Forensic Entomotoxicology: The Effects of Drugs on the Life Cycle and Succession of Forensically Important Insects

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Abstract

Forensic entomotoxicology is a rising branch of forensic science that is utilized with the intent of adapting current methodologies to reflect the effects of drugs more accurately on forensically important insects' life cycles and succession patterns. This branch of forensic science is often used for post-mortem interval estimation and, as such, has a large impact on the course of a criminal investigation. It is also utilized to determine toxins in corpses that are further along in decomposition or lacking enough tissue to run a toxicological analysis.

Several studies have been conducted to determine how certain drugs will affect the life cycle and insect succession. However, many inconsistencies arise from the lack of replicated data, which arises from the lack of replicated methodologies. Despite this, the information thus far gathered has some useful in-field applications.

Forensic Entomotoxicology: The Effects of Drugs on the Life Cycle and Succession of Forensically Important Insects

Throughout history, numerous methods have been utilized in order to generate information or evidence to aid criminal investigations. Entomology, the study of insects, is one such method. Applied forensically, entomology has many potential applications that allow for a better understanding in investigations and criminal cases.

Forensic Entomology

Forensic entomology is a branch of entomology in which insects are used in a variety of ways in order to help with legal matters (Amendt et al., 2011). For example, insects can be used to identify pollutants, proof of contaminated foodstuffs, or to help with the determination of the post-mortem interval (Voss et al., 2009). While all of these are important aspects of forensic entomology, emphasis will be placed on the post-mortem interval (PMI) since it relies heavily on insect development and succession.

Not all insects are of forensic importance. The type of case being investigated will dictate which are most useful. For instance, sawtooth beetles may get into and contaminate food products like rice (Hashem et al., 2021), whereas decreased numbers of native insects in an area may indicate pollution (Hodecek, 2020). Additionally, flies and certain types of beetles are most useful in death cases, as these are the insects that are attracted to decomposing corpses (Chophi et al., 2019). However, most of these species are extensively studied to help forensic entomologists determine the post-mortem interval (PMI).

Post-Mortem Interval

The PMI is the amount of time elapsed since death. Factors such as body temperature, rigor mortis, and bloating could help determine this. However, insects are an extremely useful tool, especially if the corpse is beyond 72 hours since death (Substance Abuse and Mental Health Services Administration, 2023). This PMI estimate is performed through two primary methods: insect development and insect succession rates.

Insect Development and Succession

Insect development involves known cycles of the growth of specific insects based primarily on temperature and species (Voss et al., 2009). In death cases, the development rates of Diptera (flies) are beneficial since flies are typically some of the first, if not the first, insects to arrive at a corpse. Insect succession is the pattern through which insects will arrive at a decomposing corpse. In death cases flies will arrive, typically the Calliphoridae first followed by other species, and lay eggs (Alifia et al., 2020). Occasionally, beetles will come in the early stages of decay to feed on fly larvae (Thümmel et al., 2023). By the dry stage, most larvae have grown into adult flies and beetles will become the dominant insects. However, there are several factors that could contribute to changes in established patterns within these insects.

As previously mentioned, the temperature is a large factor when dealing with insects. Many have a specific temperature minimum and maximum at which they will be active to lay eggs and at which they will grow. In addition to this, weather events, pH, amount of sunlight, geographical location, and condition of the remains are all factors that could increase or decrease the time for an insect to progress through its lifetime or the insect succession pattern (Ngoen-klan et al., 2011). Another factor currently under consideration is the effect of various drugs.

Forensic Entomotoxicology

One important branch of forensic entomology is entomotoxicology. This branch is defined as "[the study of] the application of toxicological analysis to carrion-feeding insects in order to identify drugs and toxins present on intoxicated tissues" as well as "the effects caused by such substances on arthropod development in order to assist the forensic PMI estimates" (Introna et al., 2001, p.42). Insects that feed on a corpse determine what toxins were within that corpse or determine if those toxins affect insect development, thus affecting PMI estimation.

There are several different approaches to determining what will be used for toxicological analysis including gas chromatography-mass spectrometry (GC-MS), immunoassays, and liquid chromatography-mass spectrometry (LC-MS) which are all quick methods to see if further testing should be performed (Chophi et al., 2019). However, it is important to remember that entomotoxicology is still a widely unresearched concept. Many aspects of entomotoxicology are not yet known such as exactly what certain toxins do in different species of insects, how the metabolism of those species affects the digestion of the toxins, and how to determine the concentration of toxin that was in the contaminated food source.

In order to continue improving the current understanding of PMI, more research will have to be done to see in what way this affects insect development and succession. One of the primary pieces of information that current entomotoxicology research is looking for is the specific effects of certain drugs or toxins on forensically important insects (Hodecek, 2020). This branch of entomology is widely understudied, and information currently varies heavily due to the lack of research in this area.

Current Research

Painkillers

Methadone

Methadone is a medication used to treat Opioid Use Disorder (OUD). It blocks the effects of opioids and is also used for pain management (Substance Abuse and Mental Health Services Administration, 2023). Although methadone overdose-related deaths have declined since 2019, more people were given access to the medication during the pandemic to reduce person-to-person contact, so that patients could take it at home rather than going to a clinic (National Institute on Drug Abuse, 2022b). Results from an experiment were published in 2011 regarding the determination and effect of this medication on *Lucilia sericata* also known as the green bottle fly. This was performed due to the fact that methadone has been used as a substitute for opioid addicts in Belgium and an increase in fatal intoxications had occurred with a rise in prescriptions (Gosselin et al., 2011).

The study using methadone and *Lucilia sericata* focused on two aspects. The first portion of the experiment focused on a method of using the puparia casings to tell if methadone was present (Gosselin et al., 2011). The second portion of the study focused on the effect of methadone on the *Lucilia sericata* with respect to PMI estimate. For the first aspect, puparia casings from the five concentrations of methadone-treated meat (0.0 μg/g, 0.2 μg/g, 0.4 μg/g, 0.8 μg/g, and 4 μg/g) were run through the ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) method. For the second, characteristics were noted as to the effect of each concentration as the flies developed. All of this was done at a constant temperature of 25 ℃ and in a laboratory, thus eliminating any effects due to weather.

Results from the first aspect showed that only at the higher concentrations of methadone (0.8 μg/g and 4 μg/g) did the drug accumulate in the puparia casings (Gosselin et al., 2011). This leads to the conclusion that if methadone is found in a puparia casing near the body, it could be a contributor to death. However, it is not so indicative as to say that if methadone is not found in puparia casings, methadone was not a contributor to death. Additionally, the study found that the amount of methadone found in the puparia casings was 60 times lower than in the third instar larvae.

The results from the second aspect showed several different pieces of information. First, it was not found that the methadone treatments biased fly growth towards one sex, which was different from previously reported data (Gosselin et al., 2011). While there was a difference in the number of blowflies that emerged on meat treated with varying concentrations of methadone, there was not a higher mortality at higher concentrations. For instance, at 0.0 μg/g methadone, about 72.8% of adult blowflies emerged but at 4 μg/g methadone, 86.4% of adults emerged. The development rate of the fly was statistically significant and showed a slightly decelerated rate at higher levels of the medication, which would affect PMI estimation until after pupation. From this, it was determined that if PMI estimation was to be done after the emergence of blowfly adults, no alterations should be made in PMI estimation when the known minimum development time of *Lucilia sericata* is used (Gosselin et al., 2011).

Tramadol

Tramadol is a medication used to help relieve pain (moderate-moderately severe; National Library of Medicine, 2022). Typically, this medication does not have a high potential for abuse (Green et al., 2021) however there is always the possibility of potential addiction. A

survey done in 2020 found that 1.6-1.8 million Americans reported misuse of the medication which is approximately 4% of the total prescriptions written (Reines et al., 2020). However, it has been suggested that when taken in combination with excess drugs that depress the central nervous system, it could be a large contributor to lethal intoxication. An experiment in 2011 was performed to determine the effects of tramadol on *Lucilia sericata* (El-Samad et al., 2011).

This study on tramadol focused both on the detection of the drug in the larvae and its effect on the development of the larvae (El-Samad et al., 2011). One control and three different concentrations of tramadol (0, 550, 1100, and 2200 mg kg^{-1}) were fed to rabbits of about the same weight which were then sacrificed and dissected. Female adult *Lucilia sericata* were placed on the meat to lay eggs. At regular time intervals (6-12 hours), random larvae were sampled and taken for toxicological analysis by high-performance liquid chromatography (HPLC).

Results from this experiment showed the control rabbit was uncontaminated and that the tissues in the second rabbit were 1.5 times higher in tramadol concentration than the first rabbit (El-Samad et al., 2011). The third rabbit's tissue was 1.2 times higher than rabbit two. From the tissue tests, it was found that the highest concentrations of tramadol for lethal and double lethal doses were found in the gastric content (124 mg kg^{-1} for rabbit three) and the lowest in the muscles (48.7 mg kg^{-1} for rabbit three). Thus, gastric content would likely be the most helpful in the determination of the time of administration and to determine if the drugs were taken orally or not. HPLC analysis showed a significantly lower concentration of tramadol in the feeding third instar of *L. sericata* than in the rabbit tissues. It was also shown that overall, the presence of tramadol significantly increased the mean minimal development times of the insects (Figure 1). This would have the ability to change PMI estimation. However, it also was mentioned that

larvae still feeding on the body would be the best to use in the determination of the concentration of toxicological data especially for bodies that were decomposed or in cases with an absence of body tissue or fluids.

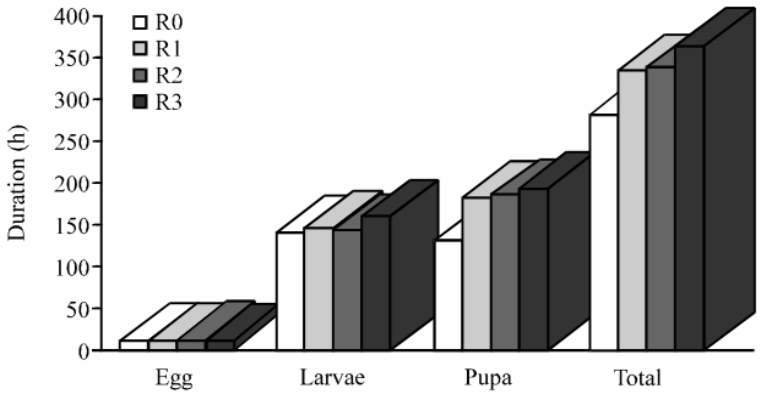

Figure 1. The duration of each stage of an insect's life cycle and R0 (0 mg kg^{-}1), R1 (550 mg kg^{-}1), R2 (1100 mg kg^{-}1), and R3 (2200 mg kg^{-}1; El-Samad et al., 2011, p. 358).

Paracetamol

Paracetamol (also known as acetaminophen) is a drug used to treat pain and fevers. It is extremely common (Tylenol is the name brand) and widely used across the U.S. as well as all over the world (Encyclopedia Britannica, 2023). A study performed in 2004 looked at the effect of paracetamol on *Calliphora vicina* larval development (O'Brien & Turner, 2004).

This study used paracetamol-treated liver to rear the insects (O'Brien & Turner, 2004). Five different concentrations of paracetamol were utilized, 0 mg/kg, 100 mg/kg, 250 mg/kg, 500 mg/kg, and 1000 mg/kg. These concentrations were specifically selected on the premise of spanning the known human fatality overdose concentrations. *Calliphora vicina* eggs were placed on the liver mixture and reared at 20°C. The day the larvae emerged was identified as day one.

Everyday 10 larvae were taken from each of the five samples, dunked in distilled water, dried, and weighed before being placed back onto the liver then repeated twice more for a total of 30 measurements for each concentration.

Results found that larvae that fed on paracetamol-treated samples did not differ significantly from each other, but all were significantly different from the control (O'Brien & Turner, 2004). Because of this, the data were plotted on a graph simply as paracetamol treated and the control (Figure 2). This graph showed that, based on differences in weight, during days 2-4 there is the possibility of a 12-hour difference in PMI estimation. However, overall, it does not appear to have a drastic effect on the growth rates of *Calliphora vicina*.

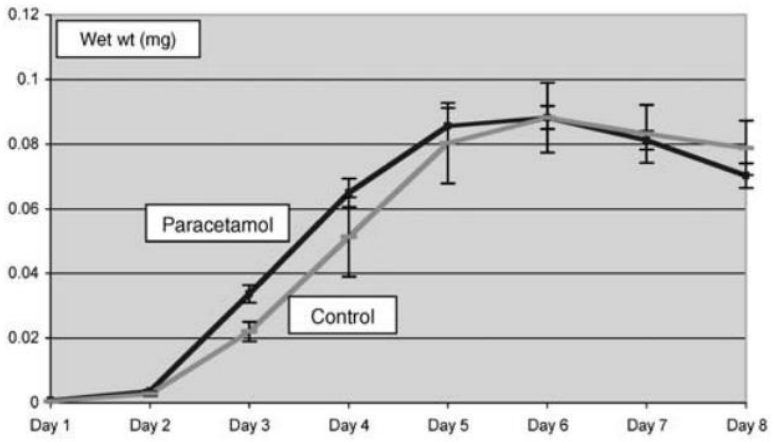

Figure 2. The average weight of the paracetamol-treated larvae in comparison with the control sample (O'Brien & Turner, 2004, p.189).

Hard Drugs

Heroin

Heroin is an opioid drug and a derivative of morphine. Heroin is extremely addictive and operates by binding to opioid receptors on the brain (National Institute on Drug Abuse, 2022a).

In 2020, in the United States alone, approximately 13,165 people died of a heroin overdose (National Institute on Drug Abuse, 2018). To investigate the impact of this drug on insect succession and carcass decomposition, a research study was performed utilizing rabbit carcasses in two different seasons (Al-Qahtni et al., 2020).

This study was done in Riyadh, Saudi Arabia which means the summer had desert-like conditions, but the winter was relatively cold (Al-Qahtni et al., 2020). Each season 12 rabbits weighing between 2.0 and 2.4 kg were used and treated in triplicates of four concentrations (0 mg, 6 mg, 12 mg, and 18 mg). An hour after the administration of the drugs, the rabbits were sacrificed and moved outside into iron cages to prevent animal scavengers and limit exposure to shade. Two traps containing water, soap, and salt were placed next to each carcass, and samples were taken at 9:00 and 5:00 every day.

Results from this experiment showed a variety of data (Al-Qahtni et al., 2020). First, the carcasses in the winter took longer to reach the dry stage than those in the summer. In the winter, carcasses treated with 18 mg of heroin took a day longer to reach the dry stage than other samples and in the summer the control carcasses entered the dry stage a day after the other carcasses. Second was the number of insects collected. During the summer period 1,161 insects were collected and in the winter 5,545 were collected. Three orders made up these insects: Diptera (flies), Coleoptera (beetles), and Hymenoptera (specifically ants in this case). The most common family of insects in both seasons was Muscidae (60.41% in winter, 54.69% in summer) followed by Calliphoridae (29.23% in winter and 16.71% in summer). Beetles were found to be the least common order in both seasons.

Additionally, Sepsidae and Milichiidae, which are two families in the order Diptera, were found only in the summer whereas three families from the Coleoptera order, Melyridae, Carabidae, and Coccinellidae were absent in the summer (Al-Qahtni et al., 2020). The Formicidae family of ants was more common in the summer than in the winter. In the winter, days 1-5 showed small numbers of insects, however from that point till days 8-11 (depending upon the treatment) there were rapid increases. Alternatively, in the summer, days 1-2 showed the least number of insects reaching a peak between days 5-6. In the winter 30 species of insects were identified and only 22 in the summer.

Overall, these results suggest that three factors play a major influence on the number of insects: season, heroin concentration, and stage of decomposition (Al-Qahtni et al., 2020). Because of this, it is implied that it will be important to consider all factors, including the effect of drugs, when trying to determine PMI from insect succession since there is some variation between seasons as well.

Cocaine

Cocaine is a potent central nervous system stimulant that is widely abused, especially in North and South America. This drug is quick acting when inhaled and can be fatal with just 20-30 mg in the nasal passages. As with many drugs, the more it is habitually abused, the more tolerant a person can become to it, although overall tolerance varies by person. This makes it an ideal drug to test for effects on forensically important insects and in 2011 it was tested on *Chrysomya albiceps* and *Chrysomya putoria* (de Carvalho et al., 2012).

Pure cocaine was used and given to rabbits in a lethal dose (de Carvalho et al., 2012). After administration of the drug, 30 minutes elapsed before control rabbits and any treated

rabbits that were not already dead were sacrificed. The livers were removed and larvae (first instar) from both *C. albiceps* and *C. putoria* were placed onto them. Larvae were left on the liver samples for 54 hours and sampling was done every six hours to gather data on average weights and mortality. After that, the flies were raised on a specific diet (beer yeast, casein, powdered milk, methylparaben, water, and agar) until they were adults, and their life cycle was timed.

Results from this research showed that at 6 hours and 18 hours after exposure, *C. albiceps* were developing significantly faster than the control and *C. putoria* significantly slower (de Carvalho et al., 2012). However, by 24 hours, the *C. albiceps* were significantly less developed than the control and there was no significant difference in the *C. putoria*. By the 54-hour mark, both species on treated samples had grown significantly more than the controls. During pupation, an approximately 13-hour difference was found between the treated and control sample (Figure 3). Additionally, it was determined that the control samples for both species had higher mortality rates except for the unemerged adult mortality of *C. albiceps*. Overall, this research demonstrates the potential possibility of incorrect PMI estimations.

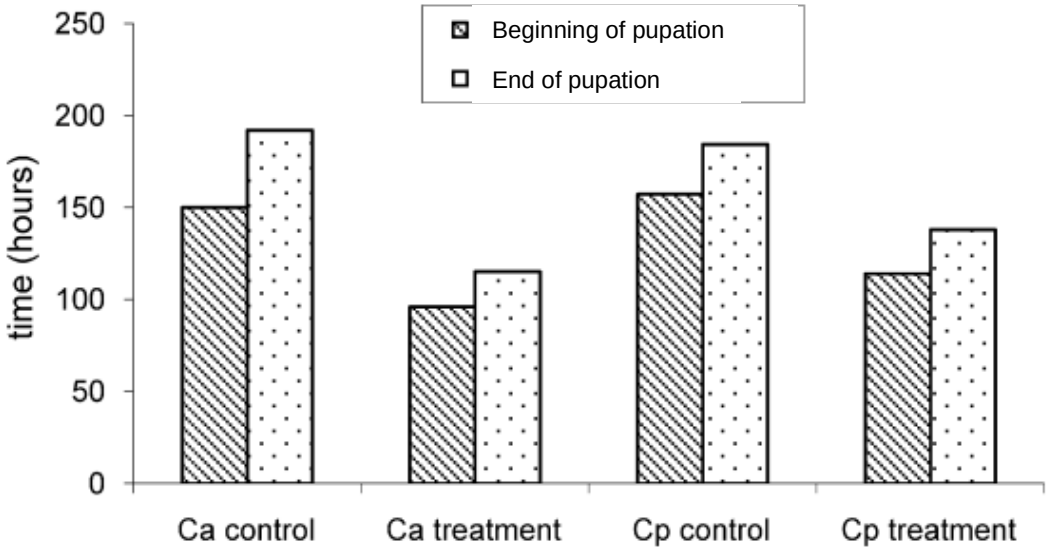

Figure 3. The time (in hours) it took larvae to begin and end pupariation. Ca is *C. albiceps* and Cp is *C. putoria* (de Carvalho et al., 2012, p. 29).

Antidepressants

Fluoxetine

Fluoxetine is a drug used for the treatment of major depressive disorder as well as other disorders such as obsessive-compulsive disorder and panic disorder (Sohel et al., 2022). This drug works by blocking the reuptake of serotonin, an amine known to impact depression when in small concentrations (Zanetti et al., 2019). While it is rarely deadly when taken in overdose alone, in combination with alcohol could be lethal (Sohel et al., 2022). Since antidepressants are among the "most widely used chemical agents in deliberate self-poisonings" (Zanetti et al., 2019, p. 1), they make the detection of this drug important.

Research was performed on the detection of fluoxetine in *Lucilia sericata* and *Sarcophaga crassipalpis* (Zanetti et al., 2019). This research was particularly focused on the detection and quantification of the drug in the flies rather than fluoxetine's effect on the

developmental aspects of the insects. Pig muscle was used as the medium with some samples treated with a lethal dose of the drug and others left untreated as a control. Then 60 *L. sericata* and 47 *S. crassipalpis* third instar larvae were reared on the samples. Ten of each were removed after 24 hours and frozen. Then 10 *L. sericata* and 9 *S. carassipalpis* pupae were removed after 6 and 8 days respectively. Another 16 *L. sericata* puparia were removed on day 13. All the samples were weighed and ground with water before being run through UV spectrometry at wavelengths of 270 and 277 nm.

Results found that it was possible to determine the presence of fluoxetine in both the larvae and pupae of both species (Zanetti et al., 2019). It was also found that the concentrations in the larvae, pupae, and puparia of *L. sericata* were not significantly different from each other. This same result was found in *S. carassipalpis* although there was a significant decrease between larvae and pupae of this species. The researchers mentioned the need for additional testing in order to confidently conclude these results.

Amitriptyline

Amitriptyline has been approved to treat depression. It works by blocking the reuptake of norepinephrine or serotonin which allows for more of these substances to remain working in the brain (Thour & Marwaha, 2022). This study utilized amitriptyline since it stated that antidepressants are one of the most commonly used drugs in the United Kingdom and that there are occasions that a long period of time could elapse between the death of people and when their bodies are found due to overdose or suicide in isolated places (Alifia et al., 2020). Insects that had been feeding on the decomposing body may be useful in the determination of toxins and PMI.

This experiment was performed using rats who were treated with one of four doses of amitriptyline (0 mg, 75 mg, 100 mg, and 125 mg; Alifia et al., 2020) The subjects then had an incision made from neck to anus in order to promote flies laying eggs on the carcasses faster before being placed in the study area. In total, 24 larvae were assessed every day and the temperatures and humidity were recorded.

Results from this experiment found that for the control, 75 mg sample, and 100 mg sample, larvae were observed on the second day (Alifia et al., 2020). For the 125 mg sample, larvae were not observed until the fourth day in the afternoon. The control sample showed an average maximum length of 16.33 mm on the fourth day in the morning and was a pupa at the same time on the fifth day. The 75 mg treated sample did not see a maximum length reached until the fifth day in the morning (maximum length of 17.33 mm) and were pupae at the same time on the sixth day. The 100 mg treated sample had a maximum length of 14.00 mm which was reached on the sixth day in the morning but took two more days to become pupae. The last group, the 125 mg treated sample, reached a maximum length of 17.33 mm on the seventh morning and did not become pupae until the morning of the tenth day.

Overall, some of the biggest impacts mentioned by the researchers were that there is potential at high doses of amitriptyline to possibly be some sort of indicator (like a smell) that makes the decomposing tissue an insufficient food source for the fly and thus caused a delay in the onset of oviposition (Alifia et al., 2020). Additionally, since the larval lengths were found to be significantly different, they could aid in detecting potential errors in PMI determinations, especially since the humidity and temperature data that was also recorded did not appear to have any large effect on the insect's growth in this case.

Antibiotics

Ceftriaxone

In 2011 an estimated 70 billion prescriptions for antibiotics, in general, were administered across the globe (Preußer et al., 2021). Ceftriaxone is an antibiotic used to treat infections like gonorrhea and meningitis (National Library of Medicine, 2016). Since this medication has a high potency and wide spectrum of activity, it is one of the most commonly used antibiotics (Ayele et al., 2018). Thus, it is important to know what effects ceftriaxone will have on forensically important insects.

A research experiment was done on the effects of both ceftriaxone and levofloxacin on the growth of *Calliphora vomitoria* (Preußer et al., 2021). Specifically for ceftriaxone, the samples were prepared on the assumption that antibiotics have a half-life of 8 hours. The regular prescribed doses of this medication are 2 g per 70 kg body weight. In order to simulate this, the amount of ceftriaxone in the samples was 0 μg, 714 μg, 357 μg, and 179 μg. These drug concentrations were minced with meat. First instar larvae (30 for each sample) were placed on the four samples and checked for the first time 48 hours after placement and every 24 hours following.

Results from this experiment found that like the control, larvae reached their maximum average length for all samples on the third day (Preußer et al., 2021). Day three was also the date that all samples reached maximum weight which was found to directly correlate with length. However, there was a significant delay in larval development found in all samples treated with this antibiotic. Thus, while it did not seem to impact weight and length, it did impact the time it took for larvae to develop.

Levofloxacin

Levofloxacin is an antibiotic typically used for pneumonia and skin infections (National Library of Medicine, 2019). While levofloxacin has a low potential for acute toxicity, it can be overdosed on (Podder & Sadiq, 2022). As mentioned previously, since antibiotics are widely used, it is important to know their exact effects on forensically important insects. A study (previously mentioned in ceftriaxone) looked at this medication's effect on C. *vomitoria* (Preußer et al., 2021).

This study found that the higher the concentration of levofloxacin in the sample, the longer it took the samples to reach their maximum length and weight (Preußer et al., 2021). However, there was no real pattern in terms of development. The highest concentration of levofloxacin caused a 24-hour difference from the control whereas the other two concentrations caused a 12-hour difference. Overall, it was determined that this could potentially cause a PMI estimation error.

Ciprofloxacin

Ciprofloxacin is an antibiotic that is commonly used to treat gonorrhea and typhoid fever and is typically prescribed when nothing else will work (National Library of Medicine, 2021). Its widespread use is to treat gram-positive and gram-negative sensitive microorganisms (Ferraz et al., 2014). This is why the medication was used to see its effects on *Chrysomya putoria* (the Old-World screwworm fly).

To perform this experiment, *C. putoria* first instar larvae were placed onto different samples of chicken gizzard homogenate which had various concentrations of ciprofloxacin (Ferraz et al., 2014). The first sample (T1) had a concentration of 3.33 μg/mL which would have

been a value between the oral and intravenous concentrations. The second sample (T2) had a concentration of 6.66 μg/mL. The third sample (T3) had a concentration of 13.33 μg/mL. Each of the three samples and a control had four replicates with 40 larvae on each replicate. The larvae were kept at a constant temperature and humidity. Observations of the larvae were made one time each day.

The results found that there was no significant difference between any of the treatments and the average larvae weight (Ferraz et al., 2014). The researchers also showed that there was no significant difference in the time it took the larvae overall to develop. When broken down into stages (duration until abandonment, larval stage, and pupal stage) there was still no significant difference to be found with any of the samples. However, despite it not being statistically significant, the raw data did show that the control larvae did on average have larger minimum weights than treated larvae and the treated larvae decreased in minimum weight with increasing concentrations of ciprofloxacin.

Sedatives

Diazepam

Diazepam is a benzodiazepine medication that can be used to treat anxiety and muscle spasms and is also used as a sedative in the ICU (Dhaliwal et al., 2022). There is a potential for overdose due to diazepam, however, overdose is most common in conjunction with other medications, such as other central nervous system depressants. This drug also has the potential for dependence/addiction in long-term use or for those who take large doses of the medication. In the research study, this compound was used to look at the effect on necrophagous flies in Brazil (Carvalho et al., 2001).

The study was performed through the use of male rabbits (Carvalho et al., 2001). The rabbits were given twice the lethal concentration (50 mg) of diazepam that had been diluted in a 2.5 mL saline solution. This solution was injected into the rabbits' ear veins and the five replicates along with the control rabbit were mechanically sacrificed. First instar larvae of *C. putoria* and *C. albiceps* were exposed to the rabbit livers of the samples. Every six hours, up to 54 hours, larvae were randomly selected and tested for each sample. After 54 hours, the larvae were removed from the liver and placed on an artificial diet to finish their life cycle and to keep a record of how long this process took. Samples taken were also run through the GC-MS in order to test for identification of the toxin.

Results showed that from 18 hours on, larvae feeding on treated samples were growing at a faster rate than those on the control for both species (Carvalho et al., 2001). Additionally, a similar pattern arose with the weight and length of the larvae with the control larvae being longer and heavier at 6 hours. By 18 hours the treated sample larvae were longer and weighed more. Not only was the larval development stage accelerated on diazepam-treated samples, but the pupariation stage was accelerated as well in both species. The study concluded that between 18 and 54 hours of larval development, there could be a significant error if diazepam was not considered.

Nordiazepam

Nordiazepam is a benzodiazepine (the main metabolite of diazepam) that is used for anxiety and sleep disorders. The study that utilized this medication looked specifically at the development and growth of post-feeding larvae as well as the ability to discern concentrations of the medication through LC-MS/MS (Pien et al., 2004).

Larvae of *Calliphora vicina* were reared on beef heart spiked with four concentrations of nordiazepam (0 μg/g, 0.5 μg/g, 1 μg/g, and 2 μg/g; Pien et al., 2004). For each concentration, 30 larvae per day were harvested, weighed, then boiled for preservation on days 4-8 of development. Another 30 were collected and frozen to run through the LC-MS/MS.

Results found that the higher the concentration of nordiazepam in the diet, the more nordiazepam was found in the insect, with the amount detectable highest on day four and a decrease occurring from that point on (Pien et al., 2004). Both nordiazepam and its metabolite (oxazepam) were detectable in empty puparia casings as well which suggested a possible metabolism and bioaccumulation of the substance rather than excretion of it which has potential uses in the determination of toxicological information.

Ketamine

Ketamine is a medication commonly used for anesthesia. It can be used alone or in conjunction with other medications and is best used for short-term medical procedures (Rosenbaum et al., 2022). It is "an NMDA receptor antagonist and a derivative of phencyclidine (PCP)" (Lü et al., 2014, p. 991). Due to an increase in deaths because of ketamine in China, researchers looked at its effect on the forensically important fly *Chrysomya megacephala* (Lü et al., 2014).

For this experiment, four concentrations of ketamine were used; 0 μg/g (control), 25 μg/g (half the lethal dose), 50 μg/g (the lethal dose), and 100 μg/g (double the lethal dose; Lü et al., 2014). *C. megacephala* eggs were placed directly onto artificial diets treated with the concentrations of ketamine. This process was done three times at constant temperatures of 32,

28, and 24 °C. While the insects were observed every hour, samples were taken every 12 hours until pupation.

Results showed that at 32°C larvae feeding on ketamine-treated samples were on average significantly shorter than the control from 16-54 hours post-hatching (Lü et al., 2014). At 28 and 24°C the average length of larvae on the ketamine-treated samples was still significantly smaller than the control but the exact hours in which this occurred varied. At 28°C this change was discernable from 16-40 hours and at 24°C was discernable from 16-76 hours. This same result was shown with average weight. At 32°C, treated insects weighed significantly less than the control from 16-52 hours and 16-64 hours at both 28°C and 24°C. However, while it did make the colonies weigh significantly less than the control, there was no significant difference found when comparing the treated colonies to each other. Overall, it was determined that the duration of development for treated samples was significantly longer than that of the control in the larvae and prepupal stage (with the exception of 32°C), however, the pupal stage of treated insects was slightly shorter (Figures 4-6).

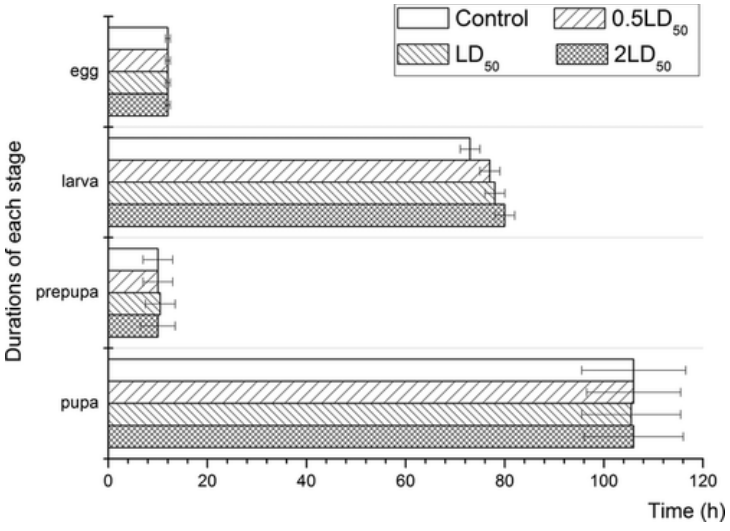

Figure 4. Duration of each stage of *C. megacephala* life cycle at 32°C (Lü et al., 2014, p. 995).

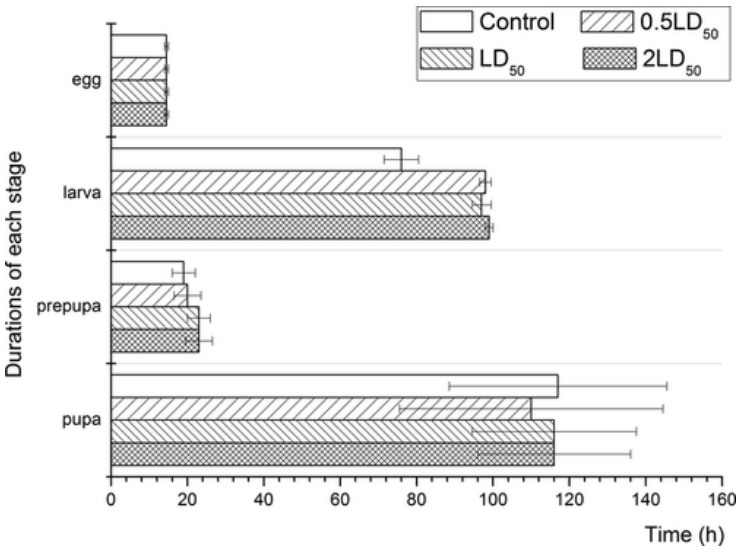

Figure 5. Duration of each stage of *C. megacephala* life cycle at 28℃ (Lü et al., 2014, p. 995).

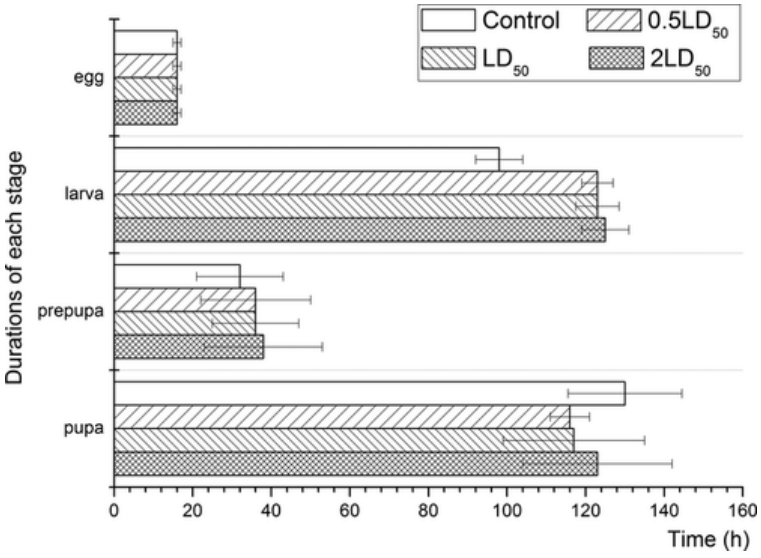

Figure 6. Duration of each stage of *C. megacephala* life cycle at 24℃ (Lü et al., 2014, p. 995).

Zolpidem Tartrate

Zolpidem tartrate is a sedative-hypnotic compound that is typically prescribed for anxiety or sleep disorders (Al-Shuraym et al., 2021). It has been estimated that in 2020, 14,272,253 prescriptions of the drug were issued. As it has been linked to use for suicide, it is important to

know its effects on forensically important insects. A research study looked specifically at its effects on the development of *Chrysomya megacephala* and *Chrysomya saffranea*.

To accomplish this, 0.05 mL, 0.1 mL, 0.15 mL, and 0.2 mL diluted solution of Zolpidem was combined with 50 g of buffalo liver tissue along with a control sample (Al-Shuraym et al., 2021). This created samples of 0, 1, 2, 3, and 4 ppm concentration. On each treatment, 60 first instar larvae from a lab-reared colony were placed. The development was observed and recorded alongside data regarding weight, length, temperature, and humidity. This was done separately for both *C. megacephala* and *C. saffranea*.

Results for *C. megacephala* showed that there was a significant difference between the amount of time it took treated colonies to develop as compared with the control (Al-Shuraym et al., 2021). The largest difference was the highest concentration of zolpidem (4ppm) which took 346.23 hours as compared to the control which took 244.44 hours (Figure 7a). Additionally, the weight, length, and width of the insects significantly decreased as the concentration of zolpidem increased. For *C. saffranea* similar results were obtained with a significant increase in time when the treatments were compared to the control. The largest difference in time of development was only 310.33 hours (at 4 ppm) as compared to 223.93 hours (control; Figure 7b). The weight, length, and width followed the same pattern as *C. megacephala*, significantly decreasing as zolpidem concentration increased.

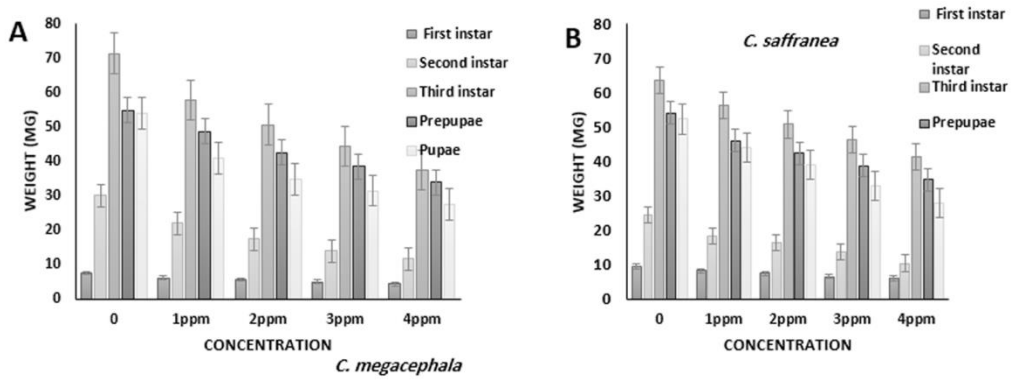

Figure 7. The effect of zolpidem concentration on the weight of larval species at different stages in their life cycle A) *C. megacephala* B) *C. saffranea* (Al-Shuraym et al., 2021, p. 2104)

Discussion

As can be seen from the aforementioned data, there are practical and useful results to be found with research of this nature, for example, the exact time alterations that would need to be made in PMI estimations. However, there are a few major limitations with this research including variables used and comparability. Future research is most assuredly required to better understand this topic.

To start, one of the most identifiable variables used was a difference in the species of forensically important insect used. For instance, the study on methadone used *Lucilia sericata* (Gosselin et al., 2011) whereas the study on paracetamol (which is also a painkiller) utilized *Calliphora vicina* (O'Brien & Turner, 2004). Because of this, the results can be compared in the sense that methadone did affect PMI until pupation (Gosselin et al., 2011) and paracetamol did not show any significant difference, but it cannot be concluded that either of these results holds true in all cases. Thus, further research would need to be done using one type of drug on multiple species or with multiple drugs on a singular species.

Another factor to consider is the types of drugs being tested. Since there is limited research on this topic, there is a broad spectrum of substances tested but even more, have not yet been. For instance, some painkillers and sedatives have been tested but not all, or even the most common drugs, have been tested. It may be beneficial to consider which drugs will be the most commonly used (which could differ in various regions) and use this preliminary research to determine which drugs would be used for testing in this field. With a wide variety of drugs available, it may not be feasible to test for every individual drug. As such, there may still be medicinal effects on the insects that are not yet understood.

Temperature is an additional aspect to consider. Many of the experiments were done at a constant temperature in a laboratory setting such as the methadone study (Gosselin et al., 2011). However, the ketamine study (Lü et al., 2014) and the heroin study (Al-Qahtni et al., 2020) both had varying temperatures which led to the results from the studies showing different results at different temperatures.

Even broader than temperature is the methodology in general used. Since a variety of methods are used, the differences this could create between research studies are still unknown. For example, in the heroin study, rabbits were fed heroin and sacrificed an hour later (Al-Qahtni et al., 2020). However, in the methadone study, beef heart was merely spiked with the concentrations (Gosselin et al., 2011). So, if the same medication and fly species had been studied, the methods could have produced different results based on the digestion effects of the rabbit versus simply treating meat with the drug.

With so many variabilities, In 2020, Bhardwaj et al., suggested that there “is a need to carry out more studies, as the statistically relevant constants are variables… can draw more

conclusive results in future work and easily extrapolate the findings to casework" (p.50). In 2022, Ghlasvand et al., suggested similar future research with an emphasis on the different geographical regions since this will affect the species of insect and other factors that contribute largely to results.

Conclusion

Overall, it has been shown that insects are beneficial in determining PMI as well as finding if toxins were within the substance they had been feeding on, but there are factors that influence the accuracy such as the drugs used, temperature, location, and type of insects. The results shown from the current research demonstrate a wide variety of findings with many limitations. More research is needed to fill in the gaps and help increase the reliability of PMI estimation and toxicology data based on insects.

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