs including hydrochlorothiazide (Cuspidi et al,
280 2012). The finding of this study could not confirm this hypothesis, which probably indicates that
281 hydrochlorothiazide is mainly used for preventative/maintenance treatment of hypertension in the
282 monitored population.

283

284 + Salicylic acid

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

294

295 4.3 Implication of the findings

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

301 degradation is quantifiable but more research is needed to quantify the degradation in different
302 sewer conditions.

303 The outcome of this study also indicated the significant seasonal variation of some chemicals such
304 as acesulfame that could affect its use as biomarker for population size estimation (O'Brien et al.,
305 2014, Lai et al., 2015), especially when it is used to estimate the population in a narrow period of
306 time (Gao et al., 2016). The knowledge of seasonal variability obtained from this study should be
307 utilised and incorporated into the model in the future to improve the accuracy of the model
308 prediction.

309

310 4.4 Limitations & recommendations

311 + Representative biomarkers of temperature-adverse health effects

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

316

317 + Stability of the PPCPs in the sewer:

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

325

326 + Variation in daily (*de facto*) population:

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

337

338 + Imputation

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

344

345 + Lacking of individual data:

346 The study had no access to individual-level data on substance usage, socio-economic, and adaptive
347 factors (e.g. air conditioning) which might reflect the actual relationship between high ambient
348 temperatures and health conditions, resulting to substance use (i.e. medication). It would be more
349 comprehensive if these factors are investigated and considered in analysis in future study.

350 There are also lack of data on sweetened drinks and drug sales to validate the measured loads from
351 the sewage system, which somewhat limited the validity of the study outcomes.

352

353 **5. Conclusions**

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

360

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366

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