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Effects of 3,4-Methylenedioxymethamphetamine in Decomposing Tissues on the Development of *Parasarcophaga ruficornis* (Diptera: Sarcophagidae) and Detection of the Drug in Postmortem Blood, Liver Tissue, Larvae, and Puparia

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ABSTRACT: Larvae of *Parasarcophaga ruficornis* (Fabricius) (Diptera: Sarcophagidae) were reared on tissues from rabbits administered different dosages of 3,4-methylenedioxymethamphetamine to study the effects of this drug on the development of this insect species. The rabbits were given 11, 22.5, and 67 mg of the drug via ear vein infusion. These dosages correspond to a 0.5, 1.0, and 2.0 median lethal dose. Larvae from colonies fed on tissues from the rabbit receiving 67 mg and the control developed more rapidly from hours 24 through 114. The period required for completion of larval development was significantly shorter for the larvae from the colony fed on liver tissues from the rabbit receiving the 67 mg dosage. This colony also had the lowest total mortality rate during development. No significant differences were observed among the colonies in the duration of the puparial period. Analyses by liquid chromatography/mass spectrometry of the larvae and empty puparial cases following base extraction detected the drug in quantities directly related to the dosage of the drug administered to the rabbits serving as a food source. These results have implications for the determination of time since death when gaged by the development of this insect species.

KEYWORDS: forensic science, entomology, Diptera, development rates, toxicology, 3,4-methylenedioxymethamphetamine, MDMA, MDA, drugs

Drug-related deaths have increased in the United States and other parts of the world in recent years. Victims are frequently not discovered for a period of several days to months and, due to decompositional changes, estimates of the postmortem interval are made using entomological techniques (1). These techniques include analyses of insect life cycles (2) and arthropod successional patterns (3). Additionally, use of maggots as alternate specimens for toxicological analyses has been well documented (4,5,6,7,8). Miller et al. (9) has demonstrated the utility of spent puparial cases as alternate toxicological specimens in a case dealing with mummified remains. Although these and other studies have death with detection of both prescription and illegal drugs and toxins, fewer studies have concerned themselves with the effects of these substances in decomposing tissues on the developmental rates and patterns of the maggots. Work by Goff et al. (10,11,12,13,14) has documented differences in growth rates for two species of flesh fly maggots (Diptera: Sarcophagidae) fed on decomposing tissues containing known amounts of cocaine, heroin, methamphetamine, amitriptyine, phencyclidine, and their metabolites. These studies have shown differences in developmental rates resulting from the presence of these substances in host tissues. Differential development may have a significant effect on the postmortem interval estimate (PMI) derived from entomological data. PMI estimates are generally based on the period of time required for a given species of insect or other arthropod, using decomposing remains as a food source, to reach a given state of development or through interpretations of successional patterns for the taxa involved (1). An example of adjustment of the PMI estimate based on the presence of cocaine in the remains is given by Lord (15).

The recent rise in use of designer-drugs has caused scientists to incorporate a wider range of drugs into the average drug screen and postmortem toxicological examination (16). The present study focuses on the effects of the 3,4-methylenedioxymethamphetamine (MDMA) and its metabolite 3,4-methylenedioxyamphethamine (MDA) in decomposing tissues on the rate of development of the sarcophagid fly *Parasarcophaga ruficornis* (Fabricius) and on extraction of MDMA from blood, postmortem hepatic tissue, fly larvae and chitinized insect puparia. MDMA is a ring substituted derivative of the central stimulant methamphetamine and has become a popular recreational drug due to its psychoactive properties with minimal hallucinogenic effects. The United States Drug Enforcement Administration has listed MDMA as Schedule I with no accepted medical application and a high abuse potential. It is know on the street as "Ecstacy, XTC, Adam, X and MDM (17). MDMA is a relatively new drug of abuse in the Hawaiian Islands, but has been widely reported from other geographic areas. *Parasarcophaga ruficornis* is an early invader of remains in the Hawaiian Islands and is frequently reported on the island of Oahu (18).

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Materials and Methods

Three domestic rabbits (4.2–4.6 kg in weight) were given dosages of 11, 22.5, and 67 mg of MDMA in 3 mL of normal saline via ear vein infusion to produce concentrations approximating 0.5, 1.0, and 2.0 median lethal dosages (MLD) of MDMA based on body weight. A third rabbit (4.2 kg in weight) received only 3 mL of normal saline via ear vein infusion. The rabbits receiving the 1.0 and 2.0 MLD of MDMA exhibited immediate stress reactions including vocalizations. The animal receiving the 0.5 MLD of the drug remained calm, although somewhat ataxic. Five minutes following administration of the drug, the animals were sacrificed in a carbon dioxide chamber.

Immediately following death, a 2-mL blood sample was taken from each rabbit and frozen for later analysis of the MDMA content. The livers and spleens were removed from all rabbits. The livers and spleens ranged from 98.5 to 140.5 g with a mean of 115.7 g. A sample of each liver was frozen for later analysis of MDMA content.

Flies used in this study were from a stock colony of *Parasarcophaga ruficornis* established from specimens collected from decomposing human remains on the island of Oahu during 1994 and reared in the laboratory for four generations prior to use in this study. Beef liver was exposed to this colony for a period of 5 min for larviposition. From this larviposition, 210 larvae were placed onto each test liver to initiate the test colonies. Colonies thus established were maintained in the laboratory at 26°C in a Labline ambi-Hi-Low environmental chamber with a 12-h photoperiod. At 6-h intervals, total body lengths were recorded from random samples of 10 larvae from each colony to indicate growth rates. At 72 h, a sample of 10 larvae was removed from each colony and frozen for later analyses for MDMA content. After completion of larval development, puparia were observed at 6-h intervals and adult emergence recorded. Emerging adults were maintained in separate colonies and provided with a standard diet of water, sugar, and protein hydrolysate. Thirteen days following emergence, liver was supplied to each colony for larviposition. Developmental data were analyzed using analysis of variance (ANOVA).

Toxicological Methods: Base Extraction

Approximately 250 mg of liver was weighed out on a Mettler MT5 balance. It was then homogenized with a Wheaton 2-mL tissue homogenizer attached to a variable speed hand-held drill set on low. The sample was homogenized for approximately 3 min with 3 mL of deionized water and then poured into a 16 × 125 mm screw cap glass culture tube that had previously been rinsed with deionized water and methanol.

Fifty microliters (50 ng) of MDMA-d5 (1 ng/uL) internal standard (Radian Corporation) was added. To this solution, 10 mL of n-butyl chloride (Baxter) and 10 drops of concentrated ammonium hydroxide (Sigma) were added. The culture tubes were capped and placed on the labquake shaker for 30 min at room temperature. Samples were then centrifuged for 5 min at 3,000 rpm.

The supernant (top layer) was collected in a clean 16 × 125 mm screw cap culture tube and back extracted with 5 mL of 1N HCL (Mallinckrodt) for 20 min on the labquake shaker. Tubes were then centrifuged for 5 min at 3,000 rpm.

The bottom layer was removed and placed into another clean 16 × 125 mm culture tube. This solution was then alkalinized with 2 mL of concentrated ammonium hydroxide. Three mL of chloroform (without preservative, Baxter) was added and the solution vortexed for 1 min. The chloroform layer (bottom) was filtered through sodium sulfate/glass wool into a 12 × 75 mm glass tube that was in a 55°C heating block. Two drops of 1N HCL/methanol (1:2) were added and the solution evaporated to dryness under nitrogen. The concentrate was then reconstituted with 50 μL of acetonitrile (Baxter, HPLC grade) for LC/MS analysis.

Liquid Chromatography Mass Spectrometry (LC/MS)

Identification and quantification of MDA and MDMA were done by LC/MS for blood, postmortem hepatic tissue, fly larvae and chitinized insect tissues (puparial cases). LC/MS analyses were preformed on a Finnigan TSQ 700 (San Jose, CA) using the Finnigan API electrospray interface at a spray voltage of 4.5 kV. The liquid chromatograph was a Hewlett Packard HP 1090 ternary pump (Palo Alto, CA) A Hamilton PRP-1.2 × 150 mm reversed-phase column (Reno, NV) was used for the analyses with a mobile phase of acetonitrile/10 mM ammonium hydroxide (75:25). Only isocratic runs were used at a flow rate of 0.3 mL/min. Test standards of MDA (Sigma) and MDMA (Radian) were used for instrument tuning and check out. The mass spectrometer was scanned from 160 to 250 in 1 sec scans. Three ions were monitored for the metabolite, parent drug, and internal standard: MDA ions were 163, 180, and 221; MDMA ions were 163, 194, and 235; MDMA-d5 ions were 165, 199, and 240. The highest mass ion for each compound was used for quantification. The MDA content was estimated based on its relative response to MDMA-d5 which was calculated to be 1.78.

Results and Discussion

The analyzed samples of blood, liver, larvae, and puparia all showed the presence of MDMA in samples from all rabbits receiving the drug and colonies fed on tissues from these animals (Table 1). In all samples, the amount of MDMA detected in samples was related directly to the dosage administered. MDA, a metabolite of MDMA, was not observed in any of the blood, liver, or larvae samples. However, MDA was detected in the three puparia samples from larvae fed livers from rabbits given MDMA. Figure 1 illustrates LC/MS ion traces for a puparial extract that was positive for both MDMA and MDA. The compounds were positively identified by the monitoring of 3 ions. The 163 ion is a fragment for both MDMA and MDA. The other 2 ions, MDMA = 194 & 235 and MDA = 180 & 221, are adduct ions of the compounds which are (M+H)+ and (M+acetonitrile+H)+.

Quantitative base extraction of both insect larvae and chitinous puparia resulted in successful isolation of MDMA and MDA. Miller et al. (9) has previously demonstrated the use of both acid and base extraction protocols for isolation of forensically important pharmaceuticals from entomological specimens. The highest concentrations of MDMA and MDA were isolated from the puparia. This probably reflects the length of time to which the developing insects were fed on the drug laden liver. It should be noted that the larvae were harvested for toxicological analysis three days post-colonization. Puparia, however, were sampled on day 22 or later (Table 2).

As previously mentioned, MDA was detected only in samples of puparia. This likely reflects the lengthy interval to which the insects were exposed to the rabbit liver as a food source. No MDA was isolated in the livers or blood of the rabbits, as they were sacrificed rapidly after infusion of the parent drug. It remains unclear as to whether the metabolism of MDMA to MDA occurred in the decomposing rabbit liver or the developing insect. In either case, this metabolism appears to be slow. A similar pattern was

TABLE 1—*Concentrations of 3,4-methylenedioxymethamphetamine (MDMA) in blood and liver tissues of rabbits administered different concentrations of the drug via ear vein infusion and from larvae and puparial cases of* Parasarcophaga ruficornis *fed on those tissues. Base extraction protocol followed by LC/MS analysis.*

MLD of MDMA Administered	MDMA Conc. in Blood (ng/mg)	MDMA Conc. in Liver (ng/mg)	MDMA Conc. in 3rd Instar Larvae (ng/mg)	MDMA Conc. in Puparia (ng/mg)	MDA Conc. in Puparia (ng/mg)	MDMA/MDA Ratio in Puparia
Control	ND*	ND	ND	0.4	ND	–
0.5	0.2	0.4	0.6	1.1	0.8	1.4
1	5	1.6	1.5	6.6	1.8	3.7
2	7.9	7.9	1.7	22.8	10.1	2.3

*ND = None detected.

TABLE 2—*Durations of larval stage, larval mortality, durations of puparial stage, puparial mortality, and total mortality for colonies of* Parasarcophaga ruficornis *reared on rabbit liver tissues containing varying amounts of 3,4-methylenedioxymethamphetamine (MDMA) at a temperature of 26°C.*

Colony by MLD of MDMA Administered	$\bar{X}$ Duration of Larval Stage	Larval Mortality	$\bar{X}$ Duration of Puparial Stage	Puparial Mortality	$\bar{X}$ Time from Larviposition to Adult Emergence	Total Mortality
Control	214.3 h(180–258)a (N = 200)	31.50%	348.6 h(342–384)a (N = 137)	7.20%	562.9 h(522–642)a (N = 127)	36.50%
0.5	216.6 h(186–258)a (N = 200)	40.50%	340.8 h(336–360)a (N = 119)	4.20%	557.4 h(522–618)a (N = 114)	43.00%
1	212.3 h(186–252)a (N = 200)	16.50%	351.7 h(336–366)a (N = 167)	4.20%	564.0 h(522–618)a (N = 160)	20.00%
2	190.9 h(162–252)b (N = 200)	1.00%	347.0 h(336–348)a (N = 198)	1.50%	537.9 h(498–600)a (N = 195)	2.50%

[a]Figures in a column followed by the same letters are not significantly different ($P > 0.05$).

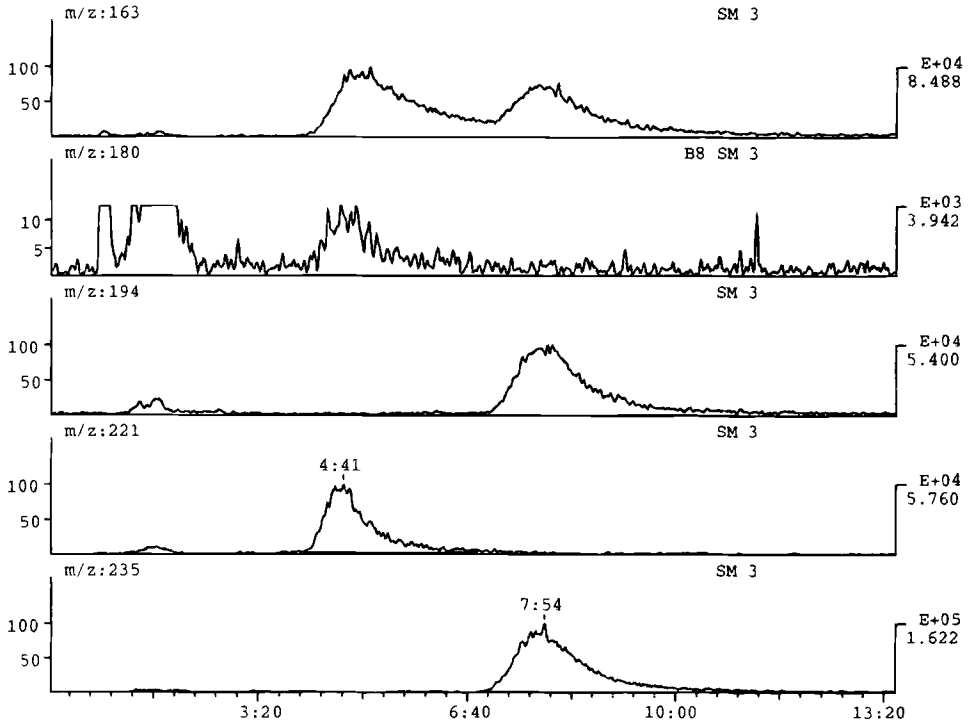

FIG. 1—*LC/MS chromatographic ion traces for MDMA (elution time 7:54) and MDA (elution time 4:41) from the 2.0 MLD puparial extract. MDMA has ions at m/z 163, 194, and 235. MDA has ions at m/z 163, 180, and 221. A three point smooth (SM 3) was performed on all ion traces and the 180 ion trace was expanded 8 times (B8).*

observed by Rohrig et al. (17) in human tissues harvested from decedents with a short and extended postmortem interval.

The ratio of MDMA to MDA (Table 1) detected in puparia was consistent with acute rather than chronic exposure (parent drug concentration > metabolite concentration) (19,20). This ratio appeared not to be correlated to insect developmental time. However, the lowest ratio corresponded to the lowest MLD. These results further demonstrate how insects and other arthropods can serve as alternative sources of toxicological information.

Rates of development were determined by increases in body length over time for larvae until maximum length was attained (Fig. 2). These rates were not significantly different from hours 0 through 24, a period roughly corresponding to the duration of the 1st instar. Between hours 24 and 114, larvae from the control colony and the 2.0 MLD colony developed more rapidly than those from the 0.5 and 1.0 MLD colonies. A maximum length of 20 mm was attained in the control colony at 84 h and in the 2.0 MLD colony at hour 108 of 19.1 mm. The 0.5 and 1.0 MLD colonies did not attain these lengths, with 18.9 mm at 132 h for the 0.5 MLD colony and 18.6 mm at 114 h for the 1.0 MLD colony. No significant differences in length among the colonies were observed after 114 h. Pupariation was first observed in the 2.0 MLD colony at 190 h (Table 2) and this was significantly less time than was required by larvae from the other colonies. Larval mortality was also lower for the 2.0 MLD colony (1.0%), followed by the 1.0 MLD colony with 16.5%. No significant differences were observed in the durations of the puparial stage among the different colonies (Table 2). As for the larval stage, puparial mortality was less for the 2.0 MLD colony with a rate of 1.5%. The control colony had the highest mortality rate. The total period required for development from larviposition to adult was significantly shorter for the 2.0 MLD colony. The shortened maturation period for the 2.0 MLD colony was primarily due to the increased rate of development during the larval stages. In addition, the 2.0 MLD colony had the lowest overall mortality (Table 2).

The above results further demonstrate the necessity for consideration of the possible effects of drugs and/or toxins present in decomposing bodies on insect development rates when estimating a postmortem interval based on entomological evidence. In addition to these applications, our results further demonstrate how insects and other arthropods may serve as reliable alternate specimens for toxicological analyses in the absence of tissues and fluids normally taken for such purposes.

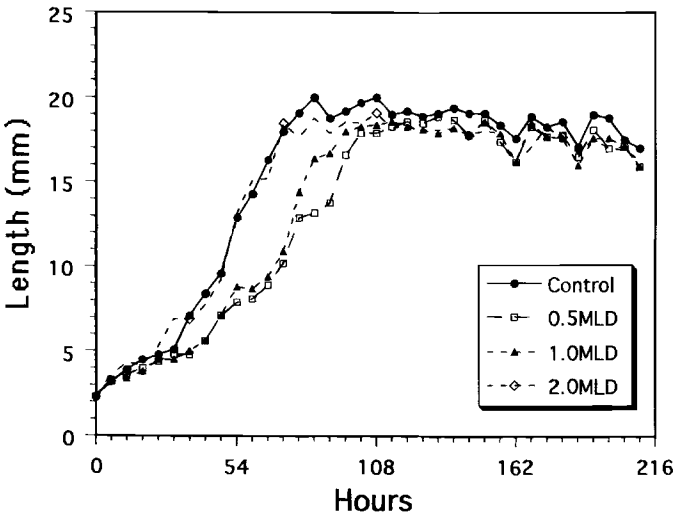

FIG. 2—*Rates of development, as indicated by total body lengths of larvae of* Parasarcophaga ruficornis *reared on rabbit liver tissues dosed with different amounts of 3,4-methylenedioxymethamphetamine. Dosage of 11 mg = 0.5 MLD; 22.5 mg = 1.0 MLD; and 67 mg = 2.0 MLD.*

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