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36

## 37 **Introduction**

38 Wastewater analysis (WWA) or Sewage epidemiology was first proposed to estimate  
39 drug consumption by US EPA environmental scientist Daughton in 2001 (Daughton,  
40 2001). The approach was based on the assumption that when particular drugs are  
41 consumed, the active parent compounds and its metabolites are excreted through urine  
42 and faeces into the sewer system, and thus enter the sewage treatment plants (STPs).  
43 By measuring levels of target parent compounds and/or metabolites, back-estimation  
44 of drug use in the population of a STP catchment area could be realised. Compared  
45 with conventional methods such as questionnaires and socio-epidemiological surveys  
46 including crime statistics, medical records, drug production and seizure rates, WWA  
47 has the advantage of providing objective, continuous, near real-time estimates of drug  
48 consumption in the population (van Nuijs et al., 2011a). Additionally, using WWA to  
49 estimate illicit drug consumption can overcome ethical issues associated with some  
50 other methods (Hall et al., 2012; Khan et al., 2014).

51 A lot of effort has been made to improve all aspects of WWA. These include  
52 sampling protocol development to get representative samples, developing robust,  
53 sensitive analytical methods and more recently normalizing chemical loads to per  
54 capita estimates that allows more accurate comparisons between different cities and  
55 even countries (Ort et al., 2010a; Ort et al., 2010c; Zuccato et al., 2011; Zuccato et al.,  
56 2005, O'Brien et al., 2014). Many researchers from a wide range of fields including  
57 but not limited to analytical chemistry, environmental science, epidemiology, forensic  
58 science, sociology and statistics from all over the world have joined the 'WWA  
59 research community' to improve the innovative approach during the past years. This  
60 is evident by a series of conferences organised by the European Monitoring Centre for  
61 Drug and Drug Addiction called *Testing the waters* starting in May 2013 in Lisbon,  
62 Portugal and the next session is planned for 2015 in Ascona, Switzerland.

63 This review article attempted to present a brief overview of the development of WWA  
64 to date with a focus on its successful application to estimate illicit drug consumption  
65 and the future applicability of this approach in China.

## 66 **1. Current state of WWA**

### 67 **1.1 Application of WWA in estimating illicit drug consumption**

68 The approach of WWA was applied for the first time in Italy in 2005 (Zuccato et al.,  
69 2005) and was soon applied in several other cities in Europe and the US (van Nuijs et  
70 al., 2011a). Since then WWA has been applied to monitor the use of the classical  
71 illicit drugs such as cocaine, heroin, amphetamines and cannabis (Thomas et al., 2012;  
72 van Nuijs et al., 2011a) and more recently to identify the use of new psychoactive  
73 substances (Reid et al., 2014; van Nuijs et al., 2014).

74 Reports of illicit drugs estimated by WWA have come from multiple countries  
75 including Australia, Belgium, Canada, Croatia, Finland, France, Italy, Ireland, The  
76 Netherlands, Sweden, the UK and the US. Estimation of illicit drug use has been  
77 performed not only in small communities such as prisons (Postigo et al., 2011), and  
78 recreational regions (Lai et al., 2012), but also in large cities like Paris and Hong  
79 Kong (Karolak et al., 2010; Lai et al., 2013). Most obtained results are in agreement  
80 with data from traditional socio-epidemiological surveys, however some  
81 underestimation and/or overestimation has been identified for some particular drug(s)  
82 (Baker et al., 2014). Thomas et al. (2012) conducted a comparison of illicit drug  
83 consumptions in 19 cities across Europe through WWA and identified distinct  
84 temporal and spatial differences in drug consumption between these cities during a  
85 single week of sampling in 2011. In 2013, Nefau et al. (2013) studied the presence of  
86 17 illicit drugs both in influent and effluent sewage from 25 French STPs.  
87 Consumption maps were drawn for cocaine, opiates, cannabis and amphetamine-like  
88 compounds. Significant geographical differences were observed which highlighted  
89 that drug consumption within a country might not be homogeneous. Similarly, Khan  
90 et al (2014) applied WWA to evaluate the use of 10 illicit drugs in 4 megacities in  
91 China and found different consumption patterns between north and south China. At  
92 the same time, Li et al. (2014) also reported the use of amphetamines across a range  
93 of communities in the metropolitan area of Beijing. A summary of WWA applications  
94 for assessing illicit drug consumption worldwide is shown in **Table 1**.

96 **1.2 Exploration in other areas**

97 In addition to illicit drugs, there are some initial attempts to estimate the use of  
98 alcohol and tobacco, the two most common substances that have potential to  
99 negatively impact population health and cause several social problems such as crime  
100 and injuries. Reid et al conducted the first study to estimate the use of alcohol in Oslo,  
101 Norway using WWA (Reid et al., 2011) where the highest consumption of the alcohol  
102 was observed during weekends. Sixty one percent of weekly alcohol consumption was  
103 reported on Friday and Saturday alone. Over the last year, two studies were carried  
104 out to estimate the total amount of tobacco use (nicotine consumption) in different  
105 communities through WWA (Castiglioni et al., 2014; Lopes et al., 2014). The  
106 findings produced by WWA were in close agreement with survey data and can  
107 differentiate the level of tobacco consumption among different populations.

108 In addition to monitoring common substances of abuse (illicit drugs, alcohol, and  
109 tobacco), WWA can be considered as Sewage chemical-information mining (SCIM).  
110 In a broader sense, because the interpretation of acquired information from WWA can  
111 measure a vast amount of chemicals, WWA can provide a variety of information  
112 about the population living in a particular STP catchment. It could also be used as a  
113 powerful tool to evaluate community-wide human health with isoprostanes (stress  
114 biomarkers) already proposed by Daughton as ideal candidates (Daughton, 2012b).  
115 Daughton also conceptualized an approach to estimate the real-time population size in  
116 the sewer catchment using coprostanol as a population biomarker (Daughton, 2012a)  
117 although further study should be conducted to validate the applicability of coprostanol  
118 in WWA (Chen et al., 2014).

119 Venkatesan and Halden have applied SCIM to forecast ecological and human health  
120 risks of manmade chemicals by analysing sewage sludge instead of wastewater for  
121 persistent organic pollutants (POPs) which are non-polar and thus less likely to be in  
122 the wastewater itself (Venkatesan and Halden, 2014). The result revealed 70%  
123 agreement between WWA and biological specimens' analysis, and suggested that  
124 analysing sewage sludge can inform human health risk assessments by providing real-  
125 time information on toxic exposures in human populations and associated body  
126 burdens of harmful, accumulative, environmental pollutants. More outcomes could be  
127 achieved if the efforts across several disciplines including clinical chemistry,

128 environmental chemistry, environmental science, medicine and microbiology, and  
129 were combined. With continuous improvement of the method, SCIM appears a  
130 feasible and effective tool to identify the connection between population health and  
131 chemical consumption and/or exposure and thus enabling better protection of the  
132 population from such hazards.

### 133 **1.3 Current research to improve the methodology**

134 Current research mostly focuses on evaluating and minimizing uncertainties of the  
135 whole WWA procedure such as collecting representative sewage samples, simplifying  
136 sample pre-treatment, selecting suitable biomarkers in terms of sensitivity and  
137 stability, optimizing instrumental analysis, and refining the back calculation of results.  
138 Castiglioni et al. integrally addressed uncertainties associated with all the steps  
139 necessary to estimate community drug consumption through WWA (Castiglioni et al.,  
140 2013). Using data gathered from 12 laboratories, the uncertainties can range from 5–  
141 10% for sampling to 1–34% for replicated chemical analysis and 26% for back-  
142 calculation of cocaine use. But the highest uncertainty comes from the estimation of  
143 population size, which varied from 7 to 55%. Based on this study, the authors also  
144 suggested a best practice protocol to minimize the overall uncertainties of the entire  
145 procedure (Castiglioni et al., 2013).

146 Several studies have attempted to address individual issues facing WWA. For instance,  
147 Martínez Bueno et al. developed a solvent-free method for simultaneous identification  
148 and quantification of 22 illicit drugs by liquid chromatography coupled to tandem  
149 mass spectrometry (LC-MS/MS), which is deemed to be a good technique for WWA  
150 due to its simplicity, cost-effectiveness and lower environmental footprint (Martínez  
151 Bueno et al., 2011). Meanwhile, Baker and Kasprzyk-Hordern evaluated the  
152 commonly used methodologies for sample collection, storage and preparation for  
153 SCIM with solid-phase extraction (SPE) and LC-MS/MS analysis (Baker and  
154 Kasprzyk-Hordern, 2011). They concluded that from the perspective of stability,  
155 composite samples are unsuitable with regards to certain compounds like heroin and  
156 6-acetylmorphine; these two drugs reported a decrease in stability of 66% and 26%  
157 respectively after 12 hours in raw sewage at 2°C. Baker and Kasprzyk-Hordern also  
158 emphasised that more rigorous reporting of method validation data are needed as  
159 underreported parameters might have major impacts on the overall performance.

160 For the estimation of consumed drug masses in the catchment using the optimum  
161 sampling method as outlined by Ort et al. (2010b) and common chemical analysis, Lai  
162 et al. calculated the overall uncertainty to be in the range of 20-30% (relative standard  
163 deviation, RSD) (Lai et al., 2011). Lai et al. also suggested using chemicals of  
164 relatively high use in the population as a basis to estimate the population size. To  
165 further address this issue, O'Brien et al. have screened wastewater samples and found  
166 14 chemicals which could be use as real-time population markers. They then  
167 developed a model to estimate the population contributing to the sewage influents  
168 based on the load of those chemicals. Through calibrating their model with mass loads  
169 of 14 chemicals with accurate population counts (the samples were taken on Census  
170 day), they found that relatively accurate population sizes can be estimated for  
171 catchment >100,000 people (O'Brien et al., 2014).

## 172 **2 General procedure of WWA**

173 WWA is generally carried out using the procedure shown below (**Fig. 1**).  
174 Simplification and standardization of the method as well as improvement of the  
175 accuracy and reliability of the final estimates are crucial in promoting WWA for  
176 routine monitoring.

### 177 **2.1 Pre-investigation**

178 A systematic and comprehensive pre-investigation about the catchment area and STP  
179 under investigation is critical for reliable and accurate WWA estimates. Socio-  
180 economic conditions of the study area, contemporary and historical environmental  
181 monitoring data, population size and mobility in and out of the STP catchment area,  
182 and crime statistics should all be put into consideration to achieve reliable results. The  
183 investigation could be carried out through multiple approaches such as literature  
184 reviews, visiting and surveying STPs, discussions with local authorities such as law  
185 enforcement officers, relevant medical staff as well as environmental officers, local  
186 and national councillors etc. The pre-investigation may strengthen the results'  
187 reliability of WWA studies particularly where drug consumption estimates are the  
188 goal. These alternate methods for assessing community drug consumption are not  
189 limited to the pre-investigation stage but are also may be relevant to reconsider during  
190 or even post the sampling period. Examples of this include combining drug seizure

191 data with loads in the wastewater and assessing the scale of the market based on the  
192 mass load of drugs removed.

## 193 **2.2 Sampling**

194 Samples are taken from the inlets of STPs since the influent can be regarded as a  
195 pooled urine sample (although diluted and contaminated) from a large population  
196 before it is altered by different treatment processes in the STPs. However, in addition  
197 to sewage influent, activated sludge from the aerobic or anaerobic tanks has also been  
198 used as samples for WWA (Venkatesan and Halden, 2014).

199 For sample volume, one litre is the most common. However, sample volumes from  
200 0.05 to 10 litres have been reported. A variety of sampling methods have also been  
201 studied. Continuous flow, volume and time proportional sampling with  
202 commercialized auto-samplers have all been used in different studies. Grab samples  
203 have also been used in several studies (Hummel D, 2006). Ort et al. (2010b) found  
204 continuous flow proportional samples collected over a 24 hour period as the optimum  
205 sampling method as these are more representative of a whole day and are better at  
206 capturing events. Samples from weekdays, weekends and public holidays across the  
207 whole year have all been investigated to reveal temporal patterns of drug consumption.  
208 While it is possible for each research group to establish a continuous flow  
209 proportional sampling system, there is a need for the development of a commercial  
210 auto-sampler that use this optimal sampling method. This would allow for a  
211 standardized sampling approach for WWA while sampling at the different STPs (Ort  
212 et al., 2010b).

213 Detailed discussion about sampling practices for wastewater has been conducted and  
214 a comprehensive sampling guide with the aim of reducing uncertainties has been  
215 proposed (Ort et al., 2010c). Evaluation of flow measurement, choice of sampling  
216 mode, determination of frequency and location have all been discussed in the  
217 abovementioned paper. For long-term routine monitoring, on-line auto-samplers are  
218 essential for representative sampling with reliability, efficiency and from economic  
219 aspects. More research should be conducted to evaluate uncertainties brought by  
220 different sampling parameters in the future.

## 221 **2.3 Biomarker selection**

222 Selection of suitable biomarkers is an important factor for WWA. There are several  
223 criteria for appropriate WWA biomarkers as suggested by Daughton (Daughton,  
224 2012b) including: produced exclusively by humans (i.e., not introduced by unrelated,  
225 exogenous mechanisms, e.g. illicit drug discharge), excreted in sufficient quantities  
226 (to allow detection in sewage), sufficiently stable in the sewer pipeline, amenable to  
227 cost-effective, reproducible analysis, and for several health status biomarkers they  
228 should be excreted at elevated levels under “stressed condition” significantly different  
229 to the baseline range of the chemicals excreted under “normal condition” .

230 While biomarkers have been one of the most popular research topics in clinical  
231 science in the past decades, there were limited studies on biomarkers that can be used  
232 in WWA. As suggested by Daughton, one should start at the list of common clinical  
233 biomarkers and test them against the appropriate criteria (Daughton, 2012b). One of  
234 the criteria that has been tested in several studies is the stability of the biomarkers in  
235 the wastewater matrix and under sewer conditions. Until recently, most parent  
236 compounds and metabolites were used as biomarkers in WWA for monitoring of  
237 illicit drug consumption with the assumption that they were stable in the sewer system.  
238 But some of these biomarkers (such as cocaine or 6 acetyl morphine) are quite  
239 unstable (Thai et al., 2014; van Nuijs et al., 2012) which means that previous studies  
240 may have underestimated the amount of drugs consumed in certain catchments. To  
241 address this, excretion profiles of biomarkers including parent to metabolite ratio  
242 should be further investigated by pharmacologists, biochemists and sewer engineers  
243 to get a better grasp of consumed load versus measured load within wastewater.

244 For WWA to reach its’ full potential, more biomarkers should be identified and tested  
245 against all of Daughton’s proposed criteria to expand the WWA application to  
246 evaluate other markers of population health, real-time population size estimation,  
247 pollutant exposure, and promote WWA as a routine monitoring approach in STPs.

## 248 **2.4 Pre-treatment**

249 Filtration or centrifugation of the collected sample is essential to remove solids in the  
250 sample. However, this step may cause loss of certain analytes due to substantial  
251 affinity for particulate for some chemicals (Baker and Kasprzyk-Hordern, 2011;



252 Plo'sz et al., 2013). Adding isotope labelled internal standards before filtration or  
253 centrifugation is an effective approach to evaluate and minimize these uncertainties.  
254 Full and accurate understanding about biomarker absorption kinetics is also useful to  
255 minimize the uncertainties associated with correction factors for the back calculation  
256 process.

257 The observed concentrations of target compounds and their metabolites in raw sewage  
258 are often at the level of ng/L or even lower and thus pre-concentration is required. In  
259 most cases solid-phase extraction (SPE) is conducted prior to LC-MS/MS analysis in  
260 order to concentrate and remove matrix interferences from the samples.

261 Baker and Kasprzyk-Hordern have critically evaluated the whole sample preparation  
262 process from sample collection to storage and preparation for analysis. This was  
263 conducted for both pharmaceuticals and illicit drugs in surface water and wastewater  
264 using SPE-LC-MS/MS techniques (Baker and Kasprzyk-Hordern, 2011). The study  
265 showed that uncertainties associated with biomarker degradation can be minimized if  
266 proper pre-treatment is applied. The current optimal method is to collect samples in a  
267 refrigerated (4°C) container, subsample them, acidify with hydrochloric acid and then  
268 either refrigerate at 4°C in the dark or freeze if the samples are to be analysed at a later  
269 date to minimize biotransformation/degradation of the biomarkers. Degradation of  
270 illicit drugs and metabolites in wastewater has been evaluated by van Nuijs et al  
271 (2012). They concluded that most parent compounds and metabolites of illicit drugs  
272 such as amphetamine, methamphetamine, ecstasy and EDDP are considerably stable  
273 for 12 hours or longer, however some drugs such as cocaine and ecgonine methylester  
274 showed a clear decrease in concentration over this period.

275 Since the SPE process is costly, time consuming and requires larger sample volumes,  
276 simpler procedures are starting to be developed. Berset et al developed a large volume  
277 direct injection method for the simultaneous analysis of licit and illicit drugs in  
278 surface water and waste water (Berset et al., 2010). It should be noted that analytical  
279 instruments are becoming more sensitive and when combined with the development  
280 of optimised methods, it seems plausible that reliable methods for analysing illicit  
281 drugs in wastewater with acceptable limits of detection (LOD) without the need for  
282 SPE is possible. This would then enhance the argument for routine WWA monitoring  
283 as a tool for measuring drug consumption. The improved sensitivity of some  
284 instruments (i.e. LC-MS/MS) is already adequate for determination of numerous

285 chemicals in wastewater using simple pre-treatment technique such as acidifying and  
286 filtering only (e.g. the pharmaceuticals in O'Brien et al., 2014).

## 287 **2.5 Instrumental Analysis**

288 Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is used  
289 in almost all WWA studies due to its high sensitivity, versatility and selectivity. A  
290 variety of mass spectrometers have been used in different WWA studies (Castiglioni  
291 et al., 2011). These include triple quadrupole mass spectrometer (QqQ), Orbitrap, and  
292 quadrupole time-of-flight mass spectrometers (QTOF). Most applications use QqQ  
293 since it provides better selectivity and thus can achieve low detection limits. For some  
294 compounds the use of QqQ may eliminate the need for sample extraction and clean-  
295 up by using direct-injection technique (Trenholm and Snyder, 2011). Since QTOF and  
296 linear ion trap fourier transform (LIT-FT) have higher mass resolution and mass  
297 accuracy than QqQ, they can be better choices for drug identification and new  
298 synthetic drug screening in samples with complex matrix (de Voogt et al., 2011;  
299 Hernández et al., 2011).

300 Multiple reaction monitoring (MRM) mode is used for both qualification and  
301 quantification. Several studies grouped illicit drugs according to their  
302 physicochemical properties and different analytical parameters (such as  
303 chromatography and ionization mode) in order to achieve optimum separation and the  
304 highest MS resolution. As newer, more sensitive LC-MS/MS, QTOF and Orbitrap  
305 instruments are developed, it is expected that these will have a significant role in  
306 WWA applications for their incomparable advantages in rapid wide-scope screening  
307 and providing accurate mass data of both parent molecules and daughter ions for  
308 identification in complex matrices. Some of these instruments such as QTOF and  
309 Orbitrap have the ability to acquire both qualitative and quantitative information from  
310 samples in one injection and thus chemicals of interest can be retrospectively identify  
311 in these samples. Once the spectra are obtained, there is no need for reanalysing the  
312 sample. This is particularly useful for emerging drugs or pollutants where the  
313 analytical chemists usually have to catch-up to those producing the chemicals. In the  
314 future, standardised configurations could be implemented for routine illicit drug  
315 consumption monitoring while customised configuration will play an important role  
316 in the expanding WWA applications.

## 317 **2.6 Back estimation of consumption/exposure data**

318 The estimation of illicit drug consumption (IDC) in the population is carried out by  
319 using the equation below (Zuccato et al., 2008):

$$320 \text{ IDC (mg/person/day)} = \frac{C_i * F * \frac{R_i}{E_i}}{P}$$

321

322 Where  $C_i$  is the concentration of a given drug residue  $i$  (parent drug or metabolite)  
323 measured in raw sewage samples (mg/L),  $F$  is the total flow during the sampling  
324 period ( $L$ , 24 hours),  $P$  is the number of people in the catchment,  $R_i$  is the ratio of  
325 molar mass of parent drug to its metabolite and  $E_i$  is the average excretion rate of a  
326 drug residue  $i$ .

327 While  $C_i$ ,  $F$  and  $R_i$  can be measured readily in the laboratory or at the STP, estimating  
328 the values of  $P$  and  $E_i$  is more challenging.  $E_i$  can be estimated through meta-analysis  
329 of clinical data (Khan and Nicell, 2011). Meanwhile, estimation of population size  
330 could be performed by using resources like census data, STP design capacity, or using  
331 wastewater parameters such as BOD, COD, total phosphorus, total nitrogen (van  
332 Nuijs et al., 2011b). O'Brien et al used a combination of 14 chemical markers of  
333 population size (most of them pharmaceuticals) to estimate the population size using a  
334 Bayesian inference model (O'Brien et al., 2014). Chen et al evaluated seven potential  
335 population biomarkers and found that 5-hydroxyindoleacetic acid and cotinine could  
336 potentially be used as biomarkers for population estimation (Chen et al., 2014). There  
337 are also attempts to evaluate real-time population size by analysing mobile phone  
338 signals in the catchment area which could also be applied for population estimation  
339 (Ran et al., 2013).

340 It should be noted that there may be some licit sources of biomarkers used to estimate  
341 illicit drugs (e.g. morphine can be generated from the consumption of both heroin and  
342 licit codeine) and hence estimates of illicit drug consumption can be affected by this  
343 phenomenon. In such cases, cautious interpretation should be taken. The typical way  
344 to solve this issue is to subtract the average amount of legal medication/pharmaceuticals  
345 that are used in the studied population from the total chemical load measured in  
346 wastewater samples. The input load coming from licit source could be better  
347 evaluated by analysing prescription data and wastewater from the hospitals in the

348 studied catchment. If the input from licit source is significant (e.g. morphine input  
349 from the use of codeine compared to morphine input from heroin), the lack of  
350 accurate data on licit input could render the WWA estimate less valid and thus WWA  
351 should not be used in such case.

352 For other chemicals, the process of back estimation is similar as long as the necessary  
353 parameters are available (especially  $E_i$  and  $P$ ). Some chemicals may also come from  
354 other sources such as dumping parent compounds into the sewer which should be  
355 taken into account when interpreting the estimated values.

## 356 **2.7 Uncertainties and Limitations**

357 Uncertainties may occur in every step from sampling to back-calculation in WWA  
358 studies. Evaluations about uncertainties related to the whole procedure and also  
359 individual aspects have been performed in previous studies. Castiglioni et al  
360 (Castiglioni et al., 2013) evaluated uncertainties associated with all the steps  
361 commonly used in WWA with optimized experimental parameters for each step  
362 defined. Plósz et al. (2013) investigated the biotransformation kinetics and sorption of  
363 cocaine and its metabolites. Factors influencing the estimation of cocaine in sewage  
364 with WWA have been evaluated. Results show that omitting in-pipe bio-  
365 transformation affects the accuracy of back-calculated cocaine use estimates. In  
366 addition, ex-vivo biotransformation of target compounds should be considered during  
367 back calculation (Plósz et al., 2013). van Nuijs et al. evaluated the stability of nine  
368 illicit drugs and metabolites in samples collected from wastewater influent. The  
369 results suggest that it is quite important to take the compounds stability into account  
370 when dealing with drugs that show significant biotransformation in sewage (van Nuijs  
371 et al., 2012).

## 372 **3 Applicability in China**

### 373 **3.1 Research related to wastewater in China**

374 China has the largest population size (1.4 billion) in the world. The total sewage  
375 created across the country is estimated as high as 280 billion litres per day  
376 (calculation based on 200 L per capita per day), and most of the populated areas are

377 sewerage and connected to STPs. WWA could thus be used in the evaluation of illicit  
378 drug consumption as well as alcohol, tobacco (Reid et al., 2011) and other chemicals  
379 which are closely related to public health and social sustainability.

380 Recently a small number of WWA studies were conducted in China for estimating  
381 illicit drug consumption. Lai et al. utilised WWA in Hong Kong in 2011 to evaluate  
382 daily and diurnal patterns of illicit drug consumption in the megacity (Lai et al., 2013).  
383 Khan et al applied WWA in mainland China for the first time to monitor the  
384 consumption of 14 illicit drugs in 4 megacities with samples from 9 STPs covering  
385 approximately 11.4 million inhabitants. The results demonstrate that China has  
386 different drug consumption patterns to European countries. Even within China, the  
387 difference in drug use between the north and south could be observed (Khan et al.,  
388 2014). It is proposed that licit manufacture of drugs is more stringent and thus  
389 distinguishing licit from illicit sources of drugs may be possible by further research on  
390 isomer production ratios of the parent compounds and conducting chiral analysis of  
391 wastewater samples. This could potentially lead to monitoring drug manufacture,  
392 formulation, distribution and consumption (Daughton, 2011).

393 Several review articles (Liu et al., 2009; Liu, 2005; Nie et al., 2011; Xie et al., 2004;  
394 Zhou et al., 2007) have presented a range of research on persistent organic pollutants  
395 (POPs) and emerging pollutants such as pharmaceuticals and personal care products  
396 (PPCPs) in the aquatic environment, particularly in regards to wastewater and how to  
397 better manage these chemicals. The articles cover studies on the occurrence of POPs  
398 and PPCPs in STPs (Fan et al., 2011; Zhao et al., 2011), pollutants removal  
399 mechanism in STPs (Jiao et al., 2012), fate and degradation of certain group of POPs  
400 or PPCPs particularly in regard to river water (Lian and Liu, 2013; Liu, 2011; Zhang,  
401 2013), development and optimization of analytical method to qualitatively and  
402 quantitatively determine pollutants in various matrices (Chen et al., 2011; Yuan et al.,  
403 2013). However, most studies work ‘downstream’ focusing on environmental  
404 outcomes rather than ‘upstream’ which could provide the ability to evaluate human  
405 exposure and the associated health risks.

### 406 **3.2 Drug consumption and control in China**

407 Illicit drug abuse in China can be traced back to the 1760s during the Qing dynasty.  
408 The number of drug users in China increased dramatically after the Opium War. Issue  
409 of drug abuse re-emerged in the last two decades which is mainly attributed to global  
410 drug trafficking activities during the implementation of governmental reform and the  
411 open-door policies of the late 1980s (Lu et al., 2008; Qian et al., 2006). Evidence has  
412 shown that over the past decade cocaine and other illicit drug abuse has increased in  
413 East and Southeast Asia. The Ministry of Public Security in China estimates that there  
414 are currently more than two million illicit drug users in China (Xinhua News, 2013).  
415 Also, cocaine seizures in mainland China and Hong Kong increased from  
416 approximately 600,000 kg in 2010 to 800,000 kg in 2011 (UNODC, 2013).  
417 Illicit drug consumption has caused significant consequences for human health and  
418 social stability. In response the Chinese government monitors illicit drug prevalence  
419 and control. However, during the past decade the number of people abusing drugs has  
420 increased significantly and younger generations have become victims of drug  
421 addiction. Synthetic psychotropic drugs (like methamphetamine) prevail among drug  
422 consumers. This situation requires the authorities to design effective policies to  
423 control drug abuse as well as monitoring the effectiveness of these policies.  
424 The ability of WWA to measure near real-time consumption of drugs can assist  
425 authorities in assessing the impact of the strategies they've adopted and thus better  
426 manage the situation. In order to develop and implement effective anti-drug strategies,  
427 authorities need information about temporal and geographical patterns of illicit drug  
428 consumption. Wastewater analysis could provide continuous and objective illicit drug  
429 consumption information to the relevant authorities.

### 430 **3.3 Overview of sewage treatment plants and analytical laboratory capabilities in** 431 **China**

432 There are more than 3,000 domestic STPs in China covering most densely populated  
433 areas. The number of STPs is still rapidly increasing with substantial investment from  
434 the Chinese government to reduce environmental impacts. Capacity of these plants  
435 range from less than 10 ML/day to more than 1,000 ML/day. Population size served  
436 differs from a few thousand to hundreds of thousands. Most STPs have online

437 monitoring of flow, pH, COD and ammonia using auto-samplers and nearly all of  
438 these plants take regular samples for compliance purposes which may make it easier  
439 to get samples for WWA applications. Therefore, WWA could potentially capture  
440 chemical consumption and/or exposure for a variety of population sizes with  
441 considerably small effort and cost compared with traditional surveys.

442 China has strong analytical chemistry capabilities with hundreds of research centres and  
443 laboratories located across the country equipped with state of the art analytical  
444 instruments. There are more than 200 laboratories equipped with LC-MS/MS  
445 instruments in various configurations and thus have sufficient analytical abilities to  
446 apply WWA at the national level. Chen et al. (2011) developed a paper strip  
447 extraction ultra-performance liquid chromatography tandem mass spectrometry (PSE-  
448 UPLC-MS/MS) method to determine 9 PPCPs in sewage sludge. With further  
449 optimization, this method could be suitable for WWA applications for drug  
450 consumption estimation as well for the measurement of other chemical biomarkers of  
451 consumption and exposure. More recently Yuan et al. (2013) developed and applied  
452 an automated solid phase extraction-high performance liquid chromatography coupled  
453 with electrospray ionization tandem mass spectrometry (ASPE-HPLC-ESI-MS/MS)  
454 method for the quantification of 13 antipsychotics. Eleven of the thirteen  
455 pharmaceuticals were detected in all 35 samples from one STP. Further studies on  
456 wastewater treatment processes, human health biomarkers and risk assessment could  
457 all benefit through promoting WWA as a feasible and powerful tool for forensic  
458 science, environmental science and epidemiology.

#### 459 **3.4 Potential issues with applying WWA/SCIM in China.**

460 There is no doubt that WWA can provide indicative information for the assessment of  
461 illicit drug consumption. By sampling a variety of STPs and collaboration with the  
462 many advanced research facilities across China, WWA/SCIM could produce valuable  
463 information on current community health which could help define key areas of  
464 concern for both community health and maintaining social justice. However,  
465 investigation and assessment about the study area and objectives should be carried out  
466 before conducting WWA to maximise results. Most STPs constructed before the  
467 1990s receive influent that is a mixture of domestic sewage, industrial wastewater and  
468 stormwater. This may make it more challenging to apply WWA in these areas as the

469 chemicals in the industrial sewage could interact with the chemicals in domestic  
470 sewage and during rainfall events chemicals of interest may become too diluted to  
471 analyse feasibly.

472 By comparing concentrations of target chemicals in ambient environmental  
473 monitoring with results of available biomonitoring studies and WWA data, chemical  
474 consumption/exposure models could be developed for pollutants chemicals,  
475 biomarkers of human health, per capita environmental impact and others. One should  
476 also consider that there are huge population relocations during certain national  
477 holidays such as Chinese spring festival. Therefore real-time population estimates of  
478 the studied catchment area is essential to reduce under/overestimation of the per  
479 capita consumption and/or exposure of chemicals. These markers would also require  
480 some form of calibration for the studied catchment such as collecting samples during  
481 a census period.

482 As most of the STPs in a given city belong to a drainage group governed by the water  
483 resource bureau or the environmental protection agency in the municipal government,  
484 there might be concerns regarding ethical issues related to WWA studies. However, it  
485 was suggested that WWA doesn't raise major ethical concerns when used for public  
486 health purposes to monitor illicit drug use in large populations (Hall et al., 2012)  
487 although ethical issues may arise from concerns about possible indirect harm from  
488 using WWA in small areas such as prisons or entertainment venues. More effort is  
489 required from the research community, industry and government departments to  
490 promote WWA as an additional tool for illicit drug consumption monitoring.

## 491 **4 Conclusions**

492 Wastewater analysis is a promising approach to estimate illicit drug consumption and  
493 consumption/exposure of other chemicals of concern at the population level. Our  
494 review suggests that WWA could be a very useful tool in China. It could provide a  
495 relatively easy approach for China to monitor drug consumption and potentially drug  
496 trafficking and manufacturing. Early adoption of WWA/SCIM and archiving samples  
497 would allow China to both make assessments using the current knowledge, as well as  
498 create a sample bank that archives and allows reassessment of samples once analytical  
499 methods are developed or new chemicals of interest are identified. Combined with



500 traditional survey methods, WWA could be a powerful tool to optimize illicit drug  
501 consumption estimates and provide near real-time and objective data for the  
502 development of strategies concerning drugs of abuse. With progress in research on  
503 other WWA biomarkers, the approach will provide useful epidemiological data for  
504 health status including levels of certain diseases in different communities and might  
505 lead to the establishment of new monitoring approaches for population health.

506

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## 515 **References**

- 516 Baker, D.R., Barron, L., Kasprzyk-Hordern, B., 2014. Illicit and pharmaceutical drug consumption  
517 estimated via wastewater analysis. Part A: Chemical analysis and drug use estimates. *Sci. Total*  
518 *Environ.* 487, 629-641.
- 519 Baker, D.R., Kasprzyk-Hordern, B., 2011. Critical evaluation of methodology commonly used in  
520 sample collection, storage and preparation for the analysis of pharmaceuticals and illicit drugs in  
521 surface water and wastewater by solid phase extraction and liquid chromatography-mass  
522 spectrometry. *J. Chromatogr. A* 1218(44), 8036-8059.
- 523 Berset, J-D., Brenneisen, R., Mathieu, C., 2010. Analysis of illicit and illicit drugs in waste, surface and  
524 lake water samples using large volume direct injection high performance liquid chromatography  
525 - Electrospray tandem mass spectrometry(HPLC-MS/MS). *Chemosphere* 81(7), 859-866.
- 526 Castiglioni, S., Bijlsma, L., Covaci, A., Emke, E., Hernández, F.L., Reid, M., et al., 2013. Evaluation  
527 of uncertainties associated with the determination of community drug use through the  
528 measurement of sewage drug biomarkers. *Environ. Sci. Technol.* 47(3), 1452-1469.
- 529 Castiglioni, S, Senta, I, Borsotti, A, Davoli, E, Zuccato, E, 2014. A novel approach for monitoring  
530 tobacco use in local communities by wastewater analysis. *Tob. Control* (in Press).
- 531 Castiglioni, S., Zuccato, E., Fanelli, R., (Eds) 2011. *Illicit Drugs in the Environment*. John Wiley &  
532 Sons, Inc., Hoboken, New Jersey, USA.
- 533 Chen, C., Kostakis, C., Gerber, J.P., Tscharke, B.J., Irvine, R.J., White, J.M., 2014. Towards finding a  
534 population biomarker for wastewater epidemiology studies. *Sci. Total. Environ.* 487, 621-628.
- 535 Chen, W., Huang, J., Sui, Q., Yu, G., Zhu, W., 2011. Determination of nine pharmaceutical and  
536 personal care products in sludge by PSE coupled to UPLC-MS/MS. *Res. Environ. Sci. (in*  
537 *Chinese)* 24(8), 925-932.

- 538 Choi, B.K., Hercules, D.M., Gusev, A.I., 2001. Effect of liquid chromatography separation of complex  
539 matrices on liquid chromatography tandem mass spectrometry signal suppression. *J.*  
540 *Chromatogr. A* 907(1-2), 337-342.
- 541 Daughton, C.G., 2001. Illicit drugs in municipal sewage: proposed new nonintrusive tool to heighten  
542 public awareness of societal use of illicit-abused drugs and their potential for ecological  
543 consequences. In: Daughton C.G. Ternes T.A. (Eds.), *Pharmaceuticals and care products in the*  
544 *environment, scientific and regulatory issues*. American Chemical Society, Washington DC, pp.  
545 348-364.
- 546 Daughton, C.G., 2011. Illicit drugs: contaminants in the environment and utility in forensic  
547 epidemiology. In: Whitacre D.M., (Eds.), *Reviews of environmental contamination and*  
548 *toxicology*. Springer, New York, USA. pp. 59-110.
- 549 Daughton, C.G., 2012a. Real-time estimation of small-area populations with human biomarkers in  
550 sewage. *Sci. Total Environ.* 414, 6-21.
- 551 Daughton, C.G., 2012b. Using biomarkers in sewage to monitor community-wide human health:  
552 Isoprostanes as conceptual prototype. *Sci. Total Environ.* 424, 16-38.
- 553 de Voogt, P., Emke, E., Helmus, R., Panteliadis, P., van Leerdam, J.A., 2011. Determination of illicit  
554 drugs in the water cycle by LC–Orbitrap MS. In: *Illicit drugs in the environment: occurrence,*  
555 *analysis, and fate using mass spectrometry* (Eds). John Wiley & Sons, Inc., Hoboken, NJ, USA.  
556 pp. 87-114.
- 557 Fan, Q., Deng, S., Zhou, Q., Sui, Q., Huang, J., Yu, G., 2011. Occurrence and removal of  
558 perfluorinated compounds in municipal wastewater treatment plants. *Environ. Pollut. Contr.* (in  
559 Chinese). 33(1), 30-35.
- 560 Hall, W., Prichard, J., Kirkbride, P., Bruno, R., Thai, P.K., Gartner, C. et al., 2012. An analysis of  
561 ethical issues in using wastewater analysis to monitor illicit drug use. *Addiction* 107(10), 1767-  
562 1773.
- 563 Hernández, F., Sancho, J.V., Bijlsma, L., 2011. Wide-Scope screening of illicit drugs in urban  
564 wastewater by UHPLC–QTOF MS. In: *Illicit drugs in the environment: occurrence, analysis*  
565 *and fate using mass spectrometry* (Eds). John Wiley & Sons, Inc., Hoboken, NJ, USA. pp. 69-85.
- 566 Hummel D., Löffler D., Fink G., Ternes T.A., 2006. Simultaneous determination of psychoactive drugs  
567 and their metabolites in aqueous matrices by liquid chromatography mass spectrometry. *Environ.*  
568 *Sci. Technol.* 40(23),7321-7328.
- 569 Jiao, N., Song, X., Shen, Y., 2012. The effectiveness and affecting factors on the removal of PPCPs by  
570 membrane bioreactor. *Environ. Eng.* (in Chinese) 30, 25-29.
- 571 Karolak, S., Nefau, T., Bailly, E., Solgadi, A., Levi, Y., 2010. Estimation of illicit drugs consumption  
572 by wastewater analysis in Paris area (France). *Forensic Sci. Int.* 200, 153-160.
- 573 Khan, U., Nicell, J.A., 2011. Refined sewer epidemiology mass balances and their application to heroin,  
574 cocaine and ecstasy. *Environ. Int.* 37, 1236-1252.
- 575 Khan, U., van Nuijs, A.L., Li, J., Maho, W., Du, P., Li, K. et al., 2014. Application of a sewage-based  
576 approach to assess the use of ten illicit drugs in four Chinese megacities. *Sci. Total Environ.* 487,  
577 710-721.
- 578 Lai, F.Y., Bruno, R., Hall, W., Gartner, C., Ort, C., Kirkbride, P. et al., 2012. Profiles of illicit drug use  
579 during annual key holiday and control periods in Australia: wastewater analysis in an urban, a  
580 semi-rural and a vacation area. *Addiction* 108, 556-565.
- 581 Lai, F.Y., Bruno, R., Leung, H.W., Thai, P.K., Ort, C., Carter, S. et al., 2013. Estimating daily and  
582 diurnal variations of illicit drug use in Hong Kong: A pilot study of using wastewater analysis in  
583 an Asian metropolitan city. *Forensic Sci. Int.* 233, 126-132.
- 584 Lai, F.Y., Ort, C., Gartner, C., Carter, S., Prichard, J., Kirkbride, P. et al., 2011. Refining the estimation  
585 of illicit drug consumptions from wastewater analysis: Co-analysis of prescription  
586 pharmaceuticals and uncertainty assessment. *Water Res.* 45, 4437-4448.
- 587 Lian, J., Liu, J., 2013. Fate and degradation of nonylphenolic compounds during wastewater treatment  
588 process. *J. Environ. Sci.* 25(8), 1511-1518.

- 589 Liu, J., 2011. Removal of typical persistent organic pollutants (POPs) in domestic wastewater and their  
590 fading in the secondary treatment process. PhD thesis. University of Architecture and  
591 Technology Xi'an, Shannxi, China.
- 592 Liu, Y., Guan, Y., Mizuno, T., Zhang, X., Tsuno, H., Zhu, W. et al., 2009. Recent research advances of  
593 pharmaceuticals and personal care pollutants. *J. Tsinghua Uni. (Sci. & Technol.)* 49(3), 368-  
594 372.
- 595 Liu, Z., 2005. Environmental behavior characteristics and research progress of persistent organic  
596 pollutants. *Res. Environ. Sci. (in Chinese)* 18(3), 93-102.
- 597 Lopes, A., Silva, N., Bronze, M.R., Ferreira, J., Morais, J., 2014. Analysis of cocaine and nicotine  
598 metabolites in wastewater by liquid chromatography–tandem mass spectrometry. Cross abuse  
599 index patterns on a major community. *Sci. Total Environ.* 487, 673-680.
- 600 Lu, L., Fang, Y., Wang, X., 2008. Drug abuse in China: Past, present and future. *Cell Mol. Neurobiol.*  
601 28, 479-490.
- 602 Martínez Bueno, M.J., Uclés, S., Hernando, M.D., Fernández-Alba, A.R., 2011. Development of a  
603 solvent-free method for the simultaneous identification/quantification of drugs of abuse and  
604 their metabolites in environmental water by LC–MS/MS. *Talanta* 85, 157-166.
- 605 Nie, Y., Qiang, Z., Zhang, H., Ben, W., 2011. Behavior and fate of endocrine disrupting chemicals  
606 in municipal sewage treatment plants: A review. *Acta Sci. Circum.* 31(7), 1352-1362.
- 607 O'Brien, J.W., Thai, P.K., Eaglesham, G., Ort, C., Scheidegger, A., Carter, S. et al, 2014. A model to  
608 estimate the population contributing to the wastewater using samples collected on census day.  
609 *Environ. Sci. Technol.* 48(1), 517-525.
- 610 Ort, C., Lawrence, M.G., Reungoat, J., Eaglesham, G., Carter, S., Keller, J., 2010a. Determining the  
611 fraction of pharmaceutical residues in wastewater originating from a hospital. *Water Res.* 44(2),  
612 605-615.
- 613 Ort, C., Lawrence, M.G., Reungoat, J., Mueller, J.F., 2010b. Sampling for PPCPs in Wastewater  
614 Systems: Comparison of Different Sampling Modes and Optimization Strategies. *Environ. Sci.*  
615 *Technol.* 44(16), 6289-6296.
- 616 Ort, C., Lawrence, M.G., Rieckermann, J., Joss, A., 2010c. Sampling for Pharmaceuticals and Personal  
617 Care Products (PPCPs) and Illicit Drugs in Wastewater Systems: Are Your Conclusions Valid?  
618 A Critical Review. *Environ. Sci. Technol.* 44(16), 6024-6035.
- 619 Plo'ysz, B.G., Reid, M.J., Borup, M., Langford, K.H., Thomas, K.V., 2013. Biotransformation kinetics  
620 and sorption of cocaine and its metabolites and the factors influencing their estimation in  
621 wastewater. *Water Res.* 47(7), 2129-2140.
- 622 Postigo, C., a, M L d A, Barceló, D., 2011. Evaluation of drugs of abuse use and trends in a prison  
623 through wastewater analysis. *Environ. Int.* 37(1), 49-55.
- 624 Qian, H., Schumacher, J.E., Chen, H.T., Ruan, Y., 2006. Injection drug use and HIV/AIDS in China:  
625 Review of current situation, prevention and policy implications. *Harm Reduct. J.* 13 (4).
- 626 Ran, B., Qiu, Z., Qiu, Y., Lu, J., Xue, M., Dong, Z. et al., 2013. Using mobile phone location data in  
627 urban planning practice with big data environment, City era, collaborative planning -2013 urban  
628 planning summit, China, Beijing.
- 629 Reid, M.J., Derry, L., Thomas, K.V., 2014. Analysis of new classes of recreational drugs in sewage:  
630 Synthetic cannabinoids and amphetamine-like substances. *Drug Testing and Anal.* 6(1-2), 72-79.
- 631 Reid, M.J., Langford, K.H., Morland, J., Thomas, K.V., 2011. Analysis and interpretation of specific  
632 ethanol metabolites, ethyl sulfate, and ethyl glucuronide in sewage effluent for the quantitative  
633 measurement of regional alcohol consumption. *Alcohol Clin. Exp. Res.* 35(9), 1593-1599.
- 634 Thai, P.K., Jiang, G., Gernjak, W., Yuan, Z., Lai, F.Y., Mueller, J.F., 2014. Effects of sewer conditions  
635 on the degradation of selected illicit drug residues in wastewater. *Water Res.* 48(1), 538-547.
- 636 Thomas, K.V., Bijlsma, L., Castiglioni, S., Covaci, A., Emke, E., Grabic R. et al., 2012. Comparing  
637 illicit drug use in 19 European cities through sewage analysis. *Sci. Total Environ.* 432, 432-439.

- 638 Trenholm, R.A., Snyder, S.A., 2011. Analysis of illicit drugs in water using direct-injection liquid  
639 chromatography-tandem mass spectrometry. In: Castiglioni S., Zuccato E., Fanelli R., (Eds.),  
640 Illicit drugs in the environment. John Wiley & Sons, Inc., Hoboken, pp. 223-232.
- 641 UNODC, 2013. World drug report. Available at:  
642 [http://www.unodc.org/unodc/secured/wdr/wdr2013/World\\_Drug\\_Report\\_2013.pdf](http://www.unodc.org/unodc/secured/wdr/wdr2013/World_Drug_Report_2013.pdf). Date  
643 accessed: 08 May 2014.
- 644 van Nuijs, A.L.N., Abdellati, K., Bervoets, L., Blust, R., Jorens, P.G., Neels, H. et al., 2012. The  
645 stability of illicit drugs and metabolites in wastewater, an important issue for sewage  
646 epidemiology? *J. Hazard. Mater.* 239-240, 19-23.
- 647 van Nuijs, A.L.N., Castiglioni, S., Tarcomnicu, I., Postigo, C., de Alda, M.L., Neels, H. et al., 2011a.  
648 Illicit drug consumption estimations derived from wastewater analysis: A critical review. *Sci.*  
649 *Total Environ.* 409(19), 3564-3577.
- 650 van Nuijs, A L N, Gheorghe, A, Jorens, P G, Maudens, K, Neels, H, Covaci, A, 2014. Optimization,  
651 validation, and the application of liquid chromatography-tandem mass spectrometry for the  
652 analysis of new drugs of abuse in wastewater. *Drug Testing Anal.* 6:861-867.
- 653 van Nuijs, A.L.N., Mougel, J-F., Tarcomnicu, I., Bervoets, L., Blust, R., Philippe G. et al., 2011b.  
654 Sewage epidemiology - A real-time approach to estimate the consumption of illicit drugs in  
655 Brussels, Belgium. *Environ. Int.* 37(3), 612-621.
- 656 Venkatesan, A.K., Halden, R.U., 2014. Wastewater treatment plants as chemical observatories to  
657 forecast ecological and human health risks of manmade chemicals. *Sci. Rep.* 4, 3731-3737.
- 658 Xie, W., Hu, Y., Liu, H., Xu, Z., 2004. Environmental issue and study progress of persistent organic  
659 pollutants (POPs). *Environ. Monit. China* 20(2), 58-61.
- 660 XinhuaNews, 2013. China's registered drug users top 2 mln. Available at:  
661 [http://news.xinhuanet.com/english/china/2013-06/25/c\\_132486055.htm](http://news.xinhuanet.com/english/china/2013-06/25/c_132486055.htm) Date accessed: 08 May  
662 2014
- 663 Yuan, S., Li, X., Jiang, X., Zhang, H., Zheng, S., 2013. Determination of 13 antipsychotics in sewage  
664 with ASPE-HPLC-ESI-MS/MS. *Chinese J Anal. Chem* 41, 49-56.
- 665 Zhang, P., 2013. Distribution and health risk assessment on PPCPs in Hai river basin, China,  
666 Department of Forestry. Central south university of forestry and technology, Changsha.
- 667 Zhao, G., Yang, L., Zhou, H., Li, K., Wu, Z., 2011. Pollution status of pharmaceuticals and personal  
668 care productions in a certain sewage plant in Beijing. *Environ. Monit. China* 27, 63-67.
- 669 Zhou, H., Huang, X., Wen, X., 2007. Progress of the studies on occurrence and fate of new emerging  
670 micro-pollutants-PPCPs in municipal wastewaters. *Chinese J. Environ. Eng.* 1(12), 1-9.
- 671 Zuccato, E., Castiglioni, S., Tettamanti, M., Olandese, R., Bagnati, R., Melis, M. et al., 2011. Changes  
672 in illicit drug consumption patterns in 2009 detected by wastewater analysis. *Drug Alcohol*  
673 *Depen.* 118(2-3), 464-469.
- 674 Zuccato, E., Chiabrando, C., Castiglioni, S., Bagnati, R., Fanelli, R., 2008. Estimating community drug  
675 abuse by wastewater analysis. *Environ. Health Persp.* 116(8), 1027-1032.
- 676 Zuccato, E., Chiabrando, C., Castiglioni, S., Calamari, D., Bagnati, R., Schiarea, S. et al., 2005.  
677 Cocaine in surface waters: a new evidence-based tool to monitor community drug abuse.  
678 *Environ. Health-Glob.* 4, 14-20.