Concise Review

Evidence for the Presence of 1,3-Dimethylamylamine (1,3-DMAA) in Geranium Plant Materials

Thomas D. Gauthier
ENVIRON International Corp., Tampa, Florida. Corresponding author email: tgauthier@environcorp.com

Abstract: 1,3-Dimethylamylamine (1,3-DMAA) is an aliphatic amine with stimulant properties that are reportedly found naturally only in geranium plants (Pelargonium graveolens). The presence of 1,3-DMAA in geranium plants was first reported in a paper published in 1996, but some have questioned the identification of 1,3-DMAA in that study. Since then, a number of additional studies have been published, largely reporting the absence of 1,3-DMAA in geranium plants and commercial geranium oils. However, in two recent studies, 1,3-DMAA was detected in geranium plant tissues and a geranium oil sample using a simplified extraction approach on tissues and oil sourced from China. Whether or not 1,3-DMAA is found naturally in plants has significant implications as to how commercial products containing 1,3-DMAA are regulated by the US Food and Drug Administration. In this paper, differences in source materials, extraction procedures, and analytical approaches are reviewed in an attempt to rationalize the apparently conflicting evidence for the presence of 1,3-DMAA in geranium plant materials.

Keywords: DMAA, geranium, Pelargonium graveolens, natural products

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**Introduction**

1,3-Dimethylamylamine (1,3-DMAA), also known as 1,3-dimethylpentylamine and methyl hexaneamine (MHA), is an aliphatic amine with stimulant properties that is reportedly found naturally only in geranium plants (*Pelargonium graveolens*). The presence of 1,3-DMAA in geranium plants was first reported in a paper published in the Journal of Guizhou Institute of Technology by Ping et al. The authors detected the presence of 1,3-DMAA at a concentration of 0.66% in geranium oil isolated from fresh stems and leaves in *P. graveolens* plant collected from the Rongjang region of Guizhou province in China. The purpose of that study was to identify the main components of the essential oil in order to provide a basis for breed selection and to evaluate the quality of the extracted oil. However, it has since become increasingly cited as evidence for the existence of 1,3-DMAA as a natural product in certain species of geranium. In the Ping et al study, the authors used gas chromatography-mass spectrometry (GC-MS) to identify the major compounds in the oil; but some have questioned the identification of 1,3-DMAA in the oil based on apparent mislabeling of the reported chromatogram, inconsistent chromatographic elution order, and the lack of confirmation using a known standard.

More recently, a number of additional studies have been undertaken with the expressed purpose of establishing whether or not 1,3-DMAA is present as a natural product in geranium plants and geranium-derived oils. The answer to this question has significant implications as to how commercial products containing 1,3-DMAA are regulated by the US Food and Drug Administration (FDA). The relevancy of this issue has increased recently since the FDA warned in April, 2012 that synthetically-produced 1,3-DMAA is not eligible to be used as an active ingredient in dietary supplement as it is not classified as a “dietary ingredient,” a category which includes botanicals.

This paper contains a review of recent studies with respect to differences in source materials, extraction procedures and analytical approaches, and an overall evaluation of the existing evidence for the presence of 1,3-DMAA in natural geranium products.

**Evaluation of Recent Studies**

In July 2012, Wiley Online published a paper by Zhang et al. The authors analyzed eight different geranium oils for the presence of 1,3-DMAA using two different mass spectrometric methods: a high performance liquid chromatography-electrospray ionization-linear ion trap (HPLC-ESI-LIT) method, and an HPLC-electrospray ionization-triple quadrupole (HPLC-ESI-QQQ) method. In the HPLC-ESI-LIT method, separation of the 1,3-DMAA from other geranium oil components was accomplished using a LARIHC CF6-P chromatography column and a 90/10 acetonitrile/methanol mobile phase containing 0.1% formic acid. The LARIHC CF6-P is an alkyl derivatized cyclofructan 6 chiral stationary phase with reported capability of separating simple aliphatic racemic amines. However, no such separation of 1,3-DMAA was observed in the chromatogram presented in the supplementary material provided by the authors.

1,3-DMAA has two chiral centers resulting in two pairs of enantiomers, with each enantiomeric pair composing a diastereomer. Enantiomers are difficult to separate and often require reaction with a chiral reagent to achieve separation. For example, Zhang et al were able to separate all four isomers of 1,3-DMAA in 13 commercially available nutritional supplements and two synthetic standards using a GC fitted with an Astec ChiralDex G-DM column and flame ionization detector (FID). The authors reported that diastereomeric ratios of 1,3-DMAA measured in
the 13 nutritional supplements ranged from 1.23 to 1.43, and were similar to the diastereomeric ratios of 1.22 ± 0.06 and 1.42 ± 0.09 reported for the two synthetic standards. In addition, the authors reported that all enantiomers in the nutritional supplements and synthetic standards were racemic.\(^3\)

However, only a single peak was detected in spiked geranium oil samples using the HPLC-ESI-LIT method. Detection of 1,3-DMAA was accomplished with a linear ion trap mass spectrometer using selected ion monitoring at a m/z of 116.2 with ESI in the positive mode. ESI is a soft ionization technique and the m/z of 116.2 corresponds to the 1,3-DMAA ammonium ion. One disadvantage of ESI is the potential for ion suppression, which was noted by the authors. Ion suppression is a matrix effect which can adversely affect system performance and detection limits. The limit of detection (LOD) reported for the spiked geranium oil using the HPLC-ESI-LIT method was 50 ppb (assumed to be by mass). The authors reported no 1,3-DMAA detected in any of the 8 geranium oil samples using this method.

A second method used for analysis of 1,3-DMAA in geranium oil involved a derivatization step with 1-dimethylamino-naphthalene-5-sulfonyl chloride (dansyl chloride). Derivatization using dansyl chloride typically offers increased sensitivity with ESI mass spectrometry methods.

Separation of the derivatized product was performed using a XB-C18 column with a 70/30 acetonitrile/water mobile phase, with the water phase containing 0.1% trifluoroacetic acid. This column should have been capable of resolving the pair of derivatized 1,3-DMAA diastereomers. However, again only a single peak was identified in the chromatogram of the spiked geranium oil sample (Zhang et al., Figure 5: Note that this figure indicates that the oil sample was spiked with 30 ppb 1,3-DMAA whereas the experimental write-up indicates samples were spiked with 100 ppb 1,3-DMAA). Detection was accomplished using multiple reaction monitoring (MRM) of the m/z 349 to 171 transition corresponding to the loss of the primary dansyl fragment. The LOD reported for the spiked geranium oil using the HPLC-ESI-QQQ method was reported to be 10 ppb (assumed to be by mass). The authors note that the LOD refers to the concentration of the neat 1,3-DMAA and not the derivatized product, and report that no 1,3-DMAA was detected in any of the 8 geranium oil samples using this method. It is unclear how the authors established a LOD for either method.

In another study, ElSohly et al\(^4\) analyzed samples of authenticated plant materials and extracted oils from \textit{P. graveolens}, as well as a number of commercial oils, using GC-MS and two different LC-MS-MS methods: HPLC-ESI-QQQ and an ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS) method.

The oil samples were mixed with hexane and extracted with 1 N HCl. The aqueous layer was adjusted to a pH of 9–10 with 10 N KOH and extracted with dichloromethane (DCM). The DCM solution was then derivatized with heptafluorobutyric anhydride (HFBA), subjected to further treatment, and then analyzed by GC-MS.

Plant materials were initially extracted with a 0.1 N HCl-methanol solution. After evaporating the initial solvent, the residue was taken up in 1 N HCl and treated as described above for the oil samples. The extraction efficiency was determined to be approximately 35% based on recovery of the internal standard (2-amino-6-methylheptane).

Separation of the 1,3-DMAA-HFB derivative was carried out on the GC using an Agilent J&W DB-5MS column. The mass spectrometer was operated in the selected ion monitoring mode at m/z 240, 282, and 296. Based on standard chromatograms reported in Figure 3 of their paper, the response factor for the m/z 240 ion is about 100 times greater than the response factors for the m/z 282 and 296 ions.\(^5\) Despite the poorer sensitivity of the m/z 282 and 296 ions, the authors relied on all three ions for positive identification, which increased the LOD to 100 ppb.\(^4\)

The method achieved excellent separation of the pair of 1,3-DMAA diastereomers with an estimated diastereomeric ratio of 1.13 based on measured peak heights.\(^4\) Although the authors reported that "none of the authenticated \textit{P. graveolens} essential oils or plant material, nor any commercial volatile oil of Pelargonium (geranium oil) contain MHA at detectable levels,"\(^4\) inspection of the selected ion chromatograms for the control and spiked geranium oil samples suggests the qualitative presence of 1,3-DMAA at concentrations below 100 ppb. Presented in Figure 1 is an overlay of selected ion chromatograms recorded at m/z 240 for a negative geranium oil control (presented in ElSohly
et al, Fig. 4) and a negative geranium oil spiked at 0.1 ppm (100 ng/mL) 1,3-DMAA (ElSohly et al, Fig. 5).

The chromatograms are displaced slightly in the overlay due to the retention time shift observed between the two runs and have different scales on the y axis. This shift in retention times is consistent with the shift in the retention times observed for the internal standard peak. As indicated in Figure 1, spiking with 1,3-DMAA causes a noticeable increase in two of the peaks observed in the geranium oil control sample, suggesting the presence of 1,3-DMAA (showing both diastereomers) in this sample. The estimated diasteriomeric ratios are similar at 1.14 and 1.22 in the spiked and un-spiked samples. The slight difference in estimated diasteriomeric ratios likely reflects the natural variability in this parameter, as suggested in the Zhang et al study and/or differences between the natural product and synthetic standard.

The chromatograms presented for the extracted plant material (ElSohly et al, Fig. 6) appear similar to the chromatograms presented for the geranium oil (ElSohly et al, Fig. 4). Overlays of the chromatograms for the extracted plant material with chromatograms for the spiked geranium oil also suggest the qualitative presence of 1,3-DMAA in the extracted plant material at m/z 240 (not shown). Note that the authors do not present chromatograms for spiked plant materials. The estimated diasteriomeric ratio in the un-spiked plant material at m/z 240 is 1.21, similar to the un-spiked geranium oil sample. Once again, monitoring at m/z 296 and 282 appears to be unsuitable for
low level (less than 100 ppb) detection of 1,3-DMAA in this study.

ElSohly et al.\textsuperscript{4} also used two different LC-MS-MS techniques for analysis of the geranium oils and authenticated plant material extracts. The first method employed an HPLC with a triple quadrupole mass spectrometer equipped with an ESI source (HPLC-ESI-QQQ)—similar to the method used by Zhang et al.\textsuperscript{3} The same extraction procedure used for GC-MS analysis was used for HPLC-ESI-QQQ analysis except that they performed no derivatization step and the final DCM solution was evaporated to dryness and taken up in methanol for injection onto the HPLC column. Again, evaporation to dryness may have adversely affected extraction efficiency.

Separation of the 1,3-DMAA was performed using a Synergi Hydro-RP C18 column and a binary solvent gradient using a water/acetonitrile mobile phase with each solvent containing 0.1% formic acid.\textsuperscript{4} Detection was accomplished using MRM of the m/z 116 to 57 and m/z 116 to 41 transitions corresponding to the loss of hydrocarbon fragments. The authors report a LOD and LOQ of 2.5 ppb for this method, although there is no description of how this LOD was determined. The MRM chromatograms for un-extracted 1,3-DMAA showed a single large peak at a retention time of 4.2 minutes, indicating no chromatographic separation of the 1,3-DMAA diastereomers.\textsuperscript{4} The authors present representative chromatograms for oil samples, plant materials, and three different commercial products, however the reproduction quality of the chromatograms in the paper is poor. Nevertheless, similar peaks at a retention time of 4.2 minutes are observed in the chromatograms of \textit{Pelargonium graveolens} and authenticated oil of \textit{P. graveolens} (ElSohly et al, Figs. 11A, 11B, and 12A)\textsuperscript{4} suggesting the qualitative presence of 1,3-DMAA in the plant materials.\textsuperscript{4}

A second LC-MS-MS method used for confirmatory analysis involved ultra performance liquid chromatography (UPLC) with an ESI source and quadrupole time-of-flight (QToF) mass spectrometer for detection (UPLC-ESI-QToF).\textsuperscript{4} Separation was performed using an UPLC BEH C18 column. Detection was accomplished using selected ion monitoring at m/z = 116.1439. The LOD for this method was
estimated at 10 ppb, although no description is given as to how this LOD was established. The authors also present no chromatograms or mass spectra for any of these analyses but report that “all authenticated *P. graveolens* plant material, authenticated *P. graveolens* volatile oils, and commercial geranium oil purchased on the open market were negative (less than 10 ppb) for MHA.”

In Table II of their paper, ElSoley et al summarized the results of their investigation and curiously distinguish between two terminologies in reporting results for the LC-MS-MS analyses: ND is defined as not detected (below LOD of 10 ng/mL or 10 ng/g), and <10 ng/mL remained undefined, but perhaps suggesting that 1,3-DMAA was detected at a concentration less than 10 ng/mL. This interpretation would be consistent with the authors’ summary of analyses of dietary supplements (Products A, B, and C) which were reported to contain 1,3-DMAA at concentrations of >10 mg/g, >2 mg/g, and <1 mg/g, respectively.

It is unclear why the authors choose 10 ng/g or 10 ng/mL as the LOD in the summary table when no chromatographic data are presented for this method (UPLC-ESI-QToF), and a lower LOD (2.5 ng/mL) was achieved using the HPLC-ESI-QQQ methodology.

More recently, Li et al reported positive detection of 1,3- and 1,4-DMAA in geranium plant tissues at 13 to 365 ng/g and 3 to 35.3 ng/g, respectively. Detection was also reported in geranium oil samples at 167 to 13,271 ng/g and 220 ng/g (in one sample), respectively. The geranium plants were obtained from three areas of China (Yunnan, Jiangsu and Guizhou) and the geranium oil was obtained from Ji’an, Jiangxi Province, China.

Similar to the Zhang et al study, Li et al used an HPLC-ESI-QQQ method for determination of 1,3-DMAA in plant extracts and geranium oils. One major difference between the two studies is in the extraction step. Li et al used a more simplified approach involving a single hexane extraction step that minimizes potential loss pathways. For geranium oils, the oil is mixed with hexane and 0.5 M HCl and shaken at high speed for 5 minutes. The aqueous layer is then separated, further diluted with HCl as necessary, and filtered with a 0.45 µm nylon filter prior to injection in the HPLC. For geranium plant materials, the leaves and stems are first ground into fine pieces and extracted by sonication with 0.5 M HCl. After centrifugation, the aqueous layer is extracted once with hexane, then separated, further diluted with HCl as necessary, and filtered with a 0.45 µm nylon filter prior to injection in the HPLC. Spiked samples of geranium plants at 5, 10, 20 and 40 ng/g were extracted with an efficiency of 85% to 105% in the Li et al study. The single liquid-liquid extraction step with hexane was found to improve method performance by reducing sample matrix and ion suppression effects.

In contrast, as noted above, Zhang et al performed five separate organic solvent extractions and evaporated the sample to dryness prior to reconstituting the sample in methanol. Because each extraction step is not 100% efficient, there is a potential loss of analyte each time an extraction is conducted and a layer is discarded. Zhang et al did not report extraction efficiency in their study. ElSohly et al performed four separate extraction steps and reported an extraction efficiency of approximately 35%.

Separation of the 1,3-DMAA was performed using a C18 column and an 85/15 water/acetonitrile mobile phase with the water phase containing 0.1% formic acid. Detection was accomplished using MRM of the m/z 116 to 57 and m/z 116 to 99 transitions corresponding to the loss of hydrocarbon fragments. Using this method, the authors were able to achieve a mass detection limit of 1 to 2 picograms (pg) corresponding to a method quantitation limit of 1 to 2 ng/g wet weight based on a 3:1 signal-to-noise ratio.

The authors achieved good separation of 1,3-DMAA diastereomers (two peaks) along with 1,4-DMAA (one peak) using this method and reported similar collision-induced dissociation (CID) spectra for the same precursor ion (m/z = 116) for all three peaks. The authors note that the 1,3-DMAA diastereomers were present in equal amounts and are identical in all tested samples, including the standard reference. Inspection of the chromatograms reported in Li et al Figures 2, 4 and 5 show, however, diastereomeric peak height ratios of 1.2 to 1.4 (for example see Li et al Fig. 2A), which is consistent with diastereomeric ratios of 1,3-DMAA reported in 13 nutritional supplements by Zhang et al and in samples analyzed by ElSohly et al. Only the chromatogram presented by Li et al in their Figure 6 suggests equal amounts of diastereomers based on peak height ratios.

There appears to be some confusion in the paper regarding interpretation of the diastereomeric
ratios and whether or not a racemic mixture of 1,3-DMAA enantiomers is present in the extracted plant materials. Because the authors did not use a chiral chromatography column to achieve complete separation of all the 1,3-DMAA isomers, they were not able to separate the two 1,3-DMAA peaks into their respective pairs of enantiomers. As Zhang et al. have shown, 1,3-DMAA can be separated into two pairs of enantiomers using a chiral chromatography column. In this study, each pair of 1,3-DMAA enantiomers appears as a single peak; thus there is no information regarding enantiomeric ratios and no information as to whether or not 1,3-DMAA is present as a racemic mixture. The authors suggest, however, that this study demonstrates the presence of a racemate in a plant tissue. As previously noted, concentrations of 1,3-DMAA in geranium oil samples ranged from 167 to 13,271 ng/g. It is unclear if the 1000-fold difference in concentrations observed for the geranium oil samples reflects a difference in type of oil, source of geranium plant material, method of processing, or other potential factors.

In a similar study, Fleming et al. analyzed geranium plants harvested during three different seasons and from three different areas of China (Changzhou, Guiyang and Kunming) for the presence of 1,3- and 1,4-DMAA using an HPLC-ESI-QQQ method. The extraction procedure was adapted from the procedure reported by Li et al. Geranium plant materials are first extracted with 0.5 N HCl by sonication. After centrifugation, the aqueous layer was extracted once with hexane, then separated, further diluted with HCl as necessary, and filtered with a 0.45 μm nylon filter prior to injection in the HPLC. Note that samples were first analyzed without the hexane extraction step. This step was later added to reduce matrix effects. Thus, results are reported for some samples with and without the hexane extraction step. In this study, the extraction efficiency of spiked geranium samples ranged from 54% to 107% for 1,3-DMAA and 63% to 86% for 1,4-DMAA.

Separation of the 1,3-DMAA was performed using a Kinetex C18 column and an 82/18 water/acetonitrile mobile phase, with the water phase containing 1% formic acid. Detection was accomplished using MRM of the m/z 116 to 57 and m/z 116 to 99.7 transitions corresponding to the loss of hydrocarbon fragments. Using this method, the authors were able to achieve method detection limits for 1,3- and 1,4-DMAA in the plant extract on the order of 0.6 to 3.2 μg/L (ppb). This corresponds to a detection limit of about 20 ng/g in the plant material.

The authors detected both 1,3-DMAA diastereomers in the chromatograms and recorded diastereomeric ratios of 1.14 ± 0.08 in the standards. Ratios of 1.10 ± 0.01 in Changzhou S11-1 sample, 1.25 ± 0.03 in Changzhou S11-2 sample, 1.02 in Changzhou 1 sample, and 1.16 ± 0.1 in Changzhou 3 sample were recorded, suggesting a natural variability in diastereomeric ratios. The Changzhou S-11 plant material was obtained from Intertek Laboratories and derived from the same Jiangsu sample reported by Li et al. For this sample, Fleming et al. reported a 1,3-DMAA concentration of 94.7 ± 15.1 ng/g without the hexane extraction step and 254 ± 17 ng/g with the hexane extraction step. In comparison, Li et al. reported 165 ng/g for a sample collected from the same plant material. Similarly, Fleming et al. reported a 1,4-DMAA concentration of 13.5 ± 1.8 ng/g without the hexane extraction step and 39.8 ng/g with the hexane extraction step, while Li et al. reported a concentration of 35.3 ng/g for a sample collected from the same plant material. The similarity in results achieved by two different laboratories analyzing subsamples from the same plant material using similar methods provides confirmatory evidence for the presence of 1,3- and 1,4-DMAA in certain Chinese species of geranium.

Other samples of plant material obtained from Changzhou, China also contained 1,3- and 1,4-DMAA. Fleming et al. detected 213 ng/g 1,3-DMAA and 52 ng/g 1,4-DMAA in one sample harvested in March, 2012 (Changzhou 1) and 68.8 ± 36.5 ng/g 1,3-DMAA and 118 ± 45 ng/g 1,4-DMAA in a second sample harvested in May, 2012 (Changzhou 3). This reflects plant-to-plant variability or possibly seasonal effects.

Some of the samples were re-analyzed using the method of standard additions, a technique which is often used to minimize matrix effects in complex samples, such as biological materials. Using the method of standard additions, Fleming et al. detected slightly higher levels of 1,3- and 1,4-DMAA in the Changzhou 3 sample at concentrations of 97 ± 20 ng/g and 162 ± 48 ng/g, respectively. Slightly higher levels of 1,3- and 1,4-DMAA were also detected in...
the Changzhou S11-2 sample at 496 ± 46 ng/g and 68 ± 7 ng/g, respectively.

In other samples of geranium plant materials obtained from China, concentrations of 1,3- and 1,4-DMAA were at or below the detection limit near 20 ng/g.\textsuperscript{6} 1,3-DMAA was detected in one geranium plant sample from Kunming, China near the detection limit of 20 ng/g. The duplicate analysis, however, was below the method detection limit. Concentrations of 1,3-DMAA and 1,4-DMAA in all other samples from Kunming and Guiyang China were below the detection limit of approximately 20 ng/g.\textsuperscript{6}

In a short paper prepared by Lisi et al,\textsuperscript{2} five different commercially available geranium oils were analyzed for the presence of 1,3-DMAA using GC-MS. The sources of geranium oil in the five samples were reported to be Egypt, France, and New Zealand. The oil samples were extracted with 1M HCl and t-butylmethyl ether (TBME). After further treatment, the aqueous layer was made basic using 6 M KOH and amended with hexane and pentafluorobenzylchloride (PFBCL) as a derivatizing agent. The hexane layer was evaporated to dryness and reconstituted in ethyl acetate for injection onto the GC column. Extraction efficiencies were not reported.\textsuperscript{2}

Separation of the 1,3-DMAA derivative was performed using an Agilent HP Ultra 2 column.\textsuperscript{2} Semi-quantitative analysis was based on selected ion monitoring at m/z 238. Separation of both 1,3-DMAA diastereomers was achieved using this method, although the authors incorrectly refer to the two peaks as representing enantiomers. The authors report that 1,3-DMAA was not found in any of the five geranium oils, however no detection limits were reported and no standard concentrations or calibration data are presented.\textsuperscript{2}

Finally, in a short communication by Di Lorenzo et al,\textsuperscript{7} stems and leaves of seven different species/cultivars of Pelargonium provided from a plant nursery in Italy were analyzed for the presence of 1,3-DMAA using an HPLC with UV detection. The stems and leaves were first extracted with methanol under reflux conditions. The methanol extracts were then filtered, evaporated to dryness, and re-suspended in 1 to 2 mL of methanol.\textsuperscript{7} Whereas all other studies reviewed in this paper use an acidic extraction medium, no acid was added here. The final methanol extracts were derivatized using o-phthalaldehyde. Separation was achieved using a Synergi 4 µm MAX-RP or Lichrocart 5 µm RP18 reverse phase column and gradient elution using a mixture of sodium acetate/tetrahydrofuran and methanol. Detection of the 1,3-DMAA-o-phthalaldehyde derivative was accomplished using a diode array detector with quantitation at 334 nm.\textsuperscript{7}

The recovery of spiked 1,3-DMAA in samples of stems and leaves was greater than 97%, but detection limits were elevated at 600 and 1,200 ng/g for stems and leaves, respectively. In comparison, Li et al\textsuperscript{5} and Fleming et al\textsuperscript{6} reported detection limits on the order of 2 to 20 ng/g. The authors report that no detectable amounts of 1,3-DMAA were found in any of the seven Pelargonium species/cultivars tested nor in a commercial geranium oil from P. graveolens grown in Africa that was also tested.\textsuperscript{7}

**Analyses of Essential Oils**

Geranium plants are primarily cultivated for production of essential oils for use in cosmetics, perfumes, and aromatherapy. Numerous studies have been published identifying primary constituents and documenting conditions affecting the yield and composition of the extracted oil including method of distillation, seasonal effects and climate conditions, \textsuperscript{11-13} sample handling (ie, age of leaves, whether they are dried prior to distillation), \textsuperscript{14} as well as soil and moisture conditions.\textsuperscript{15} These and other studies\textsuperscript{16-19} typically provide a chemical breakdown of the constituents in the oil, yet none of these studies have documented the presence of 1,3-DMAA. Some have referenced this body of work as additional supporting evidence for the absence of 1,3-DMAA in geranium plants.\textsuperscript{2,20} However, there are a number of potential reasons why these studies do not report finding 1,3-DMAA in the oil.

First, there is considerable variability in the composition of essential oils derived from geranium plants. International standards classify geranium oils into three main types: the African type including oils from Algeria, Morocco and Egypt; the Bourbon type from Reunion Island; and the Chinese type. It is reported that 90% of the essential oil is comprised by about 30 compounds which vary in relative concentrations among the various types of oils.\textsuperscript{21} The remaining compounds, which are present at trace levels, can vary significantly. For example, Lalli et al\textsuperscript{16} identified a total of 315 different compounds in essential oils distilled
from 13 different South African *Pelargonium* species (18 samples) using GC and GC-MS. However, the total number of constituents identified in any one essential oil sample ranged from 52 to 87 and nearly one third of the compounds (101 of 315) were detected in only one of the 18 samples analyzed. Jalali-Heravi et al\(^\text{19}\) identified 61 compounds in an essential oil sample derived from geranium plants cultivated in Iran using GC-MS. This list, however, was incomplete and ultimately expanded to 85 compounds with the addition of chemometric resolution techniques. Shellie and Marriott\(^\text{20}\) identified 65 different compounds in an Egyptian geranium essential oil using a two-dimensional GC-MS approach. Kulkarni et al\(^\text{17}\) identified 48 constituents in the essential oil extracted from three Bourbon clones grown in India. Clearly, there is considerable variability in the composition of essential oils extracted from the various plant types.

Second, although detection limits are typically not reported, it appears that detection limits are not low enough to detect 1,3-DMAA in the oil. Most of these essential oil studies report trace level components as representing on the order of 0.01% of the oil fraction, which is equivalent to 100,000 ng/g. In contrast, Li et al\(^\text{5}\) and Fleming et al\(^\text{6}\) reported concentrations of 1,3-DMAA on the order of 500 ng/g or less in geranium plant parts and oil, except for one oil sample with a 1,3-DMAA concentration of 13,000 ng/g or 0.0013%. The low detection limits achieved by Li et al, Fleming et al, and others\(^\text{3,4}\) requires an acid extraction and concentration step prior to analysis, which is typically not performed for analysis of essential oils. However, one study by Vernin et al\(^\text{22}\) isolated the basic fraction of a geranium Bourbon oil by extracting with a 10% solution of HCl followed by washing with diethyl ether, neutralizing with sodium hydroxide, and extraction with ether. The authors detected 2-methyl-3-amino-1-pentene at low levels along with two pyridine compounds and other unidentified amino compounds in the oil, however no 1,3-DMAA was identified. Nevertheless, this study suggests that low levels of amines that would otherwise go undetected may be found in some geranium oils after acid extraction and cleanup.

Finally, the GC methods used for analyses of essential oils in all of the above-mentioned studies may not be optimized for analysis of 1,3-DMAA, which has a relatively low boiling point and would tend to elute quickly through the column. The early eluting compounds reported in most of the essential oil analyses, such as cis-3-hexenol and α pinene, have a boiling point on the order of 155 °C to 157 °C.\(^\text{23}\) Because 1,3-DMAA has a comparatively lower boiling point of 130 °C to 135 °C,\(^\text{24}\) it would tend to appear as one of the earliest eluting peaks and potentially outside the area of interest for many of these studies, particularly if there is a programmed solvent delay.\(^\text{24}\) Vorce et al\(^\text{24}\) note that GC-MS analysis of 1,3-DMAA is feasible, but great care must be taken to optimize the GC parameters for this compound.

A recent patent application by Northern Innovations and Formulations Corp. for an herbal supplement prepared from geranium, describes an extraction method that claims to optimize the 1,3-DMAA content of the oil by extracting the oil with an alcohol/water mixture, separating the oil and water phases, concentrating and drying the aqueous phase to a powder, and then, after purifying the oil, combing the powder with the purified oil.\(^\text{25}\) This process is claimed to achieve 1,3-DMAA concentrations in the range of 1% to 3% suggesting that much of the 1,3-DMAA extracted during production of essential oils may end up in the aqueous phase. This would be another potential reason why analyses of essential oils fail to identify measurable levels of 1,3-DMAA.

**Conclusions**

The recent studies summarized in this report provide conflicting evidence that 1,3-DMAA is found naturally in certain species of geranium plants. However, differences in the samples sources, extraction procedures, and methods of analysis may account for the conflicting results. The genus *Pelargonium* includes more than 270 distinct species, most of which are indigenous to South Africa, but many of which are now widely cultivated in Russia, Egypt, India, and China, now the largest producer of geranium oil.\(^\text{16,21,26}\) Many of the cultivars grown for production of essential oil are interspecies hybrids\(^\text{21}\) and studies have shown that the composition and quality of the extracted oil depends on the variety of plant, method of extraction, type of soil, and climate conditions.\(^\text{9,16,17,21}\) Thus, it is not completely surprising that some plant materials and extracted oil samples contain 1,3-DMAA whereas others do not.
Based on the studies reviewed in this paper, processing may also play a role as 1,3-DMAA is more likely to be found in samples extracted in the lab from plant stems and leaves than in commercially available products sold for medicinal use and aromatherapy.\textsuperscript{27} The reason for this may be due to commercial extraction procedures designed to enhance recovery of essential oils rather than 1,3-DMAA. A recent patent application suggests that much of the 1,3-DMAA extracted during production of essential oils may end up in the aqueous phase.\textsuperscript{25}

Three of the studies evaluated in this report included analysis of leaves and stems from geranium plants harvested in China and India. Li et al\textsuperscript{5} detected 1,3-DMAA in authenticated plant materials collected from three areas of China (Yunnan, Jiangsu and Guizhou) and Fleming et al\textsuperscript{6} detected 1,3-DMAA in authenticated plant materials from Changzhou, China and, at low levels, in plant materials from Guiyang, China. However, no 1,3-DMAA was detected in plant materials harvested from Kunming, China. ElSohly et al\textsuperscript{4} analyzed authenticated plant materials from India and the United States (Mississippi) and reported no detectable amounts of 1,3-DMAA at a detection limit of 100 ppb. Inspection of the chromatograms, however, suggests the qualitative presence of 1,3-DMAA at levels below 100 ppb in some samples of the authenticated plant material. The authors also reported no detection of 1,3-DMAA using an HPLC technique with much lower detection limits (2.5 ppb). However, this technique was incapable of resolving the 1,3-DMAA diastereomers. A third technique (UPLC-ESI-QTOF) with an estimated detection limit of 10 ppb was also used to analyze samples. While no data were presented for this method, the summary table provided by the authors suggests that 1,3-DMAA may have been detected in dried plant stems at a level below 10 ppb. Thus, all three studies involving extraction of plant materials provide quantitative or qualitative support for the natural presence of 1,3-DMAA in geranium plants.

Four of the reviewed studies involved analysis of commercially available geranium oils. Zhang et al\textsuperscript{3} analyzed geranium oil samples sourced from China and Egypt. Lisi et al\textsuperscript{2} analyzed five geranium oils sourced from Egypt, France, and New Zealand. ElSohly et al\textsuperscript{4} analyzed 20 different samples of commercial geranium oils, but did not report sources; and Li et al\textsuperscript{5} analyzed geranium oil samples sourced from a flavor oil factory in Jiangxi Province, China. Only Li et al\textsuperscript{5} detected 1,3-DMAA in a commercial oil product.

All eight geranium oil samples analyzed by Zhang et al\textsuperscript{3} were reportedly extracted from geranium plants using steam distillation. Lisi et al\textsuperscript{2} suggest that due to the volatility of 1,3-DMAA, steam distillation may not be a suitable technique for its extraction along with the oil product and that the alternative cold-pressed process provides a better chance of retaining 1,3-DMAA in the oil. Zhang et al\textsuperscript{3} reported no detection of 1,3-DMAA in any of the geranium oil samples using an HPLC-ESI-LIT technique with a detection limit of 50 ppb, and no detection of 1,3-DMAA using an alternative HPLC-ESI-QQQ technique at a detection limit of 10 ppb. The authors used a somewhat elaborate multi-step extraction technique for the oil samples and did not report extraction efficiencies for the method. The HPLC-ESI-LIT method employed a chiral stationary phase chromatography column that is reportedly capable of adequate retention of primary amines. However, the authors were unable to achieve separation of neither 1,3-DMAA enantiomers nor diastereomers in standards. Similarly, only a single peak was recorded using the HPLC-ESI-QQQ method.\textsuperscript{3}

Lisi et al\textsuperscript{2} analyzed five geranium oil samples from Egypt, France, and New Zealand; three of the samples were extracted using steam distillation. 1,3-DMAA was not detected in any of the oil samples but no detection limits were reported. ElSohly et al\textsuperscript{4} also reported no detectable amounts of 1,3-DMAA at a detection limit of 100 ppb using one method. However, published chromatograms for one of the oil samples before and after spiking with 1,3-DMAA at the detection limit suggests the qualitative presence of 1,3-DMAA in the original sample. In addition, the summary table included in the paper suggests that 1,3-DMAA may be present in 25% (5 out of 20) commercial oil samples at levels below 10 ppb.\textsuperscript{4}

Finally, Li et al\textsuperscript{5} detected 1,3- and 1,4-DMAA in geranium oil samples obtained from a Flavor Oil Factory in Ji’an, Jiangxi Province, China. Concentrations of 1,3-DMAA ranged from 167 to 13,271 ng/g in three samples. 1,4-DMAA was only detected in one of the samples, at a concentration of 220 ng/g. Except

\textsuperscript{27} Gauthier

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for the one geranium oil sample analyzed by Li et al., concentrations of 1,3-DMAA detected in geranium plant materials and extracted oils are generally less than 500 ppb.

Only Zhang et al. report separation of 1,3-DMAA into its four stereoisomers, accomplished using a GC-FID fitted with a chiral stationary phase. This technique, however, was used to analyze nutritional supplements and not geranium plant materials. Thus, no data has been presented in any of the five studies regarding the enantiomeric purity of 1,3-DMAA extracted from geranium plants and oils. All five studies did report separation of 1,3-DMAA into its diastereomers, and diastereomeric ratios were similar for 1,3-DMAA found in commercially obtained standards, nutritional supplements, and extracted geranium plant materials and geranium oils.

Overall, these studies show that 1,3-DMAA is found naturally in some, but not all, geranium plants and extracted geranium oils. Quantitative and/or qualitative evidence for the presence of 1,3-DMAA in plant materials is more likely to be found in studies involving extraction of the plant materials in the lab rather than analysis of commercially available products sold for medicinal use and aromatherapy. This is likely due to differences in processing. Steam distillation, which appears to be a preferred extraction procedure for commercial oils, may not be suitable for retention of 1,3-DMAA. In the lab, detection of 1,3-DMAA appears to be favored by extraction procedures involving fewer extraction steps, minimizing potential for losses. In this review, studies reporting negative findings for the presence of 1,3-DMAA all include extraction procedures involving an evaporation to dryness step or solvent removal under vacuum, which can adversely affect extraction efficiency due to the volatility of 1,3-DMAA.

Finally, these studies show that there is considerable plant-to-plant variability in 1,3-DMAA content. In plant materials where 1,3-DMAA has been quantitatively detected, all of which are sourced from China, concentrations are generally less than 500 ppb. However, some plants from China contain no detectable levels of 1,3-DMAA while one geranium oil sourced from China contained over 13 ppm 1,3-DMAA.

**Author Contributions**

Analyzed the data: TDG. Wrote the first draft of the manuscript: TDG. Contributed to the writing of the manuscript: TDG. Agree with manuscript results and conclusions: TDG. Jointly developed the structure and arguments for the paper: TDG. Made critical revisions and approved final version: TDG. Author reviewed and approved of the final manuscript.

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