**Development of a GC–MS method for methamphetamine detection in Calliphora vomitoria L. (Diptera: Calliphoridae)**

## Abstract

Entomotoxicology is the study of using insects for the detection of drugs and other chemical substances in decomposing tissues. One research aspect in particular is the effects of these substances on arthropod development and morphology, and their consequences on the post mortem interval estimation. Since methamphetamine (MA) is becoming commonplace as an illegal recreational drug, a GC–MS method for the detection of MA in *Calliphora vomitoria* L. (Diptera: Calliphoridae) was developed and validated. Furthermore, the effect of MA on the development, growth rates and survival of the blowfly was investigated. Larvae were reared on liver substrates homogeneously spiked with measured amounts of MA (5 ng/g and 10 ng/g) based on typical concentrations found in human tissue in cases of death caused by MA overdose. The experimental results demonstrated that (i) MA produced a significant increase in the developmental time from egg to adult in *C. vomitoria*, (ii) approximately 60% of larvae exposed to either dose of MA died during the pupation period and (iii) the resultant lengths of larvae and pupae were on average significantly larger than the controls.

## Keywords

- Entomotoxicology;
- Methamphetamine;
- GC–MS

## 1. Introduction

Forensic entomology is generally accepted as an essential tool when investigating any homicide case, where insects and other arthropods are associated with the corpse [1]. The umbrella of activities conducted by forensic entomologists include insect contamination of food and infestation of human environments, together with the assessment of neglect and/or cruelty, and whenever insect material is found in wounds of humans, livestock, pets and wildlife [1], [2] and [3]. Over the last 30 years, insect material has been used in toxicological analyses for various purposes [4], [5], [6] and [7]. “Entomotoxicology” is defined as a branch of forensic entomology which studies the potential uses of insects for detecting drugs or other toxic substances that may otherwise not be measurable in decomposing tissues (e.g. corpse or carcasses in high decay stage of decomposition or skeletonized remains) [8]. Several studies have demonstrated that the toxicological analyses of insect material are able to provide a more reliable and sensitive result than from highly decomposed remains [9], [10] and [11]. In one case insect material found on mummified remains, demonstrated the occurrence of significant amounts of amitryptiline and nortryptiline, allegedly ingested shortly before death [12].

A major objective of forensic entomology is the determination of the time elapsed since death of a deceased human or animal. The use of insects has become the 'gold standard' [13] for this purpose, however, it has been demonstrated that their life cycle, supposedly providing insight into such time frames, may be severely affected by the presence of drugs and toxins, and subsequently a possible bias in post mortem interval calculations [5] and [7].

Many substances (drugs, pesticides, and toxic metals) have been detected in insect tissues, and a relationship between the drug concentrations found in the substrate and insects reared on that substrate have been determined [7]. While the detection of opiod residues has been consistently reported in entomotoxicological studies, the recent literature is devoid of research and/or case studies concerning the detection, effects and analytical quantification of methamphetamine (MA) on the entomofauna feeding on a corpse [14] and [15].

MA is a psychostimulant synthetic drug commonly produced by the reduction of ephedrine with iodidric acid[16], which is becoming increasingly diffuse as a recreational drug [17], with pseudonyms like meth, crystal, and ice. It is a relatively lipophilic substance with a structure similar to that of N-adrenaline. Therefore, this drug can easily pass through the blood-brain barrier producing a stimulant effect. Originally it was used as a medical treatment of narcolepsy, mild depression and chronic alcoholism, but nowadays it is instead consumed for its euphoric effects. High doses of MA can induce palpitation, convulsion, heart attack, and death. Chronic use can cause schizophrenia and psychosis [18].

In the present study, larvae of the blowfly *Calliphora vomitoria* L. (Diptera: Calliphoridae) were reared on substrates spiked with different concentrations of MA. This study investigates the developmental rate and morphological changes of blowfly larvae feeding on a decomposing beef substrate containing MA. As well, the present research provides the development of a suitable analytical method using GC–MS to detect this drug in larvae, pupae, spent pupae and adults of *C. vomitoria*.

## 2. Material and methods

### 2.1. Preparation of foodstuff and rearing of larvae

Colonies of *C. vomitoria* were reared in the Entomotoxicology Laboratory of the Department of Chemistry at University of Turin. *C. vomitoria* is a common fly species widely distributed through the Holarctic region. It represents an early coloniser of corpse and carcasses generally in the fresh stage of decomposition. It is mainly found in rural situations or in association with *Calliphora vicina* Robineau-Desvoidy [19].

Adult flies were fed with water and sugar cubes *ad libitum*. 5 days after emergence, flies were provided with fresh beef liver to allow the ovaries to develop. After 10 days fresh beef liver was placed in the cages, on water moistened paper in small plastic trays allowing females to oviposit. Fly egg batches containing approximately 1000 eggs (1.2 g) were deposited with a fine paintbrush on 250 g of beef liver [20] and [21]spiked and homogenised with two concentrations of MA (5 ng/mg, 10 ng/mg). Another beef liver containing no drug was used as a control. The appropriate MA spiking concentrations were selected from the MA levels reported from the body tissues of human fatalities involved in MA overdoses [22], [23] and [24]. Liver was homogenized with solid MA powder using a A11 basic Analytical mill (IKA®-Werke GmbH & Co.). To disperse the analytical standard, a T18 digital ULTRA-TURRAX (IKA®-Werke GmbH & Co.) was used. Each experimental liver was placed on small round plastic tray (Ø 14 cm) with high sides (10 cm) to observe the start of the larvae post-feeding instar. Each plastic tray was placed on 5 cm of sand within a larger plastic box (22 × 40 × 20) covered with a fine mesh cloth and sealed using an elastic band. Sand was used so post-feeding larvae could pupate. For the entire experimental period, the insects were reared at 23 °C with approximately 20% RH and a photoperiod (*h*) of 12:12 (*L*:*D*). The temperature was recorded by Tinytag® data-loggers every 15 min and the average temperature was calculated every hour.

### 2.2. Sample collection

Two samples, one consisting of 10 individuals and another amounting to 1 g from each treatment were collected every 6 h. The sample of 10 individuals from each time point were analysed morphologically. This included a determination of the moult of each life stage and the length of each second (L2), third (L3), postfeeding (PF) and pupal (P) stages using the standards and guidelines for the best preserving method in forensic entomology [25]. The length of each individual was measured using a stereomicroscope (Optika®SZM-2) with a graduated lens.

The second sample following feeding utilized a fixed sample weight (1 g) from L2, L3, PF instars and also from spent pupae (SP) and adults (*A*). Each sample was stored at −20 °C until the sampling period finished and then subjected to the toxicological analysis used to detect MA. Larvae collected during feeding instars (L2 and L3) were sacrificed and stored only after careful cleaning of each individual with water and neutral soap to remove any external contamination. Adults were sacrificed 1 week after their emergence. To validate the method, 100 mg of control SP were used, because of their high chitin content. This is because SP can

be found at the scene for a long time after emergence and in some circumstances they represent the only reliable sample for toxicological analyses.
When the larvae reached the PF instar, 100 individuals from each treatment were placed in separate boxes. The time to pupation, the total number of pupated individuals, as well as the time to eclosion and the total number of emerging adults were recorded. No F1 viability was considered in this study.

## 2.3. Toxicological analysis

### *2.3.1. Chemicals and reagents*

Solid MA (≥98%) and diphenylamine (≥99%) were purchased them from Sigma–Aldrich®. The purity of MA (checked by GC–MS) was 100%. Standard solution of MA in $CH_3OH$ (20 mg/L), and diphenylamine (used as the internal standard; ISTD) in $CH_3OH$ (20 mg/L) were prepared from the solid pure standards. Dichloromethane ($CH_2Cl_2$), methanol, trifluoroacetic acid (TFA), trifluoroacetic anhydride (TFAA), tert-butyl methyl ether (TBME) were also obtained from Sigma–Aldrich®.

### *2.3.2. Sample preparation for GC analysis*

Larvae, pupae, SP and adult samples were placed in a falcon tube (50 mL) and dichloromethane was added as part of the preliminary wash. The tubes with larvae and pupae were then placed in a vortex for two minutes and the organic solvent was discarded. Meanwhile, the SP were dried at room temperature under nitrogen. Following crystallisation using liquid $N_2$, they were crushed with a glass rod and a 100 mg aliquot was placed in a new tube. To validate the method, control *C. vomitoria* SP were spiked with different amounts of MA at this stage, by adding different volumes (0, 5, 7, 10, 17, 25, 50 µL) of methanol solution of MA. Further $CH_3OH$ was added to reach a final 2 mL volume. 30 µL of diphenylamine solution was also added as the ISTD. The tubes were sealed and placed in an oven at 55 °C for 15 h to extract/dissolve the matrix. After cooling at room temperature, the separated organic phase was acidified with 30 µL of TFA and dried at 70 °C under nitrogen stream. Derivatization was performed on the residue for 40 min at 80 °C by adding 30 µL of TFAA and then sealing the tubes. After drying at 80 °C, the analytes were recovered with 100 µL of TBME and injected (1 µL) into the GC–MS instrument.

### *2.3.3. Gas chromatography–mass spectrometry (GC–MS)*

Analytical determinations for the detection of metamphetamine were performed using an Agilent 6890 gas chromatograph coupled with an Agilent 5973-inert mass spectrometer operating in the electron impact ionization mode. Splitless sample injection was effected at a temperature of 250 °C and the injection volume was 1 µl. The capillary column used was a HP-5MS, 30 m × 0.25 mm (i.d.) × 0.25 µm (f.t.). The oven temperature was programmed as follows: initial column temperature was 85 °C for 0.5 min, then increased by 8 °C/min to 110 °C then increased by 30 °C/min to 300 °C for 2.04 min for a total run time of 12 min. The carrier gas was ultrapure He (1.0 mL/min; SIAD, Bergamo, Italy). During preliminary GC–MS analises, full

mass spectra were aquired. The background subtracted mass spectrum for the MA derivative (using EI in full scan mode) is given in Fig. 1. For the quantitative analysis The mass analyser was operated in the selected ion monitoring (SIM) mode. The ions selected to identify MA derivative were: *m/z* > 154, 118, 110, and 91. Diphenylamine does not react with TFAA; characteristic ions *m/z* > 265, 172, 167, and 77 were selected.

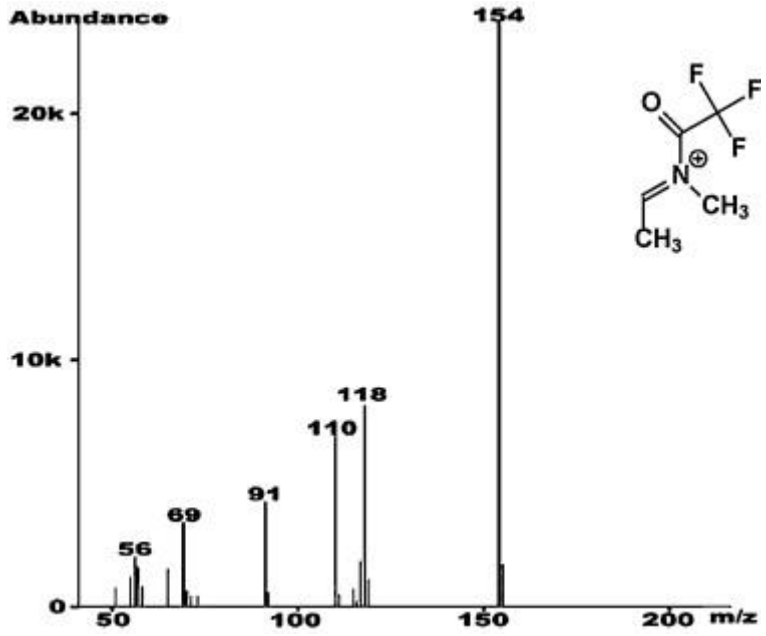


Fig. 1.
Background subtracted mass spectrum of derivatized methamphetamine (MA) obtained with electronic impact (EI) ionisation with the structure of the fragment *m/z* = 154 included. No molecular ion was observed.

### *2.3.4. Method validation*

The validation of the method was performed according to ISO/IEC 17025 requirements and ICH guidelines[26] and [27]. The validation protocol included the quantitative determination of MA in larvae, pupae and puparia: specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), extraction recovery, repeatability and carry over were determined.

### *2.3.5. Specificity*

Ten samples of the control SP were used to ascertain the method's specificity. Five of them were spiked with 30 µL of ISTD. The specificity test was successful if the S/N ratio was lower than 3 at the retention time of the target analyte, for all the specific ion chromatograms.

### *2.3.6. Linearity*

The linear calibration model was checked by analysing control SP samples (100 mg) spiked with MA solution at concentration of 1, 1.4, 2, 3.5, 5 and 10 ng/mg. The use of TFAA assures constant and high MA

derivatization yield, while diphenylamine, used as the ISTD at a final concentration of 6 ng/mg, does not undergo derivatization. The linear calibration parameters were calculated by least-squares regression, and the squared correlation coefficient ($R^2$) was used to estimate linearity. Quantitative results from area counts were corrected using the ISTD signal.

#### *2.3.7. Limit of detection and limit of quantification (LOD and LOQ)*

LOD was estimated as the analyte concentrations whose response provided a signal-to-noise (S/N) ratio of 3, as determined from the least abundant qualifier ion. The S/N ratios at the lowest concentration (LCL) were used to extrapolate the theoretical LOD and LOQ.

#### *2.3.8. Extraction recovery (ER%)*

ER% was evaluated at two different concentrations of MA in control SP: 5 ng/mg and 10 ng/mg. For each concentration, ten samples were spiked before the digestion step of the matrix and ten after the extraction. ER% was calculated by the average ratio between the analyte concentration determined after its extraction (first set) and the one determined on the spiked extract (second set).

#### *2.3.9. Repeatability (intra-assay precision)*

Repeatability was calculated as the percent coefficient of variance (CV%), after spiking ten samples of control SP with two different concentrations of MA: 5 ng/mg and 10 ng/mg. Repeatability is considered acceptable when the CV% is lower than 25% at low analyte concentrations and lower than 15% at high concentrations.

#### *2.3.10. Carry over*

Carry-over effect was evaluated by injecting an alternate sequence of five negative SP samples and five blank SP samples was spiked with MA at 10 ng/mg concentration to ensure the absence of any carry-over effect, for each transition, the signal-to-noise ratio (S/N) from negative samples had to be lower than 3.

### 2.4. Statistical analysis

Larvae and pupae length in different treatments as well as pupation and eclosion rate were analyzed by one-way ANOVA and *t*-test. The level of significance was set at $P < 0.05$. Calculations were performed using JMP 5.1 statistical package.

# 3. Results

## 3.1. Method validation

The following parameters were obtained: coefficient of linearity ($R^2 > 0.99$), detection limit (LOD = 0.10 ng/mg), quantification limit (LOQ = 0.33 ng/mg), extraction recovery (ER%), and repeatability (CV%). A summary of validation parameters are reported in Table 1. Specificity was satisfactory and no carry over effects were observed.

Table 1.
Parameters calculated for the method validation.

| Parameter | Value |
|---|---|
| LOD | 0.10 ng/mg |
| LOQ | 0.33 ng/mg |
| Extraction recovery low concentration | 78.77% |
| Extraction recovery high concentration | 88.91% |
| CV% low concentration | 19.05% |
| CV% high concentration | 5.82% |

## 3.2. MA concentration

GC–MS analyses confirmed that the MA artificially added to the food substrate was present in the different developmental stages of *C. vomitoria* as well as the SP, but not in adult samples ( Table 2). The peak of MA concentration was found in the post-feeding instar of *C. vomitoria* arising from the 5 ng/mg treatment, whereas in the treatment with 10 ng/mg MA the peak was found only in the L3 instar ( Table 2).

Table 2.
MA quantification (ng/mg ± S.E.) in samples of food substrate and *C. vomitoria* life instars (L2 = second instar, L3 = third instar, PF = post-feeding instar, *P* = pupa instar, SP = spent pupa, A = adult instar) and SP through GC–MS analysis. Measurements followed by "a" are significantly different from the control ($P < 0.05$); measurements followed by "b" are significantly different between treatments ($P < 0.05$). Quantification was calculated using 3 replicates.

| Sample | Quantification | | |
|---|---|---|---|
| | Control | MA | |
| | 0 ng/mg | 5 ng/mg | 10 ng/mg |
| Liver substrate | <0.10 | 5.01 ± 0.04 a, b | 10.07 ± 0.12 a, b |
| L2 | <0.10 | 0.45 ± 0.01 a, b | 0.67 ± 0.02 a, b |
| L3 | <0.10 | 0.78 ± 0.01 a, b | 6.70 ± 0.06 a, b |
| PF | <0.10 | 0.88 ± 0.02 a, b | 0.41 ± 0.01 a, b |
| P | <0.10 | 0.40 ± 0.01 a, b | 0.35 ± 0.01 a, b |
| SP | <0.10 | 0.39 ± 0.01 a, b | 0.71 ± 0.01 a, b |
| A | <0.10 | <0.10 | <0.10 |

### 3.3. Growth rates and survival

The duration of larval development was similar in both treatments until the L3 instar, but on reaching the PF instar it was observed that the presence of the MA was significant for fly pupation and eclosion time as well as for survival.

The time from oviposition to pupation and from oviposition to eclosion was significantly shorter for control larvae than for larvae feeding on liver containing MA (Table 3). Treatment groups time to pupation and eclosion were not significantly different (Table 3).

Table 3.

Time (hour mean ± S.E.) from ovoposition to pupation and to eclosion of *C. vomitoria* larvae, which were exposed to either liver containing different amount of MA, or to the control liver containing no drug. Measurements followed by "a" are significantly different from the control ($P < 0.05$). The table shows also the number of larvae death prior to pupation, the number of not emerged adults and the number of survivals.

| | Control | MA | |
|---|---|---|---|
| | 0 ng/mg | 5 ng/mg | 10 ng/mg |
| Time (*h*) from oviposition to pupation | *N* = 100 | *N* = 100 | *N* = 100 |
| | 165.54 ± 1.09 | 170.79 ± 1.23 a | 170.56 ± 1.39 a |
| Larvae dead prior to pupation | 0 | 1 | 4 |
| Total pupae | 100 | 99 | 96 |
| Time (*h*) from oviposition to eclosion | *N* = 100 | *N* = 99 | *N* = 96 |
| | 470.62 ± 1.73 | 482.05 ± 1.73 a | 480.00 ± 1.52 a |
| Not emerged adults | 21 | 55 | 56 |
| Survival | 79 | 44 | 40 |

The pupation and eclosion trends are depicted in Fig. 2 and Fig. 3. The pupation trend is similar between the control and treatments, however, the time to eclosion for insects that fed on liver containing MA is significantly longer than the control.

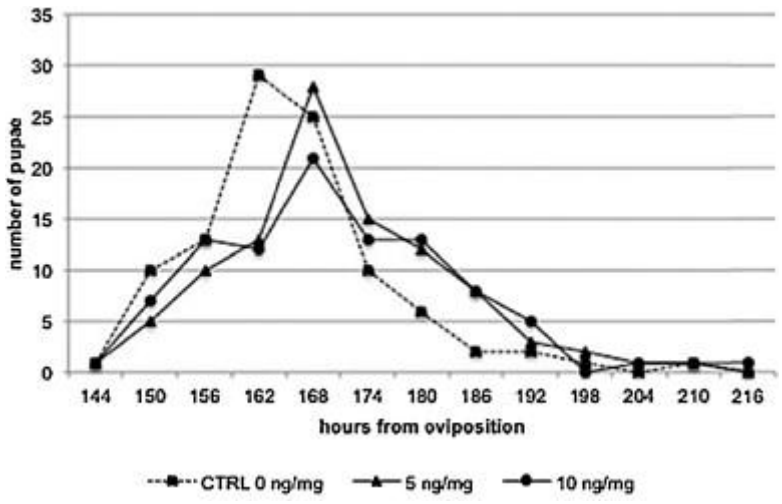


Fig. 2.

Time to pupation: the total number of *C. vomitoria* pupae observed every 6 h for the 3 treatments.

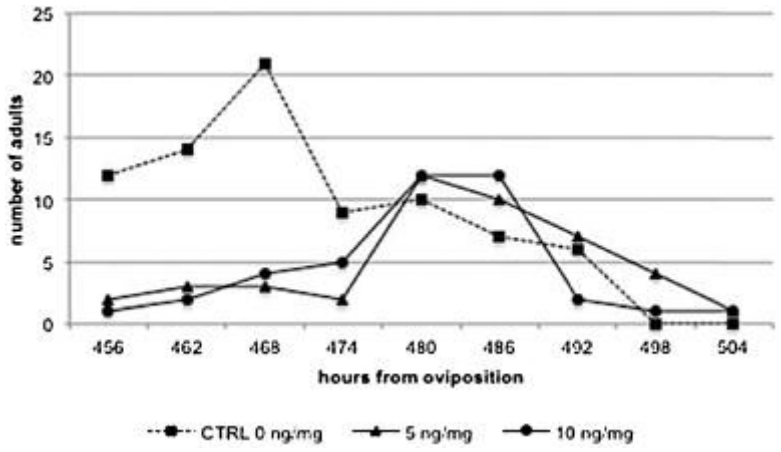


Fig. 3.
Time to eclosion: the total number of *C. vomitoria* adults emerging every 6 h for the 3 treatments.

During the PF instar only a small number of larvae died prior to pupation (0/100 in control; 1/100 in 5 ng/mg treatment; 4/100 in 10 ng/mg treatment) (Table 3). After the period of methamorphosis, less adult flies eclosed in the two treatment groups (44/99 in 5 ng/mg treatment; 40/96 in 10 ng/mg treatment) compared with the control (79/100) (Table 3). The results suggest that a diet with liver containing MA can affect *C. vomitoria* survival, especially during methamorphosis.

## 3.4. Larval and pupal length

Significant differences were observed between the length of larvae and pupae in control versus the treatment groups (Table 4). In general there was a significant increase in the average length of the larvae fed on liver containing MA compared with the control (Table 4).

Table 4.
*C. vomitoria* larvae and pupae means length (mm ± S.E.) related to time of exposure (*h*) and instar of life (L2 = second instar, L3 = third instar, PF = post-feeding instar, *P* = pupa instar). Measurements followed by "a" are significantly different from the control ($P < 0.05$); measurements followed by "b" are significantly different between treatments ($P < 0.05$). For each time of exposure (h), $N = 30$ individuals for each treatment were measured.

| Time of exposure (*h*) | Instar | CTRL | MA | |
|---|---|---|---|---|
| | | 0 ng/mg | 5 ng/mg | 10 ng/mg |
| 72 | L2 | 7.45 ± 0.39 | 8.69 ± 0.22 a | 8.65 ± 0.25 a |
| 96 | L3 | 11.78 ± 0.81 | 15.43 ± 0.36 a | 15.80 ± 1.38 a |
| 120 | L3 | 18.63 ± 0.33 | 19.44 ± 0.17 a | 20.67 ± 0.22 a, b |
| 168 | PF | 17.1 ± 0.21 | 17.71 ± 0.28 b | 18.70 ± 1.39 a, b |
| 216 | *P* | 9.17 ± 0.20 | 10.61 ± 0.13 a | 10.51 ± 0.31 a |

Significant differences occurred in every instar between control and 10 ng/mg treatment, while in 5 ng/mg treatment only the length of post-feeding larvae was not significantly different from control (Table 4). Significant differences were also observed in the length of L3 instar and PF larvae that fed on liver containing

10 ng/mg MA compared with the 5 ng/mg treatment. Therefore, these results suggest that, as the MA dosage increases in the food substrate, the average length of *C. vomitoria* immatures also increases.

## 4. Discussion

The present work as specified in the introduction demonstrated that GC–MS analyses can detect the presence of MA in *C. vomitoria* larvae and puparia reared on spiked liver containing MA. Notwithstanding that the GC–MS analyses of *C. vomitoria* immatures and SP were positive, a significant effect of the MA was an increase in the development rate of this fly species from egg to pupation and to adult.

A previous study by Goff et al. [14] involving toxicological analyses of MA using RIA (radioimmunoassay) technique in *Parasarcophagaruficornis* (Fabricius) (Diptera: Sarcophagidae) demonstrated that larvae reared at 26 °C on tissues from rabbits showed only a weak and uniform positive result on treated colonies. GC–MS technique however is far more sensitive, with positive results for immature stages and SP of *C. vomitoria* feeding on liver containing 5 ng/mg and 10 ng/mg MA at 23 °C. The inherent variability and unpredictability of the biological phenomena involved in the present study, including MA exposure and its biological incorporation, the relationship between MA toxicity and dosage, and blowfly physiology, may explain why relatively small differences in the MA concentrations were found in the instars exposed to 5 ng/mg and 10 ng/mg MA liver concentrations. This confirms that, for toxicological effects, the exposure concentration is crucial, but this is rarely correlated with the drug incorporation rate. This may also be a consequence of the environmental factors which can influence the final drug concentration detected in the insect tissue. It has been demonstrated that drug absorption, the excretion of drugs and drug stability is dependent on the ambient temperature [28] and [29].

Furthermore, the dosages of MA administered by Goff et al. [14] to live rabbits of 4.2–4.6 kg were calculated to represent sublethal (3026 ng/mL), median lethal (7594 ng/mL) and x2 median lethal (55698 ng/mL) dosages of the drug by weight (37.5 mg, 71.4 mg, 142.9 mg). These are much higher doses than those used for this study which were matched to human lethal dosages of 5 ng/mg and 10 ng/mg as detailed by Karch et al. [22], Zhu et al. [23] and Chaturvedi et al. [24].

In the current study, a comparison was also made on *C. vomitoria* SP, which were also shown to contain MA. Studies using different drugs [30], [31], [32] and [33] confirmed that larvae excrete the drug during the post-feeding instar. It appears that while the drug is excreted by larval organs, a quantity of drug is included into the cuticle of the puparium. Since puparial cases can be recovered several years and sometimes centuries after death, such samples maybe of important toxicological interest [12].

Negative results in *C. vomitoria* adults were not surprising, because it is known that upon emergence as an adult, the flies rapidly eliminate the drug [34]. Nuorteva and Nuorteva [35] observed that Calliphorids reared on fish tissues containing mercury excrete the metal into the meconium of the hindgut during the process of pupation. They also found that, two days following emergence, adult flies contained only 50% of the mercuric concentrations detected in the developing larvae. In the current study, *C. vomitoria* adults were sacrificed and analysed a week after the emergence and no metamhamphetamine was detected.

Previous studies like this current study seem to have some unexplained anomalies concerning the concentrations of MA used and the resultant effects on the development of different species of blowflies. *P.*

*ruficornis* larvae showed a similar rate of development between treatments from 0 h to 24 h (L1 instar) but the development rate from 30 h to 60 h, was significantly different in the median lethal and x2 median lethal treatments compared with sublethal and control treatments [14]. However, at 60 h only the x2 median lethal treatment was significantly different to all other treatments [14]. The emigration from the food source was firstly observed in median and x2 median lethal dosages treatments (84 h), followed by sublethal treatment (90 h) and the control treatment (96 h) [14]. Although larval development to this stage was similar in this study, the emigration of the sublethal treatments is incongruent with the emigration time of the control larvae[14].

Furthermore, in the Goff et al. study [14] the overall time of development from larva to adult was found to be greater for the control treatment (476 h) than for larvae feeding on liver containing MA (sublethal 426 h, median lethal 409 h, x2 median lethal 428 h) but again no explanation of why the highest and lowest concentrations were significantly different to the median concentration. Larval development is also accelerated by the presence of heroine, cocaine, morphine and diazepam in the food source [36], [37],[38] and [39], whereas no differences were found in the total developmental time of *P. ruficornis* larvae reared on substrates containing different concentrations of 3,4-methylenedioxymethamphetamine (MDMA) [15].

In the current study no differences in the rate of *C. vomitoria* development were observed during the feeding period (L1, L2, L3 instar) ( Table 3). Although the overall time of development for *C. vomitoria* from egg to eclosion was found to be greater for larvae that fed on liver containing MA than the control treatment ( Table 3), there is an anomaly in the MA concentration of the liver (10 ng/mg) resulting in an elevated concentration of MA in the L3 larva (6.7 ng/mg) ( Table 2). There seems to be no explanation as to why this may have occurred other than the toxicological effects experienced by the insects will depend on their exposure levels to the drug. In other fly species, the duration of the post-feeding instar was also prolonged by the presence of amitriptyline [5], but it was shorter for larvae fed on tissue containing phencyclidine [5] and [37].

In general, the present work shows that *C. vomitoria* larvae and pupae reared on liver containing MA were significantly larger in size than the control groups ( Table 4). In *C. vicina* "very large larvae" were recorded but no length or developmental data were provided when colonies of this species were fed on barbiturates (phenobarbitone and amylobarbitone) [30]. *P. ruficornis* larvae reared on control substrates and those reared on tissues from rabbits administered with 67 mg of MDMA were longer in length over a shorter time period than the larvae reared on tissues from rabbits administered with 11 mg and 22.5 mg of MDMA [15].

Likewise, larvae feeding on tissues containing higher concentrations of cocaine were longer in length between 36 h and 72 h than the control and treatment groups containing low doses of cocaine. However, at 78 h larvae of all colonies approached the maximum size and the differences between treatments were no longer significant after 84 h [36]. This was supported by Lord [40] who found *Cynomia cadaverina* Desv. and *Phaenicia sericata* (Meigen) larvae on a victim with a history of cocaine abuse. Measurements of preserved samples showed that a larva in the nasopharyngeal area was much longer (17.7 mm, no details about the species) than any other larvae (6–9 mm and smaller) found on the corpse.

In regards to the mortality of *P. ruficornis* Goff et al. [14] found an inverse relationship between pupal mortality and the dosage of MA, whereas in this research the highest pupal mortality of *C. vomitoria* was recorded for the 10 ng/mg colony. Interestingly both these studies demonstrated that a diet of liver containing MA resulted in approximately 60% larval mortality.

Other drugs such as MDMA, amitryptiline and barbiturates (thiopentone, phenobarbitone, barbitone and brallobarbitone) cause high mortality [15], [30] and [41]. On the contrary, substrates containing cocaine, hydrocortisone and sodium methohexidal did not affect the larval survival of Dipterans [36] and [42]. As a consequence the overall perspective on drug metabolism in insects is yet to be elucidated [7].

## 5. Conclusion

GC–MS is a useful technique for detecting low concentrations of MA in immature fly samples. The results presented in this study demonstrate that the developmental rate and the length of *C. vomitoria* larvae and pupae feeding on liver containing MA can be significantly affected by the presence of the drug and that some of these effects are dose-dependent.

This research highlights how the same drug can produce different effects on the development of different fly species. Therefore, and as iterated by Goff et al. [14] all care must be taken when interpreting fly developmental rates until a data base is established incorporating the effects of these drugs present in host substrates and at different temperatures.

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