Abstract: This project determined the stable isotopic compositions (δ2H / δ 13C / δ 15N) of 181 samples of high purity illicit methylamphetamine seized in Queensland between July 2015 and February 2017. The project was undertaken to augment existing stable isotopic composition data for methylamphetamine samples seized at Australian borders.

Based on a rule-of-thumb developed by the National Measurements Institute of Australia a majority of Queensland samples (~ 88%) were classified as being manufactured from synthetic ephedrine/pseudo-ephedrine (e.g. from 1-phenyl-1-propanone). A much smaller number of samples (~ 12%) were initially classified as being manufactured from natural ephedrine/pseudo-ephedrine (from Ephedra sinica). Subsequent analysis of samples classified as having a natural precursor found them to be enantiomerically pure L-methylamphetamine and, therefore, most likely manufactured from phenylpropan-2-one. In contrast to border seizures, no methylamphetamine seizures from Queensland had isotopic compositions consistent with being manufactured from semi-synthetic ephedrine/pseudo-ephedrine (fermentation of benzaldehyde and pyruvic acid).

A small number of enantiomerically pure D-methylamphetamine samples with δ15N values intermediate between natural and synthetic classification were also assumed to be derived from phenylpropan-2-one. These samples were isotopically similar to seizures reported in Japan that had been imported from Eurasia.

The combination of δ2H, δ13C and δ15N measurements was used to create a searchable database that identified a number of isotopically similar samples of methylamphetamine seized at different locations and different times. A kernel density estimate model was used to assign "moderately-strong" to "strong" support that these samples came from the same synthetic batch.
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<th>University of Otago</th>
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**Opposed Reviewers:**
Highlights

181 samples of methylamphetamine seized in Qld, Australia were analysed

Qld MA was initially classified as prepared from natural or synthetic pseudoephedrine

Samples classified as natural were subsequently found to be l-MA derived from P2P

Seizures at Australian borders were not representative of Qld seizures

Stable isotope data linked seizures from different times and locations in Qld
A stable isotope ratio approach to investigate the origins of illicit methylamphetamine in Queensland, Australia.

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Keywords:
Isotope ratio mass spectrometry
Likelihood ratios
Methamphetamine
Methylamphetamine
Probability statistics
1. Introduction

During the 1990s worldwide production of amphetamine-type stimulants (ATS) increased as they became the fashionable drugs of choice for party goers [1] and today they are the second most widely used drugs after cannabis [2]. Manufacturers or “cooks” of ATS such as methylamphetamine (MA) aka methamphetamine, methamphetamine or ice have become increasingly skilled in producing pure products and/or removing unwanted by-products and impurities so that street level drugs are now often described as “pharmaceutical grade” [3]. In many cases, low purity products sold at street level result from the addition of cutting agents, such as methylsulfonylmethane (MSM) or isopropylbenzylamine, rather than poor synthetic chemistry. One unfortunate consequence of pharmaceutical grade illicit drugs is that traditional, chromatographic methods of profiling have become ineffective as the amounts of side-products, unreacted precursors etc have decreased [4]. For this reason, researchers have started to consider other methods to characterise synthetic drugs including determining the stable isotopic composition [5].

A number of studies has have demonstrated that a combination of $\delta^{13}C / \delta^{15}N$ measurements provided a characteristic isotopic profile of synthetic drugs such as; MA, 3,4-methylenedioxymethylamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA), both commonly known as “ecstasy” [6-9]. The data generated by studies such as these have proved valuable in linking tablets or powders seized at different locations and/or at different times to a common synthetic batch [10-12]. When considering links between MA from multiple seizures, comparisons of $\delta^{13}C / \delta^{15}N$ compositions were also found to highlight different grouping patterns compared to conventional profiling techniques [13].

Early research demonstrated that batches of MA, manufactured by reductive amination of 1-phenylpropan-2-one (aka phenylacetone or P2P), using identical reagents and equipment had markedly different $\delta^{15}N$ compositions [14]. Further, systematic research into the preparation of MDMA by reductive amination found significant isotopic variation with changing reaction conditions; $\delta^2H$ values were most affected by imine stir time; $\delta^{13}C$ values were most affected by the overall efficiency (completeness) of the reaction; $\delta^{15}N$ values were most affected by an excess of methylamine [15]. This line of research concluded that the isotopic compositions of ATS...
prepared by reductive amination were characteristic of individual reaction batches more so than
the precursor materials.

In contrast, other research has found that samples of MA, prepared from ephedrine/pseudo-
ephedrine (Eph/PSE) retained the $\delta^{2}$H, $\delta^{13}$C and $\delta^{15}$N compositions of the starting materials [16-
18]. This relationship has been demonstrated for a number of synthetic routes (Nagal [hydroiodic
acid/red phosphorus], Moscow [red phosphorus/iodine], Hypo [hypophosphorus acid/iodine] or
Emde [thionyl chloride]) and has also been shown to be unaffected by variations in the reaction
conditions [16]. This line of research concluded that the isotopic composition of MA could be used
to identify the source of Eph/PSE precursors as being; natural (Ephedra sinica), semi-synthetic
(fermentation of benzaldehyde and pyruvic acid) or fully synthetic (e.g. 1-phenyl-1-propanone aka
propiophenone) [19].

Complementary research into some physiochemical processes used during MA manufacture, such
as enantiomeric resolution [20] and crystallization [21], found some evidence for isotopic
fractionation, but concluded that the isotopic profile of the final MA-HCl product retained useful
information about its provenance.

In Australia, the Australian Federal Police (AFP) Forensic Drug Intelligence team operates a drug
profiling capability through the National Measurements Institute (NMIA). The information is used
for operational intelligence, i.e. to link different seizures, with the aim of disrupting drug
trafficking networks. In 2013 the NMIA reported stable isotopic composition data ($\delta^{2}$H / $\delta^{13}$C / $\delta^{15}$N) for 782 samples of MA seized at Australian borders and compared these to samples of MA
synthesized in-house from well characterized starting materials [19]. The study presented here
aimed to compare and contrast these data with high purity MA seized within Queensland,
Australia.

2. Materials and methods

2.1. Methylamphetamine samples

A total of 181 MA samples were sub-sampled from exhibits submitted by the Queensland Police
Service (QPS) to the Queensland Health Forensic and Scientific Services (QHFSS) Illicit Drugs
Group between July 2015 and February 2017. The identity of the active ingredient (MA) was
confirmed by gas chromatography mass spectrometry (GC/MS) and the percentage purity was
determined by high-performance liquid chromatography (HPLC) according to NATA (ISO/IEC 17025:2005) accredited laboratory methods. Sample purities (expressed as free-base) ranged from 60.2 to 82.2 % with an average purity of 76.6 %, equivalent to 95.0 % expressed as MA-HCl.

The QHFSS Illicit Drugs Group does not routinely undertake drug profiling analyses, such as acquiring chemical or elemental compositions. This project was undertaken to assess what evidential and intelligence value that might be obtained from stable isotopic analysis.

2.2. Stable isotopic analysis

All isotope ratio measurements were performed using a Delta V^{PLUS} Isotope Ratio Mass Spectrometer (Thermo Scientific, Bremen, Germany) coupled to a ConFlo IV interface for working gas introduction and sample dilution. Data were collected using ISODAT 3 software.

For carbon and nitrogen analysis, samples were crimped into tin capsules (4 × 3.2 mm, IVA Analysentechnik, Meerbusch, Germany). Samples were analysed using a Flash 2000 elemental analyser (EA) (Thermo Scientific) in flash combustion mode at 950 °C, with a helium flow of 100 mL/min, followed by a post reactor GC column at 110 °C. The reactor was packed with chromium (II) oxide, electrolytic copper and silvered cobaltous (II, III) oxide (IVA). A sulfur trap (silver wool) and a water trap (magnesium perchlorate) were placed between the reactor and the GC column. For nitrogen analysis an additional CO_{2} trap (Carbosorb™ and magnesium perchlorate) was installed following the water trap. Carbon measurements were normalised to the VPDB scale using IAEA-CH-7 (International Atomic Energy Agency [IAEA], Vienna, Austria) and glucose solutions calibrated against the reference materials NBS 19 and LSVEC [22]. Nitrogen measurements were normalised to the N_{AIR} scale using ammonium sulphate reference materials IAEA-N-1, IAEA-N-2, and USGS25 (IAEA).

For hydrogen analysis, samples were crimped into silver capsules (4 × 3.2 mm, IVA). Samples were analysed using a TC/EA elemental analyser (Thermo Scientific) at 1400 °C with a helium flow of 90 mL/min via a bottom-feed adaptor. The glassy carbon reactor tube was replaced with a custom-made alumina tube (IVA) (12 mm o.d., 6 mm i.d., length 420 mm). The reactor packing comprised glassy carbon (IVA) and chromium (Aldrich, Darmstadt, Germany) in the upper 50 mm and glassy carbon and manganese (Aldrich) in the lower 50 mm. Samples were introduced into the reactor via a Zero-Blank auto-sampler (Costech Analytical Technologies Inc., Valencia, CA,
USA). Hydrogen measurements were normalised to the VSMOW scale using silver encapsulated waters GISP, VSMOW and UC03 (United States Geological Survey [USGS], Reston, VA, USA).

A sample of phenacetin (N-(4-ethoxyphenyl)-acetamide), with well characterised isotopic composition [23, 24] was analysed at the beginning of each acquisition sequence and after every five samples for quality control purposes. These data are presented in the supplementary material.

2.3 Chiral analysis

The separation and identification of MA enantiomers was achieved using the derivatizing reagent N-trifluoroacetyl-(S)-propyl chloride (Sigma, Castle Hill, NSW, Australia) [25] prepared as a 0.1 M solution in dichloromethane.

Approximately 1 mg of sample was dissolved in 1 mL of ammonia infused hexane in a 2 mL autosampler vial and 20 μL of the derivatising reagent added. Reference samples of d-MA and d,l-MA (Cerilliant, Kinesis Australia, Redland Bay, QLD, Australia) were prepared in the same way.

Samples were analysed using an Agilent 7890B GC/MS equipped with a 30m, 0.25 mm, HP-1MS column. The column was programmed from 65 °C (1 min hold) to 300 °C (3.3 min hold) at a rate of 40 °C/min. The injector and transfer line were maintained at 280 °C. The MS was operated at 70eV electron ionization with a scan range from 35-500 m/z.

2.4. Data analysis

Scatter plots and k-means clustering were performed using SYSTAT 12 statistical and graphics software (Systat Software, Inc. San Jose, CA, USA). Expectation maximization clustering was performed using SPSS Statistics 24 with AMOS 23 (IBM, Chicago, Illinois, USA). Spatial plots, kernel density estimations and likelihood ratios were calculated using R 3.3.3 software environment for statistical computing and graphics (www.r-project.org).

3. Results and discussion

3.1. Notes on δ²H analysis

Hydrogen isotopic analyses were performed using an alumina inner reactor tube packed with a mixture of glassy carbon, metallic chromium and metallic manganese as described above. This
packing was based on recently published research that addressed problems in the high temperature conversion of organic molecules containing hetero-atoms such as nitrogen, chlorine or sulfur to H₂ gas [26, 27]. High temperature conversion using only glassy carbon, was reported to form compounds such as HCN and HCl so that the final yield of H₂ did not reflect the isotopic composition of the hydrogen present in the parent molecule. Complete conversion of organic hydrogen to H₂ was considered essential for the analysis MA-HCl that contained a relatively high proportion of both nitrogen and chlorine and, potentially, sulfur from MSM as a cutting agent.

Initial trials of this reactor packing using a conventional glassy carbon inner reactor tube (12 mm o.d., 7 mm i.d., length 356 mm) produced broad, tailing peaks approximately 57 s wide. This was attributed to interactions with the chromium/manganese packing. In addition, after a period of use the chromium/glassy carbon packing became rigid and difficult to remove without damaging the glassy carbon tube. To overcome these problems, the glassy carbon tube was replaced with an alumina tube with a 6 mm i.d. (bottom drilled to 7 mm to fit the bottom feed adaptor) to increase the linear velocity of the carrier gas. Using this inner reactor tube, peak widths were typically 42 s and comparable to peak widths produced using only glassy carbon as a packing. After calibrating the instrument with silver encapsulated waters, organic reference materials IAEA-CH-7 (-100.3 ‰ ± 2.0) and NBS 22 (-116.9 ‰ ± 0.9) were analysed to test the performance of the reactor to organic materials. Both reference materials gave results within the certified uncertainties (http://www.ciaaw.org/hydrogen-references.htm accessed Feb. 2018). This reactor composition proved to have a further advantage because it did not exhibit memory effects associated with glassy carbon reactors [28] i.e. the measured isotopic composition of a sample was not affected by the isotopic composition of the previous samples. Because the alumina reactor tube had a smaller i.d. than the conventional glassy carbon tube it was necessary to reduce the size of the graphite crucibles by filing the corners using fine abrasive paper. Using this reactor configuration it was possible to analyse three batches of samples, approximately 120 individual capsules per batch, before the reactor packing had to be replaced. This was equivalent to approximately 60 samples analysed in triplicate with corresponding calibration and quality control materials per reactor.

To assess if extrinsic hydrogen was present, due to moisture absorbed or adsorbed by the samples, the δ²H compositions of five samples of MA-HCl, with purities ranging from 69 to 80.4 %, were measured before and after four days of drying under vacuum (60 °C and 10 mbar). These data are
given in Appendix A. The average change in isotopic composition over four days was -1.5 ‰ and, it was concluded that heating/vacuum treatment was not necessary for routine samples.

3.2. Synthetic origins of Queensland MA

Based on published research, summarized in Table 1, the NMIA has developed a rule-of-thumb (RoT) to characterize the Eph/PSE precursors of MA as natural (Ephedra sinica), semi-synthetic (fermentation of benzaldehyde) or fully synthetic (e.g. propiophenone). The NMIA also used k-means and expectation maximisation (EM) clustering techniques to assign seized samples to these three classes with 95.5 % agreement between the two statistical methods [19]. Both methods also showed close agreement with the RoT.

Table 2 shows the results of the three classification techniques applied to the Queensland survey data together with the original NMIA/AFP results. Initial RoT classification found that none of the Queensland survey samples had an isotopic composition consistent with manufacture from semi-synthetic Eph/PSE and, therefore, subsequent k-means and EM clustering methods were applied to the Queensland survey data using a two-class model (natural versus synthetic).
Table 1
The expected isotopic composition range for MA derived from known precursors based on a number of heuristic assessments.

<table>
<thead>
<tr>
<th>precursor</th>
<th>isotopic composition</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>natural Eph/PSE</td>
<td>(-26.5 \text{ to } -30 , \text{‰}) (&gt; +2.5 , \text{‰})</td>
<td>Salouros et al. 2013</td>
</tr>
<tr>
<td>semi-synthetic Eph/PSE(^a)</td>
<td>(&gt; -26.5 , \text{‰})</td>
<td>Makino et al. 2005</td>
</tr>
<tr>
<td>synthetic Eph/PSE</td>
<td>(&lt; -26.5 , \text{‰}) (&lt; +2.5 , \text{‰})</td>
<td>Salouros et al. 2013</td>
</tr>
<tr>
<td>P2P</td>
<td>(-32 , \text{‰}) (+5 , \text{‰})</td>
<td>Collins &amp; Salouros 2015</td>
</tr>
</tbody>
</table>

\(^a\) samples prepared from semi-synthetic Eph/PSE were also characterized by positive \(\delta^2\)H values (Collins et al. 2009)

Table 2
The percentage classification of MA precursors by rule-of-thumb (RoT), k-means and expectation maximization (EM) methods.

<table>
<thead>
<tr>
<th>Eph/PSE precursor</th>
<th>classification method</th>
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<tbody>
<tr>
<td></td>
<td>RoT</td>
</tr>
<tr>
<td>Queensland data</td>
<td></td>
</tr>
<tr>
<td>semi-synthetic</td>
<td>0</td>
</tr>
<tr>
<td>synthetic</td>
<td>88</td>
</tr>
<tr>
<td>natural</td>
<td>12</td>
</tr>
<tr>
<td>NMIA/AFP data</td>
<td></td>
</tr>
<tr>
<td>semi-synthetic</td>
<td>34</td>
</tr>
<tr>
<td>synthetic</td>
<td>24</td>
</tr>
<tr>
<td>natural</td>
<td>42</td>
</tr>
</tbody>
</table>
The results in Table 2 showed that the majority (~ 88%) of Queensland MA had isotopic compositions consistent with preparation from synthetic Eph/PSE with a much smaller number consistent with natural Eph/PSE.

Australian Government reports have identified PSE, extracted from “cold and flu” medicines, as the predominant precursor used to produce MA in Queensland for many years [2]. More recently, however, there has been a significant increase in the amount of imported MA with an estimated 70 % originating in China [29]. In addition, half of the precursor chemicals seized in Australia and New Zealand also originated in China and, to a lesser extent India [29]. Although the term Eph/PSE is used throughout this report, to denote a precursor that may be either ephedrine or pseudoephedrine, pharmaceutical PSE is typically manufactured from synthetic precursors such as propiophenone [30]. Fermentation of benzaldehyde (the semi-synthetic method) yields a mixture of Eph/PSE which may not be desirable in a commercial process.

Although the main finding from the Queensland survey was unsurprising a proportion, albeit small, of the Queensland survey MA appeared to have been manufactured from Ephedra. There was also a notable disagreement between assigned precursors using k-means or EM clustering, in contrast to the NMIA/AFP results that showed close agreement between the two methods.

To investigate these findings further, the QHFSS Illicit Drugs Group undertook analyses to determine the chiral form of MA samples with positive $\delta^{15}N$ values, which included all of the samples classified as derived from natural Eph/PSE. Eph/PSE sourced from pharmaceutical products (l-Eph or d-PSE) can be used to prepare enantiomerically pure d-MA, the physiologically active form of the drug. In Mexico, however, large quantities of MA are prepared by reductive amination of P2P which yields racemic MA that undergoes an optical resolution process to separate the d and l forms of the drug [20] (as noted above, optical resolution is reported not to have a significant effect on the isotopic composition of the final product). Although it has been reported that 80 % of precursor chemicals used by Mexican cartels are sourced in China [29] differences in the isotopic compositions of P2P and Eph/PSE derived MA should still be apparent.

The most $^{15}N$ enriched samples in the Queensland survey (+13.35 to +14.99 ‰) were all found to be enantiomerically pure l-MA (samples #108 to 121). The majority of these samples came from a single Police operation in North Booval that seized 14 exhibits, each weighing approximately one kg. Two other samples of enantiomerically pure l-MA, with similar $\delta^{15}N$ compositions
(samples #31 and #145), were seized several weeks earlier at different locations and weighed only a few grams. All other samples of MA with positive $\delta^{15}N$ values (+0.88 to +8.46 ‰) were found to be enantiomerically pure d-MA. None of the samples in this survey were found to comprise mixtures of the two enantiomers.

Samples of MA with $\delta^{15}N$ values intermediate between *synthetic* and *natural* Eph/PSE classifications (+0.88 to +8.46 ‰) were initially assumed to be mixtures of MA produced from *synthetic* and *natural* Eph/PSE. A previous report by the Japanese National Research Institute of Police Science (JRIPS) identified MA seizures derived from several batches of MA, with different sources of precursors, mixed together [31]. Considering both isotopic and chiral analysis, however, it was apparent that the samples, consistent with *natural* Eph/PSE characteristics were more likely to have been manufactured from P2P and it appeared likely that the d-MA samples with intermediate $\delta^{15}N$ values (0 to +10 ‰) were also manufactured from P2P. These samples had $\delta^{13}C$ values (approximately -30 and -28 ‰) and $\delta^2H$ values (approximately -100 to -22 ‰) consistent with data previously reported for P2P ($\delta^{13}C = -31.6$ to -28 ‰ and $\delta^2H = -71$ to -53 ‰) [32].

### 3.3. Queensland and Australian borders

The full dataset from the Queensland survey is presented in Appendix A of this manuscript. The range of isotopic compositions in this study were; $\delta^2H_{VSMOW}$ -147.6 to -22.2, $\delta^{13}C_{VPDB}$ -30.01 to -26.27 and $\delta^{15}N_{AIR}$ -10.18 to +14.99 ‰. The median standard deviations for these measurements were; $\delta^2H = 0.7$, $\delta^{13}C = 0.04$ and $\delta^{15}N = 0.09$ ‰ (n=3). The range of isotopic compositions of the Queensland survey was significantly narrower, for all three elements, than reported by NMIA ($\delta^2H_{VSMOW}$ -249.9 to +103.0, $\delta^{13}C_{VPDB}$ -31.5 to -23.4 and $\delta^{15}N_{AIR}$ -14.9 to +19.4). Notably, hydrogen and carbon data reported by NMIA spanned more than twice the range observed in the Queensland survey.

Comparisons between Queensland and NMIA/AFP data can easily be visualized in the scatter-plots shown in Figure 1. The delineation between *natural*, *semi-synthetic* and *synthetic* Eph/PSE shown in Figure 1 is based on the RoT summarized in Table 1. The size of the circles in Figure 1 has no statistical significance, they simply approximate the characteristics of the three precursors classifications.
From Figure 1a it was, again, apparent that the majority of samples in the Queensland survey had δ\(^{13}\)C values below -26.5 ‰ and δ\(^{15}\)N values below +2.5 ‰, consistent with manufacture from *synthetic* Eph/PSE. None of the Queensland survey samples had isotopic compositions consistent with having been manufacture from *semi-synthetic* Eph/PSE. This finding was in contrast to the NMIA/AFP data for which *semi-synthetic* and *natural* Eph/PSE were identified as the dominant precursors. Relatively few of the samples from the Queensland survey had δ\(^{13}\)C / δ\(^{15}\)N values consistent with manufacture from *natural* Eph/PSE and most of these samples were subsequently identified as enantiomerically pure l-MA and, therefore, most likely manufactured from P2P.

Figure 1b shows a plot of δ\(^2\)H versus δ\(^{15}\)N composition that displays similar trends to those shown in Figure 1a, *i.e.* the majority of samples from the Queensland survey were shown to be derived from *synthetic* Eph/PSE and none from *semi-synthetic* Eph/PSE. The absence of *semi-synthetic* Eph/PSE derived MA was evident as this MA precursor is characterized by positive δ\(^2\)H values [Collins et al. 2009].

In Figure 1b it was noticed that the δ\(^2\)H values for Queensland survey *synthetic* class appear depleted with respect to the equivalent NMIA/AFP data which may be a bona fide difference or may be due to different reactor materials used for measurements in the two laboratories. It has been proposed that δ\(^2\)H measurements obtained using different reactor materials can be standardized by reporting data for reference materials alongside sample data [33]. The data obtained for the QC material (phenacetin) used in this study are presented in Appendix A (this material is commercially available from LGC Standards, UK).

### 3.4. Queensland and Japanese data

Figure 2 shows a scatter plot of δ\(^{13}\)C versus δ\(^{15}\)N for MA from the Queensland survey compared with MA samples seized in Japan and analysed by the JRIPS [Iwata 2008, Tsuj 2012]. The JRIPS states that there are virtually no clandestine MA laboratories operating in Japan and, therefore, the majority of seizures come from numerous locations around the world [18]. The Japanese data, shown in Figure 2, are believed to represent MA from Eurasia although police intelligence only knows the country from which the smuggled MA departed, not the country in which it was manufactured. It is interesting to note that many of the Japanese MA samples, with δ\(^{15}\)N value intermediate between the *synthetic* and *natural* classifications, had δ\(^{13}\)C / δ\(^{15}\)N profiles very similar
to Queensland survey samples (Figure 2, triangle, green). The JRIPS state that they do not believe that these samples originated in Australia and a conclusion may be that MA was smuggled to both Japan and Australia from the same source in/or via Eurasia. Subsequent analyses of individual MA crystals by the JRIPS found that MA seizures with intermediate $\delta^{15}$N values were the result of mixing several batches of MA from different precursor sources [31]. Samples tested as part of the Queensland survey were homogenized prior to isotopic characterization and no evidence for a mixed source was found. The JRIPS attributed samples with more depleted $\delta^{13}$C values (Figure 2, square, black) to a P2P precursor.

3.5. Finding links and weighing the evidence

Although a knowledge of synthetic precursors is important in understanding the operations of clandestine MA laboratories, the stable isotopic composition of MA also provides a means to associate or differentiate batches of MA seized at different times and locations. To facilitate this, the average $\delta^2$H, $\delta^{13}$C and $\delta^{15}$N values for each sample from the Queensland survey were compiled into a spreadsheet, including; the date and location of each seizure and the weight and purity of the MA seized. These data are presented in Appendix A. Isotopic values in the “database” were auto-scaled (Eq.1) so that $\delta^2$H, $\delta^{13}$C and $\delta^{15}$N data all had a similar range. Two samples (SAMP1 and SAMP2) were then compared using a simple Euclidean distance calculation (Eq.2). The smaller the comparison distance, the greater the similarity between samples.

\[
\text{autoscaled data} = \frac{\text{data-average}}{\text{standard deviation}}
\]

\[
\text{comparison} = (\delta^2\text{H}_{\text{SAMP1}} - \delta^2\text{H}_{\text{SAMP2}})^2 + (\delta^{13}\text{C}_{\text{SAMP1}} - \delta^{13}\text{C}_{\text{SAMP2}})^2 + (\delta^{15}\text{N}_{\text{SAMP1}} - \delta^{15}\text{N}_{\text{SAMP2}})^2
\]

Applying this comparison method to the Queensland survey data identified similarities between a number of seizures from different locations, for example; Gympie (QLD 4570) and Mt. Tamborine (QLD 4271) (samples #27 and #45) separated by a distance of approximately 242 km but seized on the same date or Beerwah (QLD 4519) and Tewantin (QLD 4565) (sample #31 and #145) separated by a distance of approximately 70 km and seized two days apart.
To test the strength of evidence that might be affirmed from a match in isotopic composition the data were transformed into a three-dimensional kernel density estimate (KDE) matrix. The R script for this process is given in Appendix B to this manuscript. To explain this process Figure 3 shows an image of a simpler, two-dimensional KDE of $\delta^{13}C$ and $\delta^{15}N$ data. Each cell of this matrix represents the probability that a sample of MA has a given combination of $\delta^{13}C / \delta^{15}N$ compositions. Figure 3 has been colour coded so that combinations with low probability appear dark blue and combinations with high probability appear red. This KDE matrix is a numeric expression of the information presented in Figure 1a i.e. there is a high probability that samples are derived from synthetic Eph/PSE, with a small region of $\delta^{13}C / \delta^{15}N$ combination being extremely likely (Figure 3 “A”), and much lower probabilities for other combinations, e.g. (Figure 3 “B”). The KDE matrix allows the calculation of a likelihood ratio (LHR) based on the assumption that two samples have identical (or very similar) $\delta^{13}C / \delta^{15}N$ compositions because they are from the same batch rather than by random chance. This method was previously described for polyethylene films [34] for which only $\delta^2H$ and $\delta^{13}C$ measurements were possible. In the present study, a three-dimensional KDE matrix was calculated for $\delta^2H / \delta^{13}C / \delta^{15}N$ using bin sizes of 2.0, 0.2 and 0.2 ‰ respectively to produce a matrix with 142,884 cells.

For the examples above, seizures from Gympie and Mt. Tamborine shared a relatively common $\delta^{13}C / \delta^{15}N$ synthetic precursor composition (Figure 3 “A”) and the LHR of these samples originating from the same batch was calculated as 375. In contrast, the samples from Beerwah and Tewantin shared a less common precursor composition (Figure 3 “B”), more enriched in $^{15}N$, and the LHR of these samples originating from the same batch was calculated as 3,959. The verbal equivalent for these LHRs would be “moderately strong support” (LHR: 375) and “strong support” (LHR: 3,959) respectively for the two pairs of samples having originated from the same batch [35].

Stable isotopic compositions have been used to predict information about the geographic origins of drugs [36-38], but, to date, not synthetic drugs. It was hoped that spatial plots of $\delta^2H$, $\delta^{13}C$ or $\delta^{15}N$ values might reveal patterns in manufacture, trafficking or supply of MA within Queensland. These plots did not, however, appear to show any useful trends or distributions. Figure 4 shows a spatial plot (or ice-o-scape) of the Queensland MA seizures colour coded according to the assigned synthetic source of the precursor. The distribution of points on this map reflected the population density of Queensland i.e. largely centred in Brisbane and the immediate surrounds. One feature,
however, was that only one P2P derived MA seizures occurred outside the Brisbane metropolitan area, all other regional seizures were derived from *synthetic* Eph/PSE. Combining this type of data from other Australian states or countries in the region may provide useful information about the importation, manufacturing and trafficking of MA.

4. Conclusions

Stable isotopic analysis of 181 samples of MA seized in Queensland between July 2015 and February 2017 allowed an assessment of the synthetic precursors by comparison with published data. Excluding l-MA, the weight of the seizures in this survey ranged from 2.3 to 139.2 g and they were considered typical of dealer-level amounts in Queensland.

The majority of Queensland samples (~ 88 %) were classified as being manufactured from *synthetic* Eph/PSE including all but one seizure from regional Queensland. A smaller number of samples were most likely derived from P2P and seized mainly in the Brisbane metropolitan area.

A number of the P2P derived samples were found to be enantiomerically pure l-MA. The size and nature of these seizures (an essentially worthless enantiomer) led to speculation that this importation was used to test a smuggling route into Australia.

This survey provided some insight into the synthetic origins of MA seized in Queensland but the geographic origins of these samples remained ambiguous since a large proportion of both precursor chemicals (Eph/PSE) and finished product (MA) are reported to originate in China. It seems unlikely that isotopic analysis can distinguish MA manufactured locally from MA manufactured in China using the same precursor chemicals.

Although a large proportion of precursor chemicals available in Mexico are also reported to be sourced in China, the majority of MA is manufactured from P2P. Despite a common origin, in China, it appeared possible to distinguish MA derived from either Eph/PSE or P2P based in isotopic composition.

A searchable database created from the combination of δ²H / δ¹³C / δ¹⁵N measurements from this survey proved valuable in linking seizures from different times and locations within Queensland. Having identified possible links between MA samples, KDE provided an estimate of the LHR that these samples came from the same synthetic batch. The results from this survey showed that a
comparison of Queensland MA seizures with data from Australia border seizures would be unrepresentative.
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Appendix A. Supplementary data

Supplementary data associated with this article

Appendix B. Programming code

R script used to analyse the data for this article
References


Fig. 1. Scatter plots of MA isotopic composition (a) $\delta^{13}$C versus $\delta^{15}$N and (b) $\delta^2$H versus $\delta^{15}$N showing data from NMIA/AFP (circle, red) and Queensland (cross, blue). Circles show the proposed origin of the Eph/PSE, synthetic (solid), semi-synthetic (dashed) or natural (dotted). These circles have no statistical significance.
Fig. 2. Scatter plots of MA $\delta^{33}$C versus $\delta^{15}$N composition, showing data from Queensland (cross, blue) and Japan [Iwata 2008] (triangle, green) [Tsujikawa 2012] (square, black). Circles show the proposed origin of the Eph/PSE precursor, synthetic (solid) or natural (dotted). These circles have no statistical significance.
Fig. 3. Image of the kernel density estimate matrix of $\delta^{13}\text{C}_{\text{VPDB}}$ verses $\delta^{15}\text{N}_{\text{AIR}}$ for Queensland MA, showing the probability of a given combination of isotopic compositions (topo.colors).
Fig. 4. Spatial plot of Queensland MA seizures showing proposed synthetic precursors (green = synthetic Eph/PSE, red = P2P d-MA and black = P2P l-MA).
AUTHOR DECLARATION

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We further confirm that any aspect of the work covered in this manuscript that has involved either experimental animals or human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

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