Forensic Science International 208 (2011) 1–9

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Review article

Entomotoxicology, experimental set-up and interpretation for forensic toxicologists

Matthias Gosselin [a,b,*], Sarah M.R. Wille [a], Maria del Mar Ramírez Fernandez [a], V. Di Fazio [a], Nele Samyn [a], Gert De Boeck [a], Benoit Bourel [c,d,e]

[a] Laboratory of Toxicology, National Institute of Criminalistics and Criminology, Chaussée de Vilvorde, 100 B-1120 Brussels, Belgium
[b] Laboratory of Zoology, University of Mons, Place du parc, 20 B-7000 Mons, Belgium
[c] Univ Lille Nord de France, F-59000 Lille, France
[d] UCLille, FLST, labo Environnement & Santé, F-59000 Lille, France
[e] UDSL, IML, F-59000 Lille, France

ARTICLE INFO

Article history:
Received 12 July 2010
Received in revised form 9 December 2010
Accepted 19 December 2010
Available online 14 January 2011

Keywords:
Drugs analysis
Forensic entomology
Forensic toxicology
Insect development
Review

ABSTRACT

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.

However, several limitations of entomotoxicology have been highlighted, especially concerning interpretation of the drug concentrations in insects on human forensic cases. In addition, the lack of knowledge in pharmacokinetic of drugs in insects, large variability of experimental set-up and toxicological analysis compromise the utility of this science.

This review focuses on the current knowledge of factors influencing drug detection in insects. Reasons for the current limitations, but also recommendations for future research are discussed and proposed in this paper.

Contents

1. Introduction

From 1980 entomologists started to detect drugs in insects, hoping it would become a useful tool in forensic investigations [1,2]. Although this science has permitted to provide answers to real forensic cases in the past decades [3,4], several scientists are still skeptical of the potential value of entomotoxicology in forensic investigations, regarding it as a laboratory curiosity [5] or a scientific imposter [6].

The major interest of entomotoxicology is the determination of drug abuse just before death, especially in skeletonised remains where no tissue or fluids are left. Also in highly decomposed remains, toxicological analyses can be facilitated using insects as less interferences in the analytical run due to matrix decomposi-

* Corresponding author at: Laboratory of Toxicology, National Institute of Criminalistics and Criminology, Chaussée de Vilvorde, 100 B-1120 Brussels, Belgium. Tel.: +32 2 240 05 60; fax: +32 2 243 46 08.
E-mail address: Matthias.gosselin@just.fgov.be (M. Gosselin).

0379-0738/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.forsciint.2010.12.015

tion are observed [4,7]. Moreover, in a comparative study by Kintz et al. [8] a greater sensitivity was obtained using fly larvae instead of putrefied material. In addition, drug concentrations seem to be more stable in insects, while this is not always the case for several post-mortem tissues [7]. From a pure practical point of view, insects are of interest as they are present in high quantities and their remains (puparia or exuviae) are present for a long time, even when toxicological samples are no longer available [9]. In cases where religious and ethical beliefs result in problems collecting samples for toxicological analysis, insects could be a solution [10].

The use of necrophageous species as matrix for qualitative drug detection is well documented and generally accepted by forensic toxicologists [11]. Many compounds (drugs, metals and pesticides) have been detected in insect tissues in a forensic context (Table 1). Some animal studies have demonstrated a possible correlation between drug concentrations in the substrate and the different developmental stages of insects reared on that substrate [9,11,12]. However, the main problem for entomotoxicologists is interpretation of the results. At this point it is not possible to estimate the cause and circumstances of death from drug concentrations observed in insects reared on the corpse [6]. However, this relationship in human forensic cases is not yet established and still remains a controversy [6,13,14]. According to some authors, it seems hardly expected to find such a quantitative relationship due to a wide array of influencing factors which are largely unexplored and at the moment unpredictable [6].

The aim of this review is to give an overview of the recent knowledge in the field of entomotoxicology. In addition, several pitfalls which do not allow interpretation in forensic investigations at the moment will be discussed.

2. Interpretation of current limitations: current knowledge and limitations

2.1. Insects

2.1.1. Life traits and drug pharmacokinetics

Pharmacokinetics of drugs in insects depend on the species, the developmental stage as well as on their feeding activity [4,11].

A part from necrophageous species, bioaccumulation can also occur in parasitoids, predators or omnivorous species [15,16]. However, this drug bioaccumulation will not be similar as these species present different feeding behaviour due to their diet or life history traits [17]. 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) [18].

Not only species is of importance, but also the developmental stage and their activity. After hatching of the eggs, there are 3 larval stages. In the third larval stage, three main activities are observed: feeding, digestion and searching for food [19,20]. Once the maximum larval size is obtained, they stop feeding, walk away from the food source, searching for a place to become a pupa. Differences in drug concentrations are observed in larvae with different feeding activities [20]. Moreover, drug concentrations in puparia are lower then in larvae, but seem to be more reproducible. In highly decomposed bodies, puparia of first generation colonizing insects could be a better 'drug concentration estimator' than larvae from the second generation [21]. However, for forensic entomologists additional quantification of drugs in larvae can be important to explain alteration of blowfly development and thus PMI estimation.

While there is a difference in drug quantities observed in different larval stages or pupa, there are still a lot of questions unanswered and discussions about drug metabolism, absorption and elimination and thus drug possible drug accumulation and localisation in insects [9,11,17,22–28]. In feeding larvae, drugs could be absorbed across the midgut and distributed into the larvae [29]. However, it is unclear if drugs are stored in haemolymphs, fat bodies or the integument [29,30]. Drugs can be excreted directly via the gut [29] or via malpighian tubules after metabolism. According to Parry et al. [29], drug metabolism in insects occurs in the malpighian tubules via cytochrome P450 and glutathione transferase enzymes. However, drug metabolism in insects is not yet elucidated. Presence of metabolites could result from the action of substrate enzymes [11] and/or larvae metabolism [25,26].

Malpighian tubules do not only result in drug excretion during larval stage, but are still secreting drugs during the post-feeding stage leading to lower drug levels in comparison to actively feeding larvae for most drugs [12,28]. Once insects are in the pupal stage, the malpighian tubules are degraded and the remaining gut content will only be excreted as meconium during emergence of the adult insect [29]. As drug concentrations seem low in adults, most drugs must be excreted during the first two days of adult life [31].

It is clear that drug metabolism and excretion in the different developmental stages of insects should be studied more in detail to get an idea of how and to what extent drugs are incorporated in insect tissues. As a result, entomotoxicologists could determine which insect phase is of most interest to detect drugs, which tissues will lead to highest, most reproducible drug concentrations and thus will be the insect tissues having most change to increase the reliability of forensic entomotoxicological results.

2.1.2. Extrinsic conditions and their influence on insects

The oviposition and development of insects can be affected by bioclimatic factors such as the photoperiod and temperature [32–37]. This is of major importance for estimation of PMI by forensic entomologists. At first, these environmental factors seem to be less important for drug detection in insects. However, entomotoxicologists will use experimental data -often generated in laboratories under constant light and temperature conditions- for interpretation of real forensic cases. Therefore, entomotoxicologists must be aware of the influence of environmental factors on the insect development.

Constant light increases the variation of the time for an insect to become an adult and significantly delays the development compared to insects reared with cyclic photoperiods [32]. The development can also be accelerated or retarded according to the ambient temperature [36,37]. In addition, rain and thus the humidity influences oviposition and development of blowflies [33]. If entomological experiments are done in the lab, it is therefore important to use a realistic photoperiod 12:12 h (light/dark), and a humidity and temperature in function of the investigated insect species and the climatic conditions of the region where the forensic case is situated.

Not only the insect development can be changed due to climatic factors, but also the gut motility and thus the absorption and excretion of drugs is dependant on the ambient temperature [29]. Moreover, drug stability in tissues can also be influenced by temperature, humidity and UV-radiation [6]. As a result, environmental factors can have an influence on the final drug concentration present in the analysed insect tissue.

Because of the importance of the climatic conditions, they should be monitored and noted during the development, sampling

Table 1
List of toxic substances detected in different developmental stages of insects.

		Insect species	Developmental stages	References
Alcohol	Ethanol	Calliphoridae, Sarcophagidae	L	[13]
		Phormia regina	L	[32]
Drugs				
Antidepressants	Amitryptyline	No specified	L	[6]
		Lucilia sericata	L	[12]
		Calliphora vicina	C	[64]
			L, P	[28,39]
		Dermestes maculatus	E, F, Pu	[17]
		Megaselia scalaris	E, F, Pu	[17]
	Clomipramine	No specified	L	[6,7]
		Lucilia sericata	L	[12]
	Dothiepin	No specified	L	[6]
	Fluoxetine	No specified	L	[6]
	nortriptyline	*Lucilia sericata*	L	[12]
		Dermestes maculatus	E, F, Pu	[17]
		Megaselia scalaris	E, F, Pu	[17]
	Trazodone	*Calliphora vicina*	L	[39]
	Trimipramine	*Calliphora vicina*	L	[39]
	Venlafaxine	No specified	L	[6]
Barbiturates	Amobarbital	No specified	L	[6]
	Barbiturates	No specified	L	[6]
	Phenobarbital	*Cochliomyia macellaria*	L	[1]
		Lucilia sericata	L	[12]
		No specified	L	[6,7]
	Secobarbital	No specified	L	[65]
	Sodium amylobarbitone	*Calliphora vicina*	L, P	[51]
	Sodium barbitone	*Calliphora vicina*	L, P	[51]
	Sodium brallobarbitone	*Calliphora vicina*	L, P	[51]
	Sodium phenobarbitone	*Calliphora vicina*	L, P	[51]
	Sodium thiopentone	*Calliphora vicina*	L, P	[51]
Benzodiazepines	Alprazolam	No specified	L	[6]
		Calliphora vicina	L, P	[26]
	Bromazepam	No specified	L	[6]
		Piophila casei	L, P, A	[40]
	Clonazepam	*Calliphora vicina*	L, P, A	[26]
	Diazepam	*Calliphora vicina*	L, P, A	[26]
		Chrysomya albiceps		[27]
		Chrysomya putoria		[27]
	Flunitrazepam	*Calliphora vicina*	L, P, A	[26]
	Lorazepam	No specified	L	[6]
		Calliphora vicina	L, P, A	[26]
	Nordiazepam	No specified	L	[6]
		Calliphora vicina	L, P, A	[26]
	Oxazepam	No specified	L	[6,7]
		Calliphora vicina	L, P, A	[26]
	Prazepam	*Calliphora vicina*	L, P, A	[27]
	Temazepam	*Calliphora vicina*	L, P, A	[26,39]
	Triazolam	No specified	L	[6]
		Calliphora vicina	L, P, A	[7,26]
		No specified	L	[8]
Miscellaneous	Amphetamine	No specified	No specified	[13]
		Calliphora vicina	L	[51]
	Benzoylecgonine	No specified	L	[4]
	Cocaine	No specified	L	[4,24]
		Lucilia sericata	L	[12]
	Digoxin	No specified	L	[6]
	Meprobamate	No specified	L	[6]
	Nefopam	No specified	L	[6]
	Sodium aminohippurate	*Calliphora vicina*	L	[51]
	Sodium salicylates	*Calliphora vicina*	L	[51]
	THC-COOH	No specified	L	[6]
	11-Hydroxy-THC	No specified	L	[6]
Opioids/opiates	Codeine	*Lucilia sericata*	L, P, A	[11]
		No specified	L	[6,66]
	Methadone	*Lucilia sericata*	L	[20]
	Morphine	*Dermestes freshi*	L, P, A	[9,67]
		Thanatophilus sinuatus	L, P, A	[9,67]
		Lucilia sericata	L, P, Pu, A	[9,53]
		Calliphora stygia	L	[42]
			L, P, Pu, PP, A	[29]
		Calliphora vicina	L, Pu	[23]
		Calliphora vicina	L, P	[54]
		Protophormia terraenovae	L, P	[54]
		No specified	L	[6,66]
	Opiates	*Lucilia sericata*	L	[12,14]
	Pholcodine	No specified	L	[6]

Table 1 (*Continued*)

		Insect species	Developmental stages	References
	Propoxyphene	No specified	L	[6]
		Calliphora vicina	C	[64]
Phenothiazine	Alimezanine	No specified	L	[6,7]
	Chlorpromazine	No specified	L	[6]
	Cyamezanine	No specified	L	[6]
	Levomepromazine	No specified	L	[6]
		Lucilia sericata	L	[12]
		Piophila casei	L	[40]
	Thioridazine	*Lucilia sericata*	L	[12]
METALS	Antimony	*Calliphora dubia*	L, P, Pu, A	[22]
		Lucilia sericata	L	[68]
	Barium	*Calliphora dubia*	L, P, Pu, A	[22]
		Lucilia sericata	L	[68]
	Cadmium	*Lucilia sericata*	L, Pu, A	[69]
	Lead	*Calliphora dubia*	L, P, Pu, A	[22]
		Lucilia sericata	L	[68]
	Mercury	*Calliphoridae*	L, Pu, A	[31]
PESTICIDES				
organophosphates	Malathion	*Chrysomya megacephala*	L, P	[62]
			L, PP, P	[70]
			L	[71]
		Chrysomya ruffifacies	L	[71]
	Parathion			
		Diptera	L, P, Pu, A	[41]
		Coleoptera	A	[41]
		Hymenoptera	A	[41]

L, larva; P, pupa; PP, prepupa; Pu, puparia; A, adult; C, crop; F, frass; E, exuvia.

and storage of insects reared experimentally. In real cases, climatic conditions should be investigated via meteorological station to have as much information as possible for insect development. Concerning the influence of environmental factors on the insects gut motility and final absorption of drugs in insect tissues as well as the stability of drugs in those tissues, future research will be necessary to have an idea of the impact of environmental factors on final drug detection.

2.1.3. 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 puparia) 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 [38].

In addition, several authors [6,10,39] demonstrate the importance of collecting at different body-sites, as inter-site sampling results in a high variation of drug concentrations. 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 [13,14] 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 [15]. In older research or case reports, larvae or puparia are often removed from different areas of the corpse and pooled in one sample [7,8,40], possibly due to problems with the sensitivity of the used analytical methods. However, information concerning drug detection in different body areas as well as inter-insect variation due to differences in developmental stage or activity (see above) will be lost [6,20]. This information may not be of interest for case report interpretation at this moment, but for development of future entomotoxicological knowledge it is of most importance.

At the moment, standards and guidelines for insect sampling in forensic entomotoxicology are published by Amendt et al. [18] and Carvahlo et al. [10]. 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. [6] 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. In Fig. 1, a modified sampling protocol based on the ones from Amendt et al. [18] and Carvahlo et al. [10] is shown. The adaptations focus on the amount of specimens sampled, the sampling frequency and information concerning the insect activity. The sampling in experimental set-up can occur between different developmental stages [9,12] or at regular time intervals [11]. In the same interval time, different developmental stages or activities are observed, resulting in irreproducible quantitative results [20]. Therefore, sampling more frequently at onset of each developmental phase is recommended. We also suggest keeping larvae in the feeding phase alive during several hours before killing them, to be sure that drugs present in their digestive tract are completely excreted. These additional parameters are of most interest for research purposes and less for case report interpretation at this moment.

2.2. Analytical procedures

Reporting reliable analytical data should be the backbone of forensic research. Entomotoxicological investigators need results that are valid, reproducible and comparable to draw scientifically correct conclusions from their experiments. This is of major importance as these results will be used by others for case-report interpretation. Therefore, international agreement concerning proper insect killing methods, storage, sample handling and analysis of samples are needed. In addition, the entomotoxicologist should pay attention to validation requirements.

When reviewing the literature, the conclusion rises that there is no recommended standard protocol for killing insects, for their

Case N° ______ Localisation ______ Date/time ______

Victime ______ Age (y) ____ Sex ____ Weight (Kg) ____

Decomposition stage ______ Estimated PMI ______

Traces : Syringes ☐ (Illegal) substances ☐ Prescription ☐

Cause of death ______

Temperature (°C) ______ Climatic conditions ______

Site description ______

Sample N°	Location *	Species	Developmental stages **	N
	L☐ B☐ M☐ O☐		L☐ P☐ EP☐ A☐	
	L☐ B☐ M☐ O☐		L☐ P☐ EP☐ A☐	
	L☐ B☐ M☐ O☐		L☐ P☐ EP☐ A☐	
	L☐ B☐ M☐ O☐		L☐ P☐ EP☐ A☐	
	L☐ B☐ M☐ O☐		L☐ P☐ EP☐ A☐	
	L☐ B☐ M☐ O☐		L☐ P☐ EP☐ A☐	
	L☐ B☐ M☐ O☐		L☐ P☐ EP☐ A☐	
	L☐ B☐ M☐ O☐		L☐ P☐ EP☐ A☐	
	L☐ B☐ M☐ O☐		L☐ P☐ EP☐ A☐	
	L☐ B☐ M☐ O☐		L☐ P☐ EP☐ A☐	
	L☐ B☐ M☐ O☐		L☐ P☐ EP☐ A☐	

* L, liver; B, brain; M, muscle; O, others

** L, larvae; P, pupae; EP, empty pupae; A, adults

Fig. 1. Protocol sheet of samples for entomotoxicological analysis. (*) L, liver; B, brain; M, muscle; O, others. (**) L, larvae; P, pupae; EP, empty pupae; A, adults.

storage, and for decontamination of the samples. These steps, however, can influence the result as drug stability or contamination from the food source will alter the analytical result and thus the final conclusion.

Specimens such as larvae or adults are killed either by freezing [6,11,12,14,26,34] or boiling [22]. Thereafter, they are stored in alcohol (70%) [22] or at −20 °C [6,11,12,14,26,34]. Pupae, on the other hand, are stored under dry conditions at 2–6 °C. At the moment, we would recommend storage under dry conditions at −20 °C for all samples to ensure drug stability and to diminish possible drug extraction from the matrix when storing in alcohol. However, future research must explore drug stability using different storage methods, but also under realistic forensic circumstances. Another important step before analysis is the decontamination of insects. Higher drug concentrations were observed for unwashed specimens than washed ones due to surface contamination [39]. Insect specimens should be washed using deionized water, a physiological NaCl solution (0.9%) [11] or using methanol [9]. However, no study has yet evaluated the efficiency of those washing steps.

Once the specimens are killed and decontaminated, the samples will be prepared for analysis. The sample preparation depends on the nature of the insect tissue and on the drug of interest. Because insect specimens are solid, they will first be macerated and homogenized [41,42], or digested using strong acids, bases or enzymes [22,43], or pulverized by grinding [11,14,35]. To our experience, use of a Precellys 48 homogenizer (Bertin Technologies, Montigny-Le-Bretonneux, France) leads to pulverization of a single specimen in a separated vial, avoiding cross contamination between different samples [20]. Once the samples are pulverized, drugs are extracted from the matrix. Optimal extraction techniques lead to enhanced sensitivity of the method, but will also remove potential interfering matrix compounds, resulting in enhanced selectivity and a more reproducible method independent of variations in the sample matrix. Table 2 summarizes the detected substances, and their sample preparation/analysis observed in the literature. It is clear that entomotoxicologists still use classic extraction techniques such as protein precipitation, liquid-liquid extraction (LLE) or solid phase extraction (SPE). It is not the scope of this review to discuss the pros and cons of the different extraction techniques in detail. For this, we refer to other publications [44]. However, the aim of the final extraction technique is extracting the compounds of interest in a straightforward way, resulting in the required sensitivity, without matrix effects and with an extract that is compatible with the final analytical procedure.

Several analytical drug detection/quantification procedures have been used for the analysis of insect tissues (Table 2). The choice of technique depends on the physicochemical properties of the drugs of interest and of course on the required selectivity and sensitivity. In light of recent developments in the field of entomotoxicology, it is important to have very sensitive methods to ensure analysis of single specimens from different locations of the body for case report interpretation (see above). However, the observed techniques used in previous entomotoxicological research and case reports are not always sensitive enough and most of the time they require pools of specimens to detect any present drug. While in the past techniques such as MS/MS were not available in most laboratories, today these techniques can lead to a sensitivity which permits analysis of one single specimen [20,25,26] and will hopefully result in new insights in this field as well as better drug detection in forensic investigations. Another observation is that previously techniques that were not specific, e.g. immunoassays, were often applied to draw conclusions. Again use of MS or MS/MS methods, properly validated, can lead to more selective and specific drug detection leading to more reliable results.

Not only the choice and development of the applied technique is important to ensure a reliable drug detection and quantification; a validation according to international standards [45–47] should be obtained to ensure the validity of the results and thus the final conclusion/interpretation. It seems very plausible that a method should be ‘fit for purpose’; however, when reviewing entomotoxicological publications, the methods tend to lack proper validation. Most validation guidelines include selectivity, sensitivity, calibration, accuracy, precision, and stability as fundamental validation parameters. Although some publications discuss precision and bias of the method, only a few studies have measured the extraction recovery, matrix effects or stability of the samples [11,20,26]. For methods using LC–MS, evaluation of matrix effects is obligatory as co-eluting compounds can influence the ionization of the compounds of interest, influencing sensitivity, and quantification of the target analyte. During development of the method, the extraction technique should be evaluated to guarantee reproducible results and adequate sensitivity. However, a problem for determination of the extraction recovery in insect tissues is that often spiking drugs to blank larvae or pupae does not resemble “real cases” as the drugs will not be incorporated in the tissue but just spiked ‘onto’ the matrix. Stability is another important parameter that should be evaluated more in detail during the whole entomological study ranging from the killing procedure, to the storage and processing of the sample to ensure proper results.

2.3. Experimental set-up

The major drawback of the field of entomotoxicology at the moment is the lack of interpretation of detected drug concentrations. While this problem will not be resolved in the near future, entomotoxicologists try to establish a relationship between drug

Table 2
Comparison of sample preparation and analytical procedures for each toxic substance.

Toxic substances	Sample preparation		Analytical procedures	References
	Digestion	Extraction		
Alcohol				
Ethanol			HS GC–FID	[13,32]
Drugs				
Antidepressants				
Amitryptyline (nortryptyline)		LLE	GC–MS	[12,39]
		LLE	HPLC-UV	[64]
	Basic conditions (ammonium hydroxide) +acidic conditions (hydrochloric acid)	LLE	GC–MS	[17]
		LLE	HPLC-UV (larvae) GC–MS (pupae)	[28]
Clomipramine			HPLC	[7]
		LLE	GC–MS	[12]
Trazodone		LLE	HPLC-UV	[39]
Trimipramine		LLE	HPLC-UV	[39]
Barbiturates				
Phenobarbital			GC–MS	[1]
			HPLC	[7]
		LLE	GC–MS	[12]
Secobarbital		SLE	GC–MS	[65]
Sodium amylobarbitone		SLE	HPLC-UV	[51]
Sodium barbitone		SLE	HPLC-UV	[51]
Sodium brallobarbitone		SLE	HPLC-UV	[51]
Sodium phenobarbitone		SLE	HPLC-UV	[51]
Sodium thiopentone		SLE	HPLC-UV	[51]
Benzodiazepines				
Benzodiazepines (alprazolam, clonazepam, diazepam, flunitrazepam, lorazepam, nordiazepam, oxazepam, prazepam, temazepam, triazolam)		(1) Precipitation (2) SPE (3) LLE (4) Toxitube	LC–MS/MS	[26]
Bromazepam			HPLC	[40]
Diazepam		LLE	GC–MS	[27]
Oxazepam			HPLC	[7]
Temazepam		LLE	HPLC-UV	[39]
Triazolam			HPLC	[7,8]
Miscellaneous				
Amphetamine		SPE	GC–MS	[13]
		LLE	HPLC-UV	[51]
Benzoylecgonine		LLE	RIA, GC–MS	[4]
Cocaine		LLE	RIA, GC–MS	[4]
		LLE	GC–MS	[12]
		SPE	GC–MS	[24]
Sodium amminohippurate		SLE	HPLC-UV	[51]
Opioids/opiates				
Codeine		LLE	LC–MS	[11]
	Enzymatic conditions (β-glucoronidase)	LLE	LC–MS	[66]
Methadone		LLE	LC–MS/MS	[20]
Morphine	Enzymatic conditions (DiThioThreitol)		RIA	[9]
		LLE	LC–MS	[66]
			LC–CL	[29,42]
		precipitation	EIA	[23]
			RIA	[14,53]
Propoxyphene		SLE	GC–NPD	[64]
Phenothiazine				
Alimemazine			HPLC	[7]
Levomepromazine			HPLC	[40]
		LLE	GC–MS	[12]
Thioridazine		LLE	GC–MS	[12]
Metals				
Lead, barium, antimony	Acidic conditions (Nitric acid)		ICP-MS	[22,68]
Pesticides				
Malathion		SPE	GC–ECD	[62]
		LLE	GC–MS	[70,71]
Parathion		LLE	LC–DAD	[41]

(CL chemiluminescence, DAD diode array detector, ECD electron capture detection, EIA enzyme immunoassay, FID flame ionization detector, FPIA fluorescence polarisation immunoassay, GC gas chromatography, HS headspace, HPLC High performance liquid chromatography, ICP inductively coupled plasma, LC liquid chromatography, LLE liquid-liquid extraction, MS mass spectrometry, NPD nitrogen-phosphorus detection,, RIA radioimmunoassay, SLE supported liquid extraction, SPE solid phase extraction, UV ultraviolet). *Data coming from Tracqui et al. [4] are not mentioned because analytical methods (GC–MS and LC–MS) were not specified for each compound.

concentrations in insects and their substrates via experiments. Interpretation of these experiments are not only complicated by differences in insect species, developmental stages, activities and analytical problems as discussed above, but is also due to different experimental conditions.

Artificial foodstuff [48–51] or post-mortem spiked animal organs [9,11,22,23,25,26,32,39,42,52] are mostly used to rear insects. These substrates are widely used, as they are easy to prepare and have a low cost. Unfortunately, problems during preparation and storage such as insufficient drug homogeneity, nutritional requirements or water can occur [48]. These parameters should be monitored during the experiments and substrates with the propensity to lose water should not be frozen prior to use but stored at 4 °C [48]. One of the most important drawbacks of this type of substrate is the fact that the spiked drugs are not metabolized by a living system. This is important for interpretation as in real case scenario's the drug availability will be altered and the produced metabolites will also be consumed by the insect [50,52].

The way to get around the major drawback of post-mortem spiked substrates is the use of live-animal models, in which drugs are given ante-mortem orally or via injection. Use of live-animals gives a realistic view because the drug is metabolized. However, it is not easy to set-up statistical valid tests. In addition, experiments must be approved by an animal ethics committee [33] and the delay of acceptation can be very long. The animal is mostly sacrificed 30 min [10] after drug administration and the whole animal [9,53–55], a single organ (mostly the liver) or the muscle [14,32,56–62] are thereafter exposed to insect larvae under laboratory conditions. If the whole animal is used to rear larvae, the process is more realistic, but interpretation of the experiment is more complex due to the movement of larvae on the cadaver. Because larvae wander on the cadaver and the accurate location of feeding is not known the link between the drug concentration in actual feeding place and the insect is not possible. In addition, post-mortem redistribution may increase interpretation problems. When a single organ or muscle tissue is used, results obtained will vary according to the different type of tissue [55]. The choice of animal species will also have implications on the degree of information obtained from the ante-mortem experiment. Most of authors use small animals such as domestic rabbits [9,27,53,54,56–61] or rats [62], if possible with the same weight and sex [10] to avoid variability from these parameters. Because these animal models have limited use as drug distribution and metabolism will be different compared to humans [63], some researchers use pigs [32,35]. However we must keep in mind that an animal model never guarantees a simulation of human drug overdose due to pharmacokinetic differences [52]. Moreover, certainly if larger animals are used, the number of replicates can be very low. Three replicates is the minimum for statistical analysis [10,32,62].

Before entomo(toxico)logical experimental results can be used for case-report interpretation, possible differences in protocol such as method of injection/oral overdose, time before analysis (post-mortem redistribution), drug stability, bacterial metabolism must be studied to understand their influence on the obtained results [6,63]. In addition, more research concerning the comparison of spiked foodstuff versus live-animal models should be conducted and therefore experimental protocols should be harmonized as much as possible [48].

2.4. Interpretation in forensic cases

At the moment, there is general agreement in the forensic toxicology community that drug concentrations in insects cannot be interpreted. While entomotoxicologists try to find a relationship between drug concentrations in the substrate and the reared insects using experiments with spiked artificial foodstuff or animal models, collection of insect specimens from human post-mortem case reports are the most interesting. These insects are reared in realistic circumstances. However, there are several drawbacks. First of all, it is difficult and time-consuming to obtain a human case report database for research; e.g. Tracqui et al. [6] analyzed 29 necropsies in 15 years. Some researchers use human tissues [4,6–8,13,17,24,34] such as muscle [9,64] or liver [12,14] to rear larvae experimentally, however, this is ethically questionable. Moreover, determination of drug concentrations in decomposing tissues is not obvious and even impossible in skeletonised remains. Even though drug concentrations are determined, post-mortem drug redistribution and drug stability are not always well known, and complicate interpretation [6]. Unless enormous advances occur concerning the knowledge of factors influencing drug concentrations in insects, it is almost of no interest to have a toxicological investigation linking drug concentration to possible effect in practical casework [6]. However, for skeletonised remains, insects can be the last resort for a toxicological finding that can give information concerning possible drug use before death. Moreover, drug detection in insects can help the entomologist to determine the PMI, as insect development will be changed by drugs present in the corpse.

3. Conclusion

In the latest years more research has been conducted in the field of entomotoxicology, trying to find a relationship between drug concentrations in the substrate and insects reared on that substrate, and to increase the knowledge of insect development. A literature review demonstrates that drug detection and analytical quantification in insects is not a problem. Although the recent progress in this science, interpretation of entomotoxicological results from human cases is not yet established nor expected. The aim to establish a link between substrate and insect, and thus toxicological interpretation, is perhaps a utopia due to several unknown and complicating factors. However, when reviewing the current studies, one can not deny the fact that maybe this current limitation is partially due to insufficient knowledge of insect development and activity, of proper use and validation of analytical procedures and lack of a general consensus concerning experimental set-up and sampling. While some problems can be resolved by a standardization of methods, procedures, and validation protocols, others need further research. Thus, research in the near future should focus on physiological process during the feeding stage, drug metabolism and accumulation-excretion mechanisms in insects, but also on drug redistribution and post-mortem drug stability. After studying these parameters, a long-term comparative study could be applied on insects reared on human cases. At the moment, detection of drug in insects is certainly possible. While it can not yet be used for toxicological interpretation in most cases, when only skeletonised remains are left, puparia can be the only hope for a toxicologist to have some information concerning drug use prior to death. In addition, detection of drugs in insect tissues by the analytical toxicologist can aid the determination of the PMI by the entomologist. Hopefully, the importance of this science will increase when validated experimental protocols and methods will be systematically applied in each entomotoxicological analysis.

References

[1] J.C. Beyer, W.F. Enos, M. Stajic, Drug identification through analysis of maggots, J. Forensic Sci. 25 (2) (1980) 411–412.
[2] F. Introna, C.P. Campobasso, M.L. Goff, Entomotoxicology, Forensic Sci. Int. 120 (2001) 42–47.
[3] M.L. Goff, W.D. Lord, Entomotoxicology, A new area for forensic investigation, Am. J. Forensic Med. Pathol. 15 (1994) 51–57.
[4] K.B. Nolte, R.D. Pinder, W.D. Lord, Insect larvae used to detect cocaine poisoning in a decomposed body, J. Forensic Sci. 37 (4) (1992) 1179–1185.

[5] E. Gaudry, J.-B. Myskowiak, B. Chauvet, T. Pasquerault, F. Lefebvre, Y. Malgorn, Activity of the forensic entomology department of the French Gendarmerie, Forensic Sci. Int. 120 (1–2) (2001) 68–71.
[6] A. Tracqui, C. Keyser-Tracqui, P. Kintz, B. Ludes, Entomotoxicology for the forensic toxicologist: much ado about nothing? Int. J. Legal Med. 118 (2004) 194–196.
[7] P. Kintz, B. Godelar, A. Tracqui, P. Mangin, A.A. Lugnier, A.J. Chaumont, Fly larvae: a new toxicological method of investigation in forensic medicine, J. Forensic Sci. 35 (1990) 204–207.
[8] P. Kintz, A. Tracqui, P. Mangin, Toxicology and fly larvae on a putrefied cadaver, J. Forensic Sci. Soc. 30 (1990) 243–246.
[9] B. Bourel, G. Tournel, V. Hedouin, M. Deveaux, M.L. Goff, Morphine extraction in necrophageous insects remains for determining ante-mortem opiate intoxication, Forensic Sci. Int. 120 (2001) 127–131.
[10] L.M.L. Carvahlo, Toxicology and forensic entomology, in: J. Amendt, C.P. Campobasso, M.L. Goff, M. Grassberger (Eds.), Current Concepts in Forensic Entomology, Springer Science, 2010, pp. 163–178.
[11] H. Kharbouche, M. Augsburger, D. Cherix, F. Sporkert, C. Giroud, C. Wyss, C. Champod, P. Mangin, Codeine accumulation and elimination in larvae, pupae, and imago of the blowfly Lucilia sericata and effects on its development, Int. J. Legal Med. 122 (2008) 205–211.
[12] C.P. Campobasso, M. Gherardi, M. Caligara, L. Sironi, F. Introna, Drug analysis in blowfly larvae and in human tissues: a comparative study, Int. J. Legal Med. 118 (2004) 210–214.
[13] M. Definis-Gojanovic, D. Sutlovic, D. Britvic, K. Boze, Drug analysis in necrophageous flies and human tissues, Arh. Hig. Rada Toksikol. 58 (2007) 313–316.
[14] F. Introna, C. Lo Dico, Y.H. Caplan, J.E. Smialek, Opiate analysis in cadaveric blowfly larvae as an indicator of narcotic intoxication, J. Forensic Sci. 35 (1990) 118–122.
[15] L.D.B. Faria, L. Orsi, L.A. Trinca, W.A.C. Godoy, Larval predation by Chrysomya albiceps on Cochliomyia macellaria, Chrysomya megacephala and Chrysomya putoria, Entomol. Exp. Applic. 90 (1999) 149–155.
[16] P. Nuorteva, Sarcosaprophagous insects as forensic indicators, in: G.C. Tedeschi, W.G. Eckert, L.G. Tedeschi (Eds.), Forensic Medicine: A Study in Trauma and Environmental Hazards, W.B. Saunders, Philadelphia, 1977, pp. 1072–1095.
[17] M.L. Miller, W.D. Lord, M.L. Goff, B. Donnely, E.T. Mcdonough, J.C. Alexis, Isolation of amitriptyline and nortriptyline from fly puparia (Phoridae) and beetle exuviae (Dermestidae) with mummified human remains, J. Forensic Sci. 39 (1994) 1305–1313.
[18] J. Amendt, C. Campobasso, E. Gaudry, C. Reiter, H. Leblanc, M.J.R. Hall, Best practice in forensic entomology: standards and guidelines, Int. J. Legal Med. 121 (2) (2006) 90–104.
[19] D. Charabidze, B. Bourel, G. Morvan, V. Hedouin, D. Gosset, Forenseek, un programme de simulation dédié à l'entomologie médico-légale, J. Méd. Lég. Droit Méd. 51 (3) (2009) 131–140.
[20] M. Gosselin, M.D.M. Ramirez-Fernandez, S.M.R. Wille, N. Samyn, G.D. Boeck, B. Bourel, Quantification of Methadone and its Metabolite 2-Ethylidene-1, 5-dimethyl-3,3-diphenylpyrrolidine in Third Instar Larvae of Lucilia sericata (Diptera: Calliphoridae) Using Liquid Chromatography–Tandem Mass Spectrometry, J. Anal. Toxicol. 34 (2010) 1–7.
[21] L. Lefebvre, E. Gaudry, Forensic entomology: a new hypothesis for the chronological succession pattern of necrophageous insect on human corpses, Ann. Soc. Entomol. Fr. 45 (3) (2009) 377–392.
[22] E.M. Roeterdink, I.R. Dadour, J.R. Watling, Extraction of gunshot residues from the larvae of the forensically important blowfly *Calliphora dubia* (Macquart) (Diptera: Calliphoridae), Int. J. Legal Med. 118 (2004) 63–70.
[23] F. Introna, G. Di Vella, R. Gagliano-Candela, Identification d'opiacés sur les pupes vides: contribution expérimentale, J. Méd. Lég. Droit Méd. 44 (3) (2001) 211–215.
[24] D.T. Manhoff, I. Hood, F. Caputo, J. Perry, S. Rosen, H.G. Mirchandani, Cocaine in decomposed human remains, J. Forensic Sci. 36 (1991) 1732–1735.
[25] K. Pien, M. Laloup, M. Pipeleers-Marichal, P. Grootaert, G. De Boeck, N. Samyn, T. Boonen, K. Vits, M. Wood, Toxicological data and growth characteristics of single post-feeding larvae and puparia of Calliphora vicina (Diptera: Calliphoridae) obtained from a controlled nordiazepam study, Int. J. Legal Med. 118 (2004) 190–193.
[26] M. Wood, M. Laloup, K. Pien, N. Samyn, M. Morris, R.A.A. Maes, E.A. De Bruijn, V. Maes, G. De Boeck, Development of a rapid and sensitive method for the quantification of benzodiazepines in Calliphora vicina larvae and puparia by LC–MS-MS, J. Anal. Toxicol. 27 (2003) 505–512.
[27] L.M.L. Carvalho, A.X. Linhares, J.R. Trigo, Determination of drug levels and the effect of diazepam on the growth of necrophageous flies of forensic importance in southeastern Brazil, Forensic Sci. Int. 120 (2001) 140–144.
[28] D.W. Sadler, J. Richardson, S. Haigh, G. Bruce, D.J. Pounder, Amitriptyline accumulation and elimination in Calliphora vicina larvae, Am. J. Forensic Med. Pathol. 18 (1997) 397–403.
[29] S. Parry, S.M. Linton, P.S. Francis, M.J. O'donnell, T. Toop, Accumulation and excretion of morphine by Calliphora stygia, an Australian blow fly species of forensic importance, J. Insect Physiol. 57 (1) (2011) 62–73.
[30] B. Bourel, L. Fleurisse, V. Hedouin, C. Cailliez, C. Colette, D. Gosset, M.L. Goff, Immunohistochemical Contribution to the Study of Morphine Metabolism in Calliphoridae Larvae and Implications in Forensic Entomotoxicology, J. Forensic Sci. 46 (3) (2001) 596–599.
[31] P. Nuorteva, S.-L. Nuorteva, The fate of mercury in sarcosaprophageous flies and in eating them, Ambio 11 (1982) 34–37.
[32] D.R. Monthei, Entomotoxicological and Thermal Factors Affecting the Development of Forensically Important Flies, Faculty of Virginia Polytechnic Institute, Blacksburg, 2009, pp. 1–112.
[33] N.A. Mahat, Z. Zafarana, P.T. Jayaprakash, Influence of rain and malathion on the oviposition and development of blowflies (Diptera: Calliphoridae) infesting rabbit carcasses in Kelantan, Malaysia, Forensic Sci. Int. 192 (1–3) (2009) 19–28.
[34] D.J. Pounder, Forensic entomo-toxicology, J. Forensic Sci. Soc. 31 (1991) 469–472.
[35] K. Tabor, R.D. Fell, C.C. Brewster, K. Pelzer, G.S. Behonick, Effects of antemortem ingestion of ethanol on insect successional patterns and development of *Phormia regina* (Diptera: Calliphoridae), J. Med. Entomol. 42 (3) (2005) 481–489.
[36] L. Davies, G.G. Ratcliffe, Development rates of some preadult stages in blowflies with reference to low temperatures, Med. Vet. Ent. 8 (1994) 245–254.
[37] I.R. Dadour, D.F. Cook, N. Wirth, Rate of development of Hydrotaea rostrata under summer and winter (cyclic and constant) temperatures regimes, Med. Vet. Ent. 15 (2) (2001) 177–182.
[38] M.S. Archer, M.A. Elgar, C.A. Briggs, D.L. Ranson, Fly pupae and puparia as potential contaminants of forensic entomology samples from sites of body discovery, Int. J. Legal Med. 120 (6) (2006) 364–368.
[39] D.W. Sadler, C. Fuke, F. Court, D.J. Pounder, Drug accumulation and elimination in Calliphora vicina larvae, Forensic Sci. Int. 71 (1995) 191–197.
[40] P. Kintz, A. Tracqui, B. Ludes, J. Waller, A. Boukhabza, P. Mangin, A.A. Lugnier, A.J. Chaumont, Fly larvae and their relevance in forensic toxicology, Am. J. Forensic Med. Pathol. 11 (1990) 63–65.
[41] M. Wolff, A. Builes, G. Zapata, G. Morales, M. Benecke, Detection of parathion (O,O-diethyl O-(4-nitrophenyl) phosphorothioate) by HPLC in insects of forensic importance in Medellin, Colombia, Anil Aggrawal's Internet, J. For. Med. Toxicol. 5 (1) (2004) 6–11.
[42] J.A. Gunn, C. Shelley, S.W. Lewis, T. Toop, M. Archer, The determination of morphine in the larvae of Calliphora stygia using flow injection analysis and HPLC with chemiluminescence detection, J. Anal. Toxicol. 30 (2006) 519–523.
[43] R. Gagliano-Candela, L. Aventaggiato, The detection of toxic substances in entomological specimens, Int. J. Legal Med. 114 (2001) 197–203.
[44] S.M.R. Wille, W.E.E. Lambert, Recent developments in extraction procedures relevant to analytical toxicology, Anal. Bioanal. Chem. 388 (7) (2007) 1381–1391.
[45] US Department of Health and Human Services Food and Drug Administration-Center for Drug Evaluation and Research (Cder) Guidance for Industry, Bioanalytical Method Validation (2001).
[46] E.M.A.E. Commitee for Medicinal Products for Human Use (Chmp), Guidelines on validation of bioanalytical methods, EMEA/CHMP/EWP/192217/2009 http://www.emea.eu.int (2009).
[47] F.T. Peters, O.H. Drummer, F. Musshoff, Validation of new methods, Forensic Sci. Int. 165 (2–3) (2007) 216–224.
[48] L.D. Duke. Method validation for amitriptyline and nortriptyline in artificial foodstuff. Cprc (ed.), Ottawa, 2004, pp. 1–183.
[49] R.A. Sherman, J.M-T. Tran, A simple, sterile food source for rearing the larvae of Lucilia sericata (Diptera: Calliphoridae), Med. Vet. Entomol. 9 (1995) 393–398.
[50] H.G. Oliveira, G. Gomes, J.J. Morlin Jr., C.J. Von Zuben, A.X. Linhares, The effect of buscopan on the development of the blow fly *Chrysomya megacephala* (F.) (Diptera: Calliphoridae), J. Forensic Sci. 54 (1) (2009) 202–206.
[51] D.W. Sadler, L. Robertson, G. Brown, C. Fuke, D.J. Pounder, Barbiturates and analgesics in Calliphora vicina larvae, J. Forensic Sci. 42 (3) (1997) 481–485.
[52] K.A. George, M.S. Archer, L.M. Green, X.A. Conlan, T. Toop, Effect of morphine on the growth rate of *Calliphora stygia* (Fabricius) (Diptera: Calliphoridae) and possible implications for forensic entomology, Forensic Sci. Int. 193 (1–3) (2009) 21–25.
[53] V. Hedouin, B. Bourel, L. Martin-Bouyer, A. Becart, G. Tournel, M. Deveaux, D. Gosset, Determination of drug levels in larvae of *Lucilia sericata* (Diptera: Calliphoridae) reared on rabbit carcasses containing morphine, J. Forensic Sci. 44 (2) (1999) 351–353.
[54] V. Hedouin, B. Bourel, A. Bécart, G. Tournel, M. Deveaux, M.L. Goff, D. Gosset, Determination of drug levels in larvae of *Protophormia terraenovae* and *Calliphora vicina* (Diptera, Calliphoridae) reared on rabbit carcasses containing morphine, J. Forensic Sci. 46 (1) (2001) 12–14.
[55] V. Hedouin, B. Bourel, L. Martin-Bouyer, A. Becart, G. Tournel, M. Deveaux, D. Gosset, Morphine perfused rabbits: a tool for experiments in forensic entomotoxicology, J. Forensic Sci. 44 (2) (1999) 347–350.
[56] M.L. Goff, A.I. Omori, J.R. Goodbrod, Effect of cocaine in tissues on the rate of development of *Boettcherisca peregrina* (Diptera, Sarcophagidae), J. Med. Ent. 26 (1989) 91–93.
[57] M.L. Goff, W.A. Brown, K.A. Hewadikaram, A.I. Omori, Effect of heroin in decomposing tissues on the development rate of *Boettcherisca peregrina* (Diptera, Sarcophagidae) and implications of this effect on estimation of postmortem intervals using arthropod development patterns, J. Forensic Sci. 36 (2) (1991) 537–542.
[58] M.L. Goff, W.A. Brown, A.I. Omori, Preliminary observations of the effect of methamphetamine in decomposing tissues on the development rate of *Parasarcophaga ruficornis* (Diptera: Sarcophagidae) and implications of this effect on estimation of postmortem intervals using arthropod development patterns, J. Forensic Sci. 37 (3) (1992) 867–872.
[59] M.L. Goff, W.A. Brown, A.I. Omori, D.A. Lapointe, Preliminary observations of the effects of amitriptyline in decomposing tissues on the development of *Parasarcophaga ruficornis* (Diptera: Sarcophagidae) and implications of this effect to estimation of postmortem interval, J. Forensic Sci. 38 (2) (1993) 316–322.
[60] M.L. Goff, W.A. Brown, A.I. Omori, D.A. Lapointe, Preliminary observations of the effects of phencyclidine in decomposing tissues on the development of *Parasarcophaga ruficornis* (Diptera: Sarcophagidae), J. Forensic Sci. 39 (1) (1994) 123–128.
[61] M.L. Goff, M.L. Miller, J.D. Paulson, W.D. Lord, E. Richards, A.I. Omori, Effects of 3, 4-methylenedioxymethamphetamine in decomposing tissues on the development of *Parasarcophaga ruficomis* (Diptera: Sarcophagidae) and detection of the drug in postmortem blood, liver tissue, larvae, and puparia, J. Forensic Sci. 42 (2) (1997) 276–280.

[62] R.A. Rashid, K. Osman, M.I. Ismail, R.M. Zuha, R.A. Hassan, Determination of malathion levels and the effect of malathion on the growth of *Chrysomya megacephala* (Fabricius) in malathion-exposed rat carcass, Trop. Biomed. 25 (3) (2008) 184–190.
[63] C.S. Crandall, S. Kerrigan, R.L. Aguero, J. Lavalley, P.E. Mckinney, The influence of collection site and methods on postmortem morphine concentrations in a porcine model, J. Anal. Toxicol. 30 (2006) 651–658.
[64] Z. Wilson, S. Hubbard, D.J. Pounder, Drug analysis in fly larvae, Am. J. Forensic Med. Pathol. 14 (2) (1993) 118–120.
[65] B. Levine, M. Golle, J. Smialek, An unusual drug death involving maggots, Am. J. Forensic Med. Pathol. 21 (1) (2000) 59–61.
[66] P. Kintz, A. Tracqui, P. Mangin, Analysis of opiates in fly larvae sampled on a putrefied cadaver, J. Forensic Sci. Soc. 34 (1994) 95–97.
[67] B. Bourel, G. Tournel, V. Hedouin, M.L. Goff, D. Gosset, Determination of drug levels in two species of Necrophageous Coleoptera reared on substrates containing morphine, J. Forensic Sci. 46 (2) (2001) 600–603.
[68] L. Lagoo, S.S. Schaeffer, D.W. Szymanski, R.W. Smith, Detection of gunshot residue in blowfly larvae and decomposing porcine tissue using inductively coupled plasma mass spectrometry (ICP-MS), J. Forensic Sci. 55 (3) (2010) 624–632.
[69] K. Simkiss, S. Daniels, R.H. Smith, Effects of population density and cadmium toxicity on growth and survival of blowflies, Environ. Pollut. 81 (1993) 41–45.
[70] X. Liu, Y. Shi, H. Wang, R. Zhang, Determination of Malathion levels and its effect on the development of *Chrysomya megacephala* (Fabricius) in South China, Forensic Sci. Int. 192 (1–3) (2009) 14–18.
[71] K. Gunatilake, M.L. Goff, Detection of organophosphate poisoning in a putrefying body by analyzing arthropod larvae, J. Forensic Sci. 34 (3) (1989) 714–716.