# IMPACT OF GLYPHOSATE (HERBICIDE) AND CHLORPYRIFOS (INSECTICIDE) CONTAMINATION ON NECROPHAGOUS FLY LIFECYCLE ON PORK

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**by**

**CHANG ZHI LIN**

**Thesis submitted in partial fulfilment of the requirements**

**for the Bachelor of Science (Honours) (Forensic Science)**

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**CERTIFICATE**

This is to certify that the dissertation entitled "IMPACT OF GLYPHOSATE (HERBICIDE) AND CHLORPYRIFOS (INSECTICIDE) CONTAMINATION ON NECROPHAGOUS FLY LIFECYCLE ON PORK" is the bonafide record of research work done by Chang Zhi Lin (matric number: 157930) during the period from October 2024 to February 2025 under my supervision. I have read this dissertation and that in my opinion it confirms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation to be submitted in partial fulfilment for the degree of Bachelor of Science (Honours) (Forensic Science).

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## DECLARATION

I hereby declare that this dissertation is the result of my investigations, except where otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research, and promotional purposes.

Chang Zhi Lin

Date: 25/02/2025

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| | |
|---|---|
| % | Percentage |
| °C | Degrees Celsius |
| µL | Microlitre |
| mL | Millilitre |
| L | Litre |
| mg | Milligram |
| g | Gram |
| kg | Kilogram |
| µm | Micrometre |
| mm | Millimetre |
| m | Metre |
| mg/mL | Milligrams per millilitre |
| mL/min | Millilitres per minute |
| w/w | Weight/weight percentage |
| hrs | Hours |
| min | Minutes |

## LIST OF ABBREVIATIONS

| | |
|---|---|
| 1st | First |
| 2nd | Second |
| 3rd | Third |
| *C. megacephala* | *Chrysomya megacephala* |
| *C. rufifacies* | *Chrysomya rufifacies* |
| Chl | Chlorpyrifos |
| Con | Control |
| FAO | Food and Agriculture Organization |
| FAOSTAT | Food and Agriculture Organization Statistics |
| GC-FID | Gas Chromatography with Flame Ionisation Detector |
| Gly | Glyphosate |
| *Lucilia* sp. | *Lucilia* species |
| MET | Malaysian Meteorological Department |
| MSF | Forensic Science Laboratory |
| NPC | National Poison Centre |
| PMI | Post-mortem interval |
| PPSK | School of Health Sciences |
| RH | Relative humidity |
| *Sarcophaga* sp. | *Sarcophaga* species |
| UPMS | Science Lab Management Unit |
| USM | Universiti Sains Malaysia |
| WHO | World Health Organization |

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**IMPACT OF GLYPHOSATE (HERBICIDE) AND CHLORPYRIFOS (INSECTICIDE) CONTAMINATION ON NECROPHAGOUS FLY LIFECYCLE ON PORK**

## ABSTRAK

Entomologi forensik memainkan peranan penting dalam penyiasatan jenayah, namun perkembangan serangga boleh dipengaruhi oleh bahan toksik, yang seterusnya menjejaskan anggaran selang masa selepas kematian (PMI). Kajian ini meneliti kesan kehadiran glyphosate (herbisid) dan chlorpyrifos (insektisid) terhadap kitaran hidup lalat nekrofagus pada daging khinzir yang mereput. Eksperimen dijalankan menggunakan sampel kawalan, sampel yang dirawat dengan glyphosate, dan sampel yang dirawat dengan chlorpyrifos selama 14 hari dalam dua musim berbeza (musim hujan dan musim kering). Empat spesies lalat nekrofagus—*Chrysomya megacephala, Chrysomya rufifacies, Lucilia* sp., dan *Sarcophaga* sp.—diperhatikan dalam sampel kawalan dan yang dirawat dengan glyphosate tanpa perbezaan ketara dalam tempoh kitaran hidup, morfologi, atau komposisi spesies. Namun, kehadiran chlorpyrifos mengurangkan oviposisi secara drastik, menyebabkan kematian larva pada instar pertama dan memperlahankan proses pereputan. Glyphosate tidak memberi kesan terhadap pereputan atau aktiviti lalat, manakala chlorpyrifos melambatkan pereputan akibat kekurangan koloni lalat, yang boleh mempengaruhi anggaran PMI dalam siasatan forensik. Percubaan untuk mengesan residu racun perosak dalam larva menggunakan kromatografi gas dengan pengesan pengionan nyalaan (GC-FID) tidak berhasil kerana kegagalan kajian pemulihan, berkemungkinan akibat ketidakcekapan kaedah pengekstrakan atau kepekaan instrumen yang terhad terhadap sebatian ini. Penemuan ini menekankan keperluan untuk kaedah toksikologi yang lebih baik dalam pengesanan racun perosak dalam entomologi forensik.

**IMPACT OF GLYPHOSATE (HERBICIDE) AND CHLORPYRIFOS (INSECTICIDE) CONTAMINATION ON NECROPHAGOUS FLY LIFECYCLE ON PORK**

## ABSTRACT

Forensic entomology is crucial in criminal investigations, but insect development can be influenced by toxic substances, affecting post-mortem interval (PMI) estimations. This study examines the impact of glyphosate (herbicide) and chlorpyrifos (insecticide) contamination on necrophagous fly lifecycles on decomposing pork meat. Experiments were conducted using control, glyphosate-treated, and chlorpyrifos-treated samples over 14 days in both rainy and non-rainy seasons. Four necrophagous fly species—*Chrysomya megacephala, Chrysomya rufifacies, Lucilia* sp., and *Sarcophaga* sp.—were observed in control and glyphosate-treated samples, with no significant differences in lifecycle duration, morphology, or species composition. However, chlorpyrifos contamination drastically reduced oviposition, causing larval mortality at the first instar and slowing decomposition. Glyphosate did not affect decomposition or insect activity, whereas chlorpyrifos delayed decay due to reduced insect colonisation, potentially impacting forensic PMI estimations. Attempts to detect pesticide residues in larvae using gas chromatography-flame ionisation detection (GC-FID) were inconclusive, as the recovery study failed, possibly due to extraction inefficiencies or the instrument's limited sensitivity to these compounds. These findings highlight the need for improved toxicological methods for pesticide detection in forensic entomology.

# CHAPTER 1: INTRODUCTION

## 1.1 Background of Study

Forensic entomology is a specialised field that applies the study of insects and other arthropods to legal issues, particularly criminal investigations. This discipline is crucial for estimating the postmortem interval (PMI), which is the time elapsed since death, by analysing insect activity on decomposing bodies (Catts & Goff, 1992). Entomological data estimate PMI in two ways: by using the development time of insects, typically maggots, in early decomposition, and by analysing the arthropod community in advanced decomposition. Both methods assume insects discover the corpse soon after death, but this assumption must be carefully evaluated, especially in indoor or extreme conditions.

Insects are attracted to a body shortly after death due to factors like ammonia and moisture (Mahat & Jayaprakash, 2013). Buckland and Smith (1986) classifies insects into four categories: necrophagous species (e.g., Calliphoridae, Dermestidae, Silphidae) that feed on the corpse and are key for estimating PMI; predators and parasites of necrophagous species (e.g., Silphidae, Staphylinidae); omnivorous species (e.g., wasps, ants); and adventive species (e.g., springtails, spiders) that use the corpse as an extension of their environment. Insect developmental data, particularly from Calliphoridae, is widely used for accurate post-mortem interval (PMI) estimation, a method first popularized in Malaysia by Lee (1996).

The *Chrysomya megacephala* and *Chrysomya rufifacies* are consistent prevalence in forensic investigations across various ecological habitats in Malaysia. Research spanning over three decades has shown that these species are most commonly found on cadavers, as confirmed by multiple studies, including those by Lee et al. (2004) and Kavitha et al. (2013). Further corroboration from Mahat & Jayaprakash (2013) and Syamsa et al. (2017), highlight their dominance in diverse environments such as mangroves, peat swamps, and aquatic areas. The adaptability of these blowflies to different habitats and their frequent association with

decomposing bodies underscore their forensic significance, particularly for post-mortem interval estimation and contamination detection in forensic science.

Forensic entomotoxicology is a specialized field that detects toxic substances by analysing necrophagous insects at crime scenes. It not only confirms the presence of toxicants in insects feeding on cadavers but also studies their effects on insect bio-morphometry and growth rates (Bhardwaj et al., 2020). This approach is particularly valuable when traditional toxicological samples are unavailable, as insects like Calliphoridae can indicate environmental toxins. By understanding how these substances influence insect development, forensic entomotoxicology aids in accurate post-mortem interval (PMI) estimations and provides critical insights for legal investigations (Hodecek, 2020; Ugalde et al., 2022).

In Malaysia, pesticide poisoning is a significant public health concern, particularly among vegetable farmers who are often overexposed to chemicals like organophosphates. A decade-long study by the National Poison Centre identified herbicides, especially glyphosate, and insecticides such as chlorpyrifos as major contributors to pesticide-related poisoning incidents. These widely used agricultural chemicals pose risks of environmental contamination, especially in farming regions. Cadavers found in these areas are likely exposed to these toxicants, which can alter the lifecycle and morphology of cadaveric insects, particularly *Chrysomya* species that are crucial for estimating the PMI in forensic investigations.

Understanding how exposure to glyphosate and chlorpyrifos affects the development of these insects is essential for improving the reliability of forensic entomological methods. Contamination can lead to erroneous PMI estimations and complicate the detection of toxic substances in forensic cases. Despite the importance of this issue, the specific impacts of glyphosate and chlorpyrifos on necrophagous flies have not been extensively studied. This research aims to address this gap by investigating how these chemicals influence the development of these flies, focusing on their implications for PMI estimation and forensic toxicology.

## 1.2 Problem Statement

Applying insecticides and herbicides in agriculture is a widespread practice aimed at improving crop yields and managing pest populations. However, the environmental impacts of these chemicals, particularly in areas where human remains are discovered, are frequently overlooked in forensic science. Understanding how pesticide contamination affects decomposing bodies and the insect communities associated with them is crucial to ensuring accurate PMI estimations.

Most existing research on pesticide poisoning primarily focuses on cases of direct ingestion, highlighting acute toxicity and immediate health effects. These studies often neglect the significant scenario where an individual dies in an environment already contaminated with these substances. As a result, there is a substantial gap in understanding how environmental pesticide residues influence the decomposition process and the behaviour of cadaveric insects in Malaysia. This lack of research limits forensic practitioners' ability to accurately determine PMI in complex cases involving pesticide exposure.

This study aims to investigate the effects of glyphosate (herbicide) and chlorpyrifos (insecticide) contamination on the lifecycle, development, and morphology of necrophagous fly. By examining how these environmental contaminants impact insect behaviour and decomposition dynamics, this research will provide valuable insights into how pesticide residues may alter forensic outcomes.

The anticipated findings will address a critical knowledge gap by clarifying the relationship between pesticide contamination and cadaveric insect behaviour. By offering forensic insights into how these environmental factors may influence PMI estimations, this study aims to enhance the accuracy of forensic investigations in cases involving pesticide exposure, ultimately contributing to more reliable practices in forensic science.

## 1.3 Objective

### 1.3.1 General Objective

To investigate the effects of glyphosate (herbicide) and chlorpyrifos (insecticide) contamination on the lifecycle, development, and morphology of necrophagous flies on pork, and to assess the implications of contamination for forensic PMI estimation and the detection of toxic substances in cadaveric insects.

### 1.3.2 Specific Objective

1. To investigate the effects of glyphosate and chlorpyrifos contamination on the lifecycle, development, and morphology of necrophagous flies on pork.
2. To assess the implications of glyphosate and chlorpyrifos contamination on the accuracy of postmortem interval (PMI) estimations
3. To detect glyphosate and chlorpyrifos residues in the necrophagous flies.

## 1.4 Significance of Study

This research holds significant implications for forensic entomology by enhancing our understanding of how pesticide contamination affects the accuracy of post-mortem interval (PMI) estimations and by advancing methodologies in forensic entomotoxicology. Firstly, it contributes to the field by demonstrating the influence of environmental contaminants, such as pesticides, on PMI assessments, which is a critical aspect of determining the time of death. Recognizing these effects is vital for forensic investigators operating in agricultural or rural settings where pesticide exposure is more prevalent. Improved methods for estimating PMI in cases involving pesticide exposure will bolster the reliability of forensic evidence in legal contexts. By documenting the lifecycle and morphological changes in cadaveric insects exposed to glyphosate and chlorpyrifos, this study will provide valuable data on potential delays or abnormalities in insect development caused by these chemicals.

Additionally, it enhances entomotoxicological analysis by exploring the possibility of detecting pesticide residues in insect samples, which may offer insights into the presence of toxic substances at crime scenes. Consequently, this research can contribute to the development of more precise PMI estimation techniques and expand toxicological assessment tools used in forensic investigations. The findings from this study could serve as a foundation for future research initiatives that explore the intersections of environmental science, entomotoxicology, and forensic entomology. By establishing a framework for understanding pesticide contamination within forensic contexts, this study may encourage further interdisciplinary research efforts. Finally, the lifecycle of necrophagous fly species on pork meat can be determined as there is no study in Malaysia yet that uses pork meat.

# CHAPTER 2: LITERATURE REVIEW

## 2.1 Diptera Flies in Malaysia

The selection of *Chrysomya megacephala (C. megacephala)* and *Chrysomya rufifacies (C. rufifacies*) as the focus of this study is based on their consistent prevalence in forensic investigations and their adaptability to diverse ecological habitats, as demonstrated in multiple studies conducted in Malaysia. Over three decades of forensic entomological research (1972–2002) reviewed by (Lee et al. (2004) revealed that *C. megacephala* and *C. rufifacies* were the most commonly found species on cadavers from various ecological settings. *Sarcophaga* sp. and *Lucilia* sp. were also included. Similarly, forensic specimens collected during crime scene investigations between 2005 and 2010 were reviewed by Kavitha et al. (2013), confirming the dominance of *C. megacephala* and *C. rufifacies* in rural, residential, and aquatic habitats. Together, these reviews spanning several decades demonstrate the continued dominance of *C. megacephala* and *C. rufifacies* in forensic cases in Malaysia.

The prevalence of *C. megacephala* and *C. rufifacies* has also been corroborated by other studies. A review paper by Mahat & Jayaprakash (2013) examining forensic entomology studies in Malaysia further highlighted that *C. megacephala* is the most prevalent Calliphorid species on cadavers and carcasses, followed by C. rufifacies. Syamsa et al. (2017) confirmed the dominance of *C. megacephala* and *C. rufifacies*, reporting that *C. megacephala* maggots were the most frequently observed, followed by *C. rufifacies*, in an analysis of 34 human remains over three years at Universiti Kebangsaan Malaysia Medical Centre. Additionally, these species (*C. megacephala, C. rufifacies,* and *Sarcophaga* sp.) have been found in various habitats, including mangroves (Azmi & Lim, 2013), mangroves and peat swamps (Maramat & Rahim, 2015a; Maramat & Rahim, 2015b), aquatic areas (Abdullah et al., 2022) and coastal regions (Musa et al., 2024),

These findings collectively highlight the forensic significance of *C. megacephala* and *C. rufifacies* and their adaptability to a wide range of environments. Their consistent

association with cadavers across diverse ecological settings and their dominance in forensic entomological cases make them ideal subjects for studying the effects of environmental contaminants such as pesticides and herbicides. Moreover, their prevalence ensures that the findings of this research will have broad applicability in forensic science, particularly in the context of post-mortem interval estimation and contamination detection.

### 2.2 Life Cycle of *Chrysomya megacephala* and *Chrysomya rufifacies*

The life cycles of *Chrysomya megacephala* and *Chrysomya rufifacies* are significantly influenced by environmental factors, particularly temperature and humidity. These factors dictate the duration of their developmental stages from egg to adult emergence, with higher temperatures generally accelerating development and colder temperatures prolonging it.

Rainy Season

During the rainy season, the life cycles of both species take longer compared to summer but are faster than in winter. For *C. megacephala*, the complete life cycle was reported to last approximately 237–265 hours, depending on the specific temperature and humidity conditions (Abd-AlGalil & Zambare, 2015a; Siddiki & Zambare, 2017). Similarly, *C. rufifacies* required around 239–275 hours to complete its development during this season (Abd-AlGalil & Zambare, 2015b; Siddiki & Zambare, 2017). The relatively cooler temperatures during the rainy season slow down larval development compared to the summer.

Summer Season

In the summer, when temperatures and evaporation rates are high, the life cycles of *C. megacephala* and *C. rufifacies* are significantly shorter. *C. megacephala* completed its development in approximately 211 hours under average temperatures of 32.5°C (Siddiki &

Zambare, 2017), and *C. rufifacies* required around 216–241 hours in similar conditions (Abd-AlGalil & Zambare, 2015b; Siddiki & Zambare, 2017).

Winter Season

Winter conditions, characterized by lower temperatures and higher humidity, lead to the longest life cycle durations for both species. *C. megacephala* took approximately 263 hours to develop fully at average temperatures of around 22.7°C (Siddiki & Zambare, 2017). Similarly, *C. rufifacies* required 286–318 hours for complete development under cooler conditions (Abd-AlGalil & Zambare, 2015b; Siddiki & Zambare, 2017).

Table 2.1 summarizes the life cycle stages of *Chrysomya megacephala* and *Chrysomya rufifacies* based on the study by Siddiki & Zambare (2017). It highlights the effects of temperature and humidity on the duration of each life cycle stage during different seasons (Rainy, Summer, and Winter).

Table 2.1 The life cycle stages of Chrysomya megacephala and Chrysomya rufifacies based on the study by Siddiki & Zambare (2017)

| **Species** | **Season** | **Temperature (°C)** | **Humidity (%)** | **Total Life Cycle Duration** | **Egg Incubation** | **1st Instar** | **2nd Instar** | **3rd Instar** | **Pre-pupal Stage** | **Pupal Stage** |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Chrysomya megacephala*** | Summer | 32.5 | 21.5 | 211 hrs 13 min | 18 hrs 8 min | 26 hrs 5 min | 26 hrs 45 min | 27 hrs 5 min | 20 hrs 10 min | 93 hrs |
| | Rainy | 24.1 | 49.6 | 237 hrs 47 min | 18 hrs 37 min | 25 hrs 45 min | 26 hrs 35 min | 29 hrs | 22 hrs 40 min | 115 hrs 10 min |
| | Winter | 22.7 | 35.8 | 263 hrs 51 min | 20 hrs 36 min | 26 hrs 30 min | 28 hrs 30 min | 48 hrs | 21 hrs 50 min | 118 hrs 25 min |
| ***Chrysomya rufifacies*** | Summer | 32 | 22.07 | 216 hrs 26 min | 19 hrs 21 min | 25 hrs 45 min | 28 hrs | 48 hrs 40 min | 20 hrs 10 min | 74 hrs 30 min |
| | Rainy | 24 | 42.1 | 239 hrs 14 min | 22 hrs 38 min | 25 hrs 6 min | 27 hrs 35 min | 51 hrs 5 min | 41 hrs 50 min | 71 hrs |
| | Winter | 25 | 46.8 | 286 hrs 2 min | 19 hrs 2 min | 46 hrs 15 min | 28 hrs 25 min | 50 hrs 5 min | 24 hrs 35 min | 117 hrs 40 min |

The development of *Chrysomya megacephala* is significantly influenced by temperature and humidity. Temperature also affects larval development, as increasing ambient temperature decreases larval development time (Barrett et al., 2018; Gruner et al., 2017; Pereira et al., 2023). According to Ngando et al. (2024), the total development time from oviposition to adult eclosion at constant temperatures of 15, 20, 25, 30, and 35°C were 858.1 ± 69.2, 362.3 ± 5.9, 289.6 ± 17.8, 207.3 ± 9.3, and 184.7 ± 12.1 hours, respectively. Specifically, when daily average temperatures ranged from 25.4 to 27.6°C, the life cycle was completed in 8 days, whereas at temperatures of 23.1 to 25.1°C, the life cycle took 8 to 11 days (Barrett et al., 2018). Adult longevity is impacted by relative humidity (RH); at 40% RH, adults lived for a mean of 64 days, with a maximum of 105 days, while at 75% RH, the lifespan decreased to 54 and 95 days, respectively (Badenhorst & Villet, 2018).

Similarly, temperature significantly affects the development of *Chrysomya rufifacies*. The studies reported that the growth and development of *C. rufifacies* accelerated with higher temperatures, while colder temperatures in winter prolonged developmental stages (Bansode & More, 2024; Yanmanee et al., 2016). For example, Barrett et al. (2018) found that the life cycle of *C. rufifacies* was shorter at higher temperatures, completing in 13 days at average daily temperatures of 25.4 to 27.6°C and in 11 days at temperatures ranging from 26.8 to 29.9°C. Byrd & Butler (1997) observed a similar trend, where developmental times from egg to adult ranged from 190 to 598 hours across different temperature regimes from 15.6, 21.1, 25.0, 26.7, to 32.2°C, with shorter developmental times at higher temperatures.

## 2.3 Morphological Identification of Necrophagous Fly

### 2.3.1 *Chrysomya megacephala*

The eggs of *C. megacephala* are 1.5–1.6 mm long, sausage-shaped, whitish, and turn cream as they mature (Badenhorst & Villet, 2018). Greenberg and Kunich (2005) noted that the eggs are larger than 1.35 mm in length, with arms of the flanges curving halfway around the micropylar collar and peg-like struts on the anterior third of the plastron, which are blunt at the apex and mostly not anastomosed. Palavesam et al. (2022) further identified a narrow 'Y'-shaped plastron as a characteristic feature of *Chrysomya spp.* Eggs.

The first instar larvae of *C. megacephala* measure 1.7–3.5 mm in length and have posterior spiracles with one slit (Badenhorst & Villet, 2018). The second instar larvae grow to 6–8 mm in length and have posterior spiracles with two slits (Badenhorst & Villet, 2018). By the third instar, larvae can reach up to 16 mm but contract just before pupariation. These larvae have posterior spiracles with three slits with mildly sclerotized peritreme, an incomplete dorsal band of spinules, and anterior spiracles with 11–13 branches (Badenhorst & Villet, 2018; Greenberg & Kunich, 2005). The absence of conical tubercles on body segments, the presence of a dorsal arch with a 'dot or club-shaped' cephalopharyngeal skeleton, anterior spiracles composed of papillae, incomplete posterior spiracular peritremes, and the lack of large elongate tubercles on abdominal segments have been described (Abass & Ali, 2024; Greenberg & Kunich, 2005; Omar, 2002; Sukontason et al., 2004).

The puparium of *C. megacephala* is brown with yellow anterior spiracles and is formed from the exoskeleton of the third instar larvae, retaining the same identifying surface structures (Badenhorst & Villet, 2018). Mouth hooks can usually be found adhering to the inside of the eclosed puparium (Badenhorst & Villet, 2018).

Adults of *C. megacephala* are 7–12 mm in length. They have a greenish-blue thorax with two narrow longitudinal stripes and a greenish-blue abdomen with a purple reflection. The wings are hyaline, and the legs are black (Sawaby et al., 2018). Prothoracic spiracles are dark brown to blackish (Kurahashi et al., 1997; Musa et al., 2024). The gena and postgenal

areas are orange-yellow with pale yellow hairs except near the vibrissae (Kurahashi et al., 1997; Sawaby et al., 2018). Male specimens exhibit enlarged eye facets in the upper portion of the eyes, sharply demarcated from smaller facets below. The frontal stripe is the broadest in the middle (Greenberg & Kunich, 2005).

### 2.3.2 *Chrysomya rufifacies*

The eggs of *C. rufifacies* are also larger than 1.35 mm in length. The arms of the flanges curve halfway around the micropylar collar, and the anterior third of the plastron has peg-like struts (Greenberg & Kunich, 2005). Palavesam et al. (2022) identified a narrow 'Y'-shaped plastron as a characteristic feature of *Chrysomya sp.* eggs.

The larvae of *C. rufifacies* display unique features. First instar larvae have posterior spiracles with one slit, while second instar larvae have two slits. Third instar larvae have posterior spiracles with three slits and with incomplete heavily sclerotized peritremes and a row of conical tubercles present on segments 4 to 12, giving them a "hairy" appearance (Greenberg & Kunich, 2005; Sukontason et al., 2004). The cephalopharyngeal skeleton lacks a distinct dorsal arch, and the anterior spiracles have 10–12 papillae (Abass and Ali, 2024; Greenberg & Kunich, 2005; Sukontason et al., 2004) described large, elongate, fleshy tubercles on each segment, crowned with spines, and the presence of spinnulation.

Adults of *C. rufifacies* measure 6–12 mm in length and have a stout body. The thorax is greenish-blue, and the abdomen is also greenish-blue, while the eyes are prominent and red (Sawaby et al., 2018). The gena and postgenal areas are silvery white, and the mesothoracic spiracle is white. Tergite 5 has white hairs among black hairs, while tergites 3 and 4 display broad marginal bands. The aedeagus is funnel-shaped, and the hypophallus is sclerotized (Greenberg & Kunich, 2005; Kurahashi et al., 1997; Musa et al., 2024).

### 2.3.3 *Lucilia* species

The genus *Lucilia* belongs to the family Calliphoridae, subfamily Luciliinae. The first, second, and third instar larvae of *Lucilia* species (*Lu*cilia sp.) exhibit posterior spiracle slits 1, 2, and 3, respectively. The posterior spiracle is not situated in a deep cavity, and the spiracular slits point towards the opening in the peritreme. These slits are straight and narrow, and the peritreme is complete, enclosing the button area (Cheong et al., 1971).

In adult *Lucilia* flies, the body is slender, the head is small, and the cheeks are silvery and smooth (Holloway, 1991). According to Greenberg & Kunich (2005), the adult fly has a posterior part of the suprasquamal carina with a posterior parasquamal tuft of black, erect hairs on a small, well-defined black sclerite. The thoracic squama is quite bare on the upper surface. The body is predominantly metallic green to blue, and the supraspiracular convexity may be either bare or pubescent.

*Lucilia sericata* exhibits males with sternites that lack a tuft of long hairs, and their abdomen is not conspicuously arched in profile. The female body is usually metallic green, with some specimens showing a coppery tinge. The cerebrale in the male bears five to eight hair-like setae on each side. In contrast, *Lucilia cuprina* males have sternites with a tuft of long hairs, and their abdomen is typically arched in profile. The female body is usually brassy or coppery, set against a greenish background, with dense pruinosity. In the male, the cerebrale bears a single occipital hair-like seta on each side.

### 2.3.4 *Sarcophaga* species

The family of *Sarcophaga* species (*Sarcophaga* sp.) is Sarcophagidae, the subfamily is Sarcophaginae, and the genus is *Sarcophaga*. In the first instar larvae, there is one slit in the posterior spiracle. The second instar larvae have posterior spiracles with two slits, while the third instar larvae exhibit three slits. The posterior spiracle is located in a deep cavity, with

straight and broad spiracular slits that do not point towards the opening in the peritreme. The peritreme itself is incomplete and does not enclose the button area (Cheong et al., 1971).

The hind coxa is hairy on the posterior surface, with two strong primary bristles and two smaller subprimary bristles. Sternites 3 and 4 are fully exposed, overlapping the ventral margins of the corresponding tergites (Greenberg & Kunich, 2005). The body of the *Sarcophaga* species is dull grey or black, with the thorax featuring three prominent black stripes. The abdomen is checkered, often with a red tip, and the sides lack pale coloration (Communicable Disease Center (U.S.), 1966). Males and females can be distinguished by the structure of their abdomen that males had arched abdomens in profile.

**2.4 Definition and Global Usage of Pesticides**

The World Health Organization (WHO) defines pesticides as chemical compounds used to eliminate various pests, including insects, rodents, fungi, and unwanted plants. Over 1,000 different types of pesticides are utilized globally, especially in agriculture and public health, where they play a crucial role in protecting crops and controlling vectors of diseases such as mosquitoes. According to the Food and Agriculture Organization (FAO), pesticides are essential in modern agriculture because they protect crops from pests, weeds, bacteria, and fungi, ensuring higher yields and food security (FAO, 2024)

Statistics from the FAOSTAT Pesticide Use database reveal that between 1990 and 2022, global pesticide use per cropland area increased by 94%, while per capita use rose by 35% (FAO, 2024). In 2022 alone, 3.70 million tonnes of pesticides were applied in agricultural activities, reflecting a 4% increase from the previous year (FAO, 2024). This widespread application amounts to nearly 3 billion kilograms of pesticides annually worldwide, with an approximate market value of USD 40 billion (Sharma et al., 2020).

## 2.5 Types of Pesticides

Pesticides are classified into several main types based on the target species they control, with insecticides, herbicides, and fungicides being the most widely used in agricultural and urban environments (Syafrudin et al., 2021). FAOSTAT's Pesticides Use database details the application of these categories worldwide, noting the prevalence of these chemical groups across various ecosystems (FAO, 2024). Common pesticide types utilized globally include organophosphates, organochlorines, carbamates, and pyrethroids (Sharma et al., 2020).

Insecticides, used to combat insects, are varied and include chlorinated hydrocarbons, organophosphates, carbamates, pyrethroids, and biological products (FAO, 2024; Sharma et al., 2020; Syafrudin et al., 2021). Herbicides target weeds and include chemicals like glyphosate, triazines, phenoxy hormone products, and uracil. Additionally, fungicides and bactericides help control fungi and bacteria through compounds such as inorganic chemicals, dithiocarbamates, and benzimidazoles.

## 2.6 Pesticide Poisoning in Global and Malaysia

Pesticides are integral to modern agriculture, enabling pest control to boost crop yields and food supply. However, their widespread and prolonged use poses notable environmental and health risks. Epidemiological research has linked pesticide exposure to adverse effects on human organs, such as the liver, brain, lungs, and colon, and chronic exposure may increase the risk of life-threatening conditions like cancer. Globally, pesticide poisoning results in approximately 300,000 deaths each year, with exposure primarily categorized as occupational or accidental (Sharma et al., 2020). Occupational exposure is prevalent among those who regularly handle pesticides, including agricultural workers and pesticide manufacturers, and occurs mainly through skin contact, inhalation, or ingestion (Sharma et al., 2020).

In Malaysia, pesticide poisoning remains a serious public health concern. A study reports that 23.3% of vegetable farmers in Kundasang are overexposed to organophosphate (OP) pesticides based on blood cholinesterase testing (Botinggo, et al., 2021). Additionally, a 10-year study by the National Poison Centre (NPC) from 2006 to 2015 documented 39,088 poisoning calls, with pesticides constituting the second-largest category of toxic substances at 28.4% (Kamaruzaman et al., 2020a). Within this data, herbicides caused the highest number of pesticides poisoning cases (43.6%), followed by agricultural insecticides (34.4%), rodenticides (9.9%), and household insecticides (9.5%).

Among herbicides, glyphosate accounted for 53% of cases, while organophosphates like chlorpyrifos represented the leading cause of poisoning within the agricultural insecticide category, at 40% (Kamaruzaman et al., 2020). Glyphosate and chlorpyrifos poisoning cases are of particular interest in the context of Malaysia, with both substances being predominant contributors to pesticide-related poisoning.

The current study focuses on glyphosate (a herbicide) and chlorpyrifos (an organophosphate insecticide) to explore how contamination with these pesticides impacts cadaveric insects. This examination is intended to reveal insights into postmortem interval (PMI) estimation accuracy, considering the interference from such contaminants.

### 2.7 Pesticides and Cadaveric Insects in PMI Estimation

The presence of pesticides in cadaveric insects significantly influences post-mortem interval (PMI) estimations by affecting insect life cycles, behaviour, and overall decomposition processes. This relationship has been explored in forensic research to understand how pesticide exposure impacts insect colonization, development, and the decomposition of remains, especially in cases of suicide, homicide, accidental ingestion, and instances where a person has died in pesticide-contaminated areas like farmlands.

Studies investigating this relationship typically employ one of four approaches: oral administration of pesticides to animals, mixing pesticides with minced meat, enema administration into animals, or direct pesticide spraying on carcasses. Each methodology offers insights into how different exposure routes affect insect activity on decomposing bodies, ultimately aiding PMI estimation in forensic contexts.

One approach involves different concentrations of oral pesticide administration to different types of animals, which helps in understanding how ingestion influences decomposition and insect colonization (Abdul Rahim et al., 2024; Jales et al., 2021; Kianoush Ghiasvand et al., 2022; Musyaffa et al., 2021; Widyana et al., 2023). For instance, Abdul Rahim et al. (2024) observed a decrease in insect species richness in glyphosate- and chlorpyrifos-treated rabbit carcasses compared to control carcasses, where decomposition proceeded more quickly without pesticides.

Similarly, Jales et al. (2021) reported that organophosphate terbufos influenced insect succession patterns, larval dispersion, species development rates, and pupal mortality in Calliphoridae and *Sarcophaga* flies. Kianoush Ghiasvand et al. (2022) also observed that diazinon poisoning repelled necrophagous insects from cadavers, while Musyaffa et al., (2021) noted delayed decomposition in pyrethroid-treated bird cadavers, Pyrethroid insecticides also affect the growth and development of exposed decomposer insect larvae in the cadaver's body.

The second approach involves mixing pesticides with minced meat to study how contamination affects insect life cycles. These studies demonstrated that the organophosphate dimethoate extended the life cycle duration of Calliphoridae and *Sarcophaga* flies on the minced liver (Abd Al Galil et al., 2021a; Abd Al Galil et al., 2021b). Higher pesticide concentrations correlated with slower carrion fly development, resulting in prolonged PMI estimations.

A systematic review further supports these findings, analysing 21 studies focused on the relationship between pesticide exposure via oral administration and mixed meat

contamination and PMI determination (Widyana et al., 2023). This review covered various animals, including rabbits, pigs, dogs, birds, and hamsters, and documented how different types of pesticides impact the decomposition process and the development of necrophagous insects. Findings indicate that pesticide contamination can skew PMI estimations, as insect activity and decomposition rates are often altered by pesticide toxicity. While some pesticides accelerated decomposition, others slowed it, often stunting or halting fly development.

Enema administration has been used to investigate how pesticides affect internal tissues and insect colonization. Liu et al. (2009) administered diluted malathion to rabbits via enema at varying lethal doses. The results revealed prolonged larval and pupal stages in *Chrysomya megacephala*, with malathion residues in muscle and liver retarding normal growth rates. Shi et al. (2010) reported similar findings, with treated carcasses showing altered PMI estimates by 12–36 hours and a notable absence of *Chrysomya rufifacies* on treated carcasses, regardless of the pesticide concentration.

The fourth approach involves spraying pesticides on animal carcasses to simulate environmental contamination. Medeiros de Moura Eulalio et al. (2023) applied a commercial insecticide containing thiamethoxam to pig carcasses and found that decomposition phases were longer in treated cadavers than in control groups. The insecticide disrupted insect colonization and feeding patterns, with most eggs failing to hatch and larvae failing to mature. Additionally, contamination altered the abundance and composition of insect species, complicating PMI estimates.

## 2.8 Research Gaps and Rationale

The reviewed methodologies highlight a significant gap in understanding how environmental contamination with pesticides—specifically through spraying—affects cadaveric insects and PMI estimation. Pesticide spraying, common in agricultural settings, exposes surfaces and remains to uneven pesticide distributions, altering decomposition and insect behaviour in ways that challenge forensic investigations.

Most prior studies focus on direct ingestion of pesticides, such as poisoning cases, rather than on external contamination scenarios. This limits their applicability to real-world cases where deaths occur in pesticide-sprayed environments. Addressing this gap by studying pesticide spraying replicates environmental exposure patterns, providing critical insights into how residues affect insect colonisation and decomposition. Such research enhances the accuracy of PMI estimation in pesticide-contaminated settings.

This research investigates how glyphosate (herbicide) and chlorpyrifos (insecticide) contamination affect the lifecycle, development, and morphology of necrophagous flies on pork meat, with implications for forensic post-mortem interval (PMI) estimation and toxic substance detection. Given the widespread use of these pesticides, understanding their influence on forensic entomology is crucial. The study aims to bridge knowledge gaps by analysing how these chemicals impact insect growth and decomposition dynamics. Accurate PMI estimation is essential in forensic investigations, and contamination-induced alterations could lead to miscalculations. By refining forensic entomotoxicology methods and improving toxicological detection, this research enhances the reliability of forensic evidence in cases involving pesticide exposure.

# CHAPTER 3: METHODOLOGY

## 3.1 Material and Apparatus

For this study, materials and apparatus are divided into two categories: one for forensic entomology (life cycle observation) and another for forensic entomotoxicology (GC-FID analysis).

### 3.1.1 Life Cycle Observation

For each life cycle experiment, 1.5 kg of pork was obtained from a local supplier in Kota Bharu, Kelantan. The meat was divided into three portions of 500 g each: control sample (untreated meat), chlorpyrifos-treated sample (meat sprayed with chlorpyrifos), and glyphosate-treated sample (meat sprayed with glyphosate). Both pesticides, chlorpyrifos and glyphosate, were purchased from an online local shop. The chlorpyrifos used in this study is of the brand Zagro, while the glyphosate is of the brand Roundup.

The laboratory apparatus and equipment required for this study were procured from the Science Lab Management Unit (UPMS) at the School of Health Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan. The chemicals used included 65% ethanol, which was also provided by the Forensic Science Laboratory (MSF) at the same institution. Detailed lists of the chemicals, reagents, materials, equipment, and instruments used for this study are presented in Tables 3.1, 3.2, and 3.3.

Table 3.1 Chemicals and reagents used in this study

| No | Chemicals and reagents |
|---|---|
| 1 | Chlorpyrifos (Insecticide) |
| 2 | Ethanol (65%) |
| 3 | Glyphosate (Herbicide) |

Table 3.2 Material used in this study

| No | Material |
|---|---|
| 1 | Pork meat (500 grams per location) |

Table 3.3 Equipment and instruments used in this study

| No | Equipment |
|---|---|
| 1 | Latex gloves |
| 2 | Face mask |
| 3 | Falcon tube (15 mL & 50 mL) |
| 4 | Test tube racks |
| 5 | Spatula |
| 6 | Forceps |
| 7 | Measuring cylinders |
| 8 | Petri dish |
| 9 | Beakers |
| 10 | Plastic cups |
| 11 | Fabric mesh |
| 12 | Rubber bands |
| 13 | Stones / Bricks |
| 14 | Cable ties |
| 15 | Spray pump |
| 16 | Plastic tray |
| 17 | Reagent bottle |
| 18 | Labelling paper |
| 19 | Sands |
| 20 | Dropper |
| 21 | Pearl head pin |
| 22 | Styrofoam board |
| 23 | Cage |
| 24 | SMZ168 Stereo Zoom microscope |
| 25 | Hygrometer thermometer |
| 26 | Electronic analytical balance |
| 27 | Lab freezer |

### 3.1.2 Gas Chromatography with Flame Ionisation Detector (GC-FID) Analysis

The laboratory apparatus and equipment required for the GC-FID analysis were also requested and obtained from the Science Lab Management Unit (UPMS). Chemicals, reagents, and instruments were provided by the Analytical Laboratory and Forensic Science Laboratory (MSF) at the School of Health Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan. The detailed lists of chemicals, reagents, equipment, and instruments used for this part of the study are provided in Tables 3.4 and 3.5.

Table 3.4 Chemicals and reagents used in this study

| No | Chemicals and reagents |
|---|---|
| 1 | Chlorpyrifos (Insecticide) (Zagro, Zagro Chemicals Sdn. Bhd., Malaysia) |
| 2 | Methanol (65%) |
| 3 | Glyphosate (Herbicide) (Roundup, Monsanto (Malaysia) Sdn.Bhd., Malaysia) |
| 4 | Anhydrous magnesium sulphate (Merck KGaA, EMD Millipore Corporation, Germany) |

Table 3.5 Equipment and instruments used in this study

| No | Equipment |
|---|---|
| 1 | Latex gloves |
| 2 | Face mask |
| 3 | Falcon tube (15 mL) |
| 4 | Test tube racks |
| 5 | Spatula |
| 6 | Forceps |
| 7 | Glass pipette |
| 8 | Volumetric flask (100 mL) |
| 9 | Beakers |
| 10 | Scott bottle |
| 11 | Reagent bottle |
| 12 | Labelling paper |
| 13 | 2 mL GC vial (Agilent Technologies, Santa Clara, CA, USA) |
| 14 | Dropper |
| 15 | Glass wool |
| 16 | 0.45 µm PTFE syringe filter (Cronus, Cronus Technologies, United Kingdom) |
| 17 | Syringe (5 mL) |
| 18 | Centrifuge tube (2 mL) |
| 19 | 10 µL syringe (Agilent Technologies, Santa Clara, CA, USA) |
| 20 | Glass rod |
| 21 | Gas Chromatography with Flame Ionisation Detector (GC-FID) (Agilent 7890A, Agilent Technologies, Santa Clara, CA, USA) |
| 22 | Vortex mixer (EVM-6000 ERLA, ERLA Technologies (M) Sdn. Bhd., Malaysia) |
| 23 | Lab freezer |

### 3.2 Location and Study Site

The study was conducted in Kelantan, Malaysia, a state located in the northeastern corner of Peninsular Malaysia. The specific study site was within the Health Campus of Universiti Sains Malaysia (USM), located in Kubang Kerian, Kelantan. The research activities took place in the compound of the School of Health Sciences (PPSK) building. The geographical coordinates of the study site are 6.1006° N, 102.2851° E, and the location is shown in both the default Google Maps view and satellite view in Figure 3.1. Three distinct locations within the PPSK building compound were chosen to place the pork meat samples for the forensic entomology study.

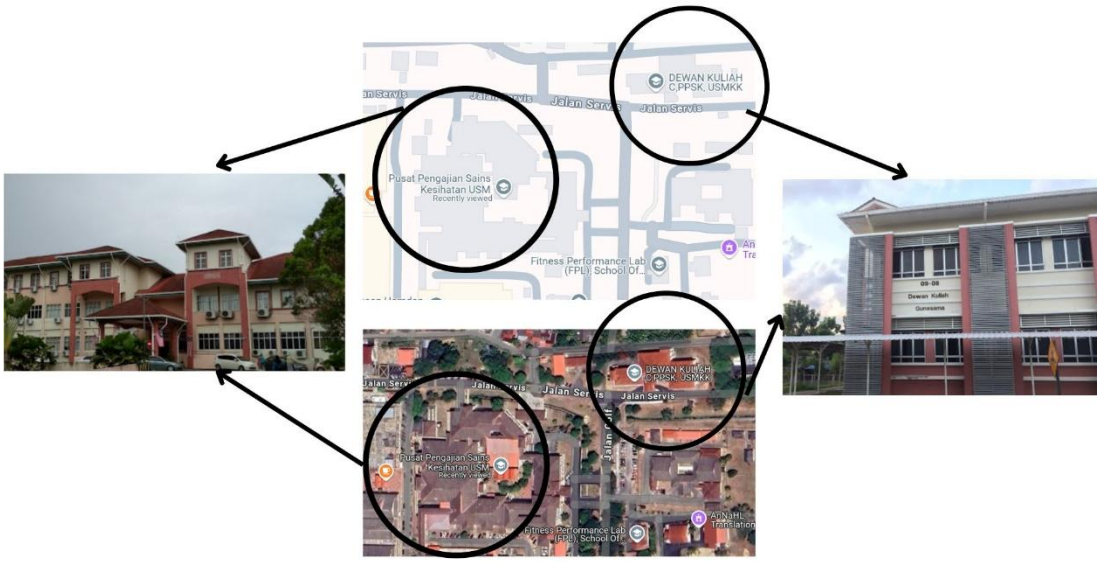


Figure 3.1 PPSK was shown in both the default Google Maps view and satellite view

Location 1 was situated in the basement car parking area under the front entrance ramp of the PPSK building. This location was designated for the control sample, which consisted of pork meat that was not sprayed with any pesticide. The setup and placement of Location 1 are shown in Figure 3.2.

Figure 3.2 Control sample situated in the basement car parking area under the front entrance ramp of the PPSK building