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Entomotoxicology☆

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Abstract

Entomotoxicology is a relatively new branch of forensic entomology. The potential use of insects for detecting drugs and other toxins in decomposing tissues has been widely demonstrated. In death investigations, Diptera and other arthropods can be reliable alternate specimens for toxicological analyses in the absence of tissues and fluids normally taken for such purposes. Entomotoxicology also investigates the effects caused by drugs and toxins on arthropod development in order to assist the forensic postmortem interval estimates. However, several remarks on the limitations of entomotoxicology have been highlighted recently. In this paper, the implications for the practice of this forensic procedure are fully reviewed.

Keywords: Forensic entomology; Forensic toxicology; Drug analysis; Insect development; Postmortem interval

1. Introduction

Entomotoxicology studies the application of toxicological analysis to carrion-feeding insects in order to identify drugs and toxins present on intoxicated tissues. Entomotoxicology also investigates the effects caused by such substances on arthropod development in order to assist the forensic PMI estimates [1]. The increase in drug-related deaths (mainly heroin and cocaine) or deaths somehow connected to accidental or suicidal consumption of poisoning and/or toxic substances justifies the great interest aroused by this discipline in forensic medicine.

In skeletonised bodies or bodies in advanced decomposition, where more traditional sources, such as blood, urine or internal organs are not available, insects may serve as reliable alternate specimens for toxicological analyses. Insects can be analysed quite easily after homogenisation of the most representative specimens by common toxicological procedures such as radio-immune analysis (RIA), gas chromatography (GC), thin layer chromatography (TLC), high pressure liquid chromatography (HPLC–MS) and gas–mass analysis (GC–MS). Diptera larvae-feeding on intoxicated human tissues introduce into their own metabolism drugs and toxins taken by the person when still alive. The transfer of these substances from the human organism to Diptera is not accomplished only at this level of the food chain but continues also in beetles predating on blow fly larvae. Also Coleoptera can in their turn be submitted to toxicological analysis for forensic purposes. A secondary bioaccumulation has been noted in these predatory beetles.

2. Detection of drugs and toxicological analyses

Already in the late 1970s, Sohal and Lamb [2,3] demonstrated the accumulation of various metals including copper, iron and zinc in adults of *Musca domestica* Linnaeus (Muscidae). Similarly, Nuorteva [4] reported the presence of mercury in larvae, puparia and adults of Calliphoridae reared on fish-containing mercury in methylated form. Mercury was also detected in Staphylinidae predating on Diptera larvae reared on fish. These entomotoxicological experiences were applied to the forensic case of a female cadaver found in advanced decomposition in a rural area of Finland and extensively colonised by Diptera larvae [5]. The low concentration of mercury measured by the toxicological analysis of adult flies made it possible to locate the geographical area where the victim came from, an area relatively free from mercury pollution.

Concerning the detection of poisons, as early as in 1958 Utsumi [6] observed that Diptera were attracted in a different manner by rat carcasses depending on the poison causing

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death. In 1985, Leclercq and Brahy [7] first demonstrated the presence of arsenic in Diptera from the families of Piophilidae, Psychodidae and Fanniidae in a case of arsenic poisoning occurred in France. In a suicidal poisoning, Gunatilake and Goff [8] detected organophosphates (malathion) in maggots of *Chrysomya megacephala* (Fabricius) (Calliphoridae) and *Chrysomya rufifacies* (Macquart) (Calliphoridae) submitted to toxicological analysis by using GC.

Regarding the detection of prescription drugs, Beyer et al. [9] illustrated the suicide with barbiturates of a 22-year-old woman found in initial skeletonisation, 14 days after she had last been seen alive. On account of the advanced decomposition, no organic fluids and/or tissues were available for toxicological analysis. The most representative *Cochliomyia macellaria* (Fabricius) (Calliphoridae) larvae were analysed by GC and TLC; the results revealed the presence of phenobarbital. Other cases illustrating the potential of entomotoxicology in forensic cases are described by Kintz et al. [10]. In a corpse, found approximatively 2 months after death, toxicological analysis by liquid chromatography of some organs (heart, lungs, liver and kidney) and of Calliphoridae larvae showed the presence of five prescription drugs among which benzodiazepines (triazolam, oxazepam), barbiturates (phenobarbital) and tricyclic antidepressants (alimemazine and clomipramine). Comparative analysis of toxicological findings showed greater sensitivity of the method using Diptera larvae as samples rather than cadaver tissues. Triazolam, in fact, was not detected in the spleen and kidney but only in maggots. In other cases, Kintz et al. [11] established a correlation between concentrations of the drugs in maggots and human tissues. They detected bromazepam and levomepromazine in cerebral material, clavicle and *Piophila casei* (Linnaeus) (Piophilidae) larvae found in completely decayed human remains. The same authors [12] detected morphine and phenobarbital from Calliphoridae larvae which had developed on the cadaver of a chronic heroin abuser found putrefied about 10 days after death and from internal organs (blood, liver, heart, kidney and brain). Both substances were analysed by using liquid GC and fluorescence polarisation immuno-assay (FPIA). Always by FPIA, Introna et al. [13] obtained positive results on empty puparia of *Calliphora vicina* (Robineau-Desvoidy) (Calliphoridae) which had been reared on substrates containing known concentrations of morphine (greater than 10 mcg/g).

Wohlenberg et al. [14] identified by GC–MS nortriptyline from larvae found on the skeletonised remains of a 40-year-old man and on fragments of muscle, bone, skin and hair. Similarly, Goff et al. [15] demonstrated amitriptyline and nortriptyline from maggots and empty pupariae of Diptera which had developed on rabbit carcasses administered with different dosages of amitriptyline when still alive. On the mummified remains of a woman whose death had occurred 2 years before the finding of the body, Miller et al. [16] showed the presence of amitriptyline and nortriptyline in desiccated cerebral fragments and from stomach contents as well as from Phoridae puparial cases, cast beetle (Dermestidae) skins and beetle fecal material; the frass was found copiously near the corpse. In this study, the authors presented two extraction techniques (strong acid and strong base) modifying the common extraction protocols from hair [17,18] as these were applied to an analysis of material having similar characteristics. Insect puparial cases consist largely of chitin (a complex polysaccharide composed essentially of *n*-acetylglucosamine and glucosamine), similar to that of human hair accounting for 25–50% of exoskeleton dry weight; the other half being protein complexes. Results showed that amitriptyline concentrations were greater in puparia than exuviae or frass. This most likely reflects the food source preferences characteristic of the carrion flies and beetles examined. Phoridae have a propensity for soft tissues where drug concentrations are likely to be higher, while Dermestidae feed primarily on dried integument.

Regarding narcotic intoxications, Introna et al. [19] demonstrated with the RIA, that the presence of opiates (morphine) in larvae developed on liver collected from bodies in which the cause of death was identified as opiate intoxication. Regression analysis comparing the concentrations of opiates found in the larvae with those found in the liver tissues resulted in a significant correlation of $r = 0.790$. Similar results on opiates were also illustrated by Goff et al. [20,21] who administered varied dosages of cocaine and heroin to laboratory rabbits. Opiate toxicological analysis (codeine and morphine) yielded positive results also on Calliphoridae larvae developed on a decomposed cadaver [22].

Although, several of these studies describe a correlation between drug concentrations in larvae and in human tissues on which they were feeding, other studies have not observed any correlation or have found the concentrations in larvae to be significantly lower than those detected in tissues [10,20–23]. For instance, concentrations of morphine in larvae reared on rabbit carcasses previously intoxicated were 30–100 times lower than the concentrations found in the tissues based on the results illustrated by Hedouin et al. [24].

Nolte et al. [23] used toxicological analysis of Diptera larvae to determine cocaine intoxication in an almost completely skeletonised cadaver of a 29-year-old intravenous drug abuser whose body was found 5 months after he had last been seen alive. Although, cocaine is generally labile and rapidly broken down by both enzymatic [25] and nonenzymatic [26] mechanisms, the authors were able to detect cocaine and its major metabolite (benzoylecgonine) both in larvae associated with human remains and in decomposing skeletal muscle using GC and GC–MS techniques. However, quantitiuation by GC was not possible in muscle samples because of interference by tissue-decomposition products. As previously illustrated by Kintz et al. [12], the larvae in this case too provided a more suitable specimen without any decomposition interference.

Manhoff et al. [27] were able to detect by GC–MS cocaine in mummified tissues, in bloody decomposition fluids and also in Calliphoridae larvae and beetle faeces collected from a set of decomposed human remains. Cocaine and other drugs have been identified in the protein matrix of human hair of drug abusers [17,18] and they may be detectable for years following death (even in mummies thousands of years old). These substances can be deposited even in the protein matrix of the puparial cases. In our experience (Introna et al., data not published) empty puparia were also positive in cocaine analysis.

3. Effect of drugs on Diptera development

Previous studies focused on the potential use of insects as alternate specimens for toxicological analyses; the results demonstrate the usefulness of testing insects associated with decomposed remains. A drug or toxin can be detected in the larvae when its rate of absorption exceeds the rate of elimination, but it is not yet known exactly how larvae bioaccumulate or eliminate drugs, and how these affect larval development.

The effects of drugs and toxins on the rate of Diptera development is a paramount matter to solve before using maggots for PMI determination. For instance, in the case of malathion poisoning reported by Gunatilake and Goff [8], the development stages of both *C. megacephala* and *C. rufifacies* were indicative of a minimum postmortem interval of 5 days, whereas the victim had last been seen alive 8 days prior to the discovery of the body. In outdoor Hawaiian situations and for a postmortem interval of 1 week, many more species of flies and predatory beetles (such as Staphylinidae and Histeridae) would have been expected in association with human remains. The presence of only two species of fly larvae on the corpse supported the conclusions of the authors that the malathion in the tissues delayed invasion of the remains by various arthropod taxa and thus oviposition for several days.

Goff et al. [15,20,21,28–30] have investigated in detail the effects of amitriptyline, cocaine, heroin, methamphetamine, phencyclidine and 3,4-methylendioxyamethamphetamine on the growth of *Boettcherisca peregrina* (Robineau-Desvoidy) and *Parasarcophaga ruficornis* (Fabricius), two species of Sarcophagidae very common in Hawaii.

Studying the effects of cocaine on rate of development in Sarcophagidae (*B. peregrina*) Goff et al. [20] demonstrated that maggots develop more rapidly 36 h after hatching if reared on liver and/or spleen of rabbits previously administered with a lethal dose of cocaine or twice such a dose. The acceleration of larval development continued for the following 76 h after hatching. Total development times required for pupariation and adult emergence were shortened correspondingly. Regarding the development of intoxicated larvae Lord [31] describes the case of a 20-year-old woman found in the early bloated stage colonised by maggots of *Lucilia sericata* (Meigen) (Calliphoridae) and *Cynomyopsis cadaverina* (Robineau–Desvoidy) (Calliphoridae) on the face and upper torso. Most of the maggots were 6–9 mm in total length or smaller indicating a PMI of 7 days, while just a single maggot from the nasopharyngeal area measured 17.7 mm in total length indicating a period of 3 weeks. The fast growth of this big larva appeared to be dependent on the amount of cocaine in the nasal region. Subsequent investigation showed the victim was a cocaine abuser who had snorted cocaine shortly before death.

Studying the effects of heroin on development of Sarcophagidae (*B. peregrina*) fed on intoxicated rabbit tissues Goff et al. [21] observed that maggots grow at rates significantly faster from 18 to 96 h, when larvae reached their maximum length. The difference observed in the rates of development were sufficient to alter postmortem interval estimates, if the effect of heroin on the Diptera growth cycle is not taken into consideration, based on larval development by up to 29 h and estimates based on puparial development by 18–38 h.

Based on the results of Bourel et al. [32] if the presence of morphine in the tissues is not considered then an underestimation of the postmortem interval of 24 h is possible for larvae of *L. sericata* measuring from 8 to 14 mm total length. The authors observed larvae of *L. sericata* developing at a slower rate than those reared on rabbit carcass receiving less than 50.0 mg/h of morphine.

Regarding the effects of methamphetamine (sympathomimetic substance active on the central system) on the developmental patterns of *P. ruficornis*, Goff et al. [28] illustrated substantial analogies with the studies carried out on heroin [21] and cocaine [20] as well as significant differences. An accelerated rate of development was observed from 24 to 60 h only in maggots reared on rabbit tissues containing lethal doses; following 60 h, the rate of growth for the median lethal dosage colony slowed down. Unlike the situation with heroin [21] and cocaine [20], larvae from all colonies fed on rabbits receiving methamphetamine were smaller at maximum length (attained earlier) than those from the control colony. As observed for heroin [21], but not for cocaine [20], there was a relationship between the concentration of methamphetamine (and amphetamine) in tissues and the duration of the puparial stage. Finally, it was demonstrated that differences observed in the rates of development were sufficient to alter postmortem interval estimates based on larval development by up to 18 h and estimates based on puparial development by up to 48 h.

The study carried out by Goff et al. [15] on the effects of amitriptyline (a tricyclic antidepressant) always on the Sarcophagid fly *P. ruficornis* showed no significant differences among colonies in the rate of development to maximum size. Once maximum size had been attained, a prolonged postfeeding period was recorded and thus duration of the larval stage was significantly longer. In colonies reared on tissues receiving the 600 and 1000 mg dosages of amitriptyline (producing concentrations corresponding

approximately to median lethal and 2.0 times median lethal dosages based on body weight) puparia were significantly greater both in terms of length and weight. Results of this study indicate that an estimate of PMI based on the duration of the puparial stage could be in error by up to 47 h; when this is combined with the error possibly resulting from the increased duration of the larval stage, the total error could be up to 77 h.

Goff et al. [29] also investigated the effects of another commonly abused drug (phencyclidine) on the development of the *P. ruficornis*. Phencyclidine was introduced in the 70 s as a smoking or snorting drug and is actually a tranquilizer, easily found on the market mainly for veterinary use. Unlike earlier studies dealing with cocaine [20], heroin [21] and methamphetamine [28], there was not a direct relationship between the dosage of phencyclidine administered and the concentration of the drug detected in the tissues. As observed with amitriptyline [15] no significant differences in larval growth rate were observed among the colonies, although the duration of the postfeeding period was shorter for larvae fed on tissues containing the drug. Mean differences in duration of the larval stage in treated colonies ranged from 3 to 17 h less than required by the larvae in the control colony. Similar to heroin, duration of the puparial stage was longer for colonies fed on tissues containing the drug.

In 1997, Goff et al. [30] focused the effects of the 3,4-methylendioxyamethamphetamine (MDMA, an hallucinatory substance derived from metamphetamine) on the rate of development of *P. ruficornis* reared on decomposing liver tissues of intoxicated rabbits. Following base extraction, analyses of the larvae and empty puparial cases detected by liquid chromatography/mass spectrometry (LC/MS) MDMA and its metabolite 3,4-methylendioxyamphetamine (MDA) in quantities directly related to the dosage of the drug administered to the rabbits serving as a food source. Larvae from colonies reared on tissues receiving the 67 mg (2.0 times the median lethal dosage) and the control developed more rapidly from 24 h through 114 h. A maximum length of 20 mm was attained in the control colony at 84 h and in the 2.0 times median lethal dosage colony at 108 h of 19.1 mm. Pupariation was first observed in this latter colony at 190 h.

4. Discussion

All these investigations have demonstrated the possibility of qualitative and quantitative correlations between drug concentrations found in tissues, in developing Diptera larvae, in puparial cases and in insect fecal material since the process of bioaccumulation is common in a wide variety of insects [4]. However, although the use of arthropods as alternate specimens for toxicological analyses is well documented in literature, there are also remarks on its limitations. Pounder [33] found no correlation between the drug concentration in the larvae and in the tissues on which the larvae were feeding. He also stated that although larvae are useful as qualitative toxicological specimens, they appear to be of limited quantitative value as the current state of research does not allow for accurate quantitative assessments. In this respect, Wilson et al. [34] analysed by HPLC *C. vicina* larvae reared on human skeletal muscle obtained from cases of suicidal overdose with co-proxamol (propoxyphene and acetaminophen) and amitriptyline. Amitriptyline, nortriptyline and propoxyphene were all detected in third-instar larvae in concentrations below that of the muscle food source. Analyses on puparia and adults were totally negative. These results demonstrate that drugs do not bioaccumulate throughout larval life-cycle, suggesting an efficient elimination through the Malpighian tubules and the ''nephrocytes'' of Diptera maggots [35]. A drug, indeed, can be detected from larvae when its rate of absorption exceeds the rate of elimination.

Similarly, Sadler et al. [36] focused on drug accumulation and elimination in *C. vicina* larvae fed on drug-laden muscle from three suicides involving amitriptyline, temazepam and a combination of trazodone and trimipramine. The pattern seen was a gradual rise in larval drug concentration to a peak at about 7–8 days (associated with postfeeding stage and pupariation) which then decreased to zero by pupariation at 16 days. These drugs were undetectable in puparia using routine toxicological techniques. The authors observed that larvae metabolise and eliminate drugs with varying levels of efficiency since larval drug concentrations vary considerably throughout larval development with a clear decrease in drug concentrations measured in non-feeding larvae and at pupariation. The precipitous decrease in drug concentrations observed in non-feeding larvae and at pupariation also suggested that only larvae actively feeding on a corpse and fully developed should be sampled for toxicological analysis because they represent the best source of drug residues. In another experiment, Sadler et al. [37] investigated amitriptyline accumulation and elimination in *C. vicina* larvae. The results showed a large degree of biological variation in larval drug concentrations indicating unreliable quantitative extrapolation and unpredictable larval drug accumulation when maggots encounter more than one drug or different concentrations of a single drug.

These studies provide very important information, namely the absence of a drug from larvae does not necessarily indicative that a drug is not present in the food source. In this respect, Sadler et al. [38] reared *C. vicina* larvae on a substrate consisting of a mixture of aspirin (acetylsalicylic acid), sodium salicylate, paracetamol, sodium aminohippurate, amphetamine sulphate and barbiturates (thiopentone, phenobarbitone, amilobarbitone, barbitone and brallobarbitone) in concentrations equivalent to those accumulated in skeletal muscle from fatal human overdoses. The toxicological analysis by HPLC of larvae developed on this food source yielded a negative result for paracetamol, aspirin, amilobarbitone and thiopentone (efficiently eliminated by *C. vicina*). For the other drugs, concentrations in larvae were

found to be significantly lower than in their food source. Barbiturates sharing the same basic chemical structure based on a pyrimidine ring but differing in their side chain structure were observed to be metabolised differently by larvae. The authors' comments were that it is impossible to predict which drugs are likely to be detected in maggots, on the basis of the chemical structure. Furthermore, it was confirmed that the absence of a drug in feeding larvae does not necessarily imply its absence in the food source. In another experiment, Sadler et al. [39] reared *C. vicina* larvae on a substrate containing four common benzodiazepines (bromazepan, diazepam, flurazepam and loprazolam). Results of the toxicological analysis by HPLC were negative for loprazolam (rapidly eliminated by larvae) while both bromazepam and diazepam were detectable but the relationship between larval drug concentration and foodstuff concentration differed. The authors demonstrated that the benzodiazepine group of drugs show unpredictable patterns of drug accumulation in larvae, as well as that of barbiturates.

Based on the results of these studies, Sadler et al. [37] also found that drug concentrations in larval and pupal samples which were left unwashed prior to analysis are significantly higher than in adequately washed samples due to surface contamination. Consequently unwashed larvae can be useful just for qualitative detection of drugs but adequate washing of larval samples is required before any quantitative assumptions can be made.

On account of the above remarks, further entomotoxicological research should be carried out focusing on bioaccumulation, insect metabolism of drugs and test study data. Much more has to be investigated even on the correlation between drug concentrations in larvae and the human tissues on which these larvae have fed. However, all the papers reviewed show that prescription and illegal drugs and toxins can be detected in arthropods. Diptera larvae, in particular, those that are actively feeding on human bodies provide a potentially valuable source of information in forensic investigations especially in the absence of tissues and fluids normally taken for toxicological analyses (see badly decomposed bodies or skeletonised human remains).

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