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Use of entomotoxicology in estimating post-mortem interval and determining cause of death

Summary

Entomotoxicology allows the estimation of the post-mortem interval and the determination of the cause of death in cases in which the corpse has decomposed and the tissues necessary for toxicological analysis are no longer available. Obtaining information about toxic substances potentially present in the body is possible by isolation of larvae and pupae of true flies (Diptera) and/or adult forms of, e.g., beetles (Coleoptera) present on or near the corpse. This article was intended to summarize the current knowledge in the field of entomotoxicology, including examples from the literature, and to present the impact of selected toxic substances and medicines on the development of necrophagous larvae of insects.

Keywords: forensic entomology, toxicology, chromatography, entomofauna, post-mortem interval

Forensic entomology is a field of science that employs insects to determine the time, causes and even place of death (using entomofauna specific for a certain location) (Gennard, 2007). For the estimation of post-mortem interval (PMI), observation of the time needed for the development of pre-imago forms (larvae and pupae) under the observed conditions (the so-called developmental method) and the pattern of occurrence of certain insect species on the body (the so-called succession method) are used (Kaczorowska et. al., 2002, 2004; Matuszewski et. al., 2010ab, 2011). The rate of decomposition changes according to the temperature (the most important variable in all developmental models), environment (soil or vegetation in an open-air setting or conditions in an enclosed space), weather, as well as corpse condition and exposure (exposed or buried, naked or dressed, intact or wounded) that occur at the site of discovery of the body (Gennard, 2007; Matuszewski et. al., 2013, 2016).

Another important factor in estimating PMI and determining the cause of death is the occurrence of toxic substances in or on the body, e.g., as a result of poisoning. In the case of a fresh corpse, when it is still possible to sample the tissues, toxicological analyses are conducted. To this end, samples are taken during the autopsy, including, e.g., stomach along with its content, fragments of liver, kidney, lung and brain, as well as blood and urine samples. If puncture marks are visible, fragment of the skin is taken (Raszeja et. al., 1990; Seńczuk, 2012). Due to the different possible routes of poisoning and non-specific action of toxins, detection of the latter is difficult. Poisoning can occur by oral intake of a toxic substance, as well as through the skin, by inhalation or by the above mentioned direct injection. However, detecting the toxin is extremely important in order to further determine whether the poisoning was a result of murder, suicide or unintended intake of the detected substance. Some changes in the body are so characteristic that they allow determining which toxic substances caused them as early as on the first body examination at the site of death. Carbon monoxide poisoning results in the occurrence of, e.g., raspberry-red patches or blisters on the skin. A yellow color of the skin may indicate liver damage (as a result of, e.g., mushroom poisoning), while rough skin with rash may be a result of opium alkaloid poisoning (Raszeja et. al., 1990; Seńczuk, 2012). However, the discovery of the corpse is not always possible immediately after death. A corpse at the end of the active stage or at an advanced stage of decomposition may be missing tissues that could be used for toxicological analysis and thus it may not be possible to determine the possible presence of toxic substances in the body. It is possible, however, to carry out this type of analysis with the use of insects that fed on the discovered body. This is enabled by the methods of entomotoxicology, a field of forensic entomology. Entomotoxicology uses toxicological analyses to identify toxins and pharmacological agents potentially present in the body at the time of death, that were consumed along with soft tissues by necrophagous insects. Entomotoxicology also examines the effect of these substances on the development of insects feeding on corpses and thus the way in which the presence of such substances affects the possibility to estimate the time of death using the methods of forensic entomology (Goff, Lord, 1994).

Beetles and larvae of true flies feeding on the tissues of corpses at the various stages of decomposition (mainly active and advanced stage) consume the same substances that were present in the body before death. The concentration of these substances in the tissues of those insects is lower than in the source tissues (the substance and the products of its metabolism are gradually excreted with time), but this is not an obstacle to their identification (Carvalho et. al., 2001). Such bioaccumulation of toxic substances allows effective use of the methods of entomotoxicology in the course of investigation to determine the cause of death. This article is intended to summarize the current knowledge of the applications of entomotoxicology in forensic investigation, including examples from the literature, and to present the impact of selected toxic substances and medicines on the development of necrophagous larvae of insects.

Initial entomotoxicological research

In 1958, Utsumi noticed that true flies (Diptera) demonstrate a different degree of interest in rat corpses, depending on the type of poison that caused the death of the animal. In the 1970s, accumulation of various metals (zinc, copper and calcium) in the tissues of adult flies (*Musca domestica*, Linnaeus, 1758) was observed for the first time (Sohal, Lamb, 1977, 1979). Beyer et al., (1980) described the accumulation of phenobarbital in larvae collected from a completely decomposed corpse of a 22-year-old woman. Despite the lack of tissues for toxicological analysis, it could be established that the death occurred as a result of suicide. The woman was seen alive for the last time two weeks before the discovery of the body. In turn, Nuorteva and Nuorteva (1982) demonstrated the accumulation of mercury in the larvae, pupae and adult forms of true flies of the Calliphoridae family (blow flies) feeding on fish containing this metal. Further reports came from France, where in 1985 Leclercq and Brahy demonstrated the accumulation of arsenic in true flies of the Fanniidae, Piophilidae and Psychodidae families. Gunatilake and Goff (1989) described the accumulation of the insecticide malathion in the larvae of *Chrysomya megacephala* (Fabricius, 1794) and *Chrysomya rufifacies* (Macquart, 1842).

Identification of toxic substances bioaccumulated in corpse entomofauna

The first insects to colonize a corpse are true flies of the Calliphoridae family and their larvae have the largest share in active decomposition (Gennard, 2007). In optimal thermal conditions, thousands of larvae are capable of decomposing soft tissues very quickly, before the discovery of the corpse and collection of samples for toxicological analysis (Campobasso et. al., 2004). If tissues are not available, it is possible to identify chemical compounds in the insects feeding on the corpse (larvae, pupae, adult forms) or even in the exuviae or excreta of those insects (Gagliano-Candela, Aventaggiato, 2001). Larvae feeding on a tissue containing toxic substances introduce these substances into their metabolic system. If such a larva is ingested by a beetle hunting on carrion (e.g., *Creophilus maxillosus*, Linnaeus, 1758; Matuszewski 2012), the substances will be passed further and introduced into the beetle at an appropriately lower concentration. This phenomenon is known as secondary bioaccumulation. According to the literature data, insects most frequently used in entomotoxicology include true flies of the Calliphoridae, Sarcophagidae and Muscidae families and beetles of the Dermestidae family (see Gagliano-Candela, Aventaggiato, 2001). The detected substances are of various types (organic and inorganic), e.g. medicines, poisons, drugs or pesticides.

The main method of identification of xenobiotics (medicines, drugs, alcohol) in larval tissues is chromatography that allows separation and analysis of the chemical composition of the mixture of various compounds. The separation is conducted by passing the mixture (larval tissue after homogenization, i.e. grinding and forming a homogeneous blend) through the so-called stationary (separation) phase–resin. The resin is composed of substances endowed with sorption properties (capacity of absorbing) in relation to the chemical compounds flowing through it. Subsequently, various chemical compounds bound to the resin are eluted using the mobile phase (eluent). Depending on the intensity of interaction between each component of the mixture and the mobile phase, some components are retained in the solid phase longer than the other, and thus they become separated. Among the methods used in entomotoxicology are gas chromatography (GC), thin layer chromatography (TLC), high pressure liquid chromatography-mass spectrometry (HPLC-MS), as well as radioimmunoassay (RIA) and gas chromatography-mass spectrometry (GC-MS) (Campobasso et. al., 2004; Kaczorowska, Draber-Mońko 2010).

Using liquid chromatography, Kintz et al., (1990) analyzed heart, liver, lung and kidney samples, as well as larvae of blow flies taken from corpses two months after death. This allowed the detection of five medicines (barbiturates, benzodiazepines and tricyclic antidepressants). The study also showed that better results and greater sensitivity of identification are achieved by isolating chemical substances from larvae and not from corpse tissues, as one might anticipate.

Campobasso et al., (2004) conducted a study of the correlation level of accumulation of various toxic substances in human tissue and the larvae of *Lucilia sericata* (Meigen, 1826) feeding on that tissue. A total of 18 cases were analyzed. The tissue initially underwent toxicological analysis and was subsequently examined for the concentrations of chemical compounds detectable in the larvae of true flies feeding on it. The analysis demonstrated that only in the case of cocaine, the concentration was similar in both tissues (human and larval), while other substances were found at much lower concentrations in the larval tissues than in the control tissue.

A similar study was conducted by Aguiar França et al., (2015). The authors analyzed 11 substances (6 medicines, cocaine and its metabolites, and a popular insecticide and its metabolites) in 28 corpses originating from formal police investigations. The effectiveness of identification of the analyzed substances was almost 70%. The study also confirmed that this type of analysis is quick and easy to conduct (with an exceptionally simple procedure), requires low concentrations of the identified compounds and allows analyzing more than ten substances at the same time.

The correct identification of chemical compounds present in or on corpses not only allows determining the cause of death, but is also important for determining the time elapsed since the death. Therefore, not knowing the potential presence of a specific chemical compound and its potential impact on the development of pre-imago forms of insects can cause significant errors in estimating the time of death and thus cannot constitute reliable evidence in the investigation.

Effect of selected compounds on the development of larvae on corpses

Currently, there are many studies on the impact of various substances on the rate of development of insect larvae on carrion available in the literature. Due to their large number, only selected examples will be presented in this article. Among the selected substances are those that (1) have been experimentally tested and the course of these analyses has been described in the literature; (2) have a confirmed impact on the rate of development of larvae; (3) have been isolated from corpses in the course of investigative procedures. The vast majority of chemical compounds accelerate the development of larvae, however, it is not difficult to find substances that delay their development or demonstrate no effect on this process. Table 1 summarizes the current knowledge in entomotoxicology, not only presenting the substances that are most often described in the literature and have been selected for this paper, but also extending the presented information on other xenobiotics.

Diazepam is a readily available substance exerting a sedative, anxiolytic, spasmolytic and hypnotic effect. Carvalho et al (2001), using GS-MS, studied its impact on the development of *Chrysomya albiceps* (Wiedemann, 1819) and *Chrysomya putoria* (Wiedemann, 1830) larvae that were cultured on liver containing diazepam. Initially, no changes in the rate of development of the larvae were noticed, but after 18 hours of the development, the larvae turned out larger than expected. The study demonstrated that the growth acceleration observed in the larvae was due to the bioaccumulation of the drug that was easily detectable not only in the larvae, but also in the pupae and adult forms. It was found that diazepam can lead to an error in the estimation of the age of larvae (and thus the time of death) of up to 54 hours.

Other substances that accelerate the development of true flies larvae are cocaine, heroin, methamphetamine and ketamine (Goff et. al., 1989, 1991, 1992; Mullany et. al., 2014; Zou et. al., 2013).

Larvae of true flies cultured on a tissue containing a lethal dose of cocaine develop much faster 36 hours after hatching (reaching maximum length after approx. 76 hours), which reduces the duration of the whole development process (Goff et. al., 1989). Lord (1990) described a case of a body of a 20-year-old woman on which larvae of *Lucilia sericata* and *Cynomyopsis cadaverina* (Robineau-Desvoidy, 1830) of different length were found. The larvae of those blow flies collected from the torso were 6–9 mm in length, which indicated that the death had occurred 7 days before, while the larvae collected from the nasal cavities were considerably longer (17.7 mm), which suggested a much longer PMI (3 weeks). However, the differences in the development of larvae collected from various parts of the body were due to the fact that, shortly before her death, the woman had taken a nasal dose of cocaine whose remains significantly accelerated the growth of the larvae found on her face.

In the case of *Sarcophaga peregrina* (Robineau-Desvoidy, 1830) larvae developing on a tissue containing heroin, Goff et al., (1991) observed a faster growth of larvae between 18th and 96th hour of development, as well as slower pupation. Larvae feeding on a tissue containing methamphetamine showed a faster growth between 24th and 60th hour of development, but subsequently their development slowed down resulting in a smaller final size of these larvae than those from the control sample, i.e. cultured on a tissue without the lethal dose of the drug (Goff et. al., 1992). The acceleration of larval development following methamphetamine intake was also confirmed in another study by Mullany et al. (2014).

A similar effect can be exerted by the anesthetic ketamine. Zou et al. (2013) demonstrated that culturing *Lucilia sericata* larvae on a tissue containing ketamine reduces the duration of the larval stage. However, Lü et. al. (2014) reached different conclusions. When culturing *Chrysomya megacephala* larvae on tissues containing different concentrations of this substance, they did not observe any significant correlation between the dose of ketamine and the difference in growth rate.

Not all chemical compounds accelerate larval development; some of them can slow it down significantly. This is well illustrated by carbon monoxide. This compound delays the colonization of corpses by binding hemoglobin in blood and thus deterring insects (Smith, 1986). An interesting example is morphine, which in one experiment noticeably delayed the growth of *Lucilia sericata* larvae, while accelerated the growth of *Chrysomya megacephala* larvae (Bourel et. al., 1999). This case is another example that a particular compound can affect different species of Calliphoridae in different ways. A similar correlation was observed in the same species in the case of ketamine (Zou et. al., 2013; Lü et. al., 2014).

Other chemicals than can delay larval development are, e.g., the psychotropic amitriptyline, hydrocortisone (used inter alia in the treatment of skin conditions), the insecticide malathion (that delays laying eggs by insects) or ethanol (Gagliano-Candela, Aventaggiato, 2001; Lü et. al., 2014). In the case of hydrocortisone, Musvasva et al. (2001) demonstrated that it accelerated the development of *Sarcophaga tibialis* (Macquart, 1851) larvae.

In turn, larvae of Calliphoridae cultured on tissues containing, e.g., phencyclidine (formerly used for preoperative anesthesia; Goff et. al., 1994) or paracetamol (O'Brien, Turner 2004) showed no differences in the growth rate compared to the control sample. In the case of phencyclidine, longer pupation periods were observed (Goff et. al., 1994).

Conclusions

Examples presented in this article demonstrate that determining the cause and the time of death requires analysis of tissues of insect larvae (mainly those of true flies) collected from the corpse for the presence of pharmacological substances. What is more, larvae of necrophagous insects introduce into their bodies not only the potential toxins, but also tissues containing DNA. Wells et al. (2001) demonstrated that it is possible to isolate human DNA from the digestive tract of larvae of true flies, while Chávez-Briones et al. (2013) successfully identified a corpse using microsatellite DNA isolated from the digestive tract content of larvae of true flies collected from the body. In turn, Curic et al. (2014) suggested that it is possible to identify a person based on DNA recovered from mosquitoes. In their study, they hypothesized that a mosquito could feed on the victim or the perpetrator at the crime scene, and that isolation of DNA from its digestive tract could be a potential tool to establish a link between a person and a place, even after removal of the body. This is useful in the investigation as some mosquito species do not fly away from their 'feeding' site at greater distances (while others can fly away as far as 30 kilometers).

Some insects can also be used as the so-called biological sensors. Frederickx et al. (2014) described the potential use of the wasp *Nasonia vitripennis* (Walker, 1836) to detect volatile compounds released by corpses (which enables their discovery) or illegal drugs. Culturing insects is cheap and teaching them to respond to a certain substance is straightforward.

Undoubtedly, forensic entomology and its subfield–entomotoxicology–are helpful in criminal investigation, and the methodologies they involve should be included in the standard investigative procedures. Further enhanced studies, analyzing the effect of various chemical compounds on the development of different species of true flies and beetles inhabiting corpses, will allow a more precise understanding of the mechanism of bioaccumulation and a more efficient estimation of the post-mortem interval.

Table 1. Summary of the current knowledge based on selected entomotoxicological analyses, taking into account the type of the substance and its effect on necrophagous insects.

Chemical substance	Species and stage of development	Effect on insect metabolism	References
Diazepam	larvae, pupae and adult forms of *Chrysomya albiceps* (Wiedemann, 1819) and *Chrysomya putoria* (Wiedemann, 1830)	accelerates development of larvae	Carvalho et. al., 2001
Cocaine	larvae of *Sarcophaga peregrina* (Robineau-Desvoidy, 1830)	accelerates development of larvae	Goff et. al., 1989
	larvae of *Lucilia sericata* (Meigen, 1826) and *Cynomyopsis cadaverina* (Robineau-Desvoidy, 1830)	accelerates development of larvae	Lord 1990
Heroin	larvae of *Sarcophaga peregrina*	accelerates development of larvae, but delays pupation	Goff et. al., 1991
Methamphetamine	larvae of *Sarcophaga ruficornis* (Fabricius, 1794)	accelerates development of larvae, but finally the exposed larvae are smaller than control	Goff et. al., 1992
	larvae of *Calliphora stygia* (Fabricius, 1781)	accelerates development of larvae	Mullany et. al., 2014
Ketamine	larvae of *Lucilia sericata*	accelerates development of larvae	Zou et. al., 2013
	larvae of *Chrysomya megacephala* (Fabricius, 1794)	no correlation	Lü et. al., 2014
Morphine	larvae of *Lucilia sericata*	delays development of larvae	Bourel et. al., 1999
	larvae of *Chrysomya megacephala*	accelerates development of larvae	Bourel et. al., 1999
Hydrocortisone	larvae of *Sarcophaga tibialis* (Macquart, 1851)	accelerates development of larvae	Musvasva et. al., 2001
Paracetamol	larvae of *Calliphora vicina* (Robineau-Desvoidy, 1830)	no correlation	O'Brien and Turner 2004
Phencyclidine	larvae of *Sarcophaga ruficornis*	no effect on growth of larvae, but pupation is significantly slower	Goff et. al., 1994
Malathion (insecticide)	larvae of *Chrysomya megacephala* and *Chrysomya rufifacies* (Macquart, 1842)	delays growth of larvae, delays laying eggs by adult forms	Gunatilake and Goff 1989
Ethanol	larvae of *Phormia regina* (Meigen, 1826)	delays growth of larvae	Tabor et. al., 2005
Codeine	larvae, pupae and adult forms of *Lucilia sericata*	accelerates development of larvae	Kharbouche et. al., 2008
Methadone	larvae of *Lucilia sericata*	high usefulness in detecting methadone; no data on the effect on development of larvae	Gosselin et. al., 2010
Methylphenidate	larvae of *Lucilia sericata* and *Calliphora vicina*	high usefulness in detecting methylphenidate; no data on the effect on development of larvae	Bushby et. al., 2012
Sodium pentothal	larvae of *Calliphora vicina*	causes death of larvae	Sadler et. al., 1997

Chemical substance	Species and stage of development	Effect on insect metabolism	References
Paraquat dichloride (herbicide)	larvae of Calliphoridae	high usefulness in detecting the herbicide; no data on the effect on development of larvae	Lawai et. al., 2015
Cadmium	larvae, pupae and adult forms of *Lucilia sericata*	delays development of larvae, extends pupation, reduces weight of larvae, pupae and adult forms	Simkiss et. al., 1993
Lead	larvae, pupae and adult forms of *Calliphora dubia* (Macquart, 1855)	high usefulness in detecting lead at various concentrations; no data on the effect on development of larvae	Roeterdink et. al., 2004
Zinc Iron Copper Calcium	larvae of *Musca domestica* (Linnaeus, 1758)	no data on the effect on development of larvae	Sohal and Lamb 1977, 1979
Mercury	larvae, pupae and adult forms of Calliphoridae and adult forms of *Creophillus maxillosus* (Linnaeus, 1758)	accumulation of xenobiotics in the entire food chain (not only in the tissues of larvae of true flies, but also in beetles that feed on these larvae); no data on the effect on development of larvae	Nuorteva and Nuorteva 1982
N,N-Diethyl-meta-toluamide (DEET, insecticide)	larvae and adult forms of *Lucilia sericata* and *Blaesoxipha plinthopyga* (Wiedemann, 1830)	delays laying eggs on corpses; delays development and growth of larvae	Shelomi et. al., 2012
Flunitrazepam (‘date rape drug’)	larvae, pupae and adult forms of *Chrysomya megacephala*	does not affect the growth rate of larvae; significantly affects the weight of pupae and adult forms	Baia et. al., 2016a
	larvae *Chrysomya megacephala*, *Chrysomya albiceps* and *Cochliomyia macellaria* (Fabricius, 1775)	high usefulness in the detection of flunitrazepam	Baia et. al., 2016b

Source of table: author

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