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Abstract:	This project determined the stable isotopic compositions ($\delta2H$ / $\delta 13C$ / $\delta 15N$) 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 $\delta15N$ 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 $\delta2H$, $\delta13C$ and $\delta15N$ 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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<u>Highlights</u>

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
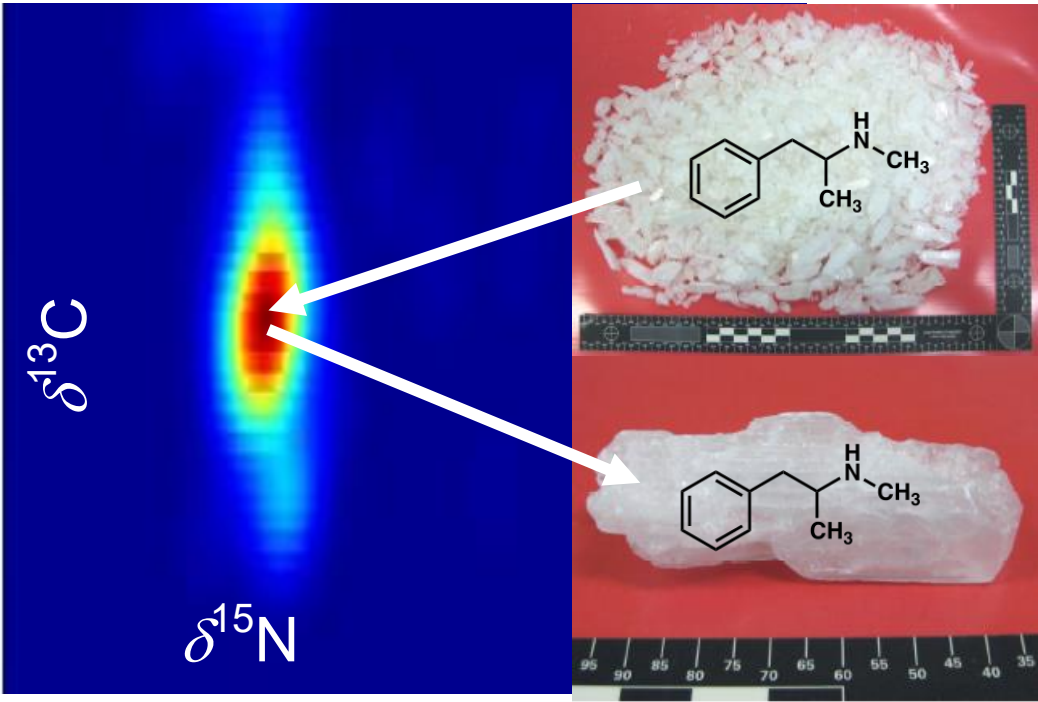
Graphical Abstract

A stable isotope ratio approach to investigate the origins of illicit methylamphetamine in Queensland, Australia.

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1   A B S T R A C T

2   This project determined the stable isotopic compositions (δ^2H / δ^{13}C / δ^{15}N) of 181 samples of high purity

3   illicit methylamphetamine seized in Queensland between July 2015 and February 2017. The project was

4   undertaken to augment existing stable isotopic composition data for methylamphetamine samples seized

5   at Australian borders.

6   Based on a rule-of-thumb developed by the National Measurements Institute of Australia a majority of

7   Queensland samples (~ 88 %) were classified as being manufactured from synthetic ephedrine/pseudo-

8   ephedrine (e.g. from 1-phenyl-1-propanone). A much smaller number of samples (~ 12 %) were initially

9   classified as being manufactured from natural ephedrine/pseudo-ephedrine (from *Ephedra sinica*).

10   Subsequent analysis of samples classified as having a natural precursor found them to be enantiomerically

11   pure l-methylamphetamine and, therefore, most likely manufactured from phenylpropan-2-one. In contrast

12   to border seizures, no methylamphetamine seizures from Queensland had isotopic compositions consistent

13   with being manufactured from semi-synthetic ephedrine/pseudo-ephedrine (fermentation of benzaldehyde

14   and pyruvic acid).

15   A small number of enantiomerically pure d-methylamphetamine samples with δ^{15}N values intermediate

16   between natural and synthetic classification were also assumed to be derived from phenylpropan-2-one.

17   These samples were isotopically similar to seizures reported in Japan that had been imported from Eurasia.

18   The combination of δ^2H, δ^{13}C and δ^{15}N measurements was used to create a searchable database that

19   identified a number of isotopically similar samples of methylamphetamine seized at different locations and

20   different times. A kernel density estimate model was used to assign "moderately-strong" to "strong" support

21   that these samples came from the same synthetic batch.

22   *Keywords:*

23   Isotope ratio mass spectrometry

24   Likelihood ratios

25   Methamphetamine

26   Methylamphetamine

27   Probability statistics

28

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, methamphet 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 δ^{13}C / δ^{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 δ^{13}C / δ^{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 δ^{15}N compositions [14]. Further, systematic research into the preparation of MDMA by reductive amination found significant isotopic variation with changing reaction conditions; δ^{2}H values were most affected by imine stir time; δ^{13}C values were most affected by the overall efficiency (completeness) of the reaction; δ^{15}N values were most affected by an excess of methylamine [15]. This line of research concluded that the isotopic compositions of ATS

30 prepared by reductive amination were characteristic of individual reaction batches more so than

31 the precursor materials.

32 In contrast, other research has found that samples of MA, prepared from ephedrine/pseudo-

33 ephedrine (Eph/PSE) retained the δ^2H, $\delta^{13}C$ and $\delta^{15}N$ compositions of the starting materials [16-

34 18]. This relationship has been demonstrated for a number of synthetic routes (*Nagal* [hydroiodic

35 acid/red phosphorus], *Moscow* [red phosphorus/iodine], *Hypo* [hypophosphorus acid/iodine] or

36 *Emde* [thionyl chloride]) and has also been shown to be unaffected by variations in the reaction

37 conditions [16]. This line of research concluded that the isotopic composition of MA could be used

38 to identify the source of Eph/PSE precursors as being; *natural* (*Ephedra sinica*), *semi-synthetic*

39 (fermentation of benzaldehyde and pyruvic acid) or fully *synthetic* (e.g. 1-phenyl-1-propanone aka

40 propiophenone) [19].

41 Complementary research into some physiochemical processes used during MA manufacture, such

42 as enantiomeric resolution [20] and crystallization [21], found some evidence for isotopic

43 fractionation, but concluded that the isotopic profile of the final MA-HCl product retained useful

44 information about its provenance.

45 In Australia, the Australian Federal Police (AFP) Forensic Drug Intelligence team operates a drug

46 profiling capability through the National Measurements Institute (NMIA). The information is used

47 for operational intelligence, i.e. to link different seizures, with the aim of disrupting drug

48 trafficking networks. In 2013 the NMIA reported stable isotopic composition data (δ^2H / $\delta^{13}C$ /

49 $\delta^{15}N$) for 782 samples of MA seized at Australian borders and compared these to samples of MA

50 synthesized in-house from well characterized starting materials [19]. The study presented here

51 aimed to compare and contrast these data with high purity MA seized within Queensland,

52 Australia.

2. Materials and methods

2.1. Methylamphetamine samples

55 A total of 181 MA samples were sub-sampled from exhibits submitted by the Queensland Police

56 Service (QPS) to the Queensland Health Forensic and Scientific Services (QHFSS) Illicit Drugs

57 Group between July 2015 and February 2017. The identity of the active ingredient (MA) was

58 confirmed by gas chromatography mass spectrometry (GC/MS) and the percentage purity was

59 determined by high-performance liquid chromatography (HPLC) according to NATA (ISO/IEC

60 17025:2005) accredited laboratory methods. Sample purities (expressed as free-base) ranged from

61 60.2 to 82.2 % with an average purity of 76.6 %, equivalent to 95.0 % expressed as MA-HCl.

62 The QHFSS Illicit Drugs Group does not routinely undertake drug profiling analyses, such as

63 acquiring chemical or elemental compositions. This project was undertaken to assess what

64 evidential and intelligence value that might be obtained from stable isotopic analysis.

2.2. Stable isotopic analysis

66 All isotope ratio measurements were performed using a Delta V^{PLUS} Isotope Ratio Mass

67 Spectrometer (Thermo Scientific, Bremen, Germany) coupled to a ConFlo IV interface for

68 working gas introduction and sample dilution. Data were collected using ISODAT 3 software.

69 For carbon and nitrogen analysis, samples were crimped into tin capsules (4 × 3.2 mm, IVA

70 Analysentechnik, Meerbusch, Germany). Samples were analysed using a Flash 2000 elemental

71 analyser (EA) (Thermo Scientific) in flash combustion mode at 950 °C, with a helium flow of 100

72 mL/min, followed by a post reactor GC column at 110 °C. The reactor was packed with chromium

73 (II) oxide, electrolytic copper and silvered cobaltous (II, III) oxide (IVA). A sulfur trap (silver

74 wool) and a water trap (magnesium perchlorate) were placed between the reactor and the GC

75 column. For nitrogen analysis an additional CO_2 trap (Carbosorb™ and magnesium perchlorate)

76 was installed following the water trap. Carbon measurements were normalised to the VPDB scale

77 using IAEA-CH-7 (International Atomic Energy Agency [IAEA], Vienna, Austria) and glucose

78 solutions calibrated against the reference materials NBS 19 and LSVEC [22]. Nitrogen

79 measurements were normalised to the N_{AIR} scale using ammonium sulphate reference materials

80 IAEA-N-1, IAEA-N-2, and USGS25 (IAEA).

81 For hydrogen analysis, samples were crimped into silver capsules (4 × 3.2 mm, IVA). Samples

82 were analysed using a TC/EA elemental analyser (Thermo Scientific) at 1400 °C with a helium

83 flow of 90 mL/min via a bottom-feed adaptor. The glassy carbon reactor tube was replaced with a

84 custom-made alumina tube (IVA) (12 mm o.d., 6 mm i.d., length 420 mm). The reactor packing

85 comprised glassy carbon (IVA) and chromium (Aldrich, Darmstadt, Germany) in the upper 50 mm

86 and glassy carbon and manganese (Aldrich) in the lower 50 mm. Samples were introduced into the

87 reactor via a Zero-Blank auto-sampler (Costech Analytical Technologies Inc., Valencia, CA,

88　USA). Hydrogen measurements were normalised to the VSMOW scale using silver encapsulated

89　waters GISP, VSMOW and UC03 (United States Geological Survey [USGS], Reston, VA, USA).

90　A sample of phenacetin (N-(4-ethoxyphenyl)-acetamide), with well characterised isotopic

91　composition [23, 24] was analysed at the beginning of each acquisition sequence and after every

92　five samples for quality control purposes. These data are presented in the supplementary material.

93　*2.3 Chiral analysis*

94　The separation and identification of MA enantiomers was achieved using the derivatizing reagent

95　N-trifluoroacetyl-(S)-propyl chloride (Sigma, Castle Hill, NSW, Australia) [25] prepared as a 0.1

96　M solution in dichloromethane.

97　Approximately 1 mg of sample was dissolved in 1 mL of ammonia infused hexane in a 2 mL

98　autosampler vial and 20 µL of the derivatising reagent added. Reference samples of d-MA and

99　d,l-MA (Cerilliant, Kinesis Australia, Redland Bay, QLD, Australia) were prepared in the same

100　way.

101　Samples were analysed using an Agilent 7890B GC/MS equipped with a 30m, 0.25 mm, HP-

102　1MS column. The column was programmed from 65 $^{\circ}$C (1 min hold) to 300 $^{\circ}$C (3.3 min hold) at

103　a rate of 40 $^{\circ}$C/min. The injector and transfer line were maintained at 280 $^{\circ}$C. The MS was

104　operated at 70eV electron ionization with a scan range from 35-500 *m/z*.

105　*2.4. Data analysis*

106　Scatter plots and k-means clustering were performed using SYSTAT 12 statistical and graphics

107　software (Systat Software, Inc. San Jose, CA, USA). Expectation maximization clustering was

108　performed using SPSS Statistics 24 with AMOS 23 (IBM, Chicago, Illinois, USA). Spatial plots,

109　kernel density estimations and likelihood ratios were calculated using R 3.3.3 software

110　environment for statistical computing and graphics (www.r-project.org).

111

3. Results and discussion

113　*3.1. Notes on δ^2H analysis*

114　Hydrogen isotopic analyses were performed using an alumina inner reactor tube packed with a

115　mixture of glassy carbon, metallic chromium and metallic manganese as described above. This

116   packing was based on recently published research that addressed problems in the high temperature

117   conversion of organic molecules containing hetero-atoms such as nitrogen, chlorine or sulfur to

118   H_2 gas [26, 27]. High temperature conversion using only glassy carbon, was reported to form

119   compounds such as HCN and HCl so that the final yield of H_2 did not reflect the isotopic

120   composition of the hydrogen present in the parent molecule. Complete conversion of organic

121   hydrogen to H_2 was considered essential for the analysis MA-HCl that contained a relatively high

122   proportion of both nitrogen and chlorine and, potentially, sulfur from MSM as a cutting agent.

123   Initial trials of this reactor packing using a conventional glassy carbon inner reactor tube (12 mm

124   o.d., 7 mm i.d., length 356 mm) produced broad, tailing peaks approximately 57 s wide. This was

125   attributed to interactions with the chromium/manganese packing. In addition, after a period of use

126   the chromium/glassy carbon packing became rigid and difficult to remove without damaging the

127   glassy carbon tube. To overcome these problems, the glassy carbon tube was replaced with an

128   alumina tube with a 6 mm i.d. (bottom drilled to 7 mm to fit the bottom feed adaptor) to increase

129   the linear velocity of the carrier gas. Using this inner reactor tube, peak widths were typically 42

130   s and comparable to peak widths produced using only glassy carbon as a packing. After calibrating

131   the instrument with silver encapsulated waters, organic reference materials IAEA-CH-7 (-100.3

132   ‰ ± 2.0) and NBS 22 (-116.9 ‰ ± 0.9) were analysed to test the performance of the reactor to

133   organic materials. Both reference materials gave results within the certified uncertainties

134   (http://www.ciaaw.org/hydrogen-references.htm accessed Feb. 2018). This reactor composition

135   proved to have a further advantage because it did not exhibit memory effects associated with glassy

136   carbon reactors [28] *i.e.* the measured isotopic composition of a sample was not affected by the

137   isotopic composition of the previous samples. Because the alumina reactor tube had a smaller i.d.

138   than the conventional glassy carbon tube it was necessary to reduce the size of the graphite

139   crucibles by filing the corners using fine abrasive paper. Using this reactor configuration it was

140   possible to analyse three batches of samples, approximately 120 individual capsules per batch,

141   before the reactor packing had to be replaced. This was equivalent to approximately 60 samples

142   analysed in triplicate with corresponding calibration and quality control materials per reactor.

143   To assess if extrinsic hydrogen was present, due to moisture absorbed or adsorbed by the samples,

144   the δ^2H compositions of five samples of MA-HCl, with purities ranging from 69 to 80.4 %, were

145   measured before and after four days of drying under vacuum (60 °C and 10 mbar). These data are

146   given in Appendix A. The average change in isotopic composition over four days was -1.5 ‰ and,

147   it was concluded that heating/vacuum treatment was not necessary for routine samples.

148   *3.2. Synthetic origins of Queensland MA*

149   Based on published research, summarized in Table 1, the NMIA has developed a rule-of-thumb

150   (RoT) to characterize the Eph/PSE precursors of MA as *natural* (*Ephedra sinica*), *semi-synthetic*

151   (fermentation of benzaldehyde) or fully *synthetic* (e.g. propiophenone). The NMIA also used k-

152   means and expectation maximisation (EM) clustering techniques to assign seized samples to these

153   three classes with 95.5 % agreement between the two statistical methods [19]. Both methods also

154   showed close agreement with the RoT.

155   Table 2 shows the results of the three classification techniques applied to the Queensland survey

156   data together with the original NMIA/AFP results. Initial RoT classification found that none of the

157   Queensland survey samples had an isotopic composition consistent with manufacture from *semi-*

158   *synthetic* Eph/PSE and, therefore, subsequent k-means and EM clustering methods were applied

159   to the Queensland survey data using a two-class model (*natural* versus *synthetic*).

160

161 **Table 1**

162 The expected isotopic composition range for MA derived from known precursors based on a
163 number of heuristic assessments.

precursor	isotopic composition		
	$\delta^{13}C_{VPDB}$	$\delta^{15}N_{AIR}$	
natural Eph/PSE	-26.5 to -30 ‰	> +2.5 ‰	Salouros *et al.* 2013
semi-synthetic Eph/PSE [a]	> -26.5 ‰		Makino *et al.* 2005
synthetic Eph/PSE	< -26.5 ‰	< +2.5 ‰	Salouros *et al.* 2013
P2P	-32 ‰	+5 ‰	Collins & Salouros 2015

164 [a] samples prepared from semi-synthetic Eph/PSE were also characterized by positive δ^2H values (Collins
165 et al. 2009)

166 **Table 2**

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

Eph/PSE precursor	classification method		
	RoT	k-means	EM
	Queensland data		
semi-synthetic	0	NA	NA
synthetic	88	66	86
natural	12	34	14
	NMIA/AFP data		
semi-synthetic	34	37	39
synthetic	24	22	23
natural	42	41	38

169

170　The results in Table 2 showed that the majority (~ 88%) of Queensland MA had isotopic

171　compositions consistent with preparation from *synthetic* Eph/PSE with a much smaller number

172　consistent with *natural* Eph/PSE.

173　Australian Government reports have identified PSE, extracted from "cold and flu" medicines, as

174　the predominant precursor used to produce MA in Queensland for many years [2]. More recently,

175　however, there has been a significant increase in the amount of imported MA with an estimated

176　70 % originating in China [29]. In addition, half of the precursor chemicals seized in Australia and

177　New Zealand also originated in China and, to a lesser extent India [29]. Although the term Eph/PSE

178　is used throughout this report, to denote a precursor that may be either ephedrine or pseudo-

179　ephedrine, pharmaceutical PSE is typically manufactured from synthetic precursors such as

180　propiophenone [30]. Fermentation of benzaldehyde (the *semi-synthetic* method) yields a mixture

181　of Eph/PSE which may not be desirable in a commercial process.

182　Although the main finding from the Queensland survey was unsurprising a proportion, albeit small,

183　of the Queensland survey MA appeared to have been manufactured from Ephedra. There was also

184　a notable disagreement between assigned precursors using k-means or EM clustering, in contrast

185　to the NMIA/AFP results that showed close agreement between the two methods.

186　To investigate these findings further, the QHFSS Illicit Drugs Group undertook analyses to

187　determine the chiral form of MA samples with positive $\delta^{15}N$ values, which included all of the

188　samples classified as derived from *natural* Eph/PSE. Eph/PSE sourced from pharmaceutical

189　products (l-Eph or d-PSE) can be used to prepare enantiomerically pure d-MA, the physiologically

190　active form of the drug. In Mexico, however, large quantities of MA are prepared by reductive

191　amination of P2P which yields racemic MA that undergoes an optical resolution process to

192　separate the d and l forms of the drug [20] (as noted above, optical resolution is reported not to

193　have a significant effect on the isotopic composition of the final product). Although it has been

194　reported that 80 % of precursor chemicals used by Mexican cartels are sourced in China [29]

195　differences in the isotopic compositions of P2P and Eph/PSE derived MA should still be apparent.

196　The most ^{15}N enriched samples in the Queensland survey (+13.35 to +14.99 ‰) were all found to

197　be enantiomerically pure l-MA (samples #108 to 121). The majority of these samples came from

198　a single Police operation in North Booval that seized 14 exhibits, each weighing approximately

199　one kg. Two other samples of enantiomerically pure l-MA, with similar $\delta^{15}N$ compositions

200 (samples #31 and #145), were seized several weeks earlier at different locations and weighed only

201 a few grams. All other samples of MA with positive δ^{15}N values (+0.88 to +8.46 ‰) were found

202 to be enantiomerically pure d-MA. None of the samples in this survey were found to comprise

203 mixtures of the two enantiomers.

204 Samples of MA with δ^{15}N values intermediate between *synthetic* and *natural* Eph/PSE

205 classifications (+0.88 to +8.46 ‰) were initially assumed to be mixtures of MA produced from

206 *synthetic* and *natural* Eph/PSE. A previous report by the Japanese National Research Institute of

207 Police Science (JRIPS) identified MA seizures derived from several batches of MA, with different

208 sources of precursors, mixed together [31]. Considering both isotopic and chiral analysis, however,

209 it was apparent that the samples, consistent with *natural* Eph/PSE characteristics were more likely

210 to have been manufactured from P2P and it appeared likely that the d-MA samples with

211 intermediate δ^{15}N values (0 to +10 ‰) were also manufactured from P2P. These samples had δ^{13}C

212 values (approximately -30 and -28 ‰) and δ^{2}H values (approximately -100 to -22 ‰) consistent

213 with data previously reported for P2P (δ^{13}C = -31.6 to -28 ‰ and δ^{2}H = -71 to -53 ‰) [32].

214 *3.3. Queensland and Australian borders*

215 The full dataset from the Queensland survey is presented in Appendix A of this manuscript. The

216 range of isotopic compositions in this study were; δ^{2}H$_{VSMOW}$ -147.6 to -22.2, δ^{13}C$_{VPDB}$ -30.01 to -

217 26.27 and δ^{15}N$_{AIR}$ -10.18 to +14.99 ‰. The median standard deviations for these measurements

218 were; δ^{2}H = 0.7, δ^{13}C = 0.04 and δ^{15}N = 0.09 ‰ (n=3). The range of isotopic compositions of the

219 Queensland survey was significantly narrower, for all three elements, than reported by NMIA

220 (δ^{2}H$_{VSMOW}$ -249.9 to +103.0, δ^{13}C$_{VPDB}$ -31.5 to -23.4 and δ^{15}N$_{AIR}$ -14.9 to +19.4). Notably,

221 hydrogen and carbon data reported by NMIA spanned more than twice the range observed in the

222 Queensland survey.

223 Comparisons between Queensland and NMIA/AFP data can easily be visualized in the scatter-

224 plots shown in Figure 1. The delineation between *natural*, *semi-synthetic* and *synthetic* Eph/PSE

225 shown in Figure 1 is based on the RoT summarized in Table 1. The size of the circles in Figure 1

226 has no statistical significance, they simply approximate the characteristics of the three precursors

227 classifications.

228 From Figure 1a it was, again, apparent that the majority of samples in the Queensland survey had

229 δ^{13}C values below -26.5 ‰ and δ^{15}N values below +2.5 ‰, consistent with manufacture from

230 *synthetic* Eph/PSE. None of the Queensland survey samples had isotopic compositions consistent

231 with having been manufacture from *semi-synthetic* Eph/PSE. This finding was in contrast to the

232 NMIA/AFP data for which *semi-synthetic* and *natural* Eph/PSE were identified as the dominent

233 precursors. Relatively few of the samples from the Queensland survey had δ^{13}C / δ^{15}N values

234 consistent with manufacture from *natural* Eph/PSE and most of these samples were subsequently

235 identified as enantiomerically pure l-MA and, therefore, most likely manufactured from P2P.

236 Figure 1b shows a plot of δ^2H versus δ^{15}N composition that displays similar trends to those shown

237 in Figure 1a, *i.e.* the majority of samples from the Queensland survey were shown to be derived

238 from *synthetic* Eph/PSE and none from *semi-synthetic* Eph/PSE. The absence of *semi-synthetic*

239 Eph/PSE derived MA was evident as this MA precursor is characterized by positive δ^2H values

240 [Collins et al. 2009].

241 In Figure 1b it was noticed that the δ^2H values for Queensland survey *synthetic* class appear

242 depleted with respect to the equivalent NMIA/AFP data which may be a bona fide difference or

243 may be due to different reactor materials used for measurements in the two laboratories. It has

244 been proposed that δ^2H measurements obtained using different reactor materials can be

245 standardized by reporting data for reference materials alongside sample data [33]. The data

246 obtained for the QC material (phenacetin) used in this study are presented in Appendix A (this

247 material is commercially available from LGC Standards, UK).

3.4. Queensland and Japanese data

249 Figure 2 shows a scatter plot of δ^{13}C versus δ^{15}N for MA from the Queensland survey compared

250 with MA samples seized in Japan and analysed by the JRIPS [Iwata 2008, Tsuj 2012]. The JRIPS

251 states that there are virtually no clandestine MA laboratories operating in Japan and, therefore, the

252 majority of seizures come from numerous locations around the world [18]. The Japanese data,

253 shown in Figure 2, are believed to represent MA from Eurasia although police intelligence only

254 knows the country from which the smuggled MA departed, not the country in which it was

255 manufactured. It is interesting to note that many of the Japanese MA samples, with δ^{15}N value

256 intermediate between the *synthetic* and *natural* classifications, had δ^{13}C / δ^{15}N profiles very similar

257 to Queensland survey samples (Figure 2, triangle, green). The JRIPS state that they do not believe

258 that these samples originated in Australia and a conclusion may be that MA was smuggled to both

259 Japan and Australia from the same source in/or via Eurasia. Subsequent analyses of individual MA

260 crystals by the JRIPS found that MA seizures with intermediate $\delta^{15}N$ values were the result of

261 mixing several batches of MA from different precursor sources [31]. Samples tested as part of the

262 Queensland survey were homogenized prior to isotopic characterization and no evidence for a

263 mixed source was found. The JRIPS attributed samples with more depleted $\delta^{13}C$ values (Figure 2,

264 square, black) to a P2P precursor.

265 *3.5. Finding links and weighing the evidence*

266 Although a knowledge of synthetic precursors is important in understanding the operations of

267 clandestine MA laboratories, the stable isotopic composition of MA also provides a means to

268 associate or differentiate batches of MA seized at different times and locations. To facilitate this,

269 the average δ^2H, $\delta^{13}C$ and $\delta^{15}N$ values for each sample from the Queensland survey were compiled

270 into a spreadsheet, including; the date and location of each seizure and the weight and purity of

271 the MA seized. These data are presented in Appendix A. Isotopic values in the "database" were

272 auto-scaled (Eq.1) so that δ^2H, $\delta^{13}C$ and $\delta^{15}N$ data all had a similar range. Two samples (SAMP1

273 and SAMP2) were then compared using a simple Euclidean distance calculation (Eq.2). The

274 smaller the comparison distance, the greater the similarity between samples.

275

276   1 $autoscaled\ data = \dfrac{data - average}{standard\ deviation}$

277

278   2 $comparison = (\delta^2H_{SAMP1} - \delta^2H_{SAMP2})^2 + (\delta^{13}C_{SAMP1} - \delta^{13}C_{SAMP2})^2 + (\delta^{15}N_{SAMP1} - \delta^{15}N_{SAMP2})^2$

279

280 Applying this comparison method to the Queensland survey data identified similarities between a

281 number of seizures from different locations, for example; Gympie (QLD 4570) and Mt. Tamborine

282 (QLD 4271) (samples #27 and #45) separated by a distance of approximately 242 km but seized

283 on the same date or Beerwah (QLD 4519) and Tewantin (QLD 4565) (sample #31 and #145)

284 separated by a distance of approximately 70 km and seized two days apart.

285 To test the strength of evidence that might be affirmed from a match in isotopic composition the

286 data were transformed into a three-dimensional kernel density estimate (KDE) matrix. The R script

287 for this process is given in Appendix B to this manuscript. To explain this process Figure 3 shows

288 an image of a simpler, two dimensional KDE of $\delta^{13}C$ and $\delta^{15}N$ data. Each cell of this matrix

289 represents the probability that a sample of MA has a given combination of $\delta^{13}C$ / $\delta^{15}N$

290 compositions. Figure 3 has been colour coded so that combinations with low probability appear

291 dark blue and combinations with high probability appear red. This KDE matrix is a numeric

292 expression of the information presented in Figure 1a *i.e.* there is a high probability that samples

293 are derived from *synthetic* Eph/PSE, with a small region of $\delta^{13}C$ / $\delta^{15}N$ combination being

294 extremely likely (Figure 3 "A"), and much lower probabilities for other combinations, *e.g.* (Figure

295 3 "B"). The KDE matrix allows the calculation of a likelihood ratio (LHR) based on the assumption

296 that two samples have identical (or very similar) $\delta^{13}C$ / $\delta^{15}N$ compositions because they are from

297 the same batch rather than by random chance. This method was previously described for

298 polyethylene films [34] for which only $\delta^{2}H$ and $\delta^{13}C$ measurements were possible. In the present

299 study, a three-dimensional KDE matrix was calculated for $\delta^{2}H$ / $\delta^{13}C$ / $\delta^{15}N$ using bin sizes of 2.0,

300 0.2 and 0.2 ‰ respectively to produce a matrix with 142,884 cells.

301 For the examples above, seizures from Gympie and Mt. Tamborine shared a relatively common

302 $\delta^{13}C$ / $\delta^{15}N$ *synthetic* precursor composition (Figure 3 "A") and the LHR of these samples

303 originating from the same batch was calculated as 375. In contrast, the samples from Beerwah and

304 Tewantin shared a less common precursor composition (Figure 3 "B"), more enriched in ^{15}N, and

305 the LHR of these samples originating from the same batch was calculated as 3,959. The verbal

306 equivalent for these LHRs would be "moderately strong support" (LHR: 375) and "strong support"

307 (LHR: 3,959) respectively for the two pairs of samples having originated from the same batch [35].

308 Stable isotopic compositions have been used to predict information about the geographic origins

309 of drugs [36-38], but, to date, not synthetic drugs. It was hoped that spatial plots of $\delta^{2}H$, $\delta^{13}C$ or

310 $\delta^{15}N$ values might reveal patterns in manufacture, trafficking or supply of MA within Queensland.

311 These plots did not, however, appear to show any useful trends or distributions. Figure 4 shows a

312 spatial plot (or *ice-o-scape*) of the Queensland MA seizures colour coded according to the assigned

313 synthetic source of the precursor. The distribution of points on this map reflected the population

314 density of Queensland i.e. largely centred in Brisbane and the immediate surrounds. One feature,

315 however, was that only one P2P derived MA seizures occurred outside the Brisbane metropolitan
316 area, all other regional seizures were derived from *synthetic* Eph/PSE. Combining this type of data
317 from other Australian states or countries in the region may provide useful information about the
318 importation, manufacturing and trafficking of MA.

4. Conclusions

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

324 The majority of Queensland samples (~ 88 %) were classified as being manufactured from
325 *synthetic* Eph/PSE including all but one seizure from regional Queensland. A smaller number of
326 samples were most likely derived from P2P and seized mainly in the Brisbane metropolitan area.
327 A number of the P2P derived samples were found to be enantiomerically pure l-MA. The size and
328 nature of these seizures (an essentially worthless enantiomer) led to speculation that this
329 importation was used to test a smuggling route into Australia.

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

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

339 A searchable database created from the combination of δ^2H / δ^{13}C / δ^{15}N measurements from this
340 survey proved valuable in linking seizures from different times and locations within Queensland.
341 Having identified possible links between MA samples, KDE provided an estimate of the LHR that
342 these samples came from the same synthetic batch. The results from this survey showed that a

343   comparison of Queensland MA seizures with data from Australia border seizures would be

344   unrepresentative.

345

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article

Appendix B. Programming code

R script used to analyse the data for this article

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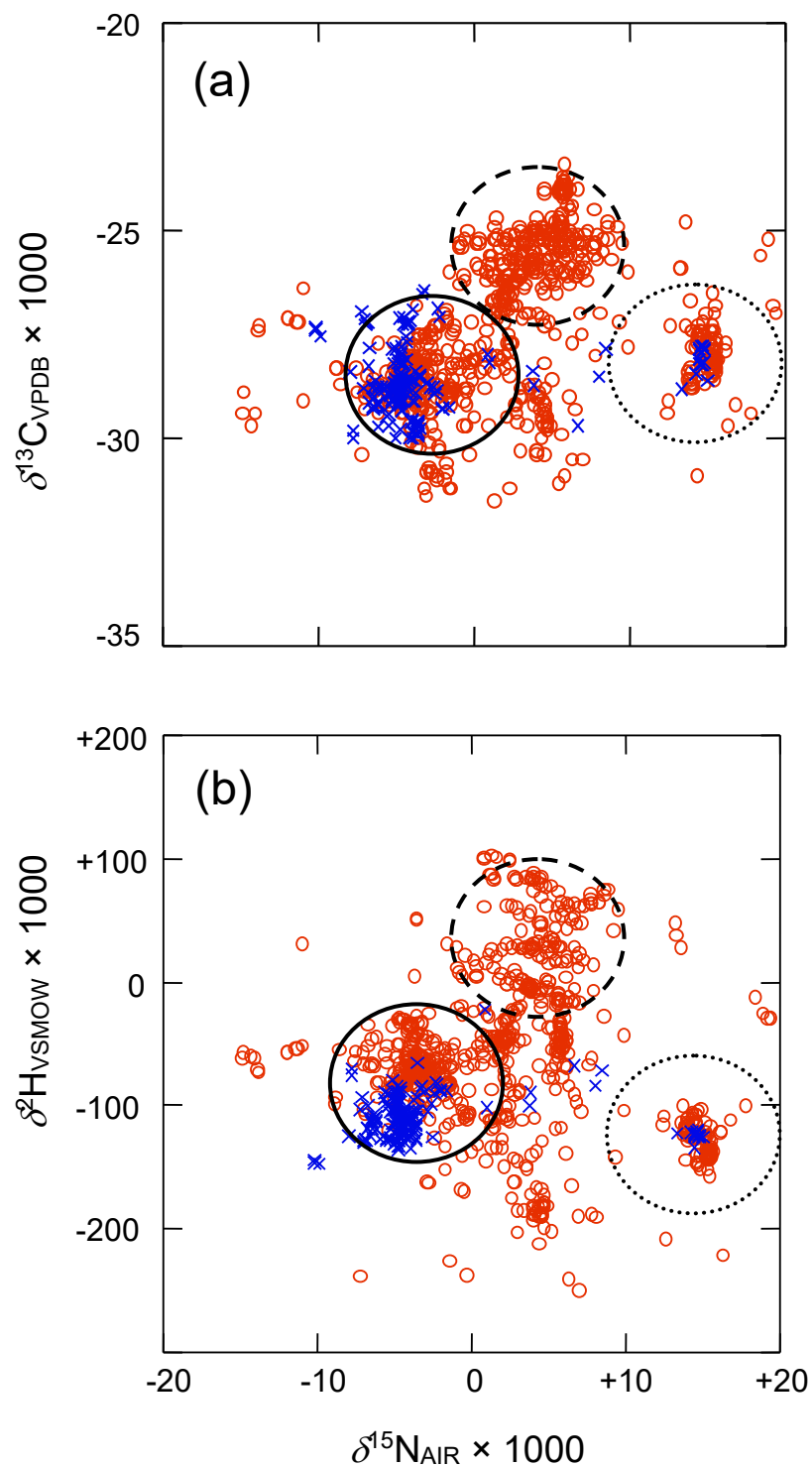
Figure 1

Fig. 1. Scatter plots of MA isotopic composition (a) $\delta^{13}C$ versus $\delta^{15}N$ and (b) δ^2H 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.

Figure 2
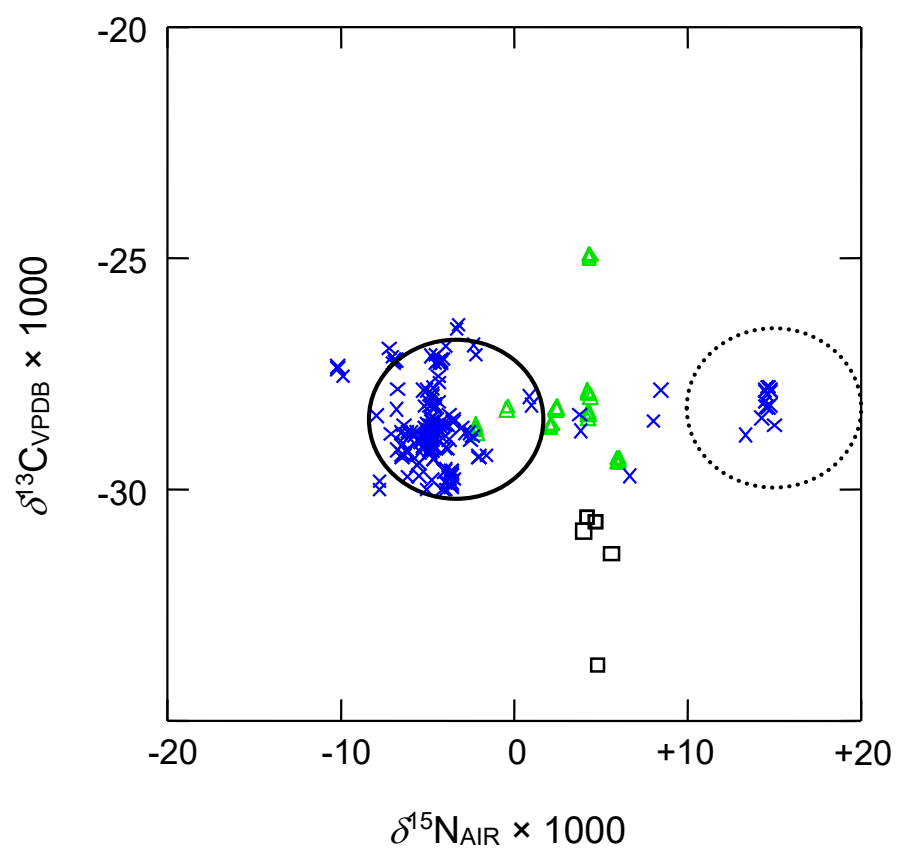
Fig. 2. Scatter plots of MA δ^{13}C versus δ^{15}N composition, showing data from Queensland (cross, blue) and Japan [Iwata 2008] (triangle, green) [Tsujikawa 2012] (square, black). Circles show the proposed origin of the Eph/PSE precursor, synthetic (solid) or natural (dotted). These circles have no statistical significance.

Figure 3
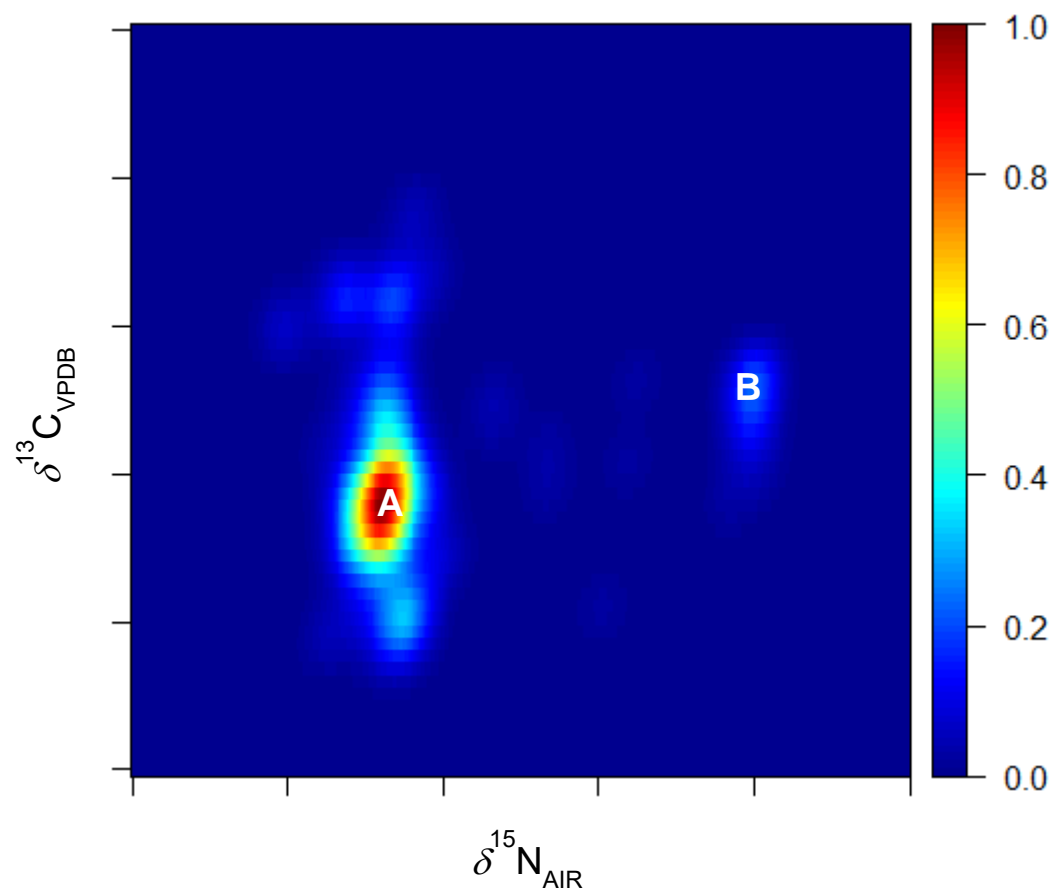
Fig. 3. Image of the kernel density estimate matrix of δ^{13}C verses δ^{15}N for Queensland MA, showing the probability of a given combination of isotopic compositions (topo.colors).

Figure 4
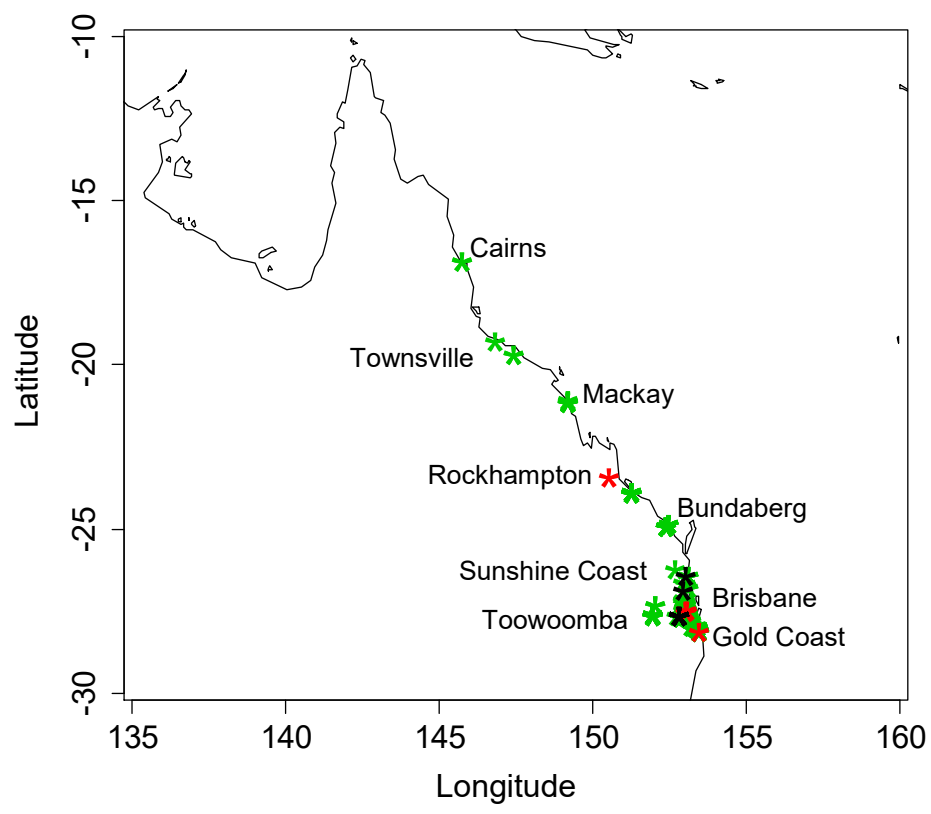
Fig. 4. Spatial plot of Queensland MA seizures showing proposed synthetic precursors (green = synthetic Eph/PSE, red = P2P d-MA and black = P2P l-MA).

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.
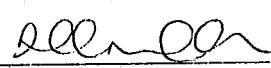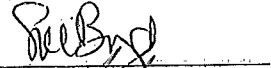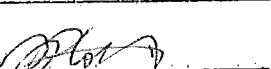
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