M. Lee Goff,[1] *Ph.D.; Wayne A. Brown,*[1] *Ph.D.;*
Alvin I. Omori,[2] *M.D.; and Dennis A. LaPointe,*[1] *M.S.*

Preliminary Observations of the Effects of Phencyclidine in Decomposing Tissues on the Development of *Parasarcophaga ruficornis* (Diptera: Sarcophagidae)

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ABSTRACT: Larvae of *Parasarcophaga ruficornis* (Fabricius) (Diptera: Sarcophagidae) were reared on tissues from rabbits administered different dosages of phencyclidine to study the effects of this drug on the development of this insect species. The rabbits were given 3.66, 7.31, and 14.62 mg of phencyclidine via ear vein infusion. No significant differences in larval growth rate were observed among the colonies, although the duration of the non-feeding portion of the third instar was shorter for larvae fed on tissues containing the drug. Mortality during the larval stage was directly related to the dosage of phencyclidine administered, ranging from 0 in the control colony to 29.0% in the colony fed on tissues from the rabbit receiving 14.62 mg of phencyclidine. Durations of the puparial stage were longer for colonies fed on tissues containing the drug. Presence of phencyclidine was detected in larvae from all colonies fed on tissues from rabbits receiving the phencyclidine using GC/MS.

KEYWORDS: toxicology, postmortem interval, entomology, Diptera, phencyclidine, drugs

Drug-related deaths are frequently not discovered for a period of several days and, due to changes associated with decompositional processes, estimates of postmortem interval are made using entomological techniques involving arthropod development combined with successional data [*1–3*]. The use of maggots as alternate specimens for toxicological analyses for drugs and toxins has been well documented [*4–7*]. While these studies have dealt with the detection of both prescription and illegal drugs and toxins, fewer studies have concerned themselves with the effects of these substances on the developmental patterns of the maggots. Work by Goff et al. [*8–11*] has documented differences in growth rates for two species of flesh fly maggots (Diptera: Sarcophagidae)

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[1]Professor of Entomology, Research Associate and Research Assistant, respectively, Department of Entomology, University of Hawaii at Manoa, Honolulu, HI.

[2]Chief Medical Examiner, City and County of Honolulu, Honolulu, HI.

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fed on tissues containing varying amounts of cocaine, heroin, methamphetamine, amitriptyline, and their metabolites. These studies demonstrate that observed differences in developmental rates 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 insects or other arthropods, using decomposing remains as a food source, to reach a given state of development or through interpretations of succession patterns for the species involved [*2,3*]. An example of adjustment of the postmortem interval estimate due to presence of cocaine has been given by Lord [*12*]. Nolte et al. [*13*] have documented a case in which detection of cocaine in Calliphoridae maggots contributed to the determination of the circumstances of death.

This study concerns the effects of phencyclidine in decomposing tissues on the development of the sarcophagid fly *Parasarcophaga ruficornis* (Fabricius). Phencyclidine is a common drug of abuse in the United States as well as being a legitimate veterinary tranquilizer. *Parasarcophaga ruficornis* is an early invader of remains and frequently encountered on the island of Oahu [*14*].

Materials and Methods

Three domestic rabbits (4.0 to 4.4 kg in weight) were given dosages of 3.66, 7.31, and 14.62 mg of phencyclidine respectively in 10 mL of normal saline via ear vein infusion to produce different concentrations of phencyclidine in tissues. These dosages were calculated to represent sublethal, mean lethal and 2.0× mean lethal dosages of the drug by body weight. A fourth rabbit (4.1 kg in weight) was used as a control and given only 10 mL of normal saline by ear vein infusion. The rabbit receiving the 2.0× mean lethal dosage exhibited immediate reactions to the drug, including vocal distress signals, labored breathing and muscular twitching. This animal expired as a result of the actions of the drug within 1 min following injection. The other animals receiving the drug exhibited lesser symptomatology, including difficulty in breathing. These rabbits and the control were killed in a carbon dioxide chamber 5 min following injection.

Immediately following death, a 2 mL blood sample was taken from each rabbit and frozen for later analysis of drug content. The livers were removed from each rabbit and a sample of tissue removed from each and frozen. Livers weighed from 31 to 57 g, with a mean of 45.5 g. Blood and liver samples were analyzed by the Chemical Toxicology Institute, Foster City, California, using gas chromatography/mass spectrometry (GC/MS).

Flies used in this study were from a stock colony of *Parasarcophaga ruficornis* (F.) established from specimens collected off a suicide case during 1990 and maintained in the laboratory for 14 generations. Beef liver was exposed to this colony for a period of 15 min to allow for larviposition. From this larviposition, approximately 220 larvae were introduced onto each test liver. 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 a random sample of 10 larvae from each colony to indicate growth rates. At 48 h, a sample of 10 larvae was removed from each colony and frozen for later analyses of drug content using GC/MS techniques. 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, protein hydrolysate, and sugar. Thirteen days following emergence, liver was supplied to each colony for larviposition. Resulting data were analyzed using analysis of variance (ANOVA) and Waller-Duncan multiple range test [*15*]. The various colonies will be referred to from this point by the relative lethality of the dosage (0.5 MLD, 1.0 MLD, 2.0 MLD, and control).

Results

The analyzed blood and liver samples showed the presence of phencyclidine in all samples from rabbits that had received the drug, except for the liver sample from the 2.0 MLD rabbit (Table 1). This sample was broken during shipping and could not be analyzed. The liver sample from the control rabbit was negative for phencyclidine. The blood sample from the control rabbit was broken and not available for analysis. Samples of blood and liver analyzed from rabbits which had received the dosages of phencyclidine did not show a direct correlation between administered dosage and concentrations in tissues (Table 1).

Analyses were made of 10 larvae from each colony collected at the 48-h sample period using GC/MS. Larvae from the control colony were negative for phencyclidine while larvae from all colonies fed on tissues from rabbits receiving dosages of phencyclidine were positive. Concentrations of phencyclidine detected in the samples of larvae ranged from 26 to 65 ng/mL. Due to breakage of the vials containing samples of blood and liver, the labels for the samples of larvae were not legible and thus no concentrations can be assigned to specific colonies, except for the control.

Rates of development were determined by increases in total body length over time until maximum size was attained. These rates were not significantly different among colonies (Fig. 1). Greatest lengths were observed in the 0.5 MLD colony at hour 78 with a mean of 19.6 mm (range 19 to 22 mm). Maximum sizes were also observed at this point in the other colonies and maximum recorded lengths ranged from 20–21 mm.

Pupariation was first observed in the 0.5 MLD colony at hour 132, followed by the 1.0 MLD colony at hour 138, 2.0 MLD colony at hour 144, and the control at hour 150. The total duration of the larval stage was significantly different between colonies. Duration was shortest for larvae from the 0.5 MLD colony with a mean of 153.3 h (range 132–180 h) (Table 2) and longest for larvae from the control colony with a mean of 170.6 h (range 150–192 h). Recorded larval mortality varied directly with the dosage of phencyclidine administered to the rabbits, with 29.0% mortality recorded for the 2.0× MLD colony (Table 2) and none for the control colony. There were significant differences observed between colonies in the duration of the puparial stage (Table 2) with the longest duration recorded for the 1.0 MLD colony and the shortest duration in the control colony. There were no significant differences between colonies in puparial weights. Puparial mortality was greatest for the control and 1.0 MLD colonies (Table 2). Total durations for the immature stages (larvae and puparia) were greatest for the 1.0 MLD colony and least for the control colony. All colonies produced viable larvae when supplied with liver for larviposition 13 days following adult eclosion and no abnormalities were observed in subsequent generations.

Discussion

Presence of phencyclidine in all samples of larvae fed on tissues from rabbits receiving dosages of phencyclidine indicates that Diptera larvae may serve as alternate toxicolog-

TABLE 1—*Concentrations of phenyclidine in blood and liver tissues from rabbits administered different dosages of phencyclidine and larvae of* Parasarcophaga ruficornis *fed on tissues from those rabbits.*

Tissue	Dosage	Control	0.5 MLD	1.0 MLD	2.0 MLD
Blood		(sample lost)	239 ng/mL	60 ng/mL	219 ng/mL
Liver		negative	18 ng/g	15 ng/g	(sample lost)
Larvae		negative	positive[a]	positive[a]	positive[a]

[a]Labels on these samples were damaged during processing. Concentrations of phencyclidine in positive samples ranged from 26 to 65 ng/g.

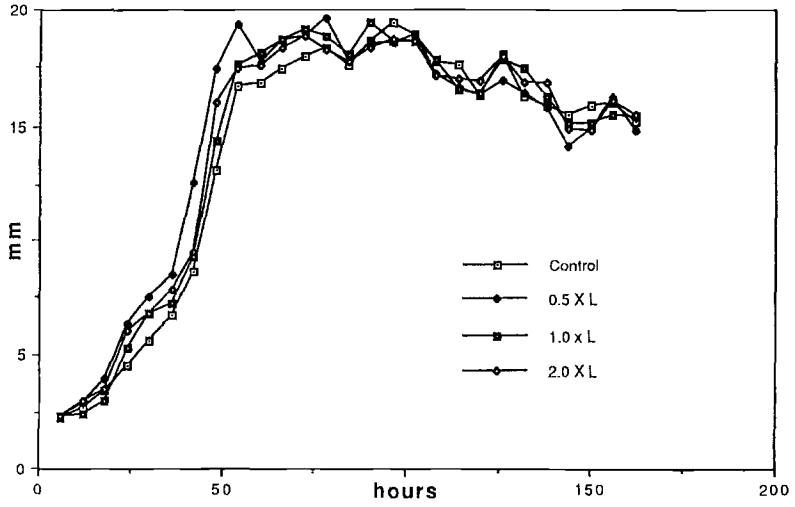

FIG. 1—*Growth rate of* P. Ruficornis *larvae fed on tissues containing phencyclidine.*

ical specimens for detection of phencyclidine in the absence of tissues normally taken for analyses. This situation is similar to that encountered in earlier studies on cocaine, heroin and amitriptyline, but not methamphetamine.

Unlike earlier studies dealing with cocaine, heroin and methamphetamine [*8–10*], there was not a direct relationship between the dosage of phencyclidine administered and the concentration of the drug detected in the tissues. This lack of correlation in concentrations found in the blood samples combined with losses of blood and liver samples allow for only general statements regarding the effects of phencyclidine in tissues on the development of these larvae.

As was observed during earlier studies on amitriptyline [*11*], there were no significant differences in the rates of growth of larvae fed on tissues containing phencyclidine and the control colony during the active feeding portions of larval development. There were differences however in the durations of the postfeeding period of larval development,

TABLE 2—*Durations of larval stage, larval mortality, puparial weights, lengths, durations and mortalities for colonies of* Parasarcophaga ruficornis *reared on rabbit liver tissues containing varying amounts of phencyclidine at a constant temperature of 26°C.*

Colony	X duration of Larval Stage (hours)	Larval Mortality	X Puparial Weight (mg)	X Duration of Puparial Stage (hours)	Total Mortality	Total Developmental Time (hours)
Control	170.6a (SD = 11.4624)	0.0%	8.6a	305.0a (SD = 13.1529)	12.0%	475.6a
0.5 MLD	153.5b (SD = 9.6534)	5.0	8.8a	327.5c (SD = 15.0412)	12.8	481.2b
1.0 MLD	166.3c (SD = 11.9989)	9.5	9.0a	340.7d (SD = 15.5531)	21.7	506.9c
2.0 MLD	167.6c (SD = 13.3914)	29.0	9.0a	310.4b (SD = 14.9311)	35.3	478.0ab

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

with larvae from the treated colonies completing larval development more rapidly than those in the control colony. Mean differences in duration of the larval stage in treated colonies ranged from 3 to 17 h less than required by the larvae in the control colony. By contrast, the duration of the puparial stage was significantly shorter for individuals in the control colony when compared to those in treated colonies. This was similar to the situation observed during earlier studies on heroin [*9*] and was sufficient increase the mean times required for total immature development (larval + puparial stages) by 5.4 to 40.7 h in treated colonies when compared to the control colony. While larval mortality can not be related to concentrations of phencyclidine in tissues for this study, there was a direct relationship (Table 2) observed between dosage administered and larval mortality. The same is true for total mortality during development, with an observed increase in mortality directly related to the dosage of phencyclidine administered. The need for additional studies to resolve questions posed by the preliminary nature of the present study must be emphasized.

Acknowledgments

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Address requests for reprints or additional information to
M. Lee Goff, Ph.D.
Dept. of Entomology
University of Hawaii at Manoa
3050 Maile Way
Honolulu, HI 96822