

Entomotoxicology

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ABSTRACT

Entomotoxicology is the analysis of toxins in arthropods (mainly flies and beetles) that feed on corpse. Forensic entomology is the use of insects in legal matters. Knowledge of the distribution, biology, ecology and behavior of insects found at crime scene can provide information on when, where and how the crime was committed. Forensic entomotoxicology studies the usefulness of insects as alternative toxicological samples. Use of insects as alternative matrix for drug detection is well documented and recommended when conventional matrices such as blood, urine or internal organs are no longer available.

The accuracy of entomological estimates in deaths involving narcotic intoxication has been subject to debate in recent years as few available studies have explored the effects of drugs contained in decomposing tissues on fly colonization and ovipositional behavior, or on the rates of development of carrion-frequenting insects feeding on such food sources.

The major lacunae in this field is the effect toxins and contaminants have on the development of immature insects feeding on the corpse containing these substances and studies have to be conducted in this grey area.

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► *Implication for health policy/practice/research/medical education:*
Entomotoxicology

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1. Introduction:

There is been an increase in drug related deaths in USA and rest of the world. Many a times these deaths are discovered after a period of time and it is not unusual that the corpse could be highly de-composed or skeletonized. Under such circumstances, there will not be sufficient tissues for toxicological analysis. Even then, it is possible to detect various toxins and controlled

substances by analysis of insects, larval skins or puparial skin present near the corpse (1). From 1980 entomologists started to detect drugs in insects, hoping it would become a useful tool in forensic investigations (2, 3). For forensic entomology to be effective in legal investigations, knowledge of local insect assemblages and their population dynamics is essential.

Entomotoxicology deals with the “analysis of toxins in arthropods (mainly flies and beetles) that feed on carrion. Using

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arthropods in a corpse or at a crime scene, investigators can determine whether toxins were present in a body at the time of death” (3) Clearly much more research is needed before the full forensic potential of Entomotoxicology is realized.

2. History of research and development of forensic entomology:

Forensic entomology as it is today is derived from a long sporadic history of research dating back to 13th century China. Insects are known to have been used in the detection of crimes for a long time and number of researchers has written about the history of forensic entomology.

During medieval times, the correlation between maggots on a corpse and the oviposition of adult flies was not recognized. However, the realistic and detailed illustration of corpses containing maggots was not unusual. The importance of insects in the decomposition of human bodies was documented, as early as the 19th century: the biologist Carl von Linné (Linnaeus) in 1767 said that “Three flies would destroy a horse as fast as a lion would”.

In the early 20th century, entomological evidence has been used sporadically in several murder cases in Europe with increased success. In the second half of the 20th century there was been an increasing interest in forensic entomology and the topic was revived by many researchers.

Forensic entomology continues to progress as a science, gaining credibility as research leads to a greater understanding of insects and refinement of methods. Employment of DNA analysis and use of scanning electron microscopy has helped improve accuracy in the classification and identification of insect species. Recent research has brought the possibility of extraction human DNA tissue and gunshot residue from the gut contents of feeding maggots. Entomotoxicology (detection of narcotics and toxins in the tissue of feeding insects on a decaying corpse) is an emerging field providing valuable tools in the investigation of homicides, suicides

and other unattended deaths where chemicals are involved.

Despite great strides having been made in fundamental and applied research, there are many questions yet to be answered. Forensic entomology is still a young discipline and there is still much room for progress. The scientific literature available on this topic, although constantly growing, remains small when compared to many other biological and legal subjects. Likewise, the number of qualified participating forensic entomologists capable of fully utilizing insect evidence is currently very small.

3. Insect sampling:

For some toxicologists, insect sampling can seem simple; just take some maggots from the corpse. However, it is a factor leading to high variability in drug detection. First of all, sampling of insects (larvae or pupa) can be carried out, around or under the body or body discovery site. When the corpse is highly decomposed, investigators must be aware that the collected insects can originate from a source other than the deceased. In addition, several authors demonstrate the importance of collecting at different body-sites, as inter-site sampling results in a high variation of drug concentrations (4-7). This observation is logic as drugs are distributed in the body according to their physicochemical properties, leading to different drug concentrations in different organs and tissues, and thus also in insects reared on these different substrates. While most investigators sample randomly, the best sampling sites for drug detection in insects are the internal organs (e.g. liver), the head-area or muscles in cases where no internal organs are left. However, in the literature, other sampling sites such as the skin surface are observed.

At the moment, standards and guidelines for insect sampling in forensic entomotoxicology are published by Amendt *et al* and Carvahlo *et al* (6). Unfortunately, the minimum number of specimens that should be sampled is not

mentioned and the differentiation between insect activities and maturity is not specified. Only Tracqui *et al* (5), describes sampling of minimal 30 specimens of the same stage and activity from each location. This is maybe not so important for real cases, but is certainly of interest for entomotoxicological experiments to ensure a good statistical analysis.

4. Sample Preservation:

Once the specimens have been removed from the body, or the crime scene, they are washed with deionizer or tap water and the specimens are then frozen for storage at a temperature ranging from -20°C to 4°C until they are needed for analyses.

Specimens are prepared for analysis in a variety of ways. They differ based upon the substance that is in question.

For the analysis of *inorganic substances*, the arthropods are taken out of storage, washed, and then dried to insure the removal of any foreign human fluids. They are then crushed and stored in a porcelain crucible at a constant temperature of 650°C for 24 hours. The resulting ash has a high concentration of metals, which are then analyzed by acid digestion using 70% HNO₃ (nitric acid).

Organic substances investigation starts up with washing and drying the specimens. 1-10 grams of larvae are finely cut and an internal standard solution is added. The specimens are then homogenized, in a 0.9% saline solution, followed by centrifuged. Strong acids or bases break down the chitinous exoskeleton to release any toxins present and the sample is allowed to extract overnight at a temperature of 65°C. The acid solution is then removed and the organic substances are available for further analyses (8-10).

Pharmacokinetics of drugs in insects depends on the species, the developmental stage as well as on their feeding activity. Apart from necrophageous species, bioaccumulations can also occur in parasitoids, predators or omnivorous species. However, this drug bioaccumulation will not be similar as

these species present different feeding behaviour due to their diet or life history traits.

For entomotoxicological investigations, use of necrophageous species belonging to Coleoptera and/or Diptera is recommended as they are the first to colonize the corpse. Necrophageous species are usually very common and abundantly present on the crime scene. Moreover, their biology and development are well-known, as they are already used in forensic entomology to estimate Post-Mortem Interval (PMI) (8).

Morphine and heroin were both believed to slow down the rate of fly development. However, closer examination of the effects of heroin on fly development has shown that it actually speeds up larval growth and then decreases the development rate of the pupal stage. This actually increases the overall timing of development from egg to adult. Cocaine and methamphetamine also accelerate the rate of fly development. Some effects of toxins on these arthropods depend on the concentration of the toxin while others simply depend on its presence. For example, cocaine (at the lethal dose) causes larvae to "develop more rapidly 36 (to 76) hours after hatching". The amount of growth depends on the concentration of cocaine in the area being fed upon. The amount of methamphetamine, on the other hand, affects the rate of pupal development (10).

5. Limitations:

Entomological specimens make excellent qualitative toxicological specimens. There is, however, a lack of research in developing an assessment to quantify the concentration of a drug in tissue using entomological evidence. One reason for this is that a drug can only be detected in larvae when the rate of absorption exceeds the rate of elimination, also samples of pupae and third instars larvae does not contain concentrations of the drugs, suggesting that drugs do not bioaccumulate over the entire life-cycle of larvae. This leads entomologists to theorize that toxins are eliminated from the

larvae's system over time if they are not receiving a constant supply of the toxin. This field of entomotoxicology is in its infantile stage, hence lot of active research is needed in this field.

6. Conclusion:

- Arthropods prove to be valuable tools in the investigation of homicides, suicides, and other unattended human deaths.
- In the latest years research has been conducted not only in the field of entomology for estimating time since death but also in the field of entomotoxicology
- Entomotoxicology can provide alternative specimens for drug detection in decomposed bodies.
- At the moment, detection of drug in insects is certainly possible. While it cannot yet be used for toxicological interpretation in most cases, when only skeletonized remains are left, pupa can be the only hope for a toxicologist to have some information concerning drug use prior to death.
- More detailed and comprehensive research is required, for the full potential of this emerging discipline can be recognized.

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