Review article:
Forensic Review of Entomology Toxicology: The Use of Insects for Toxin Detection in The Case of Human Death

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Abstract:
Determining the cause of death is an important factor in the forensic investigation process. Some aspects of death will be difficult to find if the condition of the body has suffered severe damage when found. Corpses due to drug abuse found in hidden places are often difficult to know with certainty the cause of death. Insects that eat the corpse tissue, then xenobiotics such as drugs and other toxic substances will be transferred to the larval body’s metabolic system so that it is easily detected with sophisticated equipment. Techniques such as immunoassays, High-Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectrometry (LC-MS) and Gas Chromatography-Mass Spectrometry (GC-MS) have been used routinely to detect xenobiotics from the body, larvae, and insects. The study of insects as alternative toxicological samples is known as forensic entomotoxicology

Keywords: Calliphoridae, Dermestidae, Drug use, Pesticide, Suicide

INTRODUCTION

Forensic investigation
One of the most important factors in investigating crimes of death is the cause of death. These aspects will be difficult to ascertain when the body had been badly damaged when it was founded. Case finding the bodies are often found in cases of death due to drug abuse in hidden places. When a toxicological samples such as tissues, body fluids, and internal organs have been degraded or no longer available, the alternative is to use serangga investigation. Insects are closely related to forensic science for the use of cadaver as a food source. When eating corpse network, xenobiotics such as drugs and other toxic substances will be transferred to the metabolic system larva. Insects as toxicological samples because the number of corpses that are infected, and the pupa stage will not change for the long time. The study of the use of insects as an alternative toxicology sample known as entomotoxicology. Deaths due to drug abuse, “insecticides, pesticides, and other poisons are common cases in the world. It was reported that around 450,000 people died worldwide as a result of drug abuse in 2015. It is estimated that nearly 800,000 people die each year from suicide, of which 20% are deaths from swallowing pesticides. Many deaths occur due to substance abuse, and in some cases, new dead bodies are discovered after a few weeks to 11 months. Entomotoxicology will “be a useful tool in solving cases of poisoning” when a corpse is found in advanced decay. “Detection of drugs and poisons in insects (larvae, and other arthropods) is further detected by several techniques.” A technique like immune-assays, “High-Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectrometry (LC-MS),” and “Gas Chromatography-Mass Spectrometry (GC-MS),” have been used routinely for detecting xenobiotics from bodies, larvae and insects.

History of Forensic Toxicology Entomology
Entomotoxicology relatively recent history. The first report on drug use in the field of phenobarbital was published in 1980. A 22-year-old woman

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was found in the early stages of skeletonized, no fluids or tissues were found for further decomposition. The larvae are used as samples for toxicological analysis, and phenobarbital found in the detection result. Since then, a lot of drugs and toxic substances such as amitriptyline, Procloxyphen, Acetaminophen18, Steroid19, Trazodone, Trimipramine and Temaze-pam14, benzodiazepine, barbiturates, and Meprobamate15, Methamphetamine16, Meth-amphetamine20, Clamepamber, Brom, Clamine and Opioid13; Steroid21, Nordiazepam23, Phencyclidine24, Codeine, Insecticide and Pesticide25, Mercury27, etc. have been detected in various tissues and insect larvae. **Insects as toxicological samples**

After someone dies their body undergoes a process of decay which consists of five stages: fresh, swollen, rotten, and become a skeleton. Insects are generally the first visitors to a corpse, which can lay eggs in a carcass within hours of death. Insects happen to experience several changes from one stage of life to another (metamorphosis): First, eggs are laid in large numbers on corpses; maggots emerged from eggs, ate corpses and experienced three larvae instars; On the third instar, adult maggots move from food sources to cocoons at the appropriate pupa site (usually on the ground); Adult flies appear after empty pupae and pupa pockets are sometimes seen on body clothing29. The pupa skin serves as the final step for toxicological samples if the body is found at the skeletonized stage. Medicines which accumulate in the cuticle of the larva during growth will suppress the skin of the pupa. Entomotoxicological organic materials that can be analyzed are larvae, pupae, adult insects, pupa shells, exuviae (beetle skin remnants), beetle feces, fly predators, and spoilage bacteria30. The most frequently analyzed toxicological insects are flies (Diptera) and beetles (Coleoptera). The most studied insect species are *Calliphoravicina* and *Luciliasericata*31.

**METHODS**

**Sample collection and the storage of the collection**

Good collection and storage of samples are essential for more accurate estimates of postmortem (PMI) estimates. Samples were collected from various parts of the corpse because drug redistribution caused variability in drug concentration1. Various methods of collecting and storing samples and effects on larval length have been studied to determine which media are most suitable. Experiments32 showed that larval specimens were carried out in two ways: directly put into preservatives (10% formalin, 80% ethanol, and 95% ethanol); or hot water to kill 80°C and 100°C larvae for 1, 30, 60, and 90 seconds and then soaked in preservatives. The larvae were turned off at 80°C with an immersion time of 30 seconds found to have minimal changes regarding the initial length before being killed, and therefore this method was found to be more suitable. Ethanol(80%) in beetle larvae provides a large variation in the length of beetle larvae observed33. Therefore, beetle larvae must be measured while alive44. Various methods of killing and storing samples and effects on larval length have been studied to determine which media are most suitable.

For toxicological analysis, the specimen is preserved at -4 °C, and the analysis is carried out in the same manner as human tissue or liquid30. It is recommended that specimen storage be carried out under dry conditions at -20 °C to ensure drug stability and also to reduce drug extraction from the matrix when submerged in alcohol1. **Sample Extraction**

The extraction of xenobiotic material from insect samples provides several advantages compared to human tissue12,35. Sampling is easier and faster, and the emulsion does not interfere with the results of the analysis, which sometimes occurs with human tissue 23. Several extraction techniques such as liquids extraction and solid-phase extraction are used to extract various poisons...
and drugs according to the chemical features of the material to be detected\textsuperscript{16,37}. Solid-phase extraction is known to provide the best purification of organic toxins from liquid extracts of insect specimens\textsuperscript{30}. The various types of samples and the systematic processes for which the analysis is prepared is illustrated in Figure 1.

Figure 1. The layout of insect analysis for toxicological analysis \textsuperscript{38}

**Toxicological Analysis**

Different animal models (rabbits, mice)\textsuperscript{39}, and meat substrate\textsuperscript{11,12} with medicine, as well as toxic substances are used to simulate the process of death, spoilage, and drug discovery from larvae on dead carcasses. Successful detection and discovery of toxins from insect specimen depend on the extraction technique and the effectiveness of the used technique. Figure 1 above shows the stages of systematic analysis of different chemical compounds in entomotoxicological specimens which are of interest to the analyst. Some insect species for which drugs and toxic substances can be detected are shown in Table 1.

### Qualitative Analysis

In the absence of specimens such as tissue, blood, or urine for toxicological analysis, insects can reveal the possibility of exposure to drugs that poison the related deaths. For example, amphetamines and alcohol were detected from larvae in corpses that were thought to be one month old\textsuperscript{17}. Several research articles have evidence that identification of the drug may be from insects themselves\textsuperscript{18,21}. In forensic entomotoxicology, immunoassay techniques have been mainly used to detect morphine drugs from various substrates and specimens\textsuperscript{11}. By using this technique, the distribution of morphine in various parts of the insect’s body (hemolymph, cuticle, and larval integument) was detected\textsuperscript{40}. This technique has also been useful in the qualitative and quantitative analysis of estimates of other drugs and pesticides\textsuperscript{43}.

For confirmation testing of compounds in the sample, the chromatography technique coupled with Mass Spectrometry is considered the gold standard\textsuperscript{44}. This technique is more selective, sensitive, accurate, reproductive, and requires only a few samples. In forensic analyses of drug abuse cases, this technique has been applied to detect various drugs on different matrix samples\textsuperscript{46}.

### Quantitative Analysis

The toxin concentration will be reduced in insects and drugs according to the chemical features of the material to be detected\textsuperscript{16,37}. Solid-phase extraction is known to provide the best purification of organic toxins from liquid extracts of insect specimens\textsuperscript{30}. The various types of samples and the systematic processes for which the analysis is prepared is illustrated in Figure 1.

Figure 1. The layout of insect analysis for toxicological analysis \textsuperscript{38}

**Table 1. Drugs and other toxic substances detected from several insect species**

| Insect genus | The detected drug | The detection technique |
|--------------|-------------------|-------------------------|
| Calliphora vicina | Amitriptyline, Temazepam | GC-MS \textsuperscript{3} |
| Dermestes frisco | Morphine | Immunoassay \textsuperscript{40} |
| Lucilia sericata | Morphine | Immunoassay \textsuperscript{40} |
| Chrysomya albiceps | Diazepam | GC-MS \textsuperscript{41} |
| Protophormia terraenovae | Morphine | Immunoassay \textsuperscript{42} |
| Musca domestica | Parathion | HPLC \textsuperscript{43} |
| Calliphora stygia | Morphine | HPLC \textsuperscript{44} |
| Chrysomya megacephala | Malathion | GC \textsuperscript{8} |

### Correlation Analysis

The correlation between drug concentrations in the substrate and insects is still controversial. Factors such as drug pharmacokinetics, metabolism, drug redistribution, drug accumulation, larva feeding activity, and several other factors that can influence correlation studies are not known yet. Extensive studies on 29 human bodies that were

### Extensive Studies

Extensive studies on 29 human bodies that were...
thought to have died of poisoning were analyzed, and correlation could not be concluded because there was no reproducibility. In contrast, several studies have shown that the correlation between pesticides in the substrate and larval samples. In most of these experiments, larvae are fed minced meat without allowing the drug to undergo a natural metabolic process. Therefore, the findings and results may differ from the results of actual life poisoning cases. So far, few studies have looked into metabolism, drug redistribution, accumulation, and excretion mechanisms in insects. So, until all the right factors are available, vigilance needs to be taken to interpret the results of the correlation analysis.

**CONCLUSION**

Insect and larvae of toxicological specimens can be relied upon especially when the material for toxicological analysis of the victim’s body material is no longer available. In addition to the qualitative role, the possibility of a quantitative role can also be carried out if the pharmacokinetics of the drug in insects are known.

**SUGGESTION**

Until now, the absorption-metabolism-elimination-accumulation of drugs and other toxic substances in insects has not been fully understood. The pharmacokinetics of drugs in insects depends on the species, their stage of development, and feeding activity as well. The results of the analysis are also influenced by factors such as drug stability, temperature, and humidity. Thus, the main weakness of entomotoxicology is the correlation of detected drug concentrations. To overcome these limitations, it is recommended that the development of standard protocols which must include models of organisms (e.g. available insect species), standard matrices for feeding substrates, setting sample sizes, and using sophisticated analytical methods. This will help in achieving comparative results between various studies.

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**Authors’ contribution**

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