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TECHNICAL NOTE

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Availability of Target Odor Compounds from Seized Ecstasy Tablets for Canine Detection*,†

ABSTRACT: The aim of this study was to compare seized samples of 3,4-methylenedioxy-*N*-methylamphetamine (MDMA) pills, used to train law enforcement detection canine teams, to determine what differences exist in the chemical makeup and headspace odor and their effect on detectability. MDMA solutions were analyzed by liquid chromatography–mass spectrometry. Analysis of these samples showed a wide variance of MDMA (8–25%). Headspace SPME-GC/MS analysis showed that several compounds such as 3,4-methylenedioxyphenylacetone and 1-(3,4-methylenedioxyphenyl)-2-propanol are common among these MDMA samples regardless of starting compound and synthesis procedure. However, differences, such as the level of the various methylenedioxy starting compounds, were shown to affect the overall outcome of canine detection, indicating the need for more than one MDMA training aid. Combinations of compounds such as the primary odor piperonal in conjunction with a secondary compound such as MDP-2-OH or isosafrole are recommended to maximize detection of different illicit MDMA samples.

KEYWORDS: forensic science, canine detection, 3,4-methylenedioxy-*N*-methylamphetamine, piperonal, solid-phase microextraction, gas chromatography, liquid chromatography, mass spectrometry

Detector-dog response is one of the major applications involved with odor detection studies for the determination of both the chemical signature of individual odors to which these canines are actually alerting and whether or not there is a common element within different items to support the use of contraband mimics. Recent years have seen the application of a canine's ability to expand into an increasing number of areas, including the detection of accelerants, guns, pipeline leaks, gold ore, contraband food, mold, and individual human scent (1–4).

Canine detection has been shown to rely primarily on olfaction rather than on vision (5). This can be attributed, in part, to the size of the olfactory bulbs in the canine brain, which are responsible for the increased significance in the sense of smell over the other senses. The canine detection system is the biological process of inhaling odorants followed by nerve-impulse interpretation of the odorants, considered to be a dynamic system that occurs in less than 1 sec. Because of the orientation of its nose (i.e., air is inhaled from the front and exhaled through side slits), a canine's sniffing frequency is around 5 Hz, which is *c.* 300 breaths per minute (6). This volume of air inhaled through the canine nose is around 60 mL/sec (7). At a frequency of 5 Hz, this totals to 300 mL of air sampled each second. The dynamics of the breathing combined with the large olfactory system give the canine its ability to search and identify odors quickly and efficiently. Because of these factors, a canine's olfactory sensitivity can be as high as 50–100 times that over a human's olfactory sensitivity.

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3,4-methylenedioxy-*N*-methylamphetamine (MDMA) was first developed in 1914 by the German company E. Merck as a precursor for other therapeutic drugs (8,9). Abuse in the U.S. is believed to have originated on the west coast sometime in the 1960s under the more common name "ecstasy." While it is traditionally taken in pill form, the drug is also available in powder and liquid forms. As a result of increased interest and usage, the distribution of this drug has increased in metropolitan and suburban areas across the country. MDMA is one of the top controlled substances most identified in crime laboratories, and it is the most recent drug to be added to law enforcement detection canine-training regimens.

There are over 20 published synthetic routes for the production of MDMA (10,11). Examples of the most common of these processes include the dissolving metal reduction ($Al/HgCl_2$), the cyanoborohydride reduction ($NaBH_3CN$), the borohydride reduction in low temperature ($NaBH_4$), the Leuckart reaction, and the safrole bromination (Figs 1 and 2). All of these processes begin with a methylenedioxy compound such as the commercially available compounds safrole, isosafrole, and piperonal. A common intermediate for the synthesis of MDMA is the compound 3,4-methylenedioxyphenyl-2-propanone (MDP-2-P), a controlled substance. Depending on the synthesis route, varying byproducts and degradation compounds remain in the final product.

Previous odor detection research has shown several compounds to be dominant in the headspace of ecstasy samples utilizing solid-phase microextraction in conjunction with gas chromatography–mass spectrometry (SPME-GC/MS) (12,13). Examples of identified compounds included the preliminary compounds (piperonal, isosafrole, and safrole), the intermediate compound (MDP-2-P), the alcohol version (1-(3,4-methylenedioxyphenyl)-2-propanol [MDP-2-POH]), methamphetamine HCl, 3,4-methylenedioxyacetophenone, and 3,4-methylenedioxyethamphetamine (MDEA). Of these compounds identified, the ones that dogs trained to detect ecstasy most often recognize as the dominant odorant was piperonal (12). The

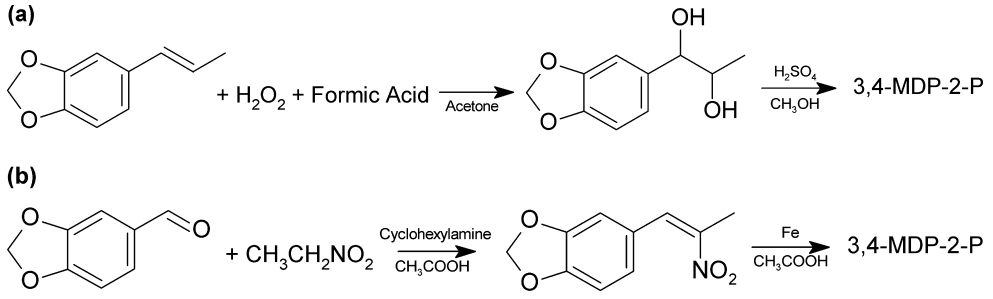

FIG. 1—*Synthesis of intermediate from methylenedioxy starting compounds (a) isosafrole and (b) piperonal.*

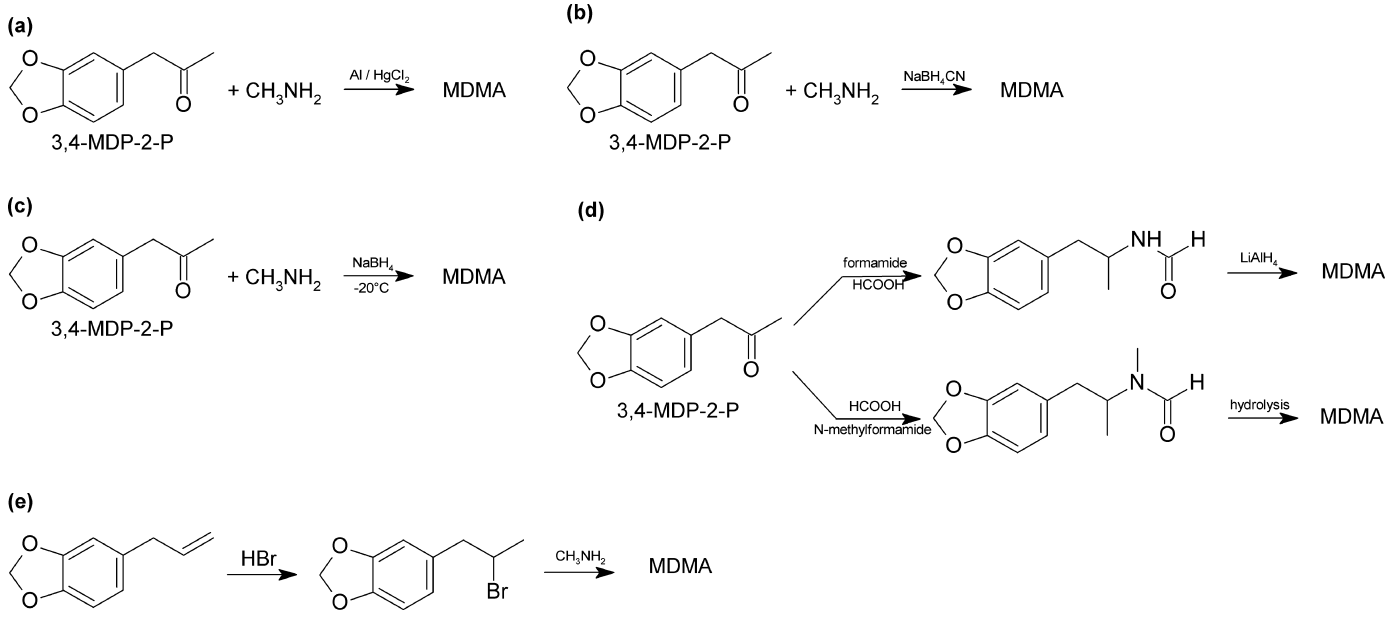

FIG. 2—*Synthesis of MDMA (a) dissolving metal reduction ($Al/HgCl_2$), (b) cyanoborohydride reduction ($NaBH_3CN$), (c) borohydride reduction in low temperature ($NaBH_4$), (d) Leuckart reaction, and (e) safrole bromination.*

identification of methamphetamine is also sometimes seen and is important as it is a drug included in most agencies' training regimens even if they do not train on MDMA.

This study addresses some of the potential complications associated with developing a universal system for training dogs to reliably detect ecstasy. Potential differences in the chemical makeup of seized drugs, as a result of synthetic route, which are used for training aids, and the lack of an optimized training aid system are shown to result in detection inconsistencies for canine teams.

Materials and Methods

Chemicals and Supplies

The chemical compounds piperonal, caffeine, and acetonitrile were obtained from Sigma Aldrich (St. Louis, MO). Drug standards were obtained from Cerilliant (Round Rock, TX) and Restek (Bellefonte, PA) including 3,4-methylenedioxymethamphetamine, MDEA, methamphetamine hydrochloride, 3,4-MDP-2-P, and 1-(3,4-methylenedioxyphenyl)-propan-2-ol. Three batches of seized ecstasy (Ex #1, #2, and #3) were provided by the Florida Highway Patrol Contraband Interdiction Program (FHP-CIP) K9 Division. StableFlex™ Carbowax®/Divinylbenzene (CW/DVB) SPME fibers (70 μm), holders for manual sampling, 10-mL headspace vials fitted with phenolic plastic caps and a PTFE/silicon septum, and clear 2-mL ABC autosampler vials with PTFE/silicone lined caps were purchased from Supelco (Bellefonte, PA). Sigma Pseudo™ Scent Cages were purchased from Sigma Aldrich. Steel electrical junction boxes, 4 in × 4 in × 2 in, were purchased from local hardware stores. Polymer, heat-seal bags were obtained in 1.5, 2.0, 3.0 and 4.0 mil low-density polyethylene (LDPE) and 2.0 mil high-density polypropylene (HDPP) from Veripak (Atlanta, GA). Heat-sealed, aluminized bags (5.75 in × 6 in and 6 in × 5.5 in) were purchased from Kapak (St. Louis, MN) and Ted Pella, Inc. (Redding, CA), respectively.

Instrumentation

Liquid Chromatography–Mass Spectrometry—The Varian ProStar Liquid Chromatography system was used in combination with the Varian Ion Trap Mass Spectrometer Model 500-MS running Varian's MS Workstation software (Version 6; Palo Alto, CA). The Varian ProStar liquid chromatography system was comprised of an autoinjector (Varian Model 230) connected in sequence with two solvent delivery modules (Varian Model 210). The LC column was fitted with a Pursuit XRs 3 C18 100 mm long × 2.0 mm wide column obtained from Varian. The mobile phase consisted of a 45:55 isocratic aqueous/organic mix. The aqueous solvent was 2 mM ammonium acetate solution with 1% formic acid. The organic

solvent was a 50:50 acetonitrile/methanol solution. The flow rate was set at 0.2 mL/min.

Gas Chromatography–Mass Spectrometry—An Agilent 6890 N Gas Chromatograph was used in combination with the Agilent 5973N Quadrupole Mass Selective Detector running Agilent Technologies MSD Productivity ChemStation software (Revision D.03.00 SP1; Santa Clara, CA). The GC was fitted with an HP5 30 m long × 0.25 mm inner diameter column with a 25-μm-thick stationary phase that was obtained from Agilent. For sample analysis, the injection port temperature was set at 235°C with a 2-mm-inner-diameter liner. The oven program consisted of a 40°C hold for 5 min, 10°C/min ramp to 280°C, and 1-min hold at 280°C with helium at a flow rate of 1.0 mL/min. The transfer line from the GC to the MS was held at a temperature of 280°C. The quadrupole temperature was held at 150°C with a scan range of 50–500 amu at a rate of 5 scans/sec.

Headspace Analysis—For each batch of seized ecstasy, eight pills (~2 g) were placed inside a 10-mL glass vial and capped with Silica/PTFE septa. The headspace was sampled for 3 h by the insertion of the SPME fiber through the septum with a previously established sampling procedure (12). The fiber was exposed *c.* 1–2 cm above the sample within the closed vial for the sample-specific adsorption time immediately prior to GC analysis.

Solutions

A standard solution of MDMA and caffeine were made by diluting the compounds in methanol at 1, 5, 10, 20, 50, and 100 μg/mL. These were used to create a calibration curve (not shown) to quantify the MDMA and caffeine present in the ecstasy batches.

For LC analysis, a 1000 μg/mL solution was created in methanol based upon sample weights of the crushed ecstasy pills. The pills' sample weights were as follows: 0.217, 0.247, and 0.122 g for the samples FHP Ex #1, #2, and #3, respectively. The reason for so high concentrations were used was that the stock concentrations were based on the mass of the entire pill, not just the MDMA concentration. The 1000 μg/mL stock solutions were each diluted to 10 μg/mL using a buffer solution (2 mM ammonium acetate with 1% formic acid). Again, a higher-than-normal concentration for this analysis was used to ensure detection because the actual MDMA concentration in the pills was unknown.

Field Trials

Field trials were performed with trained and certified local law enforcement drug detection canine teams consisting of five different breeds (10 German Shepherds, five Belgian Malinois, four black Labradors, three yellow Labradors, one Dutch Shepherd, and one Weimaraner), 20 males and four females. Piperonal odor aids were created by heat sealing piperonal into polymer bags as previously described (14). The piperonal aids were presented to the canines in electrical junction boxes or in the scent cages. Prior to use, the presentation vessels were cleaned with soap, rinsed with water, and baked at 110°C overnight. Presentation of the piperonal aids to the canines occurred as an odor lineup by placing the samples on the floor *c.* 1 m apart. The handlers were instructed to work with their detection canines to detail each sample in the lineup, utilizing their normal search pattern. The handlers had no previous knowledge of the compounds or order of placement in the lineup. Additionally, there was no marking on the containers to indicate the contents. A positive control and negative control were included at the time of testing.

Results and Discussion

Liquid Chromatography–Mass Spectrometry

Depending on the synthesis process and how much of the product has been cut, the actual MDMA present in the pill will vary. The total ion chromatograms from the LC-MS analysis for the ecstasy samples and a standard solution of MDMA and caffeine are shown in Fig. 3. MDMA was found to be present in all three samples of ecstasy. This was confirmed based on a retention time comparison to the standard solution and ions (163 and 194 [MH^+]) present in the mass spectrum (not shown). Caffeine was also identified in the last ecstasy sample (FHP Ex #3) based on a standard comparison and the ion 195 [MH^+].

The percentage of both MDMA and caffeine present in the ecstasy samples was calculated from calibration curves produced from standards (not shown); these results are given in Table 1. There is a distinct decrease in the concentration of MDMA from FHP Ex #1 to #2 to #3. This is attributed to the continual cutting of the drug with adulterants (such as caffeine) by the drug suppliers/manufacturers in an attempt to stretch the product for increased profit. As adulterants can vary depending on the origin of the ecstasy, there is no interest in using these compounds as a universal canine detection mechanism for ecstasy. Thus, these adulterants are beyond the scope of interest for this paper. In the most recent seized batch of pills (Ex #3), a higher percentage of caffeine was detected over that of the active ingredient, MDMA. This is important because a reduction in MDMA levels can have an adverse effect on the odor profiles of the samples (i.e., less MDMA translates into lower availability of detectable odor compounds). This makes it more difficult for the identification of target odors by detection systems (biological and instrumental).

Headspace Analysis

Samples of ecstasy were analyzed using HS-SPME-GC/MS to determine the dominant headspace components in the odor profile. These chromatograms are shown in Fig. 4. A summary of the relevant headspace compounds of the ecstasy samples is given in Table 2.

The parent compound MDMA was only detected in the headspace of one sample of ecstasy used by the FHP (Ex #3). In previous studies (12), MDMA was not detected in the headspace of seized ecstasy samples and similarly, in this study, MDMA was only seen in the headspace of one of three samples tested. Piperonal was detected in greatly varying abundance in the three ecstasy samples used in training by the FHP (Ex #1, #2, and #3). Among the samples tested, several other compounds were detected that are related to and/or similar in structure to piperonal and MDMA. These compounds included piperonyl alcohol, MDP-2-POH, MDP-2-P, methamphetamine hydrochloride, isosafrole, and 3,4-methylenedioxyethylamine. MDP-2-P is an immediate precursor in the manufacture of MDMA by several synthetic routes (Fig. 2). Because MDP-2-P is a controlled chemical substance, it is not considered a good universal training aid for ecstasy. Isosafrole is one of the starting compounds like piperonal and safrole used in the production of MDMA (Fig. 1). MDP-2-POH is a byproduct that develops during MDMA manufacture from the reduction and bromination synthetic routes, offering a clue as to the selected method of production for these samples. Isosafrole and MDP-2-POH are uncontrolled chemical compounds that offer potential as additional

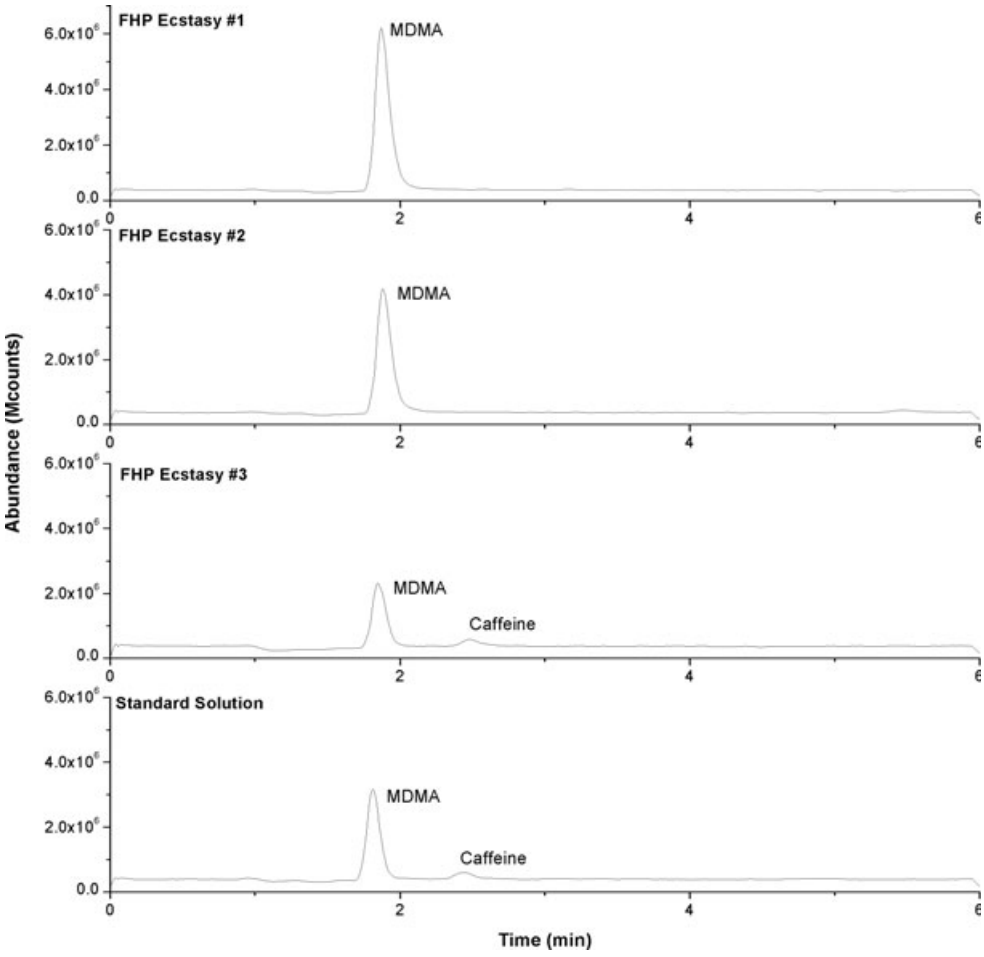

FIG. 3—*Liquid chromatograms for ecstasy samples.*

TABLE 1—*MDMA concentrations in ecstasy pills using LC/MS.*

	MDMA		Caffeine	
Sample	Concentration (μg/mL)	% of Pill (W/W)	Concentration	% of Pill (W/W)
FHP Ex #1	25	25%	–	–
FHP Ex #2	17	17%	–	–
FHP Ex #3	8	8%	100 μg/mL	10%

MDMA, 3,4-methylenedioxy-*N*-methylamphetamine.

training aids for MDMA. The detection of methamphetamine in the headspace of sample 3 is important because this is a drug generally included in training regimes of law enforcement agencies even if MDMA is not included. The significance of the methamphetamine being present is that dogs trained on methamphetamine may alert to MDMA samples such as sample 3 owing to the presence of methamphetamine.

Canine Trails

It has been shown previously that dogs trained to alert to ecstasy will alert to piperonal (12). In order to confirm the reliability and accuracy of piperonal as the primary target odor in canine training aid, previously untrained canines were first imprinted on only piperonal training aids and then they were tested with ecstasy samples. These canines were not exposed to any type of ecstasy sample prior to or during the piperonal training process. The training consisted of two sessions a day for 5–15 days (depending on the training agency) using 50 g of a piperonal training aid (1:10, piperonal: matrix). The testing phase consisted of a double-blind lineup using 25 g of blank matrix, 50 g of the piperonal aid, and 25–35 g of ecstasy tablets. For the lineup, each sample was placed in a separate scent box/electrical box. The handlers were instructed to have their canines sample the odor in each box and identify a response of alert, no-alert, or extended interest. The results of these tests are given in Fig. 5. One hundred percent of the canines (24 of 24) correctly identified the positive control (50 g of piperonal aid) to which they had been trained. Ninety-six percent of the canines (23 of 24) gave a final alert response to the ecstasy tablets after demonstrating their ability to identify the piperonal correctly. The single canine that did not alert to the ecstasy showed extended interest in the sample but did not give a final response.

As previously demonstrated, different batches of seized ecstasy pills contain different concentrations of the active ingredient MDMA (Table 1). A decreased amount of MDMA results in a decreased amount of piperonal. This identifies a potentially significant issue with regard to target odor recognition by canines. Canines of the police agencies that use seized ecstasy samples for training purposes may not utilize the same target odors. Therefore, it is important to provide laboratory testing of drugs to be used in training aids and to make adjustments, where needed, in training aids deployed and/or in training protocols such as the number of training aids employed.

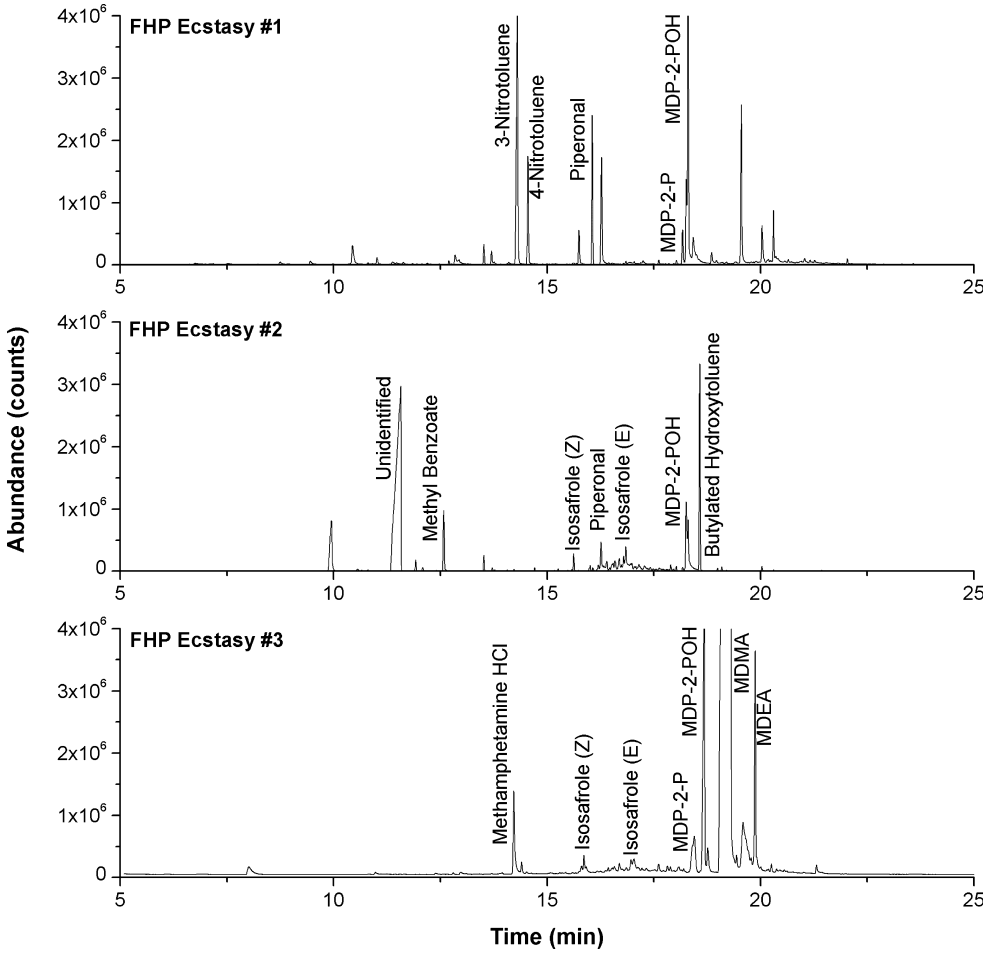

FIG. 4—*HS-SPME-GC/MS chromatograms for ecstasy samples.*

TABLE 2—*Headspace components detected in ecstasy samples.*

Detected Compound	MW	FHP Ex #1	FHP Ex #2	FHP Ex #3
Methamphetamine HCl	149			X
Isosafrole	162		X	X
Piperonal	149	X	X	
MDP-2-POH	180	X	X	X
MDP-2-P	194	X		X
MDMA	193			X
MDEA	207			X

MDMA, 3,4-methylenedioxy-*N*-methylamphetamine; MDEA, 3,4-methylenedioxyethamphetamine.

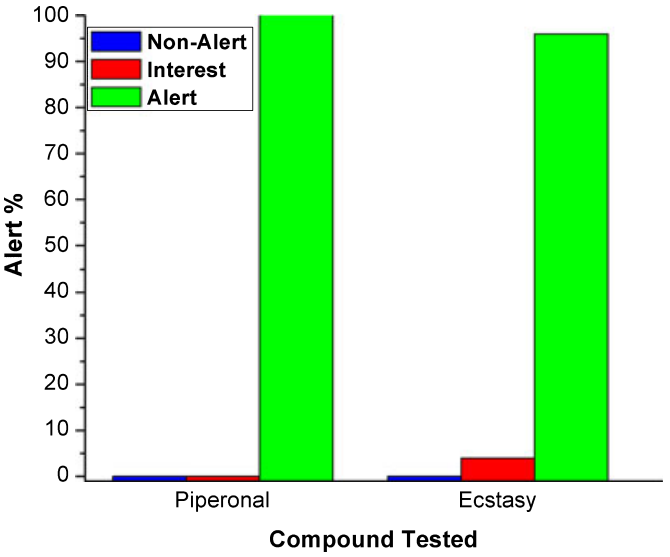

FIG. 5—*Results of ecstasy tested, piperonal imprinted canines.*

In order to determine the field threshold detection levels of piperonal dogs trained to detect ecstasy, piperonal controlled odor mimic permeation devices (COMPS) were utilized, which provided several different orders of magnitude in permeation of the target odorant. Piperonal COMPS with permeation rates of 10, 100, and 1000 ng/sec were selected. A 3 in × 3 in 2 mil HDPP with 2 g of piperonal was used for the 10 ng/sec sample and a 3 in × 3 in 1.5 mil LDPE with 500 mg of piperonal was used for the 100 ng/sec sample. Because no COMPS aid yielded a permeation rate of 1000 ng/sec, five 3 in × 3 in 1.5 mil LDPE with 2 g of piperonal were used in combination (5 × 200 ng/sec). Each aid was tested five times with five different trained and certified canines (Canines 109, 111, 131, 144, and 145). The results of the field tests are given in Fig. 6. The field detection results on the left (Fig. 6*a–c*) were for canines that were trained using training aids confirmed to contain piperonal in their headspace. Consistent

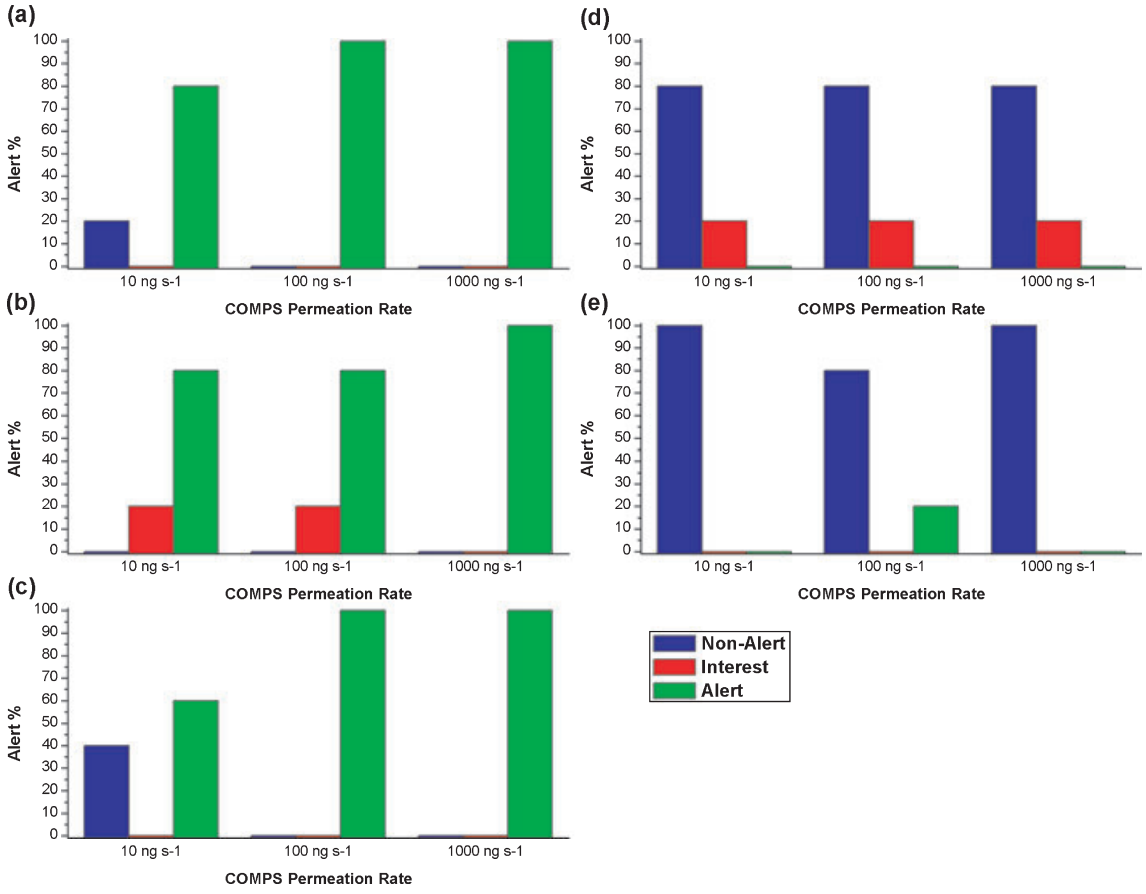

FIG. 6—*Results of piperonal threshold testing (a) Canine 109, (b) Canine 111, (c) Canine 131, (d) Canine 144, and (e) Canine 145.*

detection was observed for 55–75% of detector dogs tested at the 10 ng/sec level, increasing to nearly 100% for the 1000 ng/sec piperonal COMPS. In contrast, the results on the right (Fig. 6*d*,*e*) are for dogs trained with MDMA tablets later determined not to contain piperonal as a major volatile organic compound (VOC). Most trials with these dogs demonstrated nonrecognition to any of the piperonal COMPS used, regardless of the permeation rate.

Canines 109, 111, and 131 had also been exposed to pure piperonal during initial training scenarios, whereas Canines 144 and 145 had not been exposed to pure piperonal. The results demonstrate that the recognition of the piperonal odor is highly dependent upon on the training aids employed. The results also demonstrate that more than one training aid may be required for the complete detection of street MDMA samples owing to the variability in the VOCs present in street samples that may be chosen for training purposes.

Conclusions

As reported in previous studies, piperonal is shown to be a reliable and accurate detector-dog training aid for MDMA produced along typical synthetic routes (i.e., reduction and bromination). However, in this study, we report the discovery of training aid samples of ecstasy without detectable odor levels of piperonal, which were likely synthesized along an alternate route with different starting compounds (e.g., safrole or isosafrole). We observed a high degree of variability of MDMA levels in ecstasy pills (ranging from 8% to 25%) taken from different seized batches, which can result in variable odor thresholds for MDMA. Variations in canine training aids can play a dominant role in the level of target odor recognition by the canines. Based on the common dominant headspace odor compounds from the ecstasy samples tested, it is shown that additional training compounds may be needed in addition to piperonal to ensure reliable location of MDMA. The compounds MDP-2-POH or isosafrole are recommended as the best choices for secondary odorants for MDMA as they are noncontrolled and commercially available. The use of a two training aid system should maximize the detection potential of ecstasy samples with biological detectors. These results also highlight the need for controlled substance training aids to be laboratory-tested prior to their use in the field for the training of detection canines. Ongoing testing of seized controlled substances not only for bulk impurities but also for the most abundant VOCs present in the headspace are important for intelligence purposes as well as the intelligent development of optimal detector-dog training aids. Additionally, samples found to contain other drugs and/or drug odorants, such as methamphetamine identified here, should not be utilized as detector-dog training aids. These results clearly demonstrate the need for continued research in the odorants utilized by trained detector dogs and the development of reliable field calibrants, such as the COMPS used in this study, in order to improve the performance of detector dogs in the field.

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