# UNIVERSIDAD REGIONAL AMAZÓNICA IKIAM

## FACULTAD CIENCIAS DE LA VIDA

### CARRERA DE INGENIERÍA EN BIOTECNOLOGÍA

# ENTOMOTOXICOLOGY IN *PECKIA INTERMUTANS* (DIPTERA: SARCOPHAGIDAE) AND ITS POTENTIAL AS A MARKER FOR METHANOL DETERMINATION IN BIOLOGICAL SAMPLES

Proyecto de investigación previo a la obtención del título de:

**INGENIERA EN BIOTECNOLOGÍA**

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**TABLA DE CONTENIDO**

## TABLE INDEX

## FIGURE INDEX

**RESUMEN**

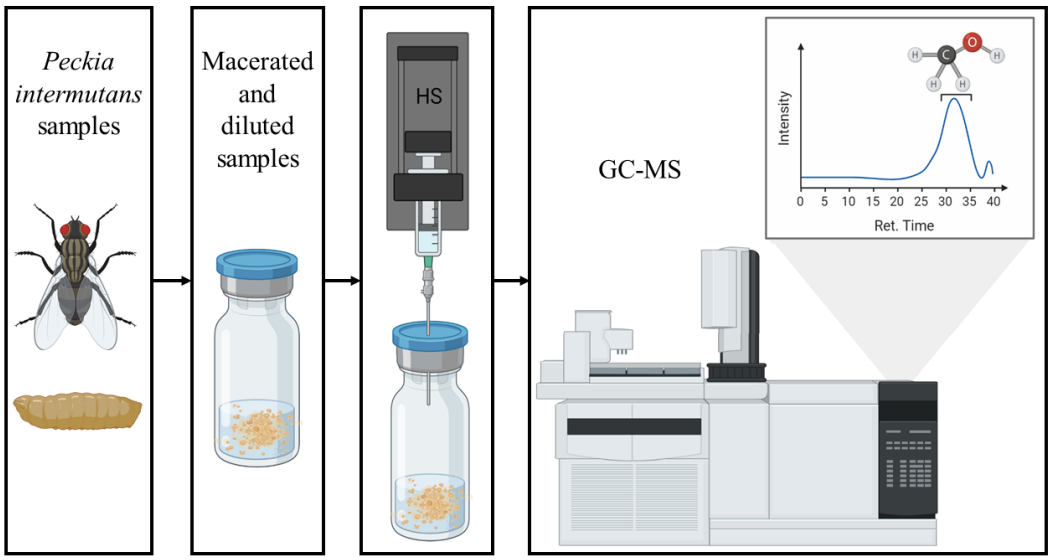
La entomotoxicología forense emplea métodos analíticos e instrumentación en insectos necrófagos que se alimentan del tejido en descomposición para detectar drogas y otros xenobióticos. Empleándose principalmente en determinar la causa de muerte y estimar el intervalo *post-mortem*. Aunque los estudios entomotoxicológicos se centran en la detección de drogas, poca información se refiere al efecto del alcohol en las moscas de la carne. El metanol, a pesar de encontrarse en niveles bajo en las bebidas alcohólicas disponibles comercialmente, su uso en bebidas adulteradas lo convierten en un potente veneno. Demostramos el potencial de *Peckia intermutans* (Diptera: Sarcophagidae) como marcador para la determinación de metanol utilizando cromatografía de gases-espectrometría de masas. Además, se estudiaron los efectos del metanol sobre el tiempo de desarrollo, la supervivencia y la morfología de las moscas de la carne. Las larvas se criaron en sustratos de hígado enriquecidos con metanol (300, 500 y 100 mg/kg) según las concentraciones encontradas en tejidos humanos implicados en la intoxicación por metanol. Los resultados demostraron que (i) HS-GC-MS detecta metanol en todos los estadios de *Peckia intermutans*; (ii) el metanol modifica el tiempo de desarrollo de las moscas de la carne; (iii) la supervivencia fue mayor a medida que aumentaba la concentración de metanol exógeno; y (iv) los cambios morfológicos fueron más evidentes en el peso. Esta investigación proporciona una valiosa adición al conocimiento actual de entomotoxicología de Ecuador. *Peckia intermutans* mostró potencial como marcador toxicológico de metanol en muestras biológicas. A pesar de esto, los cambios en la morfología y el ciclo de vida de las moscas de la carne deben investigarse y considerarse más a fondo al obtener resultados forenses. Estas variaciones pueden causar estimaciones erróneas del intervalo *post-mortem*.

*Palabras clave*: Entomotoxicología, metanol, HS-MS-GC, *Peckia intermutans*, morfología.

## ABSTRACT

Forensic entomotoxicology employs analytical methods and instrumentation on necrophagous insects feeding on decomposing tissue to detect drugs and other xenobiotics. Being used mainly to determine the cause of death and estimate the *post-mortem* interval. Although entomotoxicological studies focus on drug detection, there is little information on the alcohol effects on flesh flies. Methanol, although found at low levels in commercially available alcoholic beverages, is used in adulterated beverages making it a potent poison. We demonstrated the potential of *Peckia intermutans* (Diptera: Sarcophagidae) as a marker for methanol detection using Gas chromatography–mass spectrometry. For this, we studied the effects of methanol on developmental time, survival and morphology of flesh flies were studied. Larvae were reared on liver substrates homogeneously spiked with measured amounts of methanol (300, 500 and 100 mg/kg) based on the concentrations found in human tissues involved in methanol poisoning. Results demonstrated that (i) the HS-GC-MS method detects methanol in all instars of *Peckia intermutans*; (ii) methanol influence the developmental time of flesh flies; (iii) survival was higher as the exogenous methanol concentration increased; and (iv) morphological changes were more evident in weight than in length. This research provides a valuable addition to current knowledge in Ecuador entomotoxicology investigations. Peckia intermutans showed potential as a marker for methanol determination in biological samples, which opens the way for future research in insects as toxicological evidence. Changes in the morphology and life cycle of the flesh flies need to be further investigated and considered when making forensic results. These variations can cause erroneous estimates of the *post-mortem* interval.


### Graphical Abstract




*Keywords:* Entomotoxicology, HS-MS-GC, methanol, morphology, *Peckia intermutans.*

## INTRODUCTION

Insects are the world's most diverse group of animals, inhabiting terrestrial and marine ecosystems [1]. Around 1.1 million species have been described, and up to 10 million may exist [1,2]. The biochemical, and molecular diversity gives them a series of unique characteristics [1]. Especially, the chitin covering the insect's body keeps chemical compounds stable and unchanged for longer [3]. In certain environments, insects determine the presence of xenobiotics (drugs or other toxic substances). Serving as environmental contamination evidence to study the effect of specific xenobiotics on target insect species [4].

Forensic entomology, which handles insects' morphology, life cycle, and behavior study, is very useful in forensic medicine [5]. Arthropods of Diptera (flies, mosquitoes, among others) and Coleoptera (beetles) orders perceive the smell of gasses and fluids emanating from decomposing bodies, becoming the first visitors at a crime scene [6]. Insects that use animal carcasses as food sources acquired great forensic importance [4-6]. Insects' forensic potential is to estimate post-mortem interval (PMI), determine toxins, and the site of death [5–7].

*Ante-mortem* exposure to xenobiotics may be the sole or contributing cause of death. When scavengers feed on contaminated carcasses, xenobiotics in the tissue get transferred to their metabolic system [8,9]. The use of insects as a toxicological sample develops a new field in forensic entomology known as entomotoxicology. Entomotoxicology is a compound of ancient Greek words: "*entomon"* meaning insect, *"toxikos"* meaning poisonous, and *"logos"* meaning matter [4]. In 1991, Derrick Pounder coined the term forensic entomotoxicology, but, da Silva et al. conceive the most appropriate terminology [9,10]. Forensic entomotoxicology is the use of insect specimens as an indirect source of toxicological evidence in the absence of direct matrices, such as blood, urine, soil, or water, to determine the presence of a xenobiotic in the environment [10].

A key element in unnatural deaths is establishing the cause of death. Deaths from drug abuse, pesticides, and other toxins are common worldwide. Methanol or methyl alcohol is an alcohol that is found in all distilled alcoholic beverages [11]. Toxicologically, in

commercially available alcoholic drinks, methanol levels are rarely relevant since they conform to the permissible limits of alcoholic degree [12]. In 2018, the WHO estimated that over 23,000 deaths globally were attributed to methanol poisoning [11]. Most were related to vehicular accidents, drownings, suicides, and homicides. In 2021, the Ecuadorian Ministry of Public Health declared methyl alcohol consumption as the fourth cause of poisoning [12]. Especially, methyl alcohol abuse predominates in the Amazon region [13,14].

The small molecular size of methanol and its water-soluble nature cause its rapid absorption in tissues and body fluids. Methanol concentrates in biological compartments with high water content: urine, blood, and vitreous humor. These biological samples are the most used for alcohol *post-mortem* analysis [15]. When cases of late recovery occur, establishing the cause of death can be complicated. Depending on the body's condition, conventional toxicological samples are easily degraded [16].

Entomotoxicology analyses use immunoassay techniques, gas and liquid chromatography (GC, LC), and mass spectrometry (MS) [9,15]. In methanol analyses, these techniques have limitations. The low molecular weight and boiling point (65°C) cause the retention time to increase and overlap with other volatile organic compounds, difficult qualitative and quantitative analysis [16,17].

Among the necrophagous insects of forensic relevance, flesh flies (Diptera: Sarcophagidae) are one of the most used Diptera worldwide [18-21]. This family comprises about 3100 species, some taxa having a cosmopolitan distribution [22]. Despite being one necrophagous found on corpses, its use is limited. The lack of taxonomic information and studies on the flesh fly's development hinders its forensic entomological use [23].

*Peckia* is a New World genus of large-bodied flesh flies that comprises 67 species, found in urban and natural areas [18,24]. In particular, *Peckia (Pattonella) intermutans* (Walker) is distributed from Mexico to Paraguay, with records in Ecuador (Napo, Orellana, and Imbabura provinces) [18]. This species has before been reported to be associated with human corpses and forensic biomodels such as pigs [25,26].

In Ecuador, forensic entomology studies are scarce. Ecuadorian Amazon is the most biodiverse region, but, entomologist records are few [27]. The cadaveric entomofauna

identification and investigations focus on the Sierra region [27,28]. Besides, in the country, no records of toxins and toxic substances analysis in insects.

This research represents the first attempt to detect methanol in all instars of *P. intermutans*. Despite the importance of *Peckia* species in forensic entomology, this study aimed to determine the toxicological potential of *P. intermutans*. The present research provides an analytical method using GC-MS to detect methanol in *P. intermutans*. Furthermore, methanol effects on *P. intermutans* developmental time, survival, and morphology (length and weight) were determined.

## MATERIALS AND METHODS

### Sample selection and identification

Adult wild flies were collected according to MAATE permission (MAATE-ARSFC-2023-3006) using bottle traps containing beef offal and identified using dichotomous keys [29–31]. Six traps were hung on tree branches, in the surroundings of Ikiam University, Tena, Ecuador (0°57′01″S, 77°51′46″O). Adults identified as *Peckia (Pattonella) intermutans* (Walker) were placed in 900 $cm^2$ containers and 15 days replenished to prevent inbreeding. *P. intermutans* species used in this experiment were harvested from a third generation.

### Establishment of the colony and rearing of *P. intermutans*

Twelve hatcheries were used to get the first generation (F1) of *P. intermutans.* Each contained 10 adult flies, and for 5 days adults were supplied with water and sugar ad libitum. On day 6, flies were provided with a small plastic tray containing 200 g of fresh beef liver on water-moistened paper, to allow females larviposit. Unlike other flies, Sarcophagidae eggs hatch in the uterus of the female. The larvae deposited by *P. intermutans* were removed using a fine paintbrush. Larvae were transferred to a plastic tray containing 3 cm of moist soil (substrate for the pupation) and beef liver.

When larvae emerged as adults, the first generation (F1) was collected. F1 individuals were kept in another container. Procedures described to get F1 were replicated for the

second (F2) and third (F3) generations. *P. intermutans* were reared at a temperature of 27 ± 2°C, a relative humidity of 73 ± 6% and the photoperiod (h) was 12:12 (L:D). Relative humidity and temperature were recorded with a portable thermo hygromete, three times a day.

**Preparation of foodstuff and rearing of flesh flies**

Four experimental samples were prepared. For each sample, 200 g of ground beef liver was used, and using a spatula was homogenized with methanol. Concentrations of 300, 500, and 1000 mg/kg of methanol, referred to as M1, M2, and M3, respectively, were added. Another liver substrate containing no methanol was used as a control (M0).

Each experimental sample (M0, M1, M2, and M3) was placed in airtight containers. For 24h at 4°C, samples were left to settle. After, livers were placed on water-moistened paper to avoid desiccation, inside a plastic tray containing 3 cm of moist soil. Each tray was placed inside cages covered with a fine cloth. The larvae obtained from F3 were removed with a paintbrush and placed in samples M0, M1, M2, and M3. In each substrate, 120 larvae were added.

Liver was used because is the typical medium for forensic entomology experiments [29, 30]. The amount of methanol was based on the largest permissible limits of alcoholic degree established by NTE INEN 362:2014 [12]. The lethal dose is estimated at 300-1000 mg/kg [32,33].

**Sample collection**

Two sets of samples, from each treatment at each life stage were collected. One consisting of 10 individuals and another set of 1 g. Instars are second (L2), third (L3), post-feeding (PF), pupal (P), and adults (A). Empty puparium (EP) was also collected.

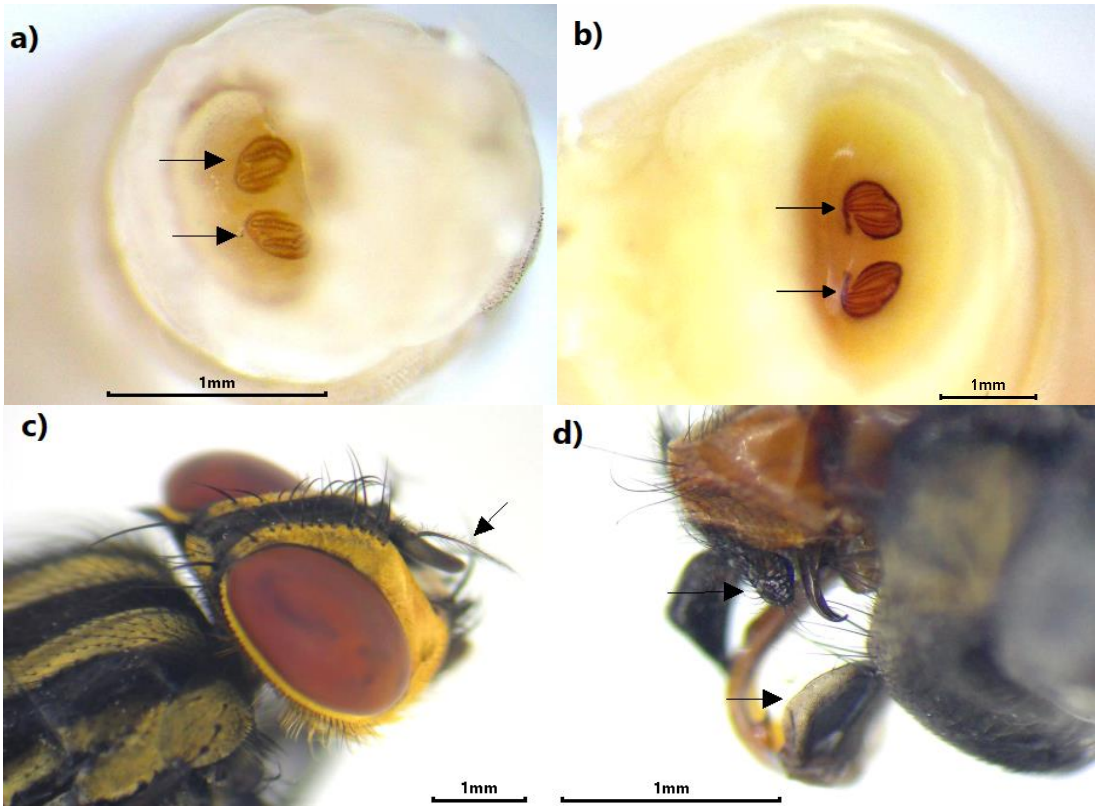
The samples of 10 individuals of each treatment were used for morphological analyses. The larvae (L2, L3, PF) and pupa from each time point were sacrificed in hot water (>80 °C) for 30 s, then stored in 70% ethanol at 4 °C [34]. The adults were sacrificed 2 days after their emergence, refrigerated, and once dead were stored. After preservation, a stereomicroscope (AmScope SM-1TSL-64S-V331) was used to determine the stage of *P. intermutans* based on taxonomic descriptions (Fig. 1) [18,35]. Besides, the length and

weight of every instar were recorded. To measure the length, a stereomicroscope, a ruler, and the ToupView program were used. An analytical balance was used for weight.

The 1 g samples were used for toxicological analysis by HS-GC-MS. After cleaning with deionized water to drop external contamination, samples were stored at -80 °C [36]. The adults were sacrificed 2 days after their emergence.

The analytical method validation was performed using 1 g of control Empty puparia. Empty puparia was chosen because of their high chitin content. EP's long lifetime in the environment provides reliable data for entomotoxicological analyses [37,38].

To consider the methanol effects on the life cycle and survival *of P. intermutans,* in separate boxes place 100 individuals in PF instar. The time to pupation and to hatching, the total number of individuals pupating and emerging adults were recorded.



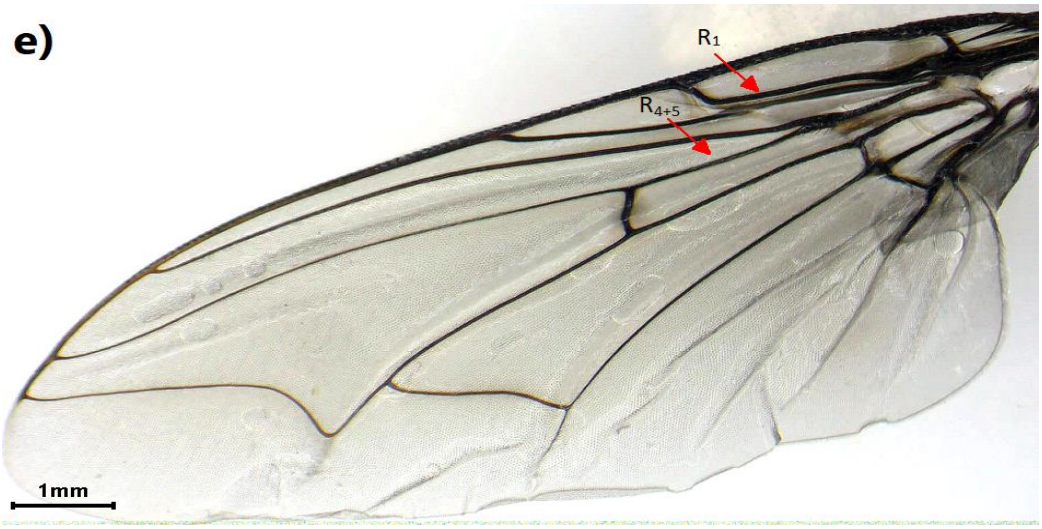



**Figure 1. Stage of *Peckia intermutans*.** (a) Second instar; (b) third instar; (c-e) adult instar, arista with long plumosity, surstylus round, and vein $R_1$ and $R_{4+5}$ bare dorsally, respectively.
**Realizado por:** Rivera, Celia, 2023.

**Toxicological Analysis**

*Chemicals and reagents* - Methanol (HPLC grade) and sodium chloride 99.9% (NaCl) were purchased by J.T Baker (USA). Deionized water for preparation of all the solution was purified (18.2 μΩ·cm) using Smart2 Pure of Thermo Scientific.

*Sample preparation for HS-GC-MS analysis* - 1 g of *P. intermutans* samples (L2, L3, PF, P, EP, and A) were placed separately in 15 ml falcon tubes, and 1 ml of ice-cold 0.9% NaCl was added as part of the preliminary wash [36]. The tubes with samples were placed in a vortex for 2 min and the solvent was discarded. Empty puparia and adults were dried at room temperature under nitrogen. Frozen samples (liquid $N_2$) were crushed using a mortar and placed in a 20 ml glass vial (Vial-20-ND18-CG-100). Pupa, empty puparia, and adults were diluted 1:4 with deionized water [39].

The methanol detection method was validated using 1 g of control *P. intermutans*. Empty puparium were used because of their high chitin content. EP can be found at the scene for a long time after emergence and may represent the only reliable sample for toxicological analysis. For the toxicological analysis, the whole specimen was used.

*Headspace conditions* - The automated headspace autosampler AOC-6000 (Switzerland) was coupled with the GC-MS for sample preparation and introduction into the GC. The samples were automatically shaken and incubated for 5 min at 80°C. Then,

300 µL headspace aliquot was sampled for the analysis with a fill speed of 35 mL/min. In order to obtain homogenous sampling, a fill stroke count of 3 was used. The sample was injected into the GC at an injection speed of 25 mL/min. After each analysis, the syringe was flushed with nitrogen for 5 min. The run cycle time was set at 15 min.

*GC-MS analysis* - Analytical determinations for the detection of methanol were performed using a gas chromatograph coupled with a mass spectrometer Shimadzu GCMS-QP2020NX fitted with a split/splitless injector and equipped with a capillary column Rtx-50 30 m x 0.25 mm i.d. x 0.25 µm df. The oven temperature was programmed as follows: initial column temperature was 45°C for 5 min, then increased by 10°C/min to 80°C for 1 min for a total run time of 9.5 min. The injection port and transfer line were kept at T = 200°C using helium ultrapure as carrier gas at an initial flow of 1 mL/min. The analyses were performed operating in the electron impact ionization mode and full mass spectra were acquired.

The MS ionization source was set to 200°C and the mass scan range was m/z 20 to 80 Da. For the quantitative analysis, the mass analyzer was operated in the selected ion monitoring (SIM) mode using the ion m/z 31 characteristic of methanol. The background subtracted mass spectrum for methanol (using EI in full scan mode) is given in Figure 2. The peak identification was performed using NIST 2020 (Wiley Registry 12th Edition/NIST 20). All the analyses were performed in triplicate.

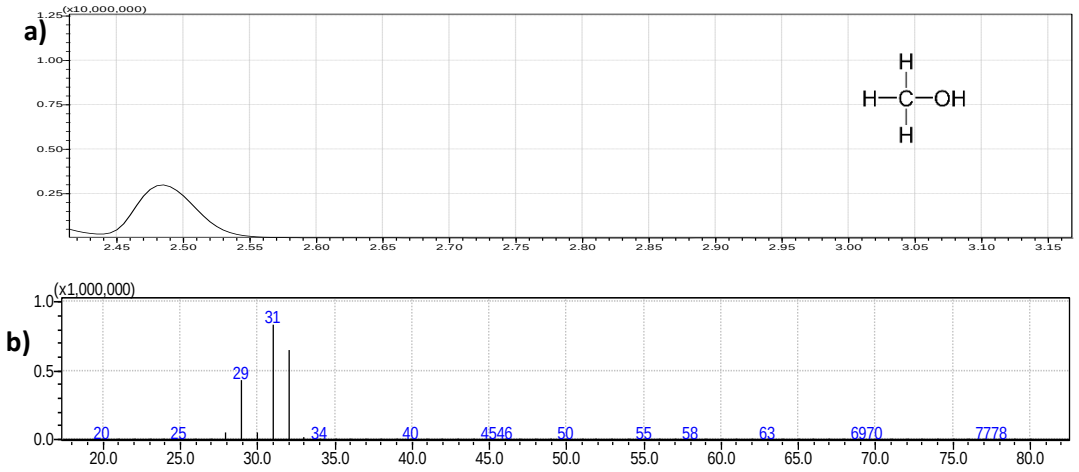



**Figure 2. Background subtracted mass spectrum of methanol.** (a) Chromatogram of methanol obtained with EI ionization; (b) full scan mass spectra of methanol, m/z for methanol is 31.
**Realizado por:** Rivera, Celia, 2023.

*Method validation* - The following parameters were obtained: coefficient of linearity ($R^2$), specificity, limit of detection (LOD), limit of quantification (LOQ), extraction recovery (ER%), repeatability and carryover (CV%). The validation included the quantitative determination of methanol in larvae, P, EP and A.

*Specificity* - A total of ten samples of the control EP were used to ascertain specificity of the method, of which five were spiked with 126 µL of methanol.

*Linearity* - Calibration models were determined from five-point calibration curves with calibrators prepared in triplicate in homogenized EP. The linear calibration model was checked by analyzing control EP samples (1 g) spiked with methanol at concentrations of 300, 400, 500, 700 and 100 mg/kg. The linear calibration parameters were calculated by least-squares regression, and the squared correlation coefficient ($R^2$) was used to estimate linearity.

*LOD and LOQ* - The LOD and LOQ were administratively set at and verified with the lowest calibrator prepared in triplicate in three lots of EP homogenates.

*Extraction recovery* - ER% was evaluated using three methanol concentrations in control EP: 50, 100 and 200 mg/mg. 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).

*Repeatability (intra-assay precision)* - Repeatability was calculated as the percent coefficient of variance (CV%), after spiking ten samples of control EP with two methanol concentrations: 350 and 900 mg/kg. Repeatability is considered acceptable when the CV% is lower than 25% at low analyte concentrations and lower than 15% at high concentration.

*Carry Over* - Carry-over effect was evaluated by injecting an alternate sequence of five negative EP samples and ten blank EP samples spiked with methanol at 1000 mg/kg concentration to ensure the absence of any carry-over effect.

The sequence was: blank, methanol, blank, methanol, methanol, blank, methanol, methanol, methanol, blank, methanol, methanol, methanol, methanol, blank.

### Statistical Analysis

Larvae and pupae length and weight in different treatments as well as eclosion rates were analyzed by one-way ANOVA and Tukey test. The level of statistical significance was set at $p < 0.05$. Calculations were using Microsoft Excel and IBM SPSS Statistics 29 software package.

## RESULTS

### Method validation

The following parameters were obtained for methanol: coefficient of linearity ($R^2$), detection limit (LOD), quantification limit (LOQ), ER%, and repeatability (CV%).

A summary of validation parameters is reported in Table 1. Specificity was satisfactory and no carry over effects were observed.

**Table 1.** Validation parameters calculated for methanol using a HS-GC-MS.

| Parameter | Value |
|---|---|
| | **Methanol** |
| Correlation coefficient, $R^2$ | 0.99 |
| LOD (mg/kg) | 50 |
| LOQ (mg/kg) | 200 |
| Extraction recovery low concentration (%) | 97.53 |
| Extraction recovery high concentration (%) | 96.91 |
| CV % low concentration | 14.70 |
| CV % high concentration | 10.57 |

LOD: detection limit; LOQ: limit of quantification.
**Realizado por:** Rivera, Celia, 2023.

### Methanol concentration

GC-MS analysis shows that methanol was not quantifiable in the PF stage of the M1 and M2 treatments. While for M3, the amount detected was lower than the LOD (Table 2).

Methanol could be quantified in the other stages of *P. intermutans* (L2, L3, P and A). In treatments M1, M2, and M3, the quantification is higher when methanol concentration increases. In M0, methanol was present in the different *P. intermutans* development instars.

**Table 2.** Methanol quantification (mg/kg ± SE) in *P. intermutans* (L2 = second instar, L3 = third instar, PF = post-feeding instar, P = pupa instar, EP = empty puparia, A = adult instar) by HS-GC-MS analysis.

| Life instar | Quantification | | | |
|---|---|---|---|---|
| | **M0** | **M1** | **M2** | **M3** |
| | **0 mg/kg** | **300 mg/kg** | **500 mg/kg** | **1000 mg/kg** |
| L2 | 122.02 ± 0.95 | 1951.81 ± 130.40 (M0) | 835.00 ± 363.22 | 908.11 ± 606.76 |
| L3 | 6325.06 ± 91.31 (M1, M2, M3) | 1150.98 ± 23.99 | 973.56 ± 35.44 | 1051.03 ± 18.38 |
| PF | 3050.68 ± 155.00 (M1, M2, M3) | NQ | NQ | < LOD |
| P | 99.49 ± 0.95 | 96.11 ± 13.02 | 115.40 ± 9.06 | 1667.05 ± 63.95 (M0, M1, M2) |
| EP | 111.91 ± 0.49 (M1) | 70.69 ± 1.29 | 380.95 ± 103. 69 | 1963.38 ± 54.55 (M0, M1, M2) |
| A | 103.20 ± 1.35 (M1) | 62.48 ± 6.66 | 181.79 ± 51.96 | 1707.51 ± 189.84 (M0, M1, M2) |

Quantification was calculated using three replicates. The groups indicated in brackets (i.e., M0, M1, M2, M3) are the ones whose results proved significantly different ($p < 0.05$) from the group indicated in the corresponding column. LOD = 50 ppm. NQ = not quantifiable.
**Realizado por:** Rivera, Celia, 2023.

**Developmental time and survival**

The presence of methanol significantly affected the survival and developmental time of *P. intermutans*. The time from oviposition to eclosion was significantly shorter for larvae feeding on the liver containing methanol than for the control (Table 3).

During the PF instar, 8/100 in 0 mg/kg treatment, 9/100 in 300 mg/kg treatment, 9/100 in 500 mg/kg treatment and 10/100 in 100 mg/kg treatment of larvae died prior to the pupation (Table 3; Fig. 3). After the period of metamorphosis, less adult flies eclosed in the M0 treatment (55/100) compared with the M3 treatment (88/100). The results suggest that a diet with liver containing methanol can affect *P. intermutan's* survival, especially during metamorphosis.

**Table 3.** Time (h mean ± S.E.) from oviposition to pupation and from oviposition to eclosion of *P. intermutans* larvae, which were exposed to either liver containing different amounts of methanol and control.

| Treatment | Concentration | | | |
|---|---|---|---|---|
| | **M0** | **M1** | **M2** | **M3** |
| | **0 mg/kg** | **300 mg/kg** | **500 mg/kg** | **1000 mg/kg** |
| Total larvae | 100 | 100 | 100 | 100 |
| Larvae third instar N= | 100 | 100 | 98 | 95 |
| Time (h) from larviposition to pupation | 264.96 ± 6.27 (M2, M3) | 250.08 ± 5.52 (M3) | 238.80 ± 4.85 (M3) | 216.00 ± 3.41 |
| Larvae died before pupation | 10 | 9 | 9 | 10 |
| Pupae | 90 | 91 | 92 | 90 |
| Time (h) from oviposition to eclosion | 546.00 ± 10.29 (M2, M3) | 513.12 ± 9.84 (M3) | 489.12 ± 9.84 (M3) | 427.68 ± 8.92 |
| Adult no emerged | 45 | 31 | 20 | 12 |
| Survival | 55% | 69% | 80% (M0) | 88% (M0, M1) |

The groups indicated in brackets (i.e., M0, M1, M2, M3) are the ones whose results proved significantly different ($p < 0.05$) from the group indicated in the corresponding column.
**Realizado por:** Rivera, Celia, 2023.

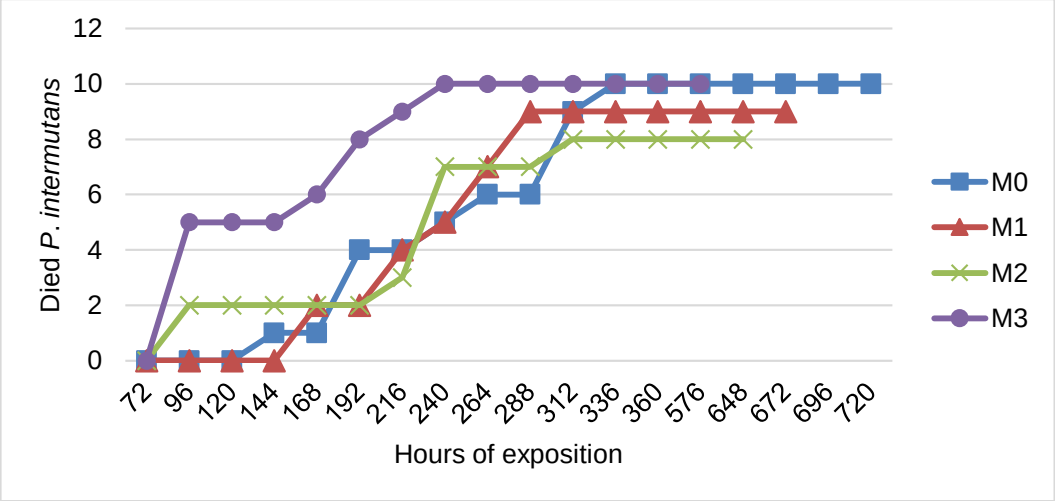



**Figure 3. Time to pupation.** Number of dead *P. intermutans* observed before.
**Realizado por:** Rivera, Celia, 2023.

Since adults were obtained from each treatment, they were again given sugar water for five days and beef liver on the sixth day. The adults of the M1 and M2 treatments died between days ten and fifteen after their emergence. M1 and M2 adults did not leave any descendants. M3 adults oviposit fifteen days after their emergence.

The larvae of the M3 adults and the adults that managed to hatch presented deformations (Fig. 4). Being swollen, the larvae presented thinner skin (Fig. 4a). Adults presented a tissue that covered the facial crest up to the fronto-clypeal suture and terminalia without segmentation (Fig. 4b and 4c).

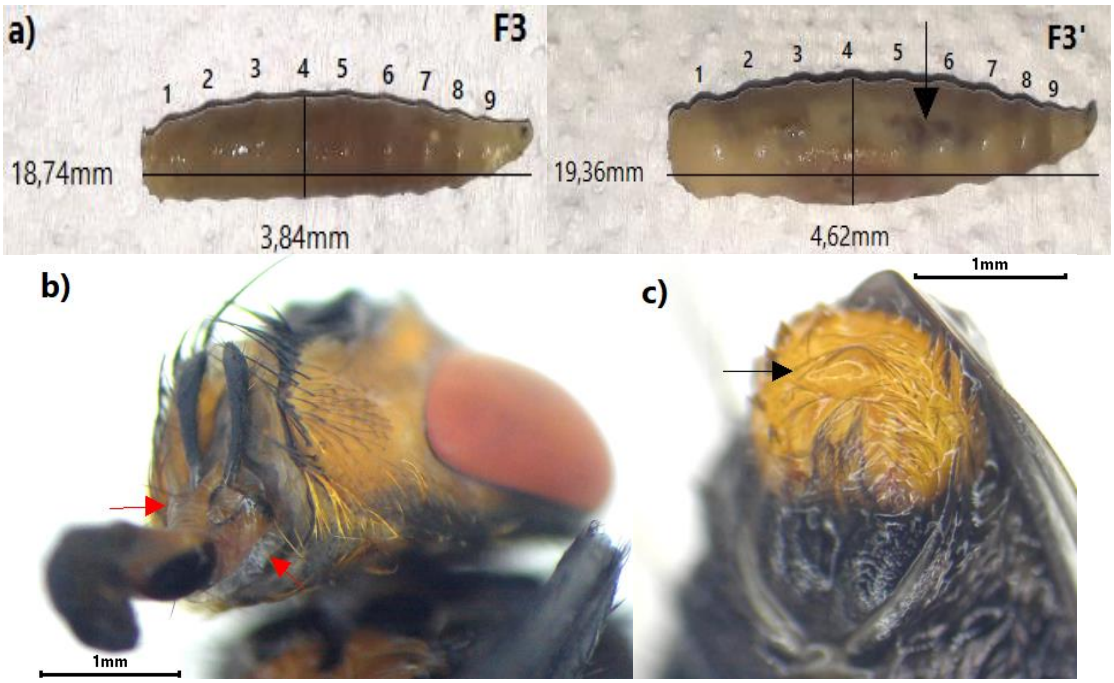



**Figure 4. Offspring of adults fed with M3.** (a) Third generation larvae vs F3 offspring larvae (F3'), (b-c) adult instars of *P. intermutans*.
**Realizado por:** Rivera, Celia, 2023.

### *Peckia intermutans* length and weight

The presence of methanol had a significant impact only on the length of pupae reared in the M3 treatment, which were notably larger than those in M0, M1, and M2 treatments. However, no significant differences were observed in the mean length of larvae or adults. The effects of methanol on the length of *P. intermutans* larvae, pupae, and adults are summarized in Table 4.

**Table 4.** Mean lengths (mm ± S.E.) of *P. intermutans* larvae, pupae, and adult related to the time of exposure (day) and instar of life (L2 = second instar, L3 = third instar, PF = post-feeding instar, P = pupa instar, A= adult instar).

| Days of exposure (instar) | Concentration | | | |
|---|---|---|---|---|
| | **M0** | **M1** | **M2** | **M3** |
| | **0 mg/kg** | **300 mg/kg** | **500 mg/kg** | **1000 mg/kg** |
| L2 - day 3 | 8.60 ± 0.56 | 8.80 ± 0.39 | 8.70 ± 0.42 | 9.00 ± 0.47 |
| L3 - day 4 | 19.30 ± 0.37 | 20.30 ± 0.30 | 19.00 ± 0.56 | 19.70 ± 0.42 |
| PF - day 6 | 15.80 ± 0.33 | 15.40 ± 0.34 | 15.30 ± 0.33 | 14.50 ± 0.37 |
| P - day 11 (M2, M3) | 12.60 ± 0.22 | 13.30 ± 0.15 | 13.10 ± 0.23 | 13.60 ± 0.22 (M0) |
| A - day 23 (M2, M3) | 14.60 ± 0.31 | 14.40 ± 0.31 | 14.70 ± 0.26 | 14.90 ± 0.23 |

The groups indicated in brackets (i.e., M0, M1, M2, M3) are the ones whose results proved significantly different ($p < 0.05$) from the group indicated in the corresponding column.
**Realizado por:** Rivera, Celia, 2023.

**Table 5.** Mean weight (mg ± S.E.) of *P. intermutans* larvae, pupae, and adult related to the time of exposure (day) and instar of life (L2 = second instar, L3 = third instar, PF = post-feeding instar, P = pupa instar, A= adult instar).

| Days of exposure (instar) | Concentration | | | |
|---|---|---|---|---|
| | **M0** | **M1** | **M2** | **M3** |
| | **0 mg/kg** | **300 mg/kg** | **500 mg/kg** | **1000 mg/kg** |
| L2 - day 3 | 49.27 ± 3.32 | 45.62 ± 2.55 | 46.55 ± 2.47 | 51.00 ± 3.51 |
| L3 - day 4 | 226.87 ± 11.50 | 271.53 ± 15.95 | 251.50 ± 14.22 | 288.71 ± 14.04 (M0) |
| PF - day 6 | 283.11 ± 10.01 | 283.47 ± 11.18 | 309.49 ± 8.34 | 304.70 ± 2.62 |
| P - day 11 (M2, M3) | 148.51 ± 3.13 | 158.18 ± 3.93 | 171.24 ± 4.05 (M0, M3) | 154.17 ± 2.51 |
| A - day 23 (M2, M3) | 85.72 ± 4.36 | 106.58 ± 2.69 (M0) | 112.88 ± 2.79 (M0) | 109.14 ± 3.97 (M0) |

The groups indicated in brackets (i.e., M0, M1, M2, M3) are the ones whose results proved significantly different ($p < 0.05$) from the group indicated in the corresponding column.
**Realizado por:** Rivera, Celia, 2023.

Furthermore, the weight of *P. intermutans* adults was found to differ significantly in samples that consumed liver with methanol. Adult flies in the M1, M2, and M3 treatments

exhibited greater weight (mg) than those in the control group. Additionally, significant differences were observed in the weight of L3 larvae that consumed liver containing 1000 mg/kg methanol, as compared to the M1, M2, and control groups. The effects of methanol on the weight of larvae, pupae, and adults are summarized in Table 5.

## DISCUSSION

Methanol or methyl alcohol, although found at low levels in commercially available alcoholic beverages, is used in adulterated beverages making it a potent poison [25]. Toxicologically, methanol levels are rarely relevant since they conform to the permissible limits of alcoholic degree [12,33]. However, cases of poisoning, either accidentally or intentionally, have been reported, with the leading cause being the intake of adulterated or illegal alcoholic drinks.

Methanol molecule is rapid absorption in tissues and body fluids. Methanol concentrates mainly in biological compartments with high water content (urine, blood, vitreous humor), making them the most used samples for *post-mortem* alcohol analysis [41]. Forensic investigations of a suspicious poisoning death need a toxicological analysis of human remains to identify the type and amount of xenobiotics that may contribute to or cause the death [42]. The use of insects as an alternative to conventional biological samples in *post-mortem* alcohol analysis is of great relevance for forensic purposes. The alcohol studies focus on the effects on insects' development and records of arthropods associated with human corpses [43,44]. Unfortunately, alcohol case studies typically involve ethanol, but no research has been done on detecting methanol in insects. The current research has demonstrated that HS-GC-MS analyses can detect the presence of lethal methanol concentrations in liver substrates, significantly affecting the developmental time, survival, and morphology (weight) of *P. intermutans* immatures and adults.

*Methanol concentration* **-** As stated, no information is available about methanol's effects on flesh flies. However, comparisons and analogies can be made with some volatile organic compounds [43,44]. The analytical method used in these research papers detects volatile organic compounds in numerous arthropods (blowflies, ants, termites, beetles) using HS-SPME-GC-MS. The toxicological effects showed that (1) detectable

amounts of volatile compounds were found in the larvae instar and adult arthropods, and (2) control samples also had alcohol concentrations. The results on methanol are similar: (1) methanol detection at each instar was higher as the concentration spread in the liver increased, with the only exception of PF in M1, M2, and M3 treatment, where the amount of methanol was lower than that in control (Table 2); (2) Methanol was detected in larvae, pupa, and adults of *P. intermutans* that consumed the control sample (M0).

The elevated methanol concentrations in L3 and PF of the control samples (M0) can be attributed to the *post-mortem* alcohol formation. Although the sample was not mixed with methanol, *post-mortem* methanol formation through endogenous metabolic processes has been recorded [41,45]. Regarding the production of alcohol *post-mortem*, liver mixed with methanol presented a lower decomposition compared to liver without methanol (M0). Studies of *ante-mortem* alcohol ingestion show that alcohol could delay corpses' first decomposition stages (fresh and bloat) [8,46,47]. Also, microbiologically, methanotrophs and certain methylotrophs can oxidize methane to methanol through oxygenase complexes [48].

*Effects of methanol on development time and survival of flies* - Experimental data demonstrate that the development time and survival of *P. intermutans* were affected by the presence of methanol in the food substrate as the concentration of exogenous methanol increased (Table 3).

Insects found in corpses are mainly used to estimate the *post-mortem* interval (PMI). Therefore, any change in developmental time can affect the calculation of the minPMI. Previous studies have shown that *ante-mortem* ingestion of a toxin or drug can alter the development rates of insects that have fed on the tissues of the carcass. In the case of alcohols, observations mention that ethanol can retard the development of *Phormia regina* [8]. Compared with ethanol, *P. intermutans* reared on a food substrate spiked with methanol show a lower development time.

Larvae do not appear to have the same level of aversion to methanol content as the adults (Table 3). Studies of *Drosophila melanogaster* mention that in media with alcohol, larvae could increase the enzymatic metabolism of ADH or the increased tolerance of larvae within anaerobic conditions compared to adults [35].

Considering the high and detectable concentration of methanol in the larvae fed on substrates spiked with methanol and the control, it is possible to speculate that *P. intermutans* may efficiently excrete the alcohol, develop and survive despite its presence since the concentration found is lower in its final stage (adults). Methanol concentration was the highest in adults fed with M3. Survival can be attributed to the tolerance caused by this toxin at its early stages. When the larvae are exposed to a high methanol concentration, various detoxification mechanisms could be activated, and lead them to adapt early to the contamination [49,50].

Previous studies note that insects may be capable of excreting drugs efficiently [20,49–53]. Malpighian tubules are insects' main excretory and osmoregulatory organs where metabolism and detoxification occur. Observations on Sarcophagidae suggest that peroxisomes found in the Malpighian tubules are involved in eliminating several toxic molecules [20].

The P450 enzymes (cytochrome P450 monooxygenase), are a diverse class of enzymes found in virtually all insect tissues that play a central role in the metabolism of foreign chemicals of natural or synthetic origin [50-52]. In the larval stages, detoxification of the larvae is given by P450 [49]. In the presence of methanol, P450 increases its activity and expression levels. Being in a medium with the highest amount of methanol (M1, M2, M3), the activity of P450 occurs at earlier times, so methanol concentrations decrease as the larval stages progress, increasing its probability of survival [52,53]. However, in the control sample (M0), alcohol is unlikely to affect insect development as it is a typical product of liver decomposition.

Despite obtaining a new generation of flesh flies exposed to M3, the individuals presented deformations. Our results suggest that methanol ingestion may affect the fertility of the flesh flies and have consequences on their offspring. However, more studies are needed to demonstrate the methanol effects on the necrophagous insect’s fertility.

*Effects of methanol on larval and pupal lengths and weights* - The length of the larvae and adults of *P. intermutans* feeding on a substrate spiked with methanol was not significantly different from the control (Table 4). Larvae with the highest methanol concentration (1000 mg/kg, M3) were visibly affected: they formed larger pupae and pupation time was the shortest (Table 3). The control pupae weights were significantly

lower than the treatment pupae, and lower adult weights would also correspond with lower pupae weights (Table 5).

The morphological changes in *P. intermutans* can be attributed to its feeding. Certain types of insects can be attracted by the odorants given off at each carcass decomposition stage.

Odorants perceived by olfactory receptors (ORs) activate sensory olfactory neurons (OSNs), modulating the response to food sources. There are three types of OSN; two dictate methanol and ethanol attraction, while the third responds primarily to methanol and controls aversion to high and potentially toxic alcohol concentrations [54]. Larvae exposed to the medium with methanol had a higher weight than larvae fed on the liver without methanol (Table 5). The difference in weight can be attributed to the fact that the OSNs responded to the liver with methanol as a more attractive food source for the larvae. In addition, in the observations made, M1, M2, and M3 were almost entirely consumed by the larvae, so the concentrations that are lethal to humans are not lethal to *P. intermutans*. Otherwise, the OSN would have reduced the attraction to the methanol media in case of detecting methanol as an aversive odorant. Under such circumstances, the presence of *P. intermutans* in deceased people who have consumed methanol should be investigated in more detail. Since larvae consume tissue faster and develop in less time, the forensic examiner must consider the context of where and how to discover the carcass was discovered before making any interference.

## CONCLUSIONS

Adulteration of alcoholic beverages with methanol is a grave public health issue that is prevalent in many countries. Methanol, a toxic alcohol, is sometimes added to counterfeit or illegally produced alcoholic drinks to increase their strength or as a substitute for ethanol, which is more expensive. Even small amounts of methanol can be hazardous and can lead to severe health problems such as blindness, coma, and death. Often, people who consume these adulterated drinks are unaware of the presence of methanol, as it is added in small amounts to evade detection. The problem is particularly widespread in low- and middle-income countries, where the regulations and enforcement of alcohol production and distribution are often inadequate. In the current study, *P. intermutans* is identified as a potential biomarker for methanol detection in biological

samples using HS-GC-MS, which is a valuable technique for detecting methanol. The study demonstrated that it was possible to detect methanol in all stages of *P. intermutans* reared on both low and high methanol concentrations. Furthermore, different concentrations of methanol were found to have adverse effects on the morphology, survival, and development time of flesh flies, including their offspring that consumed the contaminated substrate.

This research is a significant contribution to current knowledge in entomotoxicology investigations in Ecuador, emphasizing the need for more studies on the effects of methanol on flesh flies. Specifically, future research should explore: (a) how deaths caused by alcoholic beverage consumption affect the development of flesh flies, both for those that emerged and for future generations from flies that emerged after consuming the contaminated substrate and how this could impact the minimum PMI estimation; (b) the effects of higher doses of methanol in other genera of flies, as Sarcophagidae larvae can activate their resistance mechanisms earlier, and oviposition should be studied; (c) how methanol mixed with other drugs affects flesh flies; (d) quantification of the endogenous alcohol *post-mortem* formation to determine if methanol found is due to *ante-mortem* ingestion or *post-mortem* formation; and (e) the effects of methanol on the degradation of corpses.

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## REFERENCES


1. Neugebauer R, Fraunhofer-Forschungsfokus E. Biological Transformation. 2020. doi:10.1007/978-3-662-59659-3

2. Hoffmann KH. Insect biotechnology - A major challenge in the 21st century. Zeitschrift fur Naturforschung - Section C Journal of Biosciences. Walter de Gruyter GmbH; 2017. pp. 335–336. doi:10.1515/znc-2017-0145

3. Gosselin M, Wille SMR, Fernandez M del MR, Di Fazio V, Samyn N, De Boeck G, et al. Entomotoxicology, experimental set-up and interpretation for forensic toxicologists. Forensic Science International. 2011. pp. 1–9. doi: 10.1016/j.forsciint.2010.12.015

4. Hodecek J. Revisiting the concept of entomotoxicology. Forensic Science International: Synergy. Elsevier B.V.; 2020. pp. 282–286. doi: 10.1016/j.fsisyn.2020.09.003

5. José M, Pozo H. Entomotoxicología Forense En Cadáveres En Estado De Descomposición. The Ecuador Journal of Medicine. 2021;1: 17–32. doi:10.46721/TEJOM-VOL1ISSESP-2021-17-32

6. Boulkenafet F, Dob Y, Karroui R, Al-Khalifa M, Boumrah Y, Toumi M, et al. Detection of benzodiazepines in decomposing rabbit tissues and certain necrophagic dipteran species of forensic importance. Saudi J Biol Sci. 2020;27: 1691–1698. doi: 10.1016/J.SJBS.2020.04.044

7. Ganguly A. Intervention of Biotechnology in forensic entomology. The BioTalk Magazine. 2020; 1–3. Available: https://www.researchgate.net/publication/349366510_INTERVENTION_OF_BIOTECHNOLOGY_IN_FORENSIC_ENTOMOLOGY#fullTextFileContent

8. Tabor KL, Fell RD, Brewster CC, Pelzer K, Behonick GS. Effects of Antemortem Ingestion of Ethanol on Insect Successional Patterns and Development of *Phormia regina* (Diptera: Calliphoridae). J Med Entomol. 2005. Available: https://academic.oup.com/jme/article/42/3/481/850574

9. Chophi R, Sharma S, Sharma S, Singh R. Forensic entomotoxicology: Current concepts, trends and challenges. Journal of Forensic and Legal Medicine. Churchill Livingstone; 2019. pp. 28–36. doi: 10.1016/j.jflm.2019.07.010

10. da Silva EIT, Wilhelmi B, Villet MH. Forensic entomotoxicology revisited—towards professional standardization of study designs. International Journal of Legal Medicine. Springer Verlag; 2017. pp. 1399–1412. doi:10.1007/s00414-017-1603-9

11. World Health Organization. Global status report on alcohol and health 2018. Geneva; 2018. Available: https://apps.who.int/iris/bitstream/handle/10665/274603/9789241565639-eng.pdf?ua=1

12. Instituto Ecuatoriano de Normalización. Bebidas alcohólicas. Aguardiente de caña. Quito; 2014 Apr.

13. Gerstner RMF, Soriano I, Sanhueza A, Caffe S, Kestel D. Epidemiología del suicidio en adolescentes y jóvenes en Ecuador. Rev Panam Salud Publica;42, aug 2018. 2018;42. doi:10.26633/RPSP.2018.100

14. Comité Interinstitucional de Drogas. Plan Nacional de Prevención Integral y Control del Fenómeno Socio Económico de las Drogas. Quito; 2017.

15. Aly SM, Aldeyarbi H. Zastosowanie entomologii sądowej: przegląd stanu wiedzy i najnowsze doniesienia. Arch Med Sadowej Kryminol. 2020;70: 44–77. doi:10.5114/amsik.2020.97832

16. Lee JB, Jeong YA, Ahn DJ, Bang IS. SPME-GC/MS Analysis of Methanol in Biospecimen by Derivatization with Pyran Compound. Molecules 2020, Vol 25, Page 41. 2019;25: 41. doi:10.3390/MOLECULES25010041

17. Bursová M, Hložek T, Čabala R. Simultaneous determination of methanol, ethanol and formic acid in serum and urine by headspace GC-FID. J Anal Toxicol. 2015;39: 741–745. doi:10.1093/jat/bkv075

18. Buenaventura E, Pape T. Revision of the New World genus *Peckia* Robineau-Desvoidy (Diptera: Sarcophagidae). Zootaxa. 2013;3622: 1–87. doi:10.11646/zootaxa.3622.1.1

19. De Camargo SLLX, Da Silva Carvalho-Filho F, Cristina Esposito M. The genus *Peckia* Robineau-Desvoidy (Diptera: Sarcophagidae) in the Brazilian amazon: A new species, new records, descriptions of female terminalia and key to species. Zootaxa. 2018;4483: 1–35. doi:10.11646/zootaxa.4483.1.1

20. Pal R, Kumar K. Ultrastructural features of the larval Malpighian tubules of the flesh fly *Sarcophaga ruficornis* (Diptera: Sarcophagidae). Int J Trop Insect Sci. 2012;32: 166–172. doi:10.1017/S1742758412000252

21. Nusair SD, Abed SI, Rashaid AHB. Chlorpromazine Impacts on the Length and Width of Chlorpromazine Impacts on the Length and Width of *Sarcophaga haemorrhoidalis* (Diptera: Sarcophagidae) Larvae: Potential Forensic Implications. J Entomol Sci. 2017;52: 370–378. doi:10.18474/JES17-33.1

22. Pape T, Blagoderov V, Mostovski M. Order Diptera Linnaeus, 1758. In: Zhang, Z.-Q. (Ed.) Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness. 2011. Available: www.diptera.org

23. Tabor KL, Fell RD, Brewster CC, Pelzer K, Behonick GS. Effects of Antemortem Ingestion of Ethanol on Insect Successional Patterns and Development of *Phormia regina* (Diptera: Calliphoridae). J Med Entomol. 2005. Available: https://academic.oup.com/jme/article/42/3/481/850574

24. Garcés PA, Arias LN, Medina M. Sarcophagidae de interés forense en el Parque Nacional Soberanía, Provincia de Panamá. Periodicidad: Semestral. 2020;22: 2020. Available: http://portal.amelica.org/ameli/jatsRepo/224/2241149007/2241149007.pdf

25. Carvalho L, Thyssen PJ, Linhares AX, Palhares F. A Checklist of Arthropods Associated with Pig Carrion and Human Corpses in Southeastern Brazil. Mem Inst Oswaldo Cruz. 2000;95: 135–138.

26. Barros RM, Mello-Patiu CA, Pujol-Luz JR. Sarcophagidae (Insecta, Diptera) associados à decomposição de carcaças de *Sus scrofa* Linnaeus (Suidae) em área de Cerrado do Distrito Federal, Brasil. Rev Bras Entomol. 2008;52: 606–609. doi:10.1590/S0085-56262008000400011

27. Salazar F, Donoso D. Catálogo de insectos con valor forense en el Ecuador. REMCB. 2015;36: 49–59. Available: http://theantsofecuador.com/david/publications/Salazar%20&%20Donoso_2015_Catalogo%20Forense.pdf

28. García-Ruilova AB, Barragán A, Ordoñez S del C, García JF, Mazón JD, Cueva R, et al. First records of Diptera associated with human corpses in Ecuador. Neotrop Biodivers. 2020;6: 197–202. doi:10.1080/23766808.2020.1845009

29. Carvalho CJ, Mello-Patiu C. Key to the adults of the most common forensic species of Dipterain South America. Rev Bras Entomol. 2008;52: 390–406. Available: https://doi.org/10.1590/S0085-56262008000300012

30. Vairo KP, Mello-Patiu CA, Carvalho C. Pictorial identification key for species of Sarcophagidae (Diptera) of potential forensic importance in southern Brazil. Rev Bras Entomol. 2011;55: 333–347.

31. Buenaventura E, Camacho G, García A, Wolff M. Sarcophagidae (Diptera) de importancia forense en Colombia: claves taxonómicas, notas sobre su biología y distribución Sarcophagidae (Diptera) of forensic importance in Colombia: taxonomic keys, notes on biology, and distribution. Rev Colomb Entomol. 2009;35: 189–196.

32. Marín Ruiz JA. Norma de Atención de los Pacientes intoxicados por Metanol. 2011. Available: https://www.paho.org/disasters/dmdocuments/norma%20manejo%20de%20intoxicacion%20por%20metanol.pdf

33. Public Health England (PHE). Compendium of Chemical Hazards: Methanol. 2015 Aug. Available: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/456293/Methanol_TO_PHE_260815.pdf

34. Amendt J, Campobasso CP, Gaudry E, Reiter C, LeBlanc HN, J. R. Hall M. Best practice in forensic entomology - Standards and guidelines. Int J Legal Med. 2007;121: 90–104. doi:10.1007/s00414-006-0086-x

35. GBIF Backbone Taxonomy. *Peckia* Robineau-Desvoidy, 1830 in GBIF Secretariat. 2022 [cited 21 Feb 2023]. doi:https://doi.org/10.15468/39omei

36. Li H, Tennessen JM. Preparation of *Drosophila* larval samples for gas chromatography-mass spectrometry (GC-MS)-based metabolomics. Journal of Visualized Experiments. 2018;2018. doi:10.3791/57847

37. Magni PA, Pazzi M, Vincenti M, Alladio E, Brandimarte M, Dadour IR.

Development and validation of a GC-MS method for nicotine detection in *Calliphora vomitoria* (L.) (Diptera: Calliphoridae). Forensic Sci Int. 2016;261: 53–60. doi: 10.1016/j.forsciint.2015.11.014

38. Magni PA, Pacini T, Pazzi M, Vincenti M, Dadour IR. Development of a GC-MS method for methamphetamine detection in *Calliphora vomitoria* L. (Diptera: Calliphoridae). Forensic Sci Int. 2014;241: 96–101. doi: 10.1016/j.forsciint.2014.05.004

39. Chun HJ, Poklis JL, Poklis A, Wolf CE. Development and validation of a method for alcohol analysis in brain tissue by headspace gas chromatography with flame ionization detector. J Anal Toxicol. 2016;40: 653–658. doi:10.1093/jat/bkw075

40. Andresen H, Schmoldt H, Matschke J, Flachskampf FA, Turk EE. Fatal methanol intoxication with different survival Times-Morphological findings and postmortem methanol distribution. Forensic Sci Int. 2008;179: 206–210. doi: 10.1016/j.forsciint.2008.05.014

41. Savini F, Tartaglia A, Coccia L, Palestini D, D'Ovidio C, Grazia U De, et al. Ethanol Determination in Post-Mortem Samples: Correlation between Blood and Vitreous Humor Concentration. Molecules. 2020;25. doi:10.3390/molecules25122724

42. Waghmare PB, Chikhalkar B, Nanandkar S. Establishing Identity and Cause of Death in Mutilated and Un Identifiable Corpses: A Challenging Task for a Medico Legal Expert. Journal of Forensic Biomechanics. 2015;06. doi:10.4172/2090-2697.1000120

43. Chen J. Freeze-Thaw Sample Preparation Method Improves Detection of Volatile Compounds in Insects Using Headspace Solid-Phase Microextraction. Anal Chem. 2017;89: 8366–8371. doi: 10.1021/acs.analchem.7b01622

44. Cai L, Macfadyen S, Hua B, Zhang H, Xu W, Ren Y. Identification of Biomarker Volatile Organic Compounds Released by Three Stored-Grain Insect Pests in Wheat. Molecules. 2022;27. doi:10.3390/molecules27061963

45. Wunder C, Weber C, Paulke A, Koelzer SC, Holz F, Toennes SW. Endogenous formation of 1-propanol and methanol after consumption of alcoholic beverages. Forensic Sci Int. 2021;325. doi: 10.1016/j.forsciint.2021.110905

46. Ewing S, Koch M, Lee IJ, Pena A, Stroupe S. Effects of Ethanol Exposure on Decomposition and Insect Colonization. Instars: A Journal of Undergraduate Research. 2014;1: 1–4.

47. Al-Khalifa M, Mashaly A, Al-Qahtni A. Impacts of antemortem ingestion of alcoholic beverages on insect successional patterns. Saudi J Biol Sci. 2021;28: 685–692. doi: 10.1016/j.sjbs.2020.10.060

48. Stöhlmacher P. [Methanol from ethanol? On cleavage of the ethanol carbon bond and methanol formation within the scope of microbial metabolic processes]. Blutalkohol. 1995;32: 193–207.

49. Wang SP, He GL, Chen RR, Li F, Li GQ. The involvement of cytochrome p450 monooxygenases in methanol elimination in *Drosophila melanogaster* larvae. Arch Insect Biochem Physiol. 2012;79: 264–275. doi:10.1002/arch.21021

50. Nauen R, Bass C, Feyereisen R, Vontas J. The Role of Cytochrome P450s in Insect Toxicology and Resistance. Annu Rev Entomol. 2021;67: 105–124. doi:10.1146/annurev-ento-070621

51. Scott JG, Wen Z. Cytochromes P450 of insects: The tip of the iceberg. Pest Management Science. 2001. pp. 958–967. doi:10.1002/ps.354

52. Feyereisen R. Insect P450 enzymes. Annu Rev Entomol. 1999. Available: www.annualreviews.org

53. Wang SP, Hu XX, Meng QW, Muhammad SA, Chen RR, Li F, et al. The involvement of several enzymes in methanol detoxification in *Drosophila melanogaster* adults. Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology. 2013;166: 7–14. doi: 10.1016/j.cbpb.2013.05.008

54. Keesey IW, Hansson BS. Neuroecology of Alcohol Preference in *Drosophila*. Annu Rev Entomol. 2022;67: 261–279. doi:10.1146/annurev-ento-070721