Analysis of N,N-dimethylamphetamine in wastewater : a pyrolysis marker and synthesis impurity of methamphetamine

Analysis of N,N-Dimethylamphetamine in Wastewater – A Pyrolysis Marker and Synthesis Impurity of Methamphetamine

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Abstract

The increased availability of high purity crystalline methamphetamine (MA) in Australia raised concerns because of high dosages and its potential consumption through inhalation. The present work investigates the possibility of using wastewater levels of N,N-dimethylamphetamine (DMA), a pyrolysis by-product formed during smoking, as an indirect indicator of MA smoking. A dedicated liquid chromatography time-of-flight mass spectrometry (LC-QTOF-MS) method was setup to detect and quantify DMA in the wastewater samples. Wastewater samples were collected from eight locations across Australia during the period 2011-2016. Data about the abundance of DMA in MA seizures as well as in residues from drug paraphernalia (i.e., pipes) were obtained from forensic laboratories in Australia. DMA/MA ratios measured in wastewater ranged from 0.0001 to 0.09 (median 0.007). DMA/MA ratios in bulk seizures are generally below 0.0025, with a median value of 0.0004, whilst residues in paraphernalia ranged from 0.031 to 3.37. DMA/MA ratios in wastewater decreased in the investigated period, in parallel to an increase in MA loads. Furthermore, wastewater analyses highlighted a strong positive correlation between DMA/MA ratios and per capita MA use (Pearson's correlation $\rho = 0.61$, p-value < 0.001). Nonetheless, geographical specificities could be highlighted between the investigated locations. The obtained data could help authorities detect hot spots of drug use as well as to plan specific intervention campaigns to tackle the issue. In future, simultaneous analysis of DMA and MA in both wastewater and seizures could improve our understanding about MA use and its consumption patterns.

Keywords: Pyrolysis, Australia, Spatio-temporal patterns, Wastewater analysis, Impurity, Methamphetamine

1 Introduction

Consumption of methamphetamine (MA) has become a major problem in many countries due to its increasing availability and addictive properties. On a global scale, MA seizures increased by 21% in 2015, forming between 61% and 80% of the total amount of amphetamine-type stimulants seized [1]. Australia similarly appears to be particularly affected by this trend [2]. In terms of MA seizures, an increase from 2.3 to 5.4 tons per year was observed between 2013 and 2015 [1,3]. Reports from the National Wastewater Drug Monitoring Program suggest that consumption has been growing steadily since 2009, with some locations in Queensland and South Australia showing an almost five-fold increase [4,5]. However, the latest household surveys (i.e., 2010, 2013 and 2016), showed a decreasing trend in the 12-months prevalence of MA use in the general population[6,7]. Yet, this could be due to the increased stigmatisation of MA use and the difficulty to capture heavy drug users with traditional surveys[2]. In fact, when figures about injecting drug users are contemplated, a significant increase in MA use has been reported (i.e., median number of days of use of crystalline MA from 20 to 30 days per half year) [8].

In recent years, there has been a substantial increase in MA purity, in particular with the increased availability of crystalline MA [2,3]. Whilst the question of whether high purity crystalline MA caused an increase in the number of new users is still being disputed [2], evidence shows that there has been a rise in regular use among existing users [9], as well as in the generated harms [2]. High purity methamphetamine with corresponding stronger dosages, coupled with the possibility of consuming the substance through inhalation (i.e., smoking) raises particular concerns. The generated effects of smoking MA are comparable to injection routes and users have higher risks of developing dependence and psychosis compared to other non-injecting routes (e.g., ingestion and snorting) [10,11]. Recent figures have shown that the prevalence of crystalline MA use, as well as smoking as administration route, have been increasing significantly in Australia between 2007 and 2016 [7]. Because of the increased availability of high purity MA and the substantial risk associated with smoking, developing additional and complementary tools to understand and monitor MA use is highly compelling. In particular, having means to easily collect spatial and temporal data to quickly assess the current situation is important for both public health and law enforcement officials. The available data could in fact be used to trigger and guide the deployment of specific responses (preventive and/or repressive) to tackle the situation.

Smoking generally consists of placing MA hydrochloride in a glass tube (e.g., pipe), heating it using a gas lighter and inhaling the formed vapours [12]. Various research groups have shown that numerous pyrolysis products are formed, including amphetamine, phenylacetone, N,N-dimethylamphetamine (DMA), N-acetyl-, N-propionyl- and N-formyl-methylamphetamine [12,13]. DMA is particularly interesting as, together with its metabolite DMA-N-oxide, it was measured in urine samples of a user who smoked DMA-free MA [14]. Sato et al. (2004) conducted controlled experiments to assess the yield of DMA formation during MA pyrolysis and reported that approximately 5% of the initial MA was converted to DMA through methylation of the methylamino group. DMA could thus be used as a urinary marker of MA smoking.

In the past decade, wastewater has been increasingly used as a complementary matrix to obtain information about substance use at the population level [15]. In particular, licit and illicit drug metabolites have been measured in wastewater samples collected at the influent of wastewater treatment plant (WWTP) serving large communities. Following this principle, measurement of DMA in wastewater could help improving our understanding about patterns of MA use at the population level. Yet, DMA can also occur in MA powder or crystals as a synthesis impurity. In particular, it is a marker of the synthesis of MA from ephedra extracts [16], although its occurrence has also been reported in MA produced following other synthesis routes (i.e., reductive amination [17], Leuckart reaction [18], ephedrine/hydriodic acid/red phosphorus [19] and ephedrine/lithium–ammonia reduction [20]). In very few cases, DMA was found as the main illicit drug in seized products, where MA was present only in lower amounts [21]. Hence, before DMA levels measured in wastewater can be used to gain insights into MA use, DMA to MA ratios in forensic seizures need to be assessed and compared to results from wastewater analysis.

The present work aimed at determining if DMA levels measured in wastewater can be used to better understand patterns of MA use at the population level. To achieve this, an analytical workflow to measure DMA and MA in wastewater samples was developed. To exclude the possibility that DMA is formed in wastewater by methylation of MA, stability experiments in wastewater were carried out. Subsequently, forensic data about levels of DMA in bulk seizures of MA (i.e., powder or crystalline) was collected to determine the ratio of DMA to MA. Additionally, results from the analysis of residues found in drug paraphernalia (i.e., pipes) were also collected to confirm that DMA was formed in substantial amounts during pyrolysis. Levels of DMA and MA measured in wastewater, in particular the ratio between

the two compounds, were confronted to forensic data to determine if levels of DMA found in wastewater were higher compared to seizures (i.e., suggesting a contribution from pyrolysis). Finally, DMA and MA levels measured in wastewater samples collected from different time periods and locations across Australia were investigated to determine if particular trends or spatial features could be highlighted.

2 Materials and methods

2.1 Chemicals and reagents

Analytical grade formic acid, ammonium hydroxide (25-30%) and methanol were purchased from Sigma-Aldrich (Castle Hill, Australia). Analytical grade hydrochloric acid (32%) was purchased from Univar (Ingleburn, Australia). Certified reference standards of DMA, MA and MA-D_9 (used as internal standard for both DMA and MA) were purchased from Lipomed (Arlesheim, Switzerland). Water was purified using a Milli-Q system (0.22 μm filter, 18.2 $m\Omega cm^{-1}$, Millipore).

2.2 Wastewater samples

Wastewater samples were collected at the influent of eight wastewater treatment plants (WWTPs) across Australia. These consisted of four urban catchments, having a population ranging from > 100,000 up to more than 1,000,000, and four rural catchments with less than 50,000 inhabitants. Details about the sampling locations and periods are reported in Tables 1 and S1. Samples were collected over 24 hours using automatic samplers installed at the influent of the WWTPs. Samples were stored at 4 °C during collection, acidified on site to pH 2 and subsequently frozen (-20 °C) until analysis.

Samples collected over a 6-year period from location QLD1 were used to assess temporal changes in DMA and MA loads, whilst samples from the remaining locations were used to investigate geographical features.

2.3 Sample preparation

Prior to extraction, wastewater samples (100 mL) were spiked with the mass-labelled internal standard (i.e., MA-D_9, final concentration of 50 ng/L). Subsequently, samples were centrifuged at 2700 RCF for 30 min to settle all suspended solids. Extraction was carried out using Oasis MCX (3 mL, 60 mg, Waters®) solid-phase extraction (SPE) cartridges pre-conditioned with 2 mL of methanol, followed by 3 mL of Milli-Q water acidified to pH 2. After sample loading, cartridges were washed using 5 mL of Milli-Q water at pH 2 and subsequently dried under vacuum for 1 h. Elution was carried out using 5 mL of methanol

with 5% ammonium hydroxide. Eluates were evaporated to dryness at room temperature under a gentle stream of nitrogen. Dry residues were reconstituted in 250 μL of MilliQ water: methanol (9:1 v/v), centrifuged at 2700 RCF for 20 min, and the supernatant recovered to remove residual particles.

2.4 Chemical analysis

Analysis of the processed samples was carried out using an ultra-high performance liquid chromatography (UHPLC) system (Nexera, Shimadzu, Kyoto, Japan) coupled to a quadrupole time-of-flight mass spectrometer (QToF-MS, Sciex TripleTOF 5600+, Ontario, Canada). Separation of the analytes was performed on a Kinetex Biphenyl column (50 mm x 2.1 mm, 2.6 μm, 100 Å, Phenomenex) using (A) 0.1% of formic acid in ultrapure water and (B) 0.1% of formic acid in methanol. The flow rate was set to 0.3 mL min^{-1} and the following gradient was used: mobile phase B was increased from 5% to 100% in 10 min, held to 100% until 14.5 min, reversed to 5% at 14.51 min and held until 18.5 min. The injection volume was set at 4 μL. For quantification of DMA, MA-D_9 was used as internal standard. The QToF-MS was operated in product ion mode and the following transitions were monitored: 164.1>91.0554 (Q1) and 164.1>119.0864 (Q2) for DMA and 159.2>125.1234 for MA-D_9. Collision energies were set to 32 V (Q1) and 17 V (Q2) for DMA and 15 V for MA-D_9, respectively. Ion spray voltage and temperature were set to 5400 V and 480 °C, respectively. A six-point calibration curve in neat solvents (i.e., MilliQ water: methanol 9:1), ranging from 0.4 to 80 ng/mL, was used.

Quantification of MA in wastewater was carried out using an in-house fully validated method [22], which has been also externally validated through regular participation to inter-laboratory testing [23]. Briefly, a 1 mL aliquot of wastewater was filtered, transferred to amber glass vials and spiked with mass-labelled internal standards (i.e., 5 ng/mL). A six-point calibration curve ranging from 0.05 to 50 ng/mL was used to quantify MA.

2.5 Method validation of DMA

The extraction recovery was determined by spiking real wastewater samples with known amounts (i.e., 50 ng/L) of DMA and MA-D_9 before and after extraction [24]. The ratio between the pre- and post-extraction spiked samples was used to calculate the extraction recovery. Accuracy and intermediate precision of the entire method were assessed using tap water samples spiked with DMA at three concentration levels. These were extracted and analysed in triplicate over three consecutive days using the previously described sample preparation protocol. Tap water was used since blank (i.e., DMA free) wastewater was not available.

However, to mimic the extraction in real conditions, a wastewater aliquot was extracted on each day to assess the method's precision with real matrix. Method detection and quantitation limits (MDL and MQL) were determined by spiking tap water samples with decreasing concentrations of DMA and extracting them using the described protocol. MDLs and MQLs were set as the lowest analyte concentration giving a signal-to-noise ratio (S/N) of 3 and 10, respectively [25].

2.6 Stability

Stability of DMA in real wastewater samples was assessed over 24 hours at room temperature using an accepted protocol used to obtain preliminary information about the stability of biomarkers in wastewater [26–28]. In particular, raw wastewater aliquots (7 mL) were transferred to pre-cleaned (i.e., rinsed with Milli-Q, methanol and acetone) glass tubes. The first sample was spiked with DMA (final concentration of 10 ng/mL), the second with MA (final concentration of 10 ng/mL) and the third with both compounds (final concentration of 10 ng/mL). From each sample, a subsample (250 µL) was taken immediately after spiking (T_0) and after 0.5, 1, 3, 6 and 24 h. For each time period, three aliquots were taken and immediately transferred to 0.2 µm centrifugal filters, spiked with mass labelled internal standard (MA-D_9, final concentration of 10 ng/mL) and centrifuged for 5 min at 10,000 RCF. The samples were then analysed using the described LC-QToF-MS method. Relative responses of DMA and MA to MA-D_9 were then computed and changes over time were expressed relative to concentrations at T_0.

2.7 Forensic data

The results of the analysis of 794 MA seizures, carried out between 2006 and 2007, were provided by the Forensic & Analytical Science Service (FASS, New South Wales Health, Sydney), whilst the result of the analysis of residues from 9 drug paraphernalia (i.e., analysis of residual contents found in devices suspected to be used for MA smoking) carried out between 2016 and 2017, were provided by the Forensic and Scientific Services (FSS, Queensland Health, Brisbane). In both cases, the data was derived from gas chromatography mass spectrometry (GC-MS) analysis of MA seizures or paraphernalia residues processed according to their validated protocols [29]. Peak areas of MA, DMA and internal standard (IS) were provided. DMA and MA responses were normalised by the response of the IS (DMA/IS and MA/IS). Subsequently, a semi-quantitative indication of the ratio of DMA to MA in the considered samples (i.e., seizures and pipe residues) was estimated by dividing DMA by MA (DMA/MA). A semi-quantitative approach was used to obtain DMA/MA ratios in seizures

and smoking devices because forensic laboratories in Australia do not routinely quantify DMA in seizures, but use it for chemical profiling purposes (i.e., semi-quantification of impurities) [30].

2.8 Data analysis

Prior to calculating DMA to MA ratios (DMA/MA), human metabolism had to be accounted for to obtain estimates of the actual amounts of parent compounds initially consumed. In fact, after consumption, only a fraction of the initial dose of DMA and MA will be excreted unchanged in urine. Thus, concentrations of each analyte measured in wastewater were divided by their respective excretion factors. For DMA, an excretion ratio of 17.0% was used, based on results reported by Inoue and Suzuki [31] after oral administration of a single dose of DMA. For MA, Khan and Nicell [32] provided an extensive review regarding excretion rates after administration (i.e., through various routes namely intravenous, insufflation, oral and smoking) of MA. Reported excretion rates ranged from 9.6% to 55%. In a previous paper by Been et al. [33], an inverse-variance weighed average excretion rate of 28.5% for MA was calculated based on all studies reported by Khan and Nicell [32]. The latter figure was used here as the excretion factor for MA as it covers multiple administration routes. Subsequently, DMA/MA ratios were calculated by dividing the adjusted concentrations of the two compounds. Population-normalised loads of DMA and MA, expressed as mg per day per thousand inhabitants, were computed by multiplying measured concentrations (in ng/L, without correcting for excretion factors) by the corresponding daily flows (in L/day) and dividing them by the number of inhabitants served by the corresponding catchment. The latter figures were provided by the WWTP personnel (Table 1).

Temporal trends were investigated using data collected from QLD1 over the period 2011-2016. Spatial trends were investigated using data collected from all WWTP considered in this study (for QLD1 however, only data from 2016 was considered). Differences in DMA/MA ratios, DMA and MA loads between years and locations were assessed using Wilcoxon signed-rank test. Half of the MQL was used for samples which had concentrations below the MQL. Prior to statistical testing, data was log-transformed to account for deviation from normality and heteroscedasticity. All calculations and statistical tests were performed with *R Software* [34].

3 Results

3.1 Method validation

The method used for the analysis of MA was previously validated [35], also through participation to yearly inter-laboratory testing [23]. As such, no further method validation for the analysis of MA was carried out. For DMA, performances of the analytical method were assessed using tap water and wastewater samples (Table 2). MDL and MQL, assessed in spiked tap water samples, were equal to 0.1 and 0.5 ng/L. Recovery of the extraction method, which was determined by spiking real wastewater samples with DMA before and after extraction, was equal to 98%. The coefficient of determination, assessed through triplicate analysis of a complete calibration curve was equal to 0.998. Accuracy and precision, determined using tap water spiked at three concentration levels, were within the established acceptance criteria (i.e., < 15% or < 20% close to the MQL) [36]. Similarly, precision using wastewater was also within the ± 15% acceptance criteria [36].

3.2 Stability

Results of stability testing of DMA in wastewater are shown in Figure S1. In the first experiment, only DMA was added to wastewater. As can be seen, a slight increase (+20%) in response was observed after 3 h. The response then decreased to its initial value. During the whole period, MA could not be detected in any of the samples. In the second experiment, only MA was added to wastewater. No particular trend could be observed over the investigated period and DMA could not be detected at any time throughout the considered period. In the last experiment, both DMA and MA were added to wastewater. Both chemicals showed a stable profile across the considered period.

3.3 Occurrence of DMA and MA in wastewater samples

Levels of DMA and MA in wastewater were assessed using the described methods and are reported in Table S2. DMA could be detected and quantified in most of the samples, with concentrations ranging between < MQL and 39 ng/L. Results below the MQL were observed only in some samples from QLD2 (5 out of 14 samples) and TAS (6 out of 7 samples). MA could be detected and quantified in all the samples and concentrations ranged from 253 to 5400 ng/L.

3.4 Temporal trends in DMA and MA in wastewater

DMA/MA ratios in wastewater did not show any difference between week days and weekends (Figure S2). In the case of DMA and MA, a slight increase in population-

normalised loads could be observed on Saturdays, Sundays and Mondays. Yet, no statistically significant difference between days of the week could be highlighted (Tukey post-hoc analysis, *p*-value > $\alpha = 0.05$). Yearly trends in DMA/MA ratios and population-normalised loads of DMA and MA measured in QLD1 are shown in Figures 1 and S3. Substantial differences in DMA/MA ratios were observed between the periods 2011-2013 and 2014-2016. In the first three-years, substantial variations in DMA/MA ratios could be observed (range: 0.006-0.01), whilst between 2014 and 2016, the ratio decreased substantially and remained stable (range: 0.004-0.009). Significant differences in DMA/MA ratios measured between 2014-2016 and 2012-2013 were observed (Tukey post-hoc analysis, *p*-value < α = 0.05). Similarly, the variability in population-normalised DMA loads was greater in 2012 and 2013 compared to the other years. However, significant differences were found only between 2011-2012 and 2011-2013 (Tukey post-hoc analysis, *p*-value < α= 0.05). MA population normalised loads showed a steady increase over the six-year period. In 2011, mean loads were equal to 195 mg/day 1000 inhab, whilst they increased to 638 mg/day 1000 inhab in 2016.

3.5 Spatial trends in DMA and MA

To compare results from different locations as well as to assess potential correlations between DMA/MA ratios and population-normalised loads of MA, only data from 2015 and 2016 was considered (Figure 2). Highest DMA/MA ratios were measured in VIC1 (range 0.008-0.02), followed by VIC3 (0.007-0.009), QLD1 (0.004-0.008) and VIC2 (0.006-0.007), whilst the remaining locations were in the range 0.0001-0.005. Wilcoxon signed-rank test indicated that significant differences exist between various locations (Table 3). Furthermore, Wilcoxon signed-rank test indicated no significant difference between rural and urban catchments (*p*-value = 0.64 > $\alpha = 0.05$). DMA and MA population-normalised loads showed very similar patterns. Highest figures were measured in VIC1 (range: 5.8-27 and 1077-2040 mg/day 1000 inhab for DMA and MA, respectively), followed by WA (range: 1.7-3.0 and 686-1053 mg/day 1000 inhab) and SA (range: 1.5-3.2 and 518-996 mg/day 1000 inhab). Lowest population normalised loads were measured in TAS (range: 0.1-0.3 and 131-257 mg/day 1000 inhab) and VIC2 (range: 1.1-1.5 and 290-435 mg/day 1000 inhab). Regarding catchment types, DMA and MA population normalized loads appeared to be higher in rural catchments (mean DMA 4.6 mg/day 1000 inhab, MA 833 mg/day 1000 inhab) compared to urbanized catchments (mean DMA 1.6 mg/day 1000 inhab, MA 443 mg/day 1000 inhab; Wilcox signed-rank test, *p*-value < $\alpha = 0.05$). Correlation analysis between DMA/MA ratios

and MA population-normalised loads was carried out and a positive relationship between DMA/MA ratios and consumption of MA was found (Spearman's correlation $\rho = 0.61$, *p*-value < 0.001) (Figure S4).

3.6 Methamphetamine forensic data

According to the available forensic data, DMA was detected in 58% (i.e., 459 out of 794) of the seizures. The 25th and 75th percentiles of estimated DMA/MA ratios where equal to 0.00002 and 0.0013, respectively, with a median of 0.0004. Figure S5 illustrates the ranges of ratios measured in MA seizures. In 87% of seizures in which DMA was detected, the ratio to MA was less than 0.0025 (based on semi-quantitative data). The analysis of residues in seized smoking devices (n = 9) indicated ratios of DMA/MA ranging from 0.031 to 3.37, with a median of 0.1 (based on semi-quantitative data).

4 Discussion

DMA has been shown to be a potential marker of MA smoking [14] as well as a synthesis by-product of MA [16]. Data from forensic analyses of MA seizures indicate that DMA was detected in 58% of all seizures (n = 794) and that, when detected, its ratio to MA was generally below 0.0025 (87% of DMA-positive seizures). These results are line with recent findings by Li et al. [37], who quantified DMA levels in 100 MA seizures from the US. In their work, the highest concentration reported was equal to 449 ng of DMA per mg of MA hydrochloride (equivalent to a DMA/MA ratio of 0.00056 expressed as MA freebase). Contrary to MA seizures, DMA/MA ratios found in smoking devices were substantially higher (i.e., > 0.03), which is in agreement with findings by Sato et al. [14], who reported that approximately 5% of the initial amount of MA was methylated to DMA during controlled pyrolysis experiments. Similarly, DMA/MA ratios measured in wastewater samples, after taking into account excretion rates, were significantly higher compared to MA seizures (median of 0.007 versus 0.0004, respectively; Welch t-test on log-transformed data, *p*-value < 0.0001, $\alpha = 0.05$). Experiments carried out to assess the stability of DMA suggest that the compound is stable in the tested conditions. A slight increase in response between 0.5h and 3h was observed, however this could be linked to an increased variability between replicates (i.e., analytical uncertainty) compared to other time points (see Figure S1). Most importantly, however, there is no indication that DMA is formed in presence of MA in wastewater (e.g., microbial methylation). Nonetheless, it should be recognised that these preliminary results do

not contemplate the potential effect of microbial degradation and sorption. Yet, MA and 3,4-methylenedioxymethamphetamine (MDMA) have been shown to be stable in-sewer conditions [38], suggesting that DMA is likely also stable in presence of biofilm. These findings suggest that there is a substantial contribution of DMA to wastewater other than MA impurities. However, it should be considered that results from seizures analysis date back to the period 2006-2007 and that the profile of MA could have changed since. In particular, a substantial increase in MA purity has been observed in recent years (median purity 13-22% in 2005-2006 versus 74-83% in 2015-2016 across all Australian jurisdictions [39,40]). This increase in MA purity may also accompany a change in DMA levels in seizures (due to more efficient manufacturing).

Regarding weekly patterns, no particular trend (e.g., increase during the weekend) could be observed in DMA/MA ratios. Similarly, population-normalised DMA and MA loads did not show any weekend peak, which was commonly seen for cocaine and MDMA. This is in agreement with results from previous studies, which suggested that MA is consumed constantly across the whole week (i.e., regular use not limited to nightlife) [22,33,41].

Multi-year data was available for one location (i.e., QLD1). Interestingly, DMA/MA ratios varied greatly between 2011 and 2013, whilst a sharp decrease was observed from 2014. In 2015 and 2016, the ratios remained comparably low and did not vary considerably. The decrease in DMA/MA ratios could be linked to the higher purity of MA, which could contain less impurities, including DMA. Population-normalised loads seem to partly support this, as a steep increase in MA consumption was observed between 2011 and 2016 (in Figure 1C), whilst DMA remained constant (Figure 1B). Figures from the Australian Criminal Intelligence Commission also indicate that MA purity increased substantially since 2011 [39]. Unfortunately, DMA is not routinely analysed in forensic laboratories and the lack of contemporary data about levels of DMA in MA seizures limits the interpretation of temporal trends. In particular, it prevents from establishing a direct link between changes in DMA levels in wastewater and changes in prevalence of MA smoking in the investigated communities.

Comparisons between locations were limited to samples collected during 2016, except for VIC1 which was sampled in 2015. The latter catchment exhibited particularly high DMA/MA ratios. Interestingly, this location also showed the highest DMA and MA population-normalised loads. Similarly, QLD1 also showed particularly high figures for DMA/MA ratios, DMA and MA population-normalised loads (Figure 2). The relationship

between DMA/MA ratios and MA use among different locations was further investigated. Although not always linear (i.e., WA had the second to highest MA population normalised loads whilst it came only fifth in terms of DMA/MA ratios), a strong positive correlation between the two variables could be highlighted (Figure S4). This could imply that in areas with high MA consumption, prevalence of MA smoking is higher compared to regions with lower MA consumption. However, to confirm this hypothesis, measuring levels of DMA in contemporary MA seizures would be highly useful.

When compared to available forensic data, median DMA/MA ratios measured in wastewater are significantly higher. This would suggest that, although the fraction of DMA to MA in seizures can vary (e.g., changes in synthesis route and/or quality of precursors), there is evidence that additional source (s), amongst which potentially MA smoking, contribute to the bulk of DMA measured in wastewater. Still, the substantial variations in DMA/MA ratios observed in QLD1 between 2011 and 2013 compared to the period 2014-2016 indicate that making assumptions over multiple years is delicate without contemporary seizures data. However, if such data were to be available, more robust conclusions could be drawn. Nevertheless, in a context where high purity MA (i.e., containing low and stable levels of impurities) is available on the market and if comparisons between locations are conducted over a short period of time (during which the profile of MA can be assumed to be stable), DMA/MA ratios could provide some indications about areas with a higher prevalence of problematic drug users (i.e., smoking). To strengthen these assumptions, however, it would be beneficial to carry out simultaneous DMA and MA analysis in both wastewater and seizures made in the investigated catchment, thus providing direct information about the profile of MA being consumed. Comparing results of wastewater analysis from countries known to have low prevalence of MA smoking, and consequently having low levels of DMA, would also be relevant. Furthermore, additional experiments should be carried out to determine the levels of DMA generated from MA using different smoking devices.

5 Conclusion

The increasing availability of high purity crystalline MA and its consumption via smoking have raised concerns in Australia and the rest of the world. In order to better understand the phenomenon and develop specific and adequate measures to tackle it, there is a need for additional tools to help decipher MA consumption patterns at the community level. The present work aimed at exploring the possibility of using wastewater measurements of DMA,

a pyrolysis by-product and synthesis impurity of MA, as a marker to improve our understanding about MA use. Levels of DMA/MA ratios found in wastewater suggested that there is a substantial contribution to DMA in wastewater from sources other than MA impurities. Substantial changes in DMA/MA ratios in wastewater were observed between 2011 and 2016. These could be linked to the increased purity of MA available, thus explaining a decrease in DMA levels, or to changes in prevalence of consumption patterns. Yet, the lack of contemporary data about DMA levels in MA seizures hinders a more thorough interpretation of temporal trends. Geographical differences in wastewater data were observed and, in particular, a strong correlation between DMA/MA ratios and MA use was found. These findings could help authorities identify potential hotspots where MA consumption is or could become problematic. Additional research is however necessary to refine DMA/MA ratios as an additional tool to monitor MA use at the population level.

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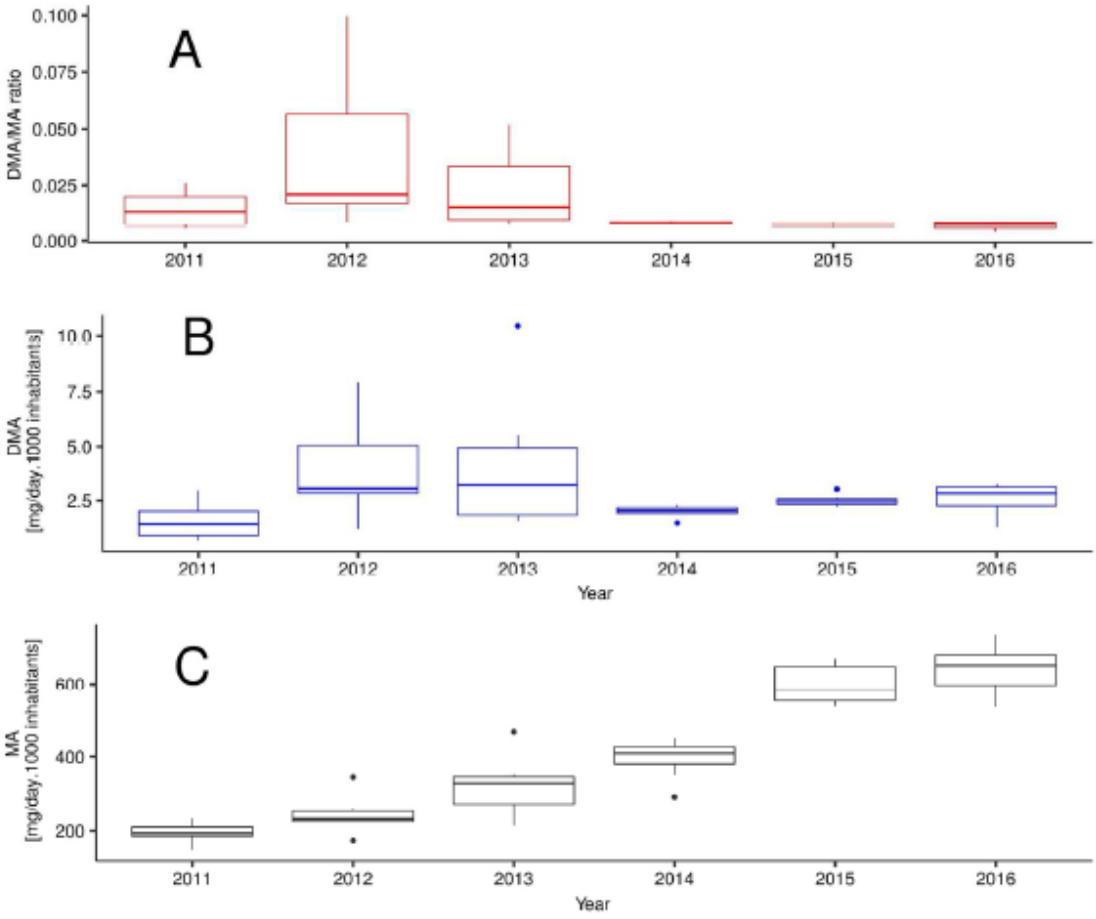

Figure 1: Yearly trends of DMA/MA ratios (A) and population-normalised DMA (B) and MA (C) loads [mg/day 1000 inhab]. Only data from QLD1 is shown.

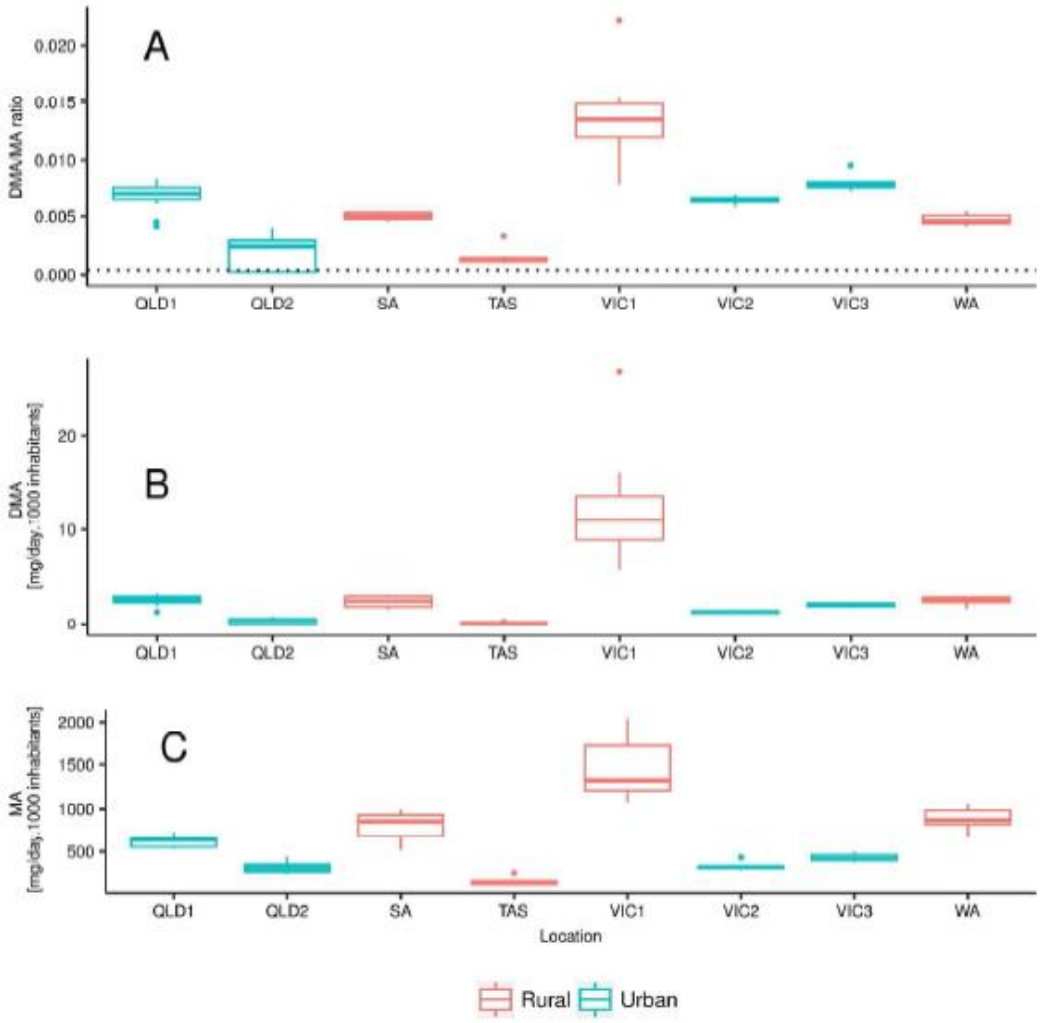

Figure 2: DMA/MA ratios (A) and population-normalised loads of DMA (B) and MA (C) [mg/day 1000 inhab]. Only data from 2015 and 2016 is reported here. The dotted line in Figure 2A represents the 0.0004 value, corresponding to the median DMA/MA ratio measured in MA seizures (see section 3.6). DMA/MA ratios were computed after correcting for the different excretion rates of DMA and MA (see section 2.8), whilst this is not the case of DMA and MA population normalised loads (charts B and C).

Table 1: Details of sampling locations. Population estimates were provided by the WWTP personnel.

Code	State	Catchment type	Population	Period	Sampling mode
QLD1	Queensland	Urban	> 100,000	2011 – 2016	Time-proportional composite, 7-8 samples per year, 42 samples in total
QLD2		Urban	> 100,000	2016	Time-proportional composite, 14 samples including 7 consecutive (i.e., 1 week)
SA	South Australia	Rural	< 50,000	2016	Time-proportional composite, 7 consecutive samples (i.e., 1 week)
TAS	Tasmania	Rural	< 50,000	2016	
VIC1	Victoria	Rural	< 50,000	2015	
VIC2		Urban	> 1,000,000	2016	
VIC3		Urban	> 1,000,000	2016	
WA	Western Australia	Rural	< 50,000	2016	

Table 2: Summary of method performances. MDL = method detection limit; MQL = method quantification limit

Compound	MDL [ng/L]	MQL [ng/L]	Recovery [%]	R^2	Concentration level [ng/L]	Tap water Accuracy [%]	Precision [%]	Wastewater Precision [%]
DMA	0.1	0.5	98	0.998	10	4.2	5.1	4.2
					35	10.9	12.0	
					75	5.9	5.4	

Table 3: Results of the pairwise comparison between DMA/MA ratios measured in the sampled locations. Values reported in red indicate locations which could not be differentiated (i.e., Wilcoxon signed-rank test, p-value > α= 0.05).

	QLD1	QLD2	SA	TAS	VIC1	VIC2	VIC3	WA
QLD1	-							
QLD2	< 0.001	-						
SA	0.07	0.004	-					
TAS	< 0.001	0.8	0.03	-				
VIC1	0.001	< 0.001	0.03	0.01	-			
VIC2	0.17	< 0.001	0.03	0.01	0.01	-		
VIC3	0.09	< 0.001	0.03	0.01	0.03	0.01	-	
WA	0.02	< 0.001	0.8	0.01	0.01	0.01	0.01	-

Analysis of N,N-Dimethylamphetamine in Wastewater – A Pyrolysis Marker and Synthesis Impurity of Methamphetamine

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