Legal Medicine 83 (2026) 102843

Contents lists available at ScienceDirect

Legal Medicine

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

Forensic entomotoxicology: Where are we going? 30 years in a review

Bianca Beltrame [a,1], Lorenzo Franceschetti [b,*,1], Giorgia Lodetti [b], Luca Manelli [c], Andrea Verzeletti [a], Valentina Bugelli [d]

[a] Forensic Medicine Unit, Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, University of Brescia, Brescia, Italy
[b] Institute of Legal Medicine, Department of Biomedical Sciences for Health, University of Milan, Milan, Italy
[c] School of Life and Environmental Sciences, Bournemouth University, Talbot Campus, Fern Barrow, BH12 5BB Poole, United Kingdom
[d] Forensic Pathology Section, Medicine and Surgery Department, University of Parma, Parma, Italy

ARTICLE INFO

Keywords:
Forensic entomotoxicology
Forensic entomology
Forensic toxicology
Postmortem interval
minPMI
Cause of death
Intoxication
Environment
Systematic review

ABSTRACT

Forensic entomotoxicology, which was first mentioned in a publication in 1994, focuses on the detection of drugs and toxins in necrophagous insects to provide valuable information in a variety of areas. This discipline faces fundamental limitations as its findings are often not easily transferable to practical contexts, thereby necessitating a case-specific approach for effective application. To overcome these challenges, a systematic review of scientific literature available on 31 December 2024 was conducted in order to summarise strengths and weaknesses of forensic entomotoxicology in accordance with the PRISMA guidelines.

After 81 relevant sources were selected, four main lines of research in entomotoxicology were identified: 1) effects of exogenous substances on larvae and, consequently, how the estimated minPMI (minimum Post-Mortem Interval) should be adjusted, 2) identification of cause of death, 3) study of the impact of exogenous substances on the environment using larval masses and 4) possible methods for analysing larvae to identify the substances they contain.

Overall, findings are heterogeneous and sometimes contradictory, indicating that exogenous substances can influence larval development and be detected in entomological samples, but in ways that are strongly species-, substance- and context-dependent and not yet robust enough for straightforward extrapolation to casework. By critically synthesising these issues, this review clarifies the main strengths and recurring limitations of forensic entomotoxicology and indicates when its use may be informative, when it should be interpreted with caution, and which methodological issues need to be addressed in future research.

1. Introduction

In 1994, Goff and Lord first mentioned entomotoxicology, a branch of science that overlaps entomology and toxicology, referring to it as 'a new area for forensic investigation' [1].

Today it is a commonly identified discipline which focuses on the detection of drugs and toxins in necrophagous insects to provide valuable information in a variety of areas, from identifying the cause of death in criminal contexts [2] to environmental [3] or veterinary investigations [4]. It offers essential information on drug-related deaths, poisonings and post-mortem interval estimations when conventional matrices are not available [5]. It is also crucial to integrate the potential of entomotoxicology in the forensic context, as the victim's toxicological pattern can have a strong impact on the larval population, thus leading to variations in the minimum Post-Mortem Interval (minPMI) estimated through entomology [6,7].

The most extensively studied arthropod taxon in entomotoxicology is the Diptera order and in particular the family Calliphoridae [8] (e.g. *Lucilia sericata* [9,10], *Calliphora vicina* [11–13], *Calliphora vomitoria* [7,14], *Chrysomya megachepala* [15,16], *Chrysomya. rufifiacies* [17]), although other families have also been investigated [18], such as Sarcophagidae [19], or Dermestidae [20,21]. The substances under investigation mainly range from drugs, such as benzodiazepines [9] or antibiotics [7], to illicit substances [14], such as cocaine or heroin, to insecticides, such as organophosphates (e.g. Terbufos [22] and Diazinon [8], which are also studied in veterinary).

This discipline faces a fundamental limitation as its findings are often not easily transferable to practical contexts, thereby necessitating a case-

* Corresponding author at: Istituto di Medicina Legale, Università degli Studi di Milano, via Luigi Mangiagalli 37, 20133 Milano, Italy.
E-mail address: lorenzo.franceschetti@unimi.it (L. Franceschetti).
[1] Bianca Beltrame and Lorenzo Franceschetti equally contributed to the manuscript.

https://doi.org/10.1016/j.legalmed.2026.102843
Received 18 January 2026; Received in revised form 28 March 2026; Accepted 5 April 2026
Available online 22 April 2026

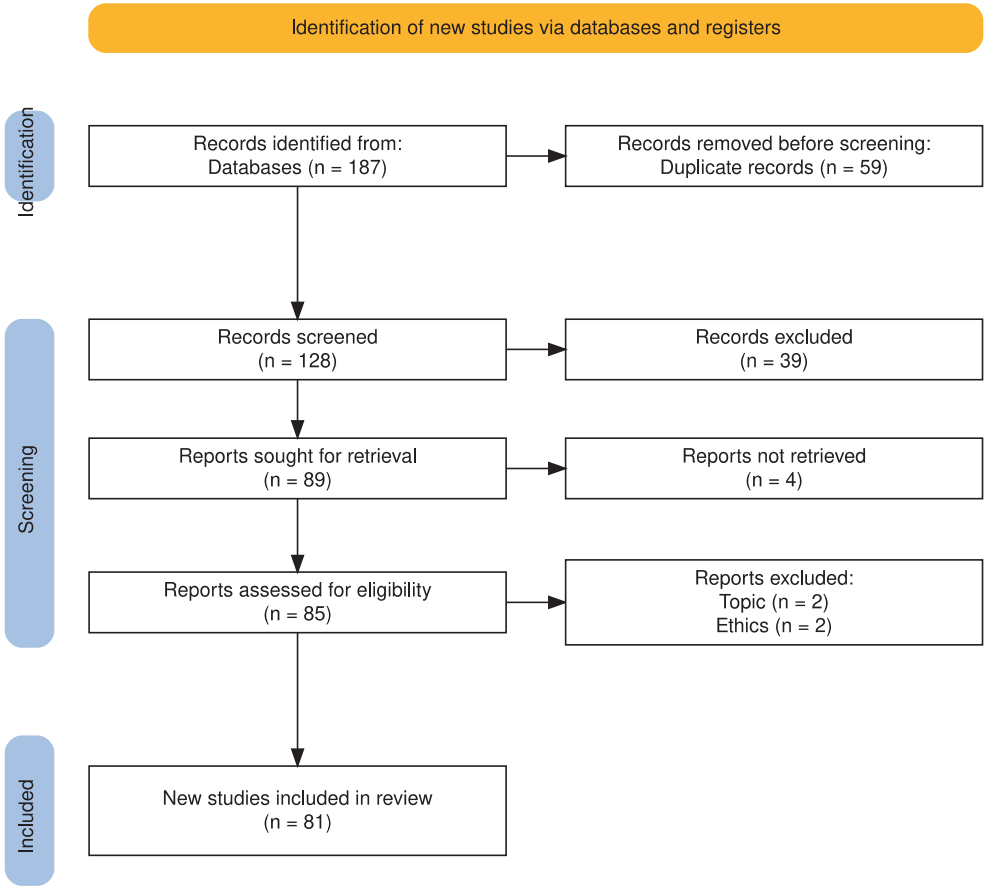

Fig. 1. PRISMA review chart.

specific approach for effective application.

To overcome these challenges, a better understanding of drug pharmacokinetics in necrophagous insects, and a standardised protocol for the application of the discipline, are needed [23].

This literature review is conducted to summarise the strengths and weaknesses of the application of forensic entomotoxicology in real cases, in the forensic, veterinary and environmental context.

Our aim is to identify elements that can be used to discriminate cases in which forensic entomotoxicology should be applied, to those in which it could be helpful, to those in which it should be avoided. Ultimately, this review is intended to support the use of forensic entomotoxicology in criminal courts.

2. Materials and methods

A systematic literature search was performed in accordance with the PRISMA (Preferred Reported Items for Systematic Reviews and Meta-Analyses) guidelines to increase comprehensiveness and transparency of reporting (Fig. 1) [24]. Studies were found using a thorough search strategy of the PubMed and Scopus Databases. No chronological or geographical restrictions were applied in our search, and only sources written in English language and with full text availability were included. As an exclusion criterion, Conference papers were excluded.

The keywords used in the search were: "Forensic" AND "Entomotoxicology".

The literature search was performed on December 31st, 2024, both on PubMed and on Scopus, resulting in 67 sources from PubMed and 120 from Scopus, for a total 187 articles and book chapters. Upon removing duplicates, articles not in the English language, and previously published literature reviews, 89 articles remained. Four articles were not fully accessible, and the remaining full texts were assessed for eligibility. Four additional sources were excluded from the final analysis based on the topic of the articles and on ethical considerations [25–28] resulting in 81 articles included in this review.

In this systematic search, no chronological or geographical limitations were applied, but it is important to note that some of the included original studies were published over 30 years ago, and analytical procedures have changed ever since. Most of the studies included in this systematic review were conducted using a case-control methodology, aiming to limit possible confounding variables; however, it should be noted that when applying the results to real cases, these variables must be taken into account.

3. Results

Among the 81 retrieved articles [4,6–10,14–17,19,21,22,29–96], 75 were original studies [4,6–9,14–17,19,21,22,30–38,40–66,68,70–94,96] and 6 cases were reports [10,29,39,67,69,95]. All the original studies were classified in a table (see Table S1 provided as Supplementary material), in which aim(s) of the study, animal model used in the study, insect species found/used in the study, substances investigated, method of analysis, results and possible limitations were outlined.

Four main research areas were identified, with few studies dealing with more than one topic. The most significant line of research concerned the effects of exogenous substances (i.e. drugs, substances of abuse and environmental pollutants) on larvae and how the estimated minPMI should be adjusted in cases in which such substances have been metabolised by the larvae feeding on the feeding substrate. This line of enquiry included 51 studies [4,6–9,14–17,22,30–48,50–57,59–66,70,71,74,77–80,82], one of which [39] was a case report. All studies were conducted using a case-control design, comparing the

development of larvae of the same species reared on contaminated versus uncontaminated artificial diet [6,33,55,56], animals (like rabbits [9,15,35,41,46,51,52,61,70], pigs [82,97] or rats [22,71,78–80]), parts from various animals (beef [17,34,37,48,53,59,66,71], buffalo liver [16,40], buffalo meat [32], pork muscles [44], pork liver [4,47], pork meat [7,9,14,30,45,54,64], porcine muscle [60], sheep liver [62], bovine rumen [74], mince containing lean kangaroo mince, lambs fry and heart [50], generic "meat" [31,57], generic "liver" [63,65], rabbit mince [36], kangaroo mince [38]) or both (non-living animal model, like pork muscle, and a living one *Sus scrofa L.* pigs [42]). Despite the fact that animal cadavers were used, the substance concentration was often related to human therapeutic [4,55,88] or lethal dose [4,35,37,42,44,47,64,68,88]. A wide variety of insect species and exogenous substances have been studied in existing literature, mostly individually. Some studies also tested a combination of different substances [7,14,30,31,38,68].

The use of entomotoxicology to determine the cause of death was investigated in 12 papers [9,10,29,69–75,98], four of which [10,29,67,69] were case reports. Two studies [74,75] are noteworthy for focusing on detecting firearm residue in larval specimens.

Entomotoxicology can also be applied at the environmental level, using larvae to study the impact of exogenous substances (particularly pesticides) on the environment. Nine studies included in this systematic review addressed this aspect [8,22,76–82]. For environmental purposes and other aims, various pesticide, insecticides and herbicides were analysed, like Terbufos [19,22,78,79], malathion [60,61], dimethoate [62,63], diazinon [8], aluminium phosphide [15], cypermethrin [15], Roundup Full® II – RFII [64], α- and β-endosulfan [65], paraquat [93,99], atrazine [74], thiamethoxam [87,88] in addition to various substances such as bleach, mosquito repellent, perfume, caustic soda, insecticide and unleaded gasoline [77].

Lastly, 38 studies [4,9,19,21,32,34–36,38,42,44,46,49–51,53,58,65,68,71–75,83–96] including one case report [95], examined potential methods for the detection and identification of substances through the analysis of larvae found on the body. In this area, the most widely used method has been GC–MS, with 11 studies [36,37,42,49,65,71,88,91–94], followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in eight studies [4,9,34,53,68,85,86,96], high performance liquid chromatography (HPLC) in seven studies [38,46,50,72,84,87,88] and other techniques less involved, like Fourier transform infrared (ATR-FTIR) spectroscopy [19], spectrophotometer [44], high-performance liquid chromatography-mass spectrometry (HPLC–MS) [32,77], ultra-high-performance liquid chromatography (UHPLC) [35], quadrupole time-of-flight mass spectrometry (QTOF-MS) [35], inductively coupled plasma mass spectrometry (ICP-MS) [67,69] and immunohistochemistry [58]. Two studies were highlighted in this review, as they represent the best practices in the toxicological analyses in entomological samples [91,92].

4. Discussion

4.1. The impact of exogenous substances on minPMI estimations

Estimating the minimum time elapsed between the discovery of the body, and the first insect colonisation on the body is one of the main fields of practical application in forensic entomology.

Exogenous substances present in the body at the time of death remain in body tissues blood and urine (indicating recent consumption), but also in lung tissue, brain, liver and kidney, and are consequently assimilated by the larvae feeding on the body, thus influencing their development. For instance, some exogenous substances may enhance larval growth by increasing larval length [14,31,32,36,38] and weight [31,32,52] throughout development, or accelerate moulting events when compared to control populations [4,6,14,31,32,35,46,47,52,53,57,64,68,71]. Conversely, other substances have been found to inhibit growth, by delaying moulting events [7,30,35,36,38,40,55,59,62,63,66] and reducing larval length [9,14,16,17,40,59–61,65,66] and weight [16,17,36,40,45,56] throughout development. In forensic practice, these developmental parameters are directly used for minPMI estimation through different approaches [100]. Larval length is routinely employed with isomegalen diagrams, in which growth curves obtained under controlled temperature conditions are used to infer the age of larvae collected on a body [101]. Conversely, moulting events and the timing of transitions between instars are pivotal for minPMI estimation based on thermal summation methods, which rely on accumulated degree-hours or degree-days required for a species to complete specific developmental stages [101,102]. Any acceleration or delay in growth, moult timing or stage transitions induced by exogenous substances may therefore lead to systematic under- or overestimation of the minPMI if such effects are not taken into account during entomological analysis.

Existing studies have investigated the effects of illicit substances [14,36–38,49,57,58,91], but also pharmaceuticals (e.g. antibiotics [7,30,31], antidepressants [6,9,16,17,21,32–34,40–44,68,85–87,89,90] and other drugs [4,14,35,45–48,50–56,83,84,94]) and field substances (e.g. insecticide [8,15,19,22,60–65,72,77–82,93]) on various insect species. A broad understanding of the possible effects of chemical substances on larval development is pivotal, considering the large numbers of deaths in which the minPMI might need to be adjusted, as shown in real cases [29]. Many of these compounds are frequently encountered in forensic casework, for example in suicides, overdoses, drug-related deaths and intoxications in agricultural or occupational settings and may therefore be present at the crime scene and in the body of the deceased. Consequently, whenever such exposure is suspected, their potential impact on larval development should be explicitly considered, when estimating and interpreting the minPMI.

The effects of substances on various species will be outlined in detail, starting with common pharmaceuticals, continuing with illegal substances and drugs of abuse and finally considering toxicants such as antifreeze, pesticides, herbicides and heavy metals.

4.2. Effects of pharmaceuticals on larval development

Several studies investigated the effect of antibiotics such as ceftriaxone and levofloxacin on the development of different fly species. Feeding on minced pork meat mixed with antibiotics (levofloxacin and ceftriaxone) was found to have a non-significant effect on the development, length, or weight of *Lucilia sericata* (Diptera: Calliphoridae) larvae, although a delay on the onset of pupation was observed [30].

In contrast, other studies conducted with *Protophormia terraenovae* (Diptera: Calliphoridae) found a significant increase in weight and length compared to a control population (without antibiotics) and an accelerated development [31].

On the contrary, considering *Calliphora vomitoria* (Diptera: Calliphoridae), development was delayed by levofloxacin and mixed antibiotics (levofloxacin and ceftriaxone) compared to control and pure ceftriaxone. Weight was significantly reduced in the mixed treatment compared to all other treatments (control and pure levofloxacin and pure ceftriaxone) [7]. Other drugs commonly used to treat many conditions have also been investigated. For instance, larvae of *Megaselia scalaris* (Diptera: Phoridae) [6] and of *Sarcophaga argyrostoma* (Diptera: Sarcophagidae) [32] that fed on a feeding substrate containing clonazepam, a drug prescribed as an anxiolytic, anticonvulsant and sedative, developed faster than control larvae, reaching their pupal stage earlier, thus posing the risk of a potential overestimation of the minPMI based on larval or pupal development.

On the other hand, lorazepam was found to have a negative impact on the growth and development of *Chrysomya rufifiacies* (Diptera: Calliphoridae) larvae, as these exhibited smaller length, weight and width of the larvae when feeding on substrates with lorazepam than populations feeding on untreated substrate [17]. In this case, an underestimation of the minPMI using larval development is possible, if

investigators fail to consider the impact of this substance.

In a single study, it was found that flunitrazepam had no significant effect on larval development or on the duration of the developmental stages in *Chrysomya megacephala* (Diptera: Calliphoridae), although the authors assumed that the dose used in their study was too low to activate any effect on larval development [33]. In yet another study, the authors found that nordiazepam (and its metabolite oxazepam) caused no significant difference in the length of *Calliphora vicina* (Diptera: Calliphoridae) larvae, although larval weight showed significant differences when compared to the control group from day 4 till day 6 [34]. Diazepam had no significant effect on larval weight and a weak effect on the length of *Lucilia sericata* (Diptera: Calliphoridae) larvae. In fact, larvae showed significantly longer length when feeding on substrate treated with 10 μg/g diazepam if compared to larvae feeding on substrates with lower doses after 48 h of development, and significantly shorter larvae when treated with 2 μg/g compared to control larvae after 120 h of development [9]. In a real case, diazepam and its main active metabolites (oxazepam and nordiazepam) were detected in the second and third instar larvae of *L. sericata* from a body almost completely burnt [39]. The administration of diazepam prior to death was established by surveillance cameras, also confirming the cause of death as carbon monoxide poisoning and fatal thermal injuries caused by fire.

Benzodiazepines (carbamazepine and clobazam) were found to accelerate development in *Chrysomya albiceps* (Diptera: Calliphoridae) larvae whereas development was slower in *L. sericata* and *Lucilia silvarum* (Diptera: Calliphoridae) larvae [35].

In *Aldrichina grahami* (Diptera: Calliphoridae) methamphetamine was found to have a negative effect on the developmental time needed to reach the pupal stage in populations feeding on contaminated tissues, when compared to larvae feeding on control tissues (slower development). In addition, the mean weight of the pupae exposed to methamphetamine was significantly lower than the control population, whereas the mean larval length population feeding on contaminated tissues was higher than in the control population [36]. Similarly, methamphetamine produced a significant increase in the developmental time from egg to adult in *C. vomitoria*, but high mortality (more than half of larvae exposed to methamphetamine) was observed during the pupariation period; similarly to the previously mentioned study, larval and pupal length was significantly higher in populations feeding on contaminated substrates than the control populations [37]. A study from Mullany et al. [38], in which methamphetamine-spiked kangaroo meat was utilised to simulate a methamphetamine overdose, found that methamphetamine and its metabolite, p-hydroxymethamphetamine significantly accelerated larval growth and increased the size of all life stages of *Calliphora stygia* (Diptera: Calliphoridae). In addition, the pupal stage lasted 78 h longer in drug-exposed samples than the controls [38].

Zolpidem tartrate, a non-benzodiazepine compound used in the treatment of insomnia was found to have a negative effect on the morphological parameters (weight, width, length and rate of development) of *C. megacephala* [16], *Chrysomya saffranea* (Diptera: Calliphoridae) [16] and also *Sarcophaga ruficornis* (Diptera: Sarcophagide) [40] in a concentration-dependent manner.

An experiment in which fluoxetine was administered to rabbits before death showed a significant acceleration of the rate of decomposition than the control group, making the turnover of larval species faster [41]. Although fluoxetine was proven to be detectable in some species (e.g. *Dermestes maculatus* (Coleoptera: Dermestidae) [21], *L. sericata* [43], *Sarcophaga Crassipalpis* (Diptera: Sarcophagidae) [44]), no detectable effect on the development of *D. maculatus* [42], *or M. scalaris* [43] was observed. The effect of codeine and diclofenac has been studied on *L. sericata,* suggesting that codeine does not affect pupal development, whereas larvae reared on liver samples treated with various concentrations of codeine developed faster than the control larvae [4]. On the other hand, calcium diclofenac, a non-steroid anti-inflammatory drug commonly used both in medicine and veterinary medicine, was found to have a negative effect on *L. sericata*, causing a reduction in body weight and a delay of 3–6 days in larval development in comparison to the control group [45].

Paracetamol, and its metabolite acetaminophen, was detected in specimens that fed on contaminated tissue, and were found to have a positive effect on the developmental rate of *C. rufifacies* [46] larvae. Likewise, paracetamol was found to have a slight impact on *C. vicina* larval development, especially during days 2–4 of its larval development, in which growth in population feeding on contaminated tissues was accelerated in comparison to the control population [47].

Cyclophosphamide and methotrexate (chemotherapeutic drugs) were found to have a negative effect on the development of *C. megacephala.* A decreased developmental rate was observed in larvae feeding on substrates contaminated with cyclophosphamide, although no significant effect was observed on larval and adult sizes, survival rate, and sex ratio [48]. On the other hand, methotrexate was observed to have a negative impact on larval and adult sizes of *C. megacephala* and survival rate, but no significant impact on the larval developmental rate [48].

4.3. Effects of illicit drugs and drugs of abuse

In experiments conducted with *C. vomitoria* with cocaine, heroin and their main metabolites (benzoylecgonine, morphine and a combination of both) [49], heroin-fed larvae were found to have smaller size and weight than controls, whereas cocaine and combination treatments (heroin alongside cocaine) prolonged the second and third instar larval stages, but also shortened the pupal stage and accelerated eclosion [14].

In an experiment conducted on *C. stygia* populations, no effects were observed in terms of developmental rate in populations feeding on morphine-contaminated tissues, even at high concentration [50]. In initial studies, no significant difference was observed in *L. sericata* populations feeding on morphine-contaminated tissues [51], but more recent research indicates that morphine has a positive effect on *L. sericata* larvae, accelerating the development and determining an increase in larval weight [52]. Methadone can be detected in larvae [29], unlike its metabolite EDDP which is rapidly metabolised [53] – but no significant effect was observed in terms of larval development in *L. sericata* populations [53].

In experiments conducted on *C. megacephala*, ketamine was found to suppress development, with a delay in larval development and pupariation, with a significantly synergistic action with low temperature (effects were enhanced in experiments conducted at lower temperatures) [54]. A similar study on the same species was conducted for the effects of butylscopolamine bromide, showing a decreased rate of development, higher mortality rate, and smaller body weight and body length throughout development [55].

Nandrolone decanoate, an anabolic androgenic steroid widely used from professional and amateur athletes in the pre-competition period, was found to have no significant effect on mean larval weight, emergence interval, or emergence rates in three species of Calliphoridae (*C. megacephala* (F.), *C. putoria* (Wiedemann), and *C. albiceps* (Wiedemann) (Diptera:Calliphoridae)) larvae fed with nandrolone decanoate at different doses or without [56].

Ethanol and cannabis were found to have a positive effect on the development of *C. rufifacies*, with much faster growth from the first larval instar to the pupal stage in comparison to control samples [57].

4.4. Effects of suicidal and environmental toxicants

In addition to the aforementioned studies on legal and illicit drugs that may commonly be present in the body at the time of death, attention has also been given to substances that can be ingested with suicidal intent. Furthermore, compounds that may be present in the environment and accidentally absorbed by individuals (such as agricultural workers) with potentially fatal consequences, were also considered.

Firstly, ethylene glycol is a soluble chemical solvent, commonly

referred to as antifreeze, widely marketed for commercial use as a household product, as well as a coolant product for automobiles and machinery; the ingestion of even small doses of this compound may lead to severe toxicity and consequentially result in death [100]. A study from Essarras et al. [59] found that in food substrate spiked with ethylene glycol at a fatal concentration in humans, eggs of *Lucilia cuprina* (Diptera: Calliphoridae) and *L. sericata* were unable to hatch if reared on a food substrate spiked with the highest concentration (T3), while lower and medium (T1 and T2) concentrations affected, but did not prevent survival and life cycle completion of these species. In addition, developmental time of both species reared onT1 and T2 concentrations was statistically slower than in control populations, while body length of the immatures of both of species reared on T1 and T2 was statistically smaller than the control.

Many studies were conducted on pesticides and herbicides (e.g. organophosphates), in order to understand their effect on colonisation, developmental rate and growth of single species but also on the physiological and biochemical aspects [15].

For example, malathion was found to have a negative effect on the development of *M. scalaris*, with significantly reduced larval length, and delayed moulting events [60], while on *C. megacephala* it was found to increase the period of larval development, maximum larval length and pupal weight at increasing concentration of the substance [61].

Similarly, dimethoate was found to have a negative effect on the development of *C. megacephala, C. saffranea, C. rufifacies* and *Chrysomya indiana* (Diptera: Calliphoridae), lengthening the feeding post-feeding, and pupal stages of development [62]. Similarly, the same impact was observed in studies on *S. ruficornis, Sarcophaga peregrine* and *Sarcophaga dux* (Diptera: Sarcophagidae). In a study, the duration of developmental stages of these species were negatively correlated with dimethoate concentration in the feeding substrate [63].

Glyphosate or N-(phosphonomethyl) glycine is a broad-spectrum systemic herbicide commonly found in soil and water, which has been known to be used in self-poisoning and accidental poisoning cases [64]. In experiments conducted with *L. sericata,* glyphosate did not have any significant impact on the duration of each developmental stage, although it reduced all size parameters in pupae, L1 larvae and adults. Similarly, on *D. maculatus* both the larval stage and total developmental duration were decreased in populations feeding on substrate with the highest dose, while size parameters remained unchanged for all development stages [64].

Endosulfan, an organochlorine insecticide, yielded a negative effect on the development of *C. vomitoria,* preventing immatures to reach the pupal stage at high concentrations, and affecting the size of immatures, resulting in significantly smaller larvae when feeding on tissues with high concentrations of this compound [65].

The effect of heavy metals (i.e. Cd, Pb, Hg, Fe, Cu, Mn, Ni, Zn) was considered in relation to the alteration of the development of *C. vicina*, with a negative effect on moulting events (delayed), larval length (maximum length delayed) and smaller pupae when development was completed on contaminated food substrates [66].

Overall, these studies highlight the effect of exogenous substances on sarcosaprophagous species, although substances could only be detected within the insects in some cases. Therefore, when investigating a case in which the minPMI has already been estimated, this should be adjusted when the presence of exogenous substances is confirmed. As a consequence, further research is needed not only to improve analytical detectability, but also to identify the substances and insect models that may offer the most reliable basis for correcting minPMI estimates. From the perspective of improving minPMI estimation, future research should prioritise substances and insect species that combine three features: forensic frequency, reproducible developmental effects, and relevance across different geographical contexts. The application of entomotoxicology relies on building reliable substance- and species-specific developmental data. On this basis, some of the most promising drug targets should be substances which are the most connected to drug-related deaths, or those substances who are not yet the most prevalent but have been observed in an increasing number of cases.

In Europe, recent data shows that the substances that are most associated with drug-induced deaths remain opioids, which appear in 70% of fatal overdoses [98]. These include heroin (although no longer dominant in most European countries) and prescription opioid painkillers such as tramadol (particularly in some African and Middle Eastern countries), morphine, oxycodone, fentanyl and derivatives. In addition, benzodiazepines (diazepam, alprazolam, etizolam), cocaine, amphetamines and methamphetamine were also often involved in drug-related deaths, often in combination with opioids [98].

Focusing on substances that increasingly appear in death investigation, an important mention goes to ultra-potent synthetic opioids (UPSOs), especially fentanyl and nitazenes. In Europe, these are found in localised outbreaks in specific countries, but there is a specific worry due to their extremely high potency, and difficulty in detection of the drug in body tissues due to their low concentration, rapid degradation during storage and limitations in routine drug testing protocols [97] (Krotulski et al., 2020). Crucially, in other countries such as the United States of America, IMF (Illegally Manufactured Fentanyl) is the leading substance associated with drug-related fatalities (Tanz et al., 2024, Holland et al., 2024) [103,104]. In the United Kingdom, increasing nitazenes-related deaths have been recorded in recent years (OHID, 2024) [105]. Other emerging classes of substances include synthetic cathinones (often called “bath salts”), synthetic cannabinoids, Neo Psychoactive Substances (NPS) such as 2C-B, and gabapentinoids [99].

Crucially, reports indicate that most drug-induced deaths are associated with polysubstance use. In these cases, death is often linked to drug interactions rather than the effects of a single drug alone. This adds a level of complexity to forensic entomotoxicological analysis. Yet, research in forensic entomotoxicology should naturally follow drug trends on a global scale and in the countries of application.

For this reason, opioids (especially morphine, heroin and methadone), cocaine, ketamine and selected benzodiazepines, appear to be the ones to focus on because these substances are recurrent in forensic casework and have already shown measurable effects on larval growth or stage transitions in blowflies. Among non-pharmaceutical toxicants, organophosphate pesticides and ethylene glycol also deserve priority, given their medico-legal relevance and their documented effects on larval survival and development. With regard to insect models, the most promising taxa remain the forensically important Calliphoridae [8], particularly *Lucilia sericata* [9,10], *Calliphora vicina* [11–13], *Calliphora vomitoria* [7,14] and *Chrysomya megacephala* [15,16], because they are among the most frequently studied species and are commonly encountered in forensic investigations. Rather than expanding the field indiscriminately to many new drug–species combinations, future studies should focus on building robust, replicated developmental datasets for a limited number of high-priority substances in these key species, under controlled temperature conditions and with comparable endpoints, so that correction factors for minPMI estimation can eventually be developed on a stronger empirical basis.

4.5. *Identification of the cause of death and toxicological condition of the victim*

When a cadaver is found in the advanced decay stage of decomposition and no other matrices are available, toxicological analysis of the larvae found on the body can provide useful indications on both the cause of death (i.e. whether it was the result of self-induced or accidental intoxication) and the toxicological state of the deceased [39,64]. The cause of death may be undetermined at the moment of burial, but toxicological analysis on the larvae can be performed even after exhumation, as shown in Aly et al., in order to determine if drugs were correctly administered during hospitalisation [67].

Analysing the literature on the cause of death, diazepam in *L. sericata* [9] and in *C. albiceps* larvae (along with amitriptyline, citalopram and

Table 1
Analytical methods used for the detection of selected xenobiotics in Calliphoridae larvae and pupae. Columns list the investigated substances (methamphetamine, α- and β-endosulfan, carbamazepine, morphine, amitriptyline and nortriptyline, ketamine, acetylsalicylic acid and related compounds, nordiazepam, lead/barium/antimony and nicotine), rows report the blowfly species examined (*C. vomitoria, C. stygia, C. albiceps, C. vicina, C. dubia*), and cells indicate the analytical techniques employed (e.g. GC–MS, HPLC-MS/MS, UHPLC–QTOF-MS, radioimmunoassay, ICP-MS).

	Methamphetamine	α- and β-endosulfan	Carbamazepine	morphine	Amitriptyline and nortriptyline	ketamine	acetylsalicylic acid and other *	nordiazepam	Heavy metals Pb, Ba and Sb	nicotine
C. vomitoria	GC–MS	GC–MS				HPLC-MS/MS				GC–MS
C. stygia	HPLC									
C. albiceps			UHPLC and QTOF-MS							
C. vicina				sensitive and specific technique (Coat-a-count Serum morphine RIA)	HPLC and GC–MS		HPLC	LC-MS/MS		
C. dubia									ICP-MS	

See other *sodium salicylate, paracetamol, aminohippuric acid, amphetamine sulfate, sodium amylobarbitone, sodium phenobarbitone, sodium thiopentone, sodium barbitone, and sodium brallobarbitone.

morphine) [68] could be detected, contributing to the identification of a drug-related death. In a real case application, the cause of death was determined as intoxication with fentanyl, which was successfully recovered from both liver samples and larvae [69].

Alcohol/ethanol is a substance commonly assumed before committing suicide, or as a cause of accidental death, so it is important to be able to identify ante-mortem alcohol consumption [10]. Cerioni et al. [10] developed an analytical method using HPLC/HR-MS for the detection and quantification of Ethyl glucuronide (EtG), a specific and stable metabolite of ethanol, in larvae of *L. sericata* collected from decomposed remains, thereby demonstrating that ante-mortem alcohol consumption can be reconstructed through entomotoxicological analysis. This is particularly important, considering how alcohol has been proved to have an effect on the successional patterns of insects in the decomposing process, as animal cadavers treated with alcohol took two days longer than the controls to reach the dry stage of decomposition in winter and one day longer in summer [70]. Methanol should also be considered, since it is a potentially lethal alcohol, often added as an adulterant in alcoholic beverages due to its low cost and similarity to ethanol [71]. Methanol was detected in some developmental stages of *Peckia intermutans* (Diptera: Sarcophagidae) using gas chromatography-mass spectrometry (HS-GC-MS) method, developed by Rivera-Puma et al. [71]. Methadone can also be detected in larvae, as demonstrated in a real case of a deceased undergoing methadone treatment [29].

Moreover, paraquat (a pesticide often used as suicide agent in many developing countries) was detected in third instar larvae of the blow fly *C. rufifacies* using HPLC, reflecting the state of intoxication of the body and indicating a previous intoxication [72]. Similarly, cadmium (Cd) and thallium (Tl) can be detected on *L. sericata*, in different growing stages, through ICP-MS [73], meaning accidental and intentional poisonings and proving the cause of death.

In addition to drug-related death, an attempt was made to identify gunshot residue (GSR) indicative particles, such as lead (Pb) [74], barium (Ba) [75] and antimony (Sb) [76]. In this study lead was detected on immature specimens of *L. cuprina*, with a low limit of detection (6.5 μg L^{-1}) [77], whereas barium and antimony were detected in larvae of *Calliphora dubia* (Diptera: Calliphoridae) using ICP-MS [78].

The first attempts to detect different substances in *C. vicina* larvae reared on artificial feeding substrates spiked with said substances gave positive results for phenobarbitone, sodium salicylate, aminohippurate, brallobarbitone, amphetamine and barbitone, even though drug concentrations in larvae were significantly lower than concentration in their food source, and the absence of a drug from feeding larvae did not necessarily imply its absence from the food source [87].

A study performed on *C. vicina* larvae feeding on a substrate contaminated with various concentrations of amitriptyline and nortriptylinein showed that drug concentration was higher in larvae reared on a substrate containing a human toxic equivalent dose when compared to a substrate containing a therapeutic dose [88]. From a forensic perspective, these findings suggest that, at least for amitriptyline and nortriptyline in *C. vicina*, larval concentrations may provide qualitative information on whether the decedent was exposed to therapeutic versus toxic doses, even when conventional matrices are no longer available. However, the marked difference between larvae reared on toxic and therapeutic substrates should not be interpreted as allowing a direct back-calculation of the ingested dose, because drug uptake, metabolism and elimination in insects are species- and stage-dependent [71,87,88]. Rather, such entomotoxicological data can support the hypothesis of overdose or poisoning when high concentrations are detected in larvae, and should be evaluated in conjunction with circumstantial, pathological and, when present, conventional toxicological findings.

In an experimental model, Hédouin et al. [89] attempted to determine whether morphine concentrations measured in *C. vicina* larvae could be quantitatively related to the drug levels in the underlying tissues. Larvae were reared on rabbit carcasses perfused with morphine at known doses, and morphine was consistently detected in the insects; however, its concentration in larvae was systematically and markedly lower than in the corresponding tissues, preventing a reliable back-calculation of tissue levels from larval data. This study relied on a previous publication by the same group [90], in which controlled morphine perfusion experiments were used to characterise the kinetics and distribution of the drug in rabbit tissues, providing the reference tissue concentrations against which the entomotoxicological findings were compared.

Nevertheless, in highly decomposed bodies, the detection of a drug or toxin in entomological specimens should not be interpreted as definitive proof that the deceased consumed that substance ante-mortem. Insects may provide a valuable alternative matrix when conventional tissues (urine and blood) are unavailable, however, positive entomotoxicological results do not necessarily indicate that the substance was consumed while the insect was alive and should therefore be interpreted with caution. The presence of a xenobiotic substance in insects suggests that the insects were exposed to substrates containing or contaminated with that substance. To determine whether the substance was actually ingested during the insect's lifetime, a coordinated

Table 2
Analytical techniques applied to entomological samples from different forensically relevant species (*A. grahami, L. sericata, L. cuprina, L. silvarum, P. terraenovae, D. maculatus*) for the detection of carbamazepine, diazepam, cadmium and thallium, fluoxetine, methamphetamine, codeine, heroin, morphine, methadone, lead and methylphenidate. Cells report the methods used in each study (e.g. UHPLC–QTOF-MS, LC–MS/MS, ICP-MS, spectrophotometry, HPLC, SWASV, radioimmunoassay).

	Carbamazepine	diazepam	Heavy metals Cd, Tl	fluoxetine	Methamphetamine	Codeine	heroin	morphine	methadone	Heavy metals Pb	methylphenidate
A. grahami					GC–MS						
L. sericata	UHPLC and QTOF-MS	LC-MS/MS	ICP-MS	spectrophotometer	GC–MS	LC/MS and HPLC with Fourier transform mass spectrometry		Coat-a-count Serum morphine RIA and HPLC with Fourier transform mass spectrometry	(LC–MS) UPLC–MS/MS and LC/MS and HPLC with Fourier transform mass spectrometry		LC–MS/MS
L. cuprina							HPLC	HPLC		SWASV	
L. silvarum	UHPLC and QTOF-MS										
P. terraenovae								Coat-a-count Serum morphine RIA			
D. maculatus				Spectrophotometer and GC–MS							

evaluation of the crime scene, the medical history, the autopsy findings, and, where available, results from other alternative matrices must be conducted. For this reason, forensic entomotoxicology is best regarded as a complementary tool that can support, but not independently establish, conclusions about intoxication-related deaths. Its evidential value is strongest when the detected substance is pharmacologically or toxicologically coherent with the circumstances of death, when metabolites or specific markers of ante-mortem exposure are identified, and when the analytical findings are consistent across specimens and with the overall forensic context.

4.6. Bio-indicators of environmental pollutants

Ecotoxicology is a well-established scientific discipline from which environmental forensic entomotoxicology is derived as a relatively new branch [76,77]. In fact, insects can be used as direct environmental bio-indicators of heavy metal contamination near industrial areas and they are important in relation to dead fauna in outdoor environments. For this reason, entomotoxicological studies can provide important information on environmental pollutants, also in relation to their role in maintaining organic balance. The presence of ubiquitous pollutants or waste products, such as insecticides or diesel fuel, can alter local biodiversity, and these effects may be further modulated by weathering process [76].

For instance, a study analysed the survival rate of larvae in presence of bleach, mosquito caustic soda, insecticide and gasoline, in dry and wet conditions [77]. This research showed a 10% larval survival rate on rat cadavers contaminated with caustic soda and insecticide and a 58% larval survival rate on cadavers contaminated with gasoline in dry conditions. Also, rain mainly had an impact on the survival rate of the larvae, especially on samples contaminated with gasoline, and the size of adult flies, probably due to the fact that precipitation washed off the chemical substances.

In another study, the effect of terbufos (a chemical compound commonly used in insecticides and nematicides) on the decomposing process was investigated [78]. Results show that the higher dose of terbufos accelerated body decomposition within the first 24 h, with a decrease in species richness and abundance of scavengers flies when compared to the control. In addition, other observations included a change in the succession pattern, a delay in the arrival of important species often used for minPMI estimations, and 8% mortality of the visiting dipterofauna [78]. In the presence of high and intermediate doses of Terbufos, *Lucilia eximia* (Diptera: Calliphoridae) larvae were more active with greater frequency of body movements and lateral contractions, whereas *Peckia chrysostoma* (Diptera: Sarcophagidae) larvae were less active, with fewer body and lateral contractions when intoxicated with the higher dose of this compound [22]. It was also found that terbufos can alter the timing of larval dispersion, modify the composition and structure of the colonisers assemblage, prolong or shorten species developmental times, and reduce emergence rates of Calliphoridae and Sarcophagidae flies, leading to increased pupal mortality in contaminated carcasses [79]. On the contrary, diazinon (an organophosphate insecticide) was found to interfere with the decomposition timeframe in carcasses, slowing the onset of the decomposition stages and affecting blow fly colonization [8].

In another study, the effect of atrazine, a common herbicide, on the decomposition process in intoxicated rat showed a significant delay in the time needed to reach full skeletonisation, taking 30 days, compared to 19 in the control samples, to reach the skeletal stage [80]. Atrazine was also found to affect the succession pattern of carrion-feeding insects, with the predominant necrophagous arthropods involved belonging to the orders Diptera and Coleoptera, rather than only Diptera in the control group [80].

Ants (Hymenoptera: Formicidae) are important components of carrion communities, acting both as scavengers and predators of other necrophagous insects, and can therefore modulate decomposition

Table 3
Overview of analytical methods used to detect amitriptyline, methanol, terbufos, diazepam, citalopram, cocaine, paracetamol, paraquat, clonazepam, morphine and asenapine maleate in larvae or puparia of sarcophagid and calliphorid flies and other carrion insects (Peckia intermutans, Chrysomya albiceps, Chrysomya rufifacies, C. megacephala, S. ruficornis, S. argyrostoma). The table summarises the techniques employed (HS-GC–MS, LC–MS/MS, HPLC with diode array detector, TLC combined with HPLC, ATR-FTIR spectroscopy, IHC, GC–MS, HPLC-MS).

	amitriptyline	methanol	Terbufos	diazepam	citalopram	cocaine	paracetamol	paraquat	clonazepam	morphine	Asenapine maleate
Peckia intermutans		HS-GC–MS)									
Chrysomya albiceps	LC/MS-MS			LC/MS-MS	LC/MS-MS	IHC				LC/MS-MS and Thin-layer Chromatography TLC and high performance liquid chromatography HPLC	
Chrysomys rufifacies							HPLC with Diode Array Detector	HPLC			
C. megacephala						IHC					
S. ruficornis						IHC					
Larvae			ATR-FTIR					GC–MS			GC–MS
S. *argyrostoma*									HPLC–MS		

dynamics [110]. Body decomposition and the associated Formicidae fauna can be affected if a body is accidentally contaminated with insecticides and the chemical compounds in them, such as terbufos [78–81]. According to a study [81], there is no difference in species composition between bodies contaminated by insecticides and non-contaminated bodies, however, the greatest abundance of ants in contaminated bodies was found in the bloating phase, whereas the greatest abundance of specimens in non-contaminated bodies was found in the advanced decomposition stage [75]. In another study investigating the effect of thiamethoxam, contaminated bodies reached the skeletal stage of decomposition in almost double the time than the control group. Moreover, only the control group showed a significant geometric regression describing changes in species richness and composition across decomposition stages, whereas this structured pattern of flies and beetles diversity was absent in thiamethoxam-contaminated carcasses [82].

4.7. Sampling, storing and analysing entomotoxicological evidence

As with any investigation in which entomological samples are collected, guidelines for the collection, preservation, analysis and storage of scientific evidence must be followed [106,107]. However, when an entomotoxicological analysis is expected to be performed within a criminal investigation, additional precautions must be taken, according to recent studies in the literature.

Collection and transport: entomotoxicological analysis can be performed on different species of cadaveric entomofauna at different life stages, therefore it is important to collect all that is found on the body, following appropriate sampling protocols by body district (e.g. different body parts, natural orifices, wounds, clothing), and to label each sampling site separately [106]. Specimens intended for entomotoxicological analysis should then be transported to the laboratory as soon as possible in sealed, chemically inert containers, preferably in cooled conditions without direct contact with ice, in order to limit degradation of both the insects and the toxicants they may contain.

Killing: it is preferable to kill larvae found on the body immediately upon collection, but this is not always possible. The two most commonly used methods for killing fly larvae are blanching and freezing. That said, a study suggested that freezing can cause drug degradation in the larvae [9], choosing blanching as the preferred method. It is also important to note that the method used for killing has an impact on larval length, therefore this should be taken into account if the isomegalen approach is chosen for the estimation of the minPMI. For instance a significant difference in size and body integrity was observed between blanched and frozen larvae of *L. sericata* which were previously affected by diazepam [9].

Storage: no preservatives should be used to preserve larvae, as these could interfere with the entomotoxicological analysis [106,107]. Immersion in ethanol, in particular, should be avoided for samples destined to entomotoxicological analysis, as the solvent may cause partial leaching of ethanol-soluble toxicants from larval tissues into the preservative and may complicate subsequent chromatographic detection [106]. Larvae should therefore be frozen and stored at −20 °C without any preservative, as in most studies investigated in this review.

Analysis: different toxicological techniques have been employed in existing literature when analysing entomological samples. The most widely used approach in literature is gas chromatography–mass spectrometry, or GC–MS [36,37,42,49,65,88,91–94]. Several studies showed better results and higher detected concentration in washed and unwashed pupae than those measured by HPLC [37,38,50,88]. For instance, Parkhideh et al. reared *L. sericata* on methamphetamine-spiked meat and showed that the drug could be detected by GC–MS in pooled larvae from all developmental stages. Derivatising the extracts by acetylation improved peak resolution and signal intensity, thereby enhancing the reliability of methamphetamine detection [91]. Similarly, GC–MS method has been used for the detection of nicotine in *C. vomitoria* larvae [92], of Paraquat in blow fly larvae [93] and of asenapine maleate in sarcophagus insects, with a detection limit of 10 μg/ml [94].

When conducting GC–MS analysis to detect heroin metabolites, it is important to consider that it might not be possible to detect the expected complete metabolites of heroin. For instance, in a study conducted with *L. cuprina* larvae, the complete heroin metabolites could not be detected in the first instar larvae and pupae, while the second and third instar larvae yielded a complete heroin metabolite profile [49]. For this reason, the absence of heroin metabolites in the first instar larvae and pupa does not necessarily mean that the drug was not present in the host [49]. In a case report, GC/MS was successfully applied in the death of a middle-aged man who committed suicide several weeks before his body was discovered, and amphetamine was detected in the larvae using GC–MS [95].

In a different study, an analytical method based on high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) was found suitable to detect ketamine in immature stages of *C. vomitoria* [86]

and methylphenidate in *L. sericata* larvae [96]. Similarly, a simple liquid–liquid extraction followed by a sensitive LC–MS analysis was proven to be sensitive enough to determine methadone and EDDP in each single larva of *L. sericata* reared on an artificial substrate spiked with methadone) [85].

To detect morphine, methadone and codeine in *L. sericata* larvae, a sensitive UHPLC-FT-MS analysis was performed. For the solid-phase extraction, a procedure already employed for keratinous matrices was followed in order to inject the samples into the UHPLC system, determining the correct identification of morphine, codeine, methadone and their metabolites in *L. sericata* [83].

Terbufos was also detected with the use of ATR-FTIR spectroscopy in fly larvae (Sarcophagidae). In a study, experimental methods of analysis in which animal cadavers were spiked with terbufos, left to be colonised, and then necrophagous larvae feeding on the contaminated tissues collected, showed sensitivity and specificity above 90% [19]. Fluoxetine was also successfully detected and quantified with a spectrophotometer at 270 nm and 277 nm from all developmental stages of *D. maculatus* and from *L. sericata* and *S. crassipalpis* [20,21,44].

In another study, TLC was used to detect morphine in *C. albiceps* and *Creophilus maxillosus* (Coleoptera: Staphylinidae) sampled from rabbits previously injected with morphine, comparing them with HPLC results from rabbit samples [84]. Liver, kidney, spleen, muscle, bile, urine tissues, peak feeding and post-feeding larvae were all positive, confirming that the analysis of the necrophagous insects tissue can detect the presence of the drug (morphine) in the body [84].

Moreover, an immunochemistry (IHC) technique was proposed as an alternative toxicological analysis to detect drugs in insects of forensic importance [100]. A test for cocaine detection performed using monoclonal benzoylecgonine antibody from mouse showed that histological procedures did not compromise antigenicity [58].

On the basis of the studies reviewed, some pragmatic indications can be provided for choosing which material to sample and which analytical approach to prioritise in different scenarios. In cases involving soft-tissue drugs (e.g. antidepressants, benzodiazepines, opioids, stimulants) during the early decomposition stages, larvae of Calliphoridae and Sarcophagidae collected from the main colonised body districts are usually the most informative matrices, and targeted GC–MS, LC–MS/MS or UHPLC–FT–MS methods validated for these families should be preferred when available. In more advanced stages, when only pupae or puparia remain, these structures can still be used to investigate previous exposure to metals, pesticides or some pharmaceuticals, particularly by ICP–MS or other elemental or high-sensitivity chromatographic techniques. For suspected environmental or agricultural toxicants (e.g. organophosphate insecticides, herbicides, paraquat), larval masses from outdoor carcasses – including both Diptera and, when present, Coleoptera – can be screened with GC–MS, HPLC or ATR-FTIR combined with chemometrics, and the results interpreted together with changes in species composition and successional patterns. These general indications are complemented by the overview reported in the three tables provided in the appendices (Table 1, 2 and 3), in which substances are cross-tabulated with the insect species and analytical techniques used in the available studies, providing a practical reference for selecting, case by case, the most appropriate combination of insect family, developmental stage and method in light of the suspected toxicant and the specific question posed by the investigation.

These considerations also suggest some practical ways to reduce one of the main barriers to real-case application, namely the accurate identification of both the insect species involved, and the xenobiotics present in the entomological samples. From a practical forensic perspective, the problem of accurate identification should be addressed through a stepwise and integrated workflow rather than by relying on a single examination. First, insect identification should be performed at the lowest possible taxonomic level, combining morphological examination with species confirmation whenever morphology is compromised by immature stages or specimen damage. In addition, toxicological analysis should follow a two-stage strategy, consisting of an initial broad screening to detect the main classes of xenobiotics, followed by targeted confirmatory and quantitative analyses on the substances most strongly suggested by the case context and by the screening results. Lastly, because drug uptake and developmental effects are strongly species- and stage-dependent, interpretation should be based on species-specific and stage-specific reference data whenever available, avoiding direct extrapolation across taxa. Finally, these analytical steps should be supported by standardized collection, killing, storage and chain-of-custody procedures, and by the parallel preservation of separate specimens for entomological and toxicological purposes. In our view, progress in applying entomotoxicology to real cases will depend primarily on the development of validated reference values for specific species at different stages of development, as well as on the specific analytical techniques used.

4.8. Limitations and strengths of the study

The limitations of this study lie in the extreme heterogeneity and specificity of the existing literature, which often focuses on a single molecule in relation to a single insect species, thus making it difficult to develop a comprehensive understanding of the topic. Conversely, this review aimed to consider only studies with robust and significant findings in terms of methodological design, in order to ensure the reliability of the reported results. The studies considered were conducted using a case-control methodology, sometimes attempting to replicate therapeutic or lethal concentrations for humans.

5. Conclusion

Forensic entomotoxicology represents a crucial field of application in cases where traditional toxicological analyses are compromised by tissue decomposition. This review highlights three main areas of application: minimum Post-Mortem Interval (minPMI) estimation, identification of the cause of death and analysis of environmental contaminants.

Evidence demonstrates that numerous substances, from antibiotics to illicit drugs, can significantly alter larval development, accelerating or slowing it down. These observations are species-specific [30,31,35]. These effects can lead to substantial errors in minPMI estimations, if not adequately considered during the entomological analysis, as discussed above.

Various methodological approaches have proven effective (GC–MS, HPLC-MS/MS, UHPLC-FT-MS, ATR-FTIR) for the toxicological analysis, enabling the identification of substances even in cadavers in advanced stages of decomposition. However, it is important to consider that the correlation between substance concentrations in larvae and tissues is not always direct and depends on the developmental stage of the insect being analysed. The methods of collection and storage of entomological samples are fundamental, to facilitate subsequent toxicological analyses.

In conclusion, over the past thirty years, significant progress has been made in the field of entomotoxicology, both in understanding the impact of substances on insect larvae and in detecting exogenous compounds in entomological samples. However, further research is still needed to integrate these two areas of knowledge. In particular, future progress will require harmonized protocols for toxicological analysis, together with species-specific reference data that make analytical findings more reliably transferable to casework. The ultimate goal is to retrospectively identify the substances ingested by the larvae, in conjunction with all other forensic findings, to refine minPMI estimation and support the reconstruction of intoxication-related deaths, leading to a routinely application of forensic entomotoxicology in future cases.

Ethics approval

N/A.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.legalmed.2026.102843.

References

[1] M.L. Goff, W.D. Lord, Entomotoxicology. a new area for forensic investigation, Am. J. Forensic Med. Pathol. 15 (1) (Mar. 1994) 51–57.
[2] J. Spicer, Forensic Entomology in Death Investigations, in: The Routledge International Handbook of Homicide Investigation, 1st ed., Routledge, London, 2023, pp. 132–149, https://doi.org/10.4324/9781003195283-13.
[3] I.A. Adedara, et al., Utility of cockroach as a model organism in the assessment of toxicological impacts of environmental pollutants, Environ. Adv. 8 (July 2022) 100195, https://doi.org/10.1016/j.envadv.2022.100195.
[4] K. Czepiel-Mil, P. Listos, R. Stryjecki, D. Kowalczyk-Pecka, M. Nieoczym, Forensic veterinary use of the fly *Lucilia sericata* (Diptera: Calliphoridae) in the aspect of determining the time of death using tissues treated with calcium diclofenac, Med. Weter. 79 (04) (2023) 6752, https://doi.org/10.21521/mw.6752.
[5] M. Gosselin, et al., Entomotoxicology, experimental set-up and interpretation for forensic toxicologists, Forensic Sci. Int. 208 (1–3) (May 2011) 1–9, https://doi.org/10.1016/j.forsciint.2010.12.015.
[6] A. Quijano-Mateos, A. Castillo-Alanis, C.S. Pedraza-Lara, M.E. Bravo-Gómez, Evaluation of the effect of clonazepam and its metabolites on the life cycle of *Megaselia scalaris* (Loew) (Diptera: Phoridae), Sci. Justice 64 (5) (Sept. 2024) 460–465, https://doi.org/10.1016/j.scijus.2024.07.002.
[7] D. Preußer, U. Bröring, T. Fischer, T. Juretzek, Effects of antibiotics ceftriaxone and levofloxacin on the growth of *Calliphora vomitoria* L. (Diptera: Calliphoridae) and effects on the determination of the post-mortem interval, J. Forensic Leg. Med. 81 (July 2021) 102207, https://doi.org/10.1016/j.jflm.2021.102207.
[8] K. Cavalcante, T. Peniche, B.L.B. Façanha, C.M. Araújo, T.A.S. Lobato, R.N. P. Souto, Effect of diazinon (organophosphate) on the composition and succession of Calliphoridae assemblages in rabbit carcasses in the Eastern Amazon, Int. J. Legal Med. 137 (4) (July 2023) 1253–1261, https://doi.org/10.1007/s00414-023-02989-0.
[9] O.C. Groth, et al., Exploring unified methods of killing and storing insect samples for forensic entomotoxicology using diazepam in *Lucilia sericata* (Meigen, 1826) (Diptera: Calliphoridae) larvae, Forensic Sci. Int. 365 (Dec. 2024) 112255, https://doi.org/10.1016/j.forsciint.2024.112255.
[10] A. Cerioni, et al., Validation of a new method for the detection of Ethyl glucuronide in larvae of Lucilia sericata as a marker of ante-mortem alcohol consumption, Heliyon 9 (10) (Oct. 2023) e20802, https://doi.org/10.1016/j.heliyon.2023.e20802.
[11] R. Chophi, S. Sharma, S. Sharma, R. Singh, Forensic entomotoxicology: current concepts, trends and challenges, J. Forensic Leg. Med. 67 (2019) 28–36, https://doi.org/10.1016/j.jflm.2019.07.010.
[12] M. Gosselin, S.M. Wille, M. Fernandez Mdel, et al., Entomotoxicology, experimental set-up and interpretation for forensic toxicologists, Forensic Sci. Int. 208 (1–3) (2011) 1–9, https://doi.org/10.1016/j.forsciint.2010.12.015.
[13] Siva Prasad, M.S., Aneesh, E.M. Tools and techniques in forensic entomology- A critical review. Int. J. Trop. Insect. Sci. 42, 2785–2794 (2022). https://doi.org/10.1007/s42690-022-00823-5. [14] T. Wood, K. Pyper, and F. Casali, 'Effects of cocaine and heroin, and their combination, on the development rate of Calliphora vomitoria (Diptera: Calliphoridae)', Sci. Justice, 62, 4, 471–475, July 2022, doi: 10.1016/j.scijus.2022.07.001.
[14] M. Tony, M. Ashry, M.M.A. Tanani, A.M.A. Abdelreheem, M.R.K. Abdel-Samad, Bio-efficacy of aluminum phosphide and cypermethrin against some physiological and biochemical aspects of Chrysomya megacephala maggots, Sci. Rep. 13 (1) (Mar. 2023) 4407, https://doi.org/10.1038/s41598-023-31349-6.
[15] L.A. Al-Shuraym, et al., Effect of Zolpidem Tartrate on the Developmental Rate of Forensically Important Flies *Chrysomya megacephala* (*Diptera: Calliphoridae*) and *Chrysomya saffranea*, J. Med. Entomol. 58 (6) (Nov. 2021) 2101–2106, https://doi.org/10.1093/jme/tjab071.
[16] S. Annasaheb Bansode and V. Ramrao More, 'Effect of Lorazepam on the Development of the Hairy Maggot Blow Fly, *Chrysomya rufifacies* (Macquart): Implication for Forensic Entomology', J. Toxicol., 2023, 1–10, July 2023, doi: 10.1155/2023/1051736.
[17] J. Kaur, Perspective of entomotoxicology in forensic investigations: a critical review, Indian J Forensic Med Pathol. 14 (3 Special) (2021) 743–748.
[18] H.K.T.D.A. Silva, et al., Detection of terbufos in cases of intoxication by means of entomotoxicological analysis using ATR-FTIR spectroscopy combined with chemometrics, Acta Trop. 238 (Feb. 2023) 106779, https://doi.org/10.1016/j.actatropica.2022.106779.
[19] S. Muskan, J. Saini, H.D. Singh, N.R. Sharma, Drugs and their effects on development rate of decomposers: an entomotoxicological approach, J. Punjab Acad. Forensic Med. Toxicol. 20 (2) (2020) 180–183, https://doi.org/10.5958/0974-083X.2020.00126.0.
[20] N.I. Zanetti, A.A. Ferrero, N.D. Centeno, Determination of fluoxetine in Dermestes maculatus (Coleoptera: Dermestidae) by a spectrophotometric method, Sci. Justice 56 (6) (Dec. 2016) 464–467, https://doi.org/10.1016/j.scijus.2016.07.005.
[21] J. T. Jales, T. M. Barbosa, V. R. F. Moreira, S. D. Vasconcelos, V. De Paula Soares Rachetti, and R. A. Gama, 'Effects of Terbufos (Organophosphate) on Larval Behaviour of Two Forensically Important Diptera Species: Contributions for Entomotoxicology', Neotrop. Entomol., 52, 6, 1155–1164, Oct. 2023, doi: 10.1007/s13744-023-01094-6.
[22] O.C. Groth, A. Pi, A.E. Jensen, F. Reckel, J. Hodecek, A. Kori Yahia, S. Rahaus, M. H. Villet, M. Graw, Evaluating the value of entomotoxicology in forensic toxicology casework using the first minipig model, Forensic Toxicol. 43 (2) (2025) 333–348, https://doi.org/10.1007/s11419-025-00728-1.
[23] N.R. Haddaway, M.J. Page, C.C. Pritchard, L.A. McGuinness, PRISMA2020: an R package and Shiny app for producing PRISMA 2020-compliant flow diagrams, with interactivity for optimised digital transparency and Open Synthesis, Campbell Syst. Rev. 18 (2) (June 2022) e1230.
[24] B. Bourel, et al., Immunohistochemical Contribution to the Study of Morphine Metabolism in Calliphoridae Larvae and Implications in Forensic Entomotoxicology, J. Forensic Sci. 46 (3) (May 2001) 596–599, https://doi.org/10.1520/JFS15009J.
[25] A. Kaczmarek, A.K. Wrońska, M. Kazek, M.I. Boguś, Metamorphosis-related changes in the free fatty acid profiles of Sarcophaga (Liopygia) argyrostoma (Robineau-Desvoidy, 1830), Sci. Rep. 10 (1) (Oct. 2020) 17337, https://doi.org/10.1038/s41598-020-74475-1.
[26] K. L. Tabor, R. D. Fell, C. C. Brewster, K. Pelzer, and G. S. Behonick, 'Effects of Antemortem Ingestion of Ethanol on Insect Successional Patterns and Development of Phormia regina (Diptera: Calliphoridae)'.
[27] D. Ganapathy, P. Ganesan, K. Murthykumar, A. Arthanari, Awareness of forensic entomotoxicology among dental students, J. Punjab Acad. Forensic Med. Toxicol. 22 (2) (2022) 68–71, https://doi.org/10.5958/0974-083X.2022.00046.2.
[28] M. Peruch, et al., Comparative toxicological analyses of traditional matrices and blow fly larvae in four cases of highly decomposed human cadavers, Insects 15 (7) (July 2024) 500, https://doi.org/10.3390/insects15070500.
[29] D. Preußer, T. Fischer, T. Juretzek, Effects of antibiotics ceftriaxone and levofloxacin on the growth of *Lucilia sericata* (Diptera: Calliphoridae), Med. Vet. Entomol. 37 (4) (Dec. 2023) 805–815, https://doi.org/10.1111/mve.12685.
[30] D. Preußer, T. Fischer, T. Juretzek, Effects of antibiotics ceftriaxone and levofloxacin on the growth of Protophormia terraenovae (Diptera: Calliphoridae), Forensic Sci. Med. Pathol. (Mar. 2024), https://doi.org/10.1007/s12024-024-00804-9.
[31] F.M. Afifi, E.A. Abdelfattah, G.M. El-Bassiony, Impact of using Sarcophaga (Liopygia) argyrostoma (Robineau-Desvoidy, 1830) as a toxicological sample in detecting clonazepam for forensic investigation, Egypt. J. Forensic Sci. 12 (1) (Aug. 2022) 37, https://doi.org/10.1186/s41935-022-00296-0.
[32] T.C. Baia, A. Campos, B.M.S. Wanderley, R.A. Gama, The effect of Flunitrazepam (Rohypnol®) on the Development of *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae) and its Implications for Forensic Entomology, J. Forensic Sci. 61 (4) (July 2016) 1112–1115, https://doi.org/10.1111/1556-4029.13104.
[33] K. Pien, et al., 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 (4) (Aug. 2004) 190–193, https://doi.org/10.1007/s00414-004-0441-8.
[34] F. Boulkenafet, et al., Detection of benzodiazepines in decomposing rabbit tissues and certain necrophagic dipteran species of forensic importance, Saudi J. Biol. Sci. 27 (7) (July 2020) 1691–1698, https://doi.org/10.1016/j.sjbs.2020.04.044.
[35] S. Wang, et al., Effects of methamphetamine on the development and its determination in Aldrichina grahami (Diptera: Calliphoridae), J. Med. Entomol. 57 (3) (May 2020) 691–696, https://doi.org/10.1093/jme/tjz239.
[36] P.A. Magni, T. Pacini, M. Pazzi, M. Vincenti, I.R. Dadour, Development of a GC–MS method for methamphetamine detection in *Calliphora vomitoria* L. (Diptera: Calliphoridae), Forensic Sci. Int. 241 (Aug. 2014) 96–101, https://doi.org/10.1016/j.forsciint.2014.05.004.
[37] C. Mullany, P.A. Keller, A.S. Nugraha, J.F. Wallman, Effects of methamphetamine and its primary human metabolite, p-hydroxymethamphetamine, on the development of the Australian blowfly Calliphora stygia, Forensic Sci. Int. 241 (Aug. 2014) 102–111, https://doi.org/10.1016/j.forsciint.2014.05.003.
[38] V. Bugelli, et al., Entomotoxicology in burnt bodies: a case of maternal filicide-suicide by fire, Int. J. Legal Med. 131 (5) (Sept. 2017) 1299–1306, https://doi.org/10.1007/s00414-017-1628-0.
[39] L. Ahmed Al-Keridis, F. M. A. Al Galil, F. A. Al-Mekhlafi, M. A. Wadaan, and M. S. Al-Khalifa, 'Impact of Hypnotic Drug Zolpidem Tartrate on the Development of Forensic Fly *Sarcophaga ruficornis* (Diptera: Sarcophagidae)', J. Med. Entomol., 59, 3, 820–825, May 2022, doi: 10.1093/jme/tjac010.
[40] F.M. Saleh, A.H. Badawy, R.M. Badawy, A.A. Rahman, E. Adly, Impact of ante-mortem fluoxetine administration on estimation of post-mortem interval and insect activity in rabbit carcasses, Egypt. J. Forensic Sci. 14 (1) (Sept. 2024) 35, https://doi.org/10.1186/s41935-024-00409-x.
[41] N.I. Zanetti, A. Costantino, N. Lazzarini, A.A. Ferrero, N.D. Centeno, *Dermestes maculatus* (Coleoptera: Dermestidae) development under fluoxetine effect using two drug administration models, J. Forensic Sci. 66 (1) (Jan. 2021) 245–254, https://doi.org/10.1111/1556-4029.14575.
[42] N.I. Zanetti, N.D. Centeno, *Megaselia scalaris* (Diptera: Phoridae) development and behaviour under fluoxetine effect and Calliphora vicina (Diptera: Calliphoridae) presence, J. Asia-Pac. Entomol. 27 (1) (Mar. 2024) 102218, https://doi.org/10.1016/j.aspen.2024.102218.

[43] N.I. Zanetti, A.A. Ferrero, N.D. Centeno, The use of two fly species to detect the anti-depressant fluoxetine post-mortem (Diptera: Calliphoridae: *Lucilia Sericata* Meigen, Sarcophagidae: *Sarcophaga Crassipalpis Macquart*), Entomol. Am. 125 (1–4) (Nov. 2019) 4, https://doi.org/10.1664/1947-5136-125.1.4.
[44] H. Kharbouche, et al., Codeine accumulation and elimination in larvae, pupae, and imago of the blowfly *Lucilia sericata* and effects on its development, Int. J. Legal Med. 122 (3) (May 2008) 205–211, https://doi.org/10.1007/s00414-007-0217-z.
[45] A. A. M. Khan, S. A. Shamsuddin, N. S. A. Zaini, K. Mohamed, and R. A. Rashid, 'Analysis of paracetamol in forensic blowfly samples from intoxicated-paracetamol carcass'.
[46] C. O'Brien, B. Turner, Impact of paracetamol on *Calliphora vicina* larval development, Int. J. Legal Med. 118 (4) (Aug. 2004) 188–189, https://doi.org/10.1007/s00414-004-0440-9.
[47] A.L. Trivia, C.J. De Carvalho Pinto, Analysis of the Effect of Cyclophosphamide and Methotrexate on *Chrysomya megacephala* (Diptera: Calliphoridae), J. Forensic Sci., 63, 5, 1413–1418, Sept. 2018, doi: 10.1111/1556-4029.13740.
[48] N. Ishak, A. H. Ahmad, S. A. Mohamad Noor, and A. Ahmad, 'Detection of heroin metabolites at different developmental stages of *Lucilia cuprina* (Diptera: Calliphoridae) reared in heroin-treated meat: a preliminary analysis', Egypt. J. Forensic Sci., 9, 1, p. 65, Dec. 2019, doi: 10.1186/s41935-019-0171-1.
[49] 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) (Dec. 2009) 21–25, https://doi.org/10.1016/j.forsciint.2009.08.013.
[50] V. Hédouin, et al., Determination of drug levels in larvae of *Lucilia sericata* (Diptera: Calliphoridae) reared on rabbit carcasses containing morphine, J. Forensic Sci. 44 (2) (Mar. 1999) 351–353, https://doi.org/10.1520/JFS14462J.
[51] B. Bourel, et al., Effects of morphine in decomposing bodies on the development of *Lucilia sericata* (Diptera: Calliphoridae), J. Forensic Sci. 44 (2) (Mar. 1999) 354–358, https://doi.org/10.1520/JFS14463J.
[52] M. Gosselin, et al., Methadone determination in puparia and its effect on the development of *Lucilia sericata* (Diptera, Calliphoridae), Forensic Sci. Int. 209 (1–3) (June 2011) 154–159, https://doi.org/10.1016/j.forsciint.2011.01.020.
[53] Z. Lü, et al., Effects of ketamine on the development of forensically important blowfly *Chrysomya megacephala* (F.) (Diptera: Calliphoridae) and its Forensic Relevance, J. Forensic Sci. 59 (4) (July 2014) 991–996, https://doi.org/10.1111/1556-4029.12430.
[54] 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) (Jan. 2009) 202–206, https://doi.org/10.1111/j.1556-4029.2008.00926.x.
[55] C.M. Souza, P.J. Thyssen, A.X. Linhares, Effect of Nandrolone Decanoate on the development of three species of *Chrysomya* (Diptera: Calliphoridae), flies of forensic importance in Brazil, J. Med. Entomol. 48 (1) (Jan. 2011) 111–117, https://doi.org/10.1603/ME09291.
[56] K. Verma, Effects of codeine, sodium pentothal and different temperature factors on the growth rate development of *Chrysomya rufifacies* for the forensic entomotoxicological purposes', J. Bioanal. Biomed., 05, 01, 2013, doi: 10.4172/1948-593X.1000074.
[57] C.M. Souza, et al., Standardization of histological procedures for the detection of toxic substances by immunohistochemistry in dipteran larvae of forensic importance, J. Forensic Sci. 58 (4) (July 2013) 1015–1021, https://doi.org/10.1111/1556-4029.12140.
[58] A. Essarras, M. Pazzi, I.R. Dadour, P.A. Magni, The effect of antifreeze (ethylene glycol) on the survival and the life cycle of two species of necrophagous blowflies (Diptera: Calliphoridae), Sci. Justice 58 (2) (Mar. 2018) 85–89, https://doi.org/10.1016/j.scijus.2017.12.008.
[59] L. A. Castillo-Alanís et al., 'Development of mixed linear models to analyze and describe the impact of malathion on the larval growth of *Megaselia scalaris* (Diptera: Phoridae) under various feeding media and environmental conditions', J. Med. Entomol., p. tjae102, Sept. 2024, doi: 10.1093/jme/tjae102.
[60] S. Yan-Wei, L. Xiao-Shan, W. Hai-Yang, Z. Run-Jie, Effects of malathion on the insect succession and the development of *Chrysomya megacephala* (Diptera: Calliphoridae) in the field and implications for estimating postmortem interval, Am. J. Forensic Med. Pathol. 31 (1) (Mar. 2010) 46–51, https://doi.org/10.1097/PAF.0b013e3181c215b4.
[61] F. M. A. A. Galil, S. P. Zambare, F. A. Al-Mekhlafi, and L. A. AL-Keridis, 'Effect of dimethoate on the developmental rate of forensic importance Calliphoridae flies', Saudi J. Biol. Sci., 28, 2, 1267–1271, Feb. 2021, doi: 10.1016/j.sjbs.2020.12.022.
[62] F. M. Abd Al Galil, S. P. Zambare, F. A. Al-Mekhlafi, M. A. Wadaan, and M. S. Al-Khalifa, 'Effects of insecticide dimethoate on the developmental rate of forensic importance sarcophagid flies', J. King Saud Univ. - Sci., 33, 2, p. 101349, Mar. 2021, doi: 10.1016/j.jksus.2021.101349.
[63] N.I. Zanetti, N.D. Centeno, Forensic significance of Roundup Full® II effect on the development of *Dermestes maculatus* (Coleoptera: Dermestidae) and *Lucilia sericata* (Diptera: Calliphoridae), J. Forensic Sci. 69 (1) (Jan. 2024) 213–221, https://doi.org/10.1111/1556-4029.15408.
[64] P.A. Magni, M. Pazzi, M. Vincenti, V. Converso, I.R. Dadour, Development and validation of a method for the detection of α- and β-endosulfan (organochlorine Insecticide) in Calliphora vomitoria (Diptera: Calliphoridae), J. Med. Entomol. 55 (1) (Jan. 2018) 51–58, https://doi.org/10.1093/jme/tjx177.
[65] A. Ferhat, K. A. Yavuz, and F. Köse, 'Effects Of Some Toxic Heavy Metals', Fresenius Environ. Bull., 19, 6, 2010.
[66] https://www.euda.europa.eu/publications/european-drug-report/2024/drug-induced-deaths_en (accessed on 20th March 2026).
[67] H.N. Açikgöz, Multiple drug analysis of Chrysomya albiceps larvae provides important forensic insignt to unravel drug-associated mortalities, Entomol. News 128 (1) (2018) 99–107, https://doi.org/10.3157/021.128.0116.
[68] O. Groth, et al., Unexpected results found in larvae samples from two postmortem forensic cases, Forensic Toxicol. 40 (1) (Jan. 2022) 144–155, https://doi.org/10.1007/s11419-021-00601-x.
[69] M. Al-Khalifa, A. Mashaly, A. Al-Qahtni, Impacts of antemortem ingestion of alcoholic beverages on insect successional patterns, Saudi J. Biol. Sci. 28 (1) (Jan. 2021) 685–692, https://doi.org/10.1016/j.sjbs.2020.10.060.
[70] Universidad Regional Amazónica Ikiam, Ecuador, et al., First sight at entomotoxicology using Peckia intermutans (Diptera: Sarcophagidae) in Ecuador and its potential as a marker for methanol determination in biological samples, Rev. Soc. Entomológica Argent. (2004), https://doi.org/10.25085/rsea.830102.
[71] W.M.A. Wan Mahmood, A. Khan, S. Shamsuddin, N. Zaini, K. Mohamed, R. Rashid, Paraquat dichloride detection from forensic blowfly samples, Malays. Appl. Biol., Jan. 44 (2015) 133–138.
[72] J. Malejko, K. Deoniziak, M. Tomczuk, J. Długokencka, B. Godlewska-Żyłkiewicz, Puparial cases as toxicological indicators: bioaccumulation of cadmium and thallium in the forensically important blowfly Lucilia sericata, Front. Chem. 8 (Nov. 2020) 586067, https://doi.org/10.3389/fchem.2020.586067.
[73] B.G.D.O. Bessa, H.D.A. Silva-Neto, W.K.T. Coltro, T.L. Rocha, W.R. Lopes, Lead toxicity in Lucilia cuprina and electrochemical analysis: a simple and low-cost alternative for forensic investigation, Anal. Bioanal. Chem. 413 (12) (May 2021) 3201–3208, https://doi.org/10.1007/s00216-021-03257-z.
[74] E.M. Roeterdink, I.R. Dadour, R.J. Watling, Extraction of gunshot residues from the larvae of the forensically important blowfly Calliphora dubia (Macquart) (Diptera: Calliphoridae), Int. J. Legal Med. 118 (2) (Apr. 2004) 63–70, https://doi.org/10.1007/s00414-003-0408-1.
[75] I. Azam, et al., Evaluating insects as bioindicators of heavy metal contamination and accumulation near industrial Area of Gujrat, Pakistan, Biomed Res. Int. 2015 (2015) 1–11, https://doi.org/10.1155/2015/942751.
[76] C. Aubernon, D. Charabidzé, C. Devigne, Y. Delannoy, D. Gosset, Experimental study of *Lucilia sericata* (Diptera Calliphoridae) larval development on rat cadavers: Effects of climate and chemical contamination, Forensic Sci. Int. 253 (Aug. 2015) 125–130, https://doi.org/10.1016/j.forsciint.2015.05.032.
[77] J.T. Jales, T.D.M. Barbosa, L.C. Dos Santos, V.D.P.S. Rachetti, R.A. Gama, Carrion decomposition and assemblage of necrophagous dipterans associated with Terbufos (Organophosphate) intoxicated rat carcasses, Acta Trop. 212 (Dec. 2020) 105652, https://doi.org/10.1016/j.actatropica.2020.105652.
[78] J.T. Jales, T.M. Barbosa, V.P. Soares, R.A. Gama, Effect of terbufos (organophosphate) on the cadaveric colonization process: implications for postmortem interval calculation, J. Med. Entomol. 58 (3) (May 2021) 1056–1063, https://doi.org/10.1093/jme/tjaa284.
[79] T.M. Saber, E.A.A. Hassanen, R.G.A. Anter, M.R. Farag, T. Saber, T.S. Imam, Identification of forensically important insects on atrazine-intoxicated rat carcasses at different decomposition stages during summer season, Slov. Vet. Res. (Dec. 2021), https://doi.org/10.26873/SVR-1457-2021.
[80] G.S. Viana, M.C.D. Paula, A.D.M.D.M. Eulalio, P.G.D. Santos, S.E. Lima-Junior, W. F. Antonialli-Junior, Formicidae fauna in pig carcasses contaminated by insecticide: implications for forensic entomology, Rev. Bras. Entomol. 66 (1) (2022) e20210085, https://doi.org/10.1590/1806-9665-rbent-2021-0085.
[81] A.D.M.D.M. Eulalio, et al., Effect of thiamethoxam (organophosphate) on the flies and beetle visitation and cadaveric decomposition process, Rev. Bras. Entomol. 67 (1) (2023) e20220049, https://doi.org/10.1590/1806-9665-rbent-2022-0049.
[82] E. Buratti, et al., Detection of three opioids (morphine, codeine and methadone) and their metabolites (6-monoacetylmorphine and 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine) in Larvae of *Lucilia sericata* species by UHPLC-TF-MS AND Validation, Molecules 28 (12) (June 2023) 4649, https://doi.org/10.3390/molecules28124649.
[83] M. Salimi, et al., Toxicological analysis of insects on the corpse: a valuable source of information in forensic investigations, J. Arthropod-Borne Dis. 12 (3) (Sept. 2018) 219–231.
[84] M. Gosselin, M.D.M.R. Fernandez, S.M.R. Wille, N. Samyn, G. De 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 (7) (Sept. 2010) 374–380, https://doi.org/10.1093/jat/34.7.374.
[85] P.A. Magni, M. Pazzi, J. Droghi, M. Vincenti, I.R. Dadour, Development and validation of an HPLC-MS/MS method for the detection of ketamine in *Calliphora vomitoria* (L.) (Diptera: Calliphoridae), J. Forensic Leg. Med. 58 (Aug. 2018) 64–71, https://doi.org/10.1016/j.jflm.2018.04.013.
[86] D. Sadler, L. Robertson, G. Brown, C. Fuke, D. Pounder, Barbiturates and Analgesics in *Calliphora vicina* Larvae, J. Forensic Sci. 42 (3) (May 1997) 481–485, https://doi.org/10.1520/JFS14151J.
[87] 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 (4) (Dec. 1997) 397–403, https://doi.org/10.1097/00000433-199712000-00015.
[88] V. Hédouin, et al., 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) (Jan. 2001) 12–14, https://doi.org/10.1520/JFS14905J.

[89] V. Hédoui, et al., Morphine perfused rabbits: a tool for experiments in forensic entomotoxicology, J. Forensic Sci. 44 (2) (Mar. 1999) 347–350, https://doi.org/10.1520/JFS14461J.
[90] S.Z. Parkhideh, et al., Preliminary analysis of methamphetamine detection in *Lucilia sericata* (Diptera: Calliphoridae) reared in methamphetamine-treated meat at various developmental stages, J. Arthropod-Borne Dis. (Feb. 2024), https://doi.org/10.18502/jad.v17i3.14984.
[91] P.A. Magni, M. Pazzi, M. Vincenti, E. Alladio, M. Brandimarte, I.R. Dadour, Development and validation of a GC–MS method for nicotine detection in *Calliphora vomitoria* (L.) (Diptera: Calliphoridae), Forensic Sci. Int. 261 (Apr. 2016) 53–60, https://doi.org/10.1016/j.forsciint.2015.11.014.
[92] V. Lawai, N. A. Abdul Rahim, Z. Ngaini, 'Blowfly larval tissues as a secondary detector for determining paraquat-related death in rabbit carcass', J. Forensic Sci., 60, 6, 1620–1624, Nov. 2015, doi: 10.1111/1556-4029.12852.
[93] Muskan, V. Chauhan, J. Singh, S. Shukla, D. Harish, 'Determination of asenapine maleate from maggots by solid-phase extraction and gas chromatography-mass spectroscopy', J. Forensic Med. Sci. Law, 32, 1, 48–53, June 2023, doi: 10.59988/jfmsl.vol.32issue1.10.
[94] M. Definis-Gojanović, D. Sutlović, D. Britvić, B. Kokan, Drug analysis in necrophagous flies and human tissues, Arch. Ind. Hyg. Toxicol. 58 (3) (Sept. 2007) 313–316, https://doi.org/10.2478/v10004-007-0022-6.
[95] S.K. Bushby, N. Thomas, P.A. Priemel, C.V. Coulter, T. Rades, J.A. Kieser, Determination of methylphenidate in Calliphorid larvae by liquid–liquid extraction and liquid chromatography mass spectrometry – Forensic entomotoxicology using an in vivo rat brain model, J. Pharm. Biomed. Anal. 70 (Nov. 2012) 456–461, https://doi.org/10.1016/j.jpba.2012.06.024.
[96] J. Amendt, C.P. Campobasso, E. Gaudry, C. Reiter, H.N. LeBlanc, M.J.R. Hall, Best practice in forensic entomology—standards and guidelines, Int. J. Legal Med. 121 (2) (Mar. 2007) 90–104, https://doi.org/10.1007/s00414-006-0086-x.
[97] L.J. Tanz, A. Stewart, R.M. Gladden, J.Y. Ko, L. Owens, J. O'Donnell, Detection of illegally manufactured fentanyls and carfentanil in drug overdose deaths — United States, 2021–2024, MMWR Morb. Mortal. Wkly Rep. 73 (2024) 1099–1105, https://doi.org/10.15585/mmwr.mm7348a2.
[98] A.J. Krotulski, D.M. Papsun, S.L. Kacinko, B.K. Logan, Isotonitazene quantitation and metabolite discovery in authentic forensic casework, J. Anal. Toxicol. 44 (6) (2020) 521–530, https://doi.org/10.1093/jat/bkaa016.
[99] S.M. Aly, et al., In the case of extensively putrefied bodies, the analysis of entomological samples may support and complement the toxicological results obtained with other alternative matrices, Leg. Med. 63 (July 2023) 102261, https://doi.org/10.1016/j.legalmed.2023.102261.
[100] M. Grassberger, C. Reiter, 'Effect of Temperature on Lucilia Sericata (Diptera: Calliphoridae) Development with Special Reference to the Isomegalen-and Isomorphen-Diagram', Forensic Sci Int, pp 32–26, 2021.
[101] L.G. Higley, N.H. Haskell, Insect Development and Forensic Entomology, in: J. H. Byrd, J.L. Castner (Eds.), Forensic Entomology: the Utility of Arthropods in Legal Investigations, 1st ed., CRC Press, Boca Raton, 2001, pp. 287–302.
[102] M.C. Paula, G.M. Morishita, C.H. Cavarson, C.R. Gonçalves, P.R. Tavares, A. Mendonça, Y.R. Súarez, W.F. Antonialli-Junior, Action of ants on vertebrate carcasses and blow flies (Calliphoridae), J. Med. Entomol. (2016) 1283–1291, https://doi.org/10.1093/jme/tjw119.
[103] A. Holland, C.S. Copeland, G.W. Shorter, D.J. Connolly, A. Wiseman, J. Mooney, K. Fentonand, M. Harris, Nitazenes – heralding a Second Wave for the UK drug-related death crisis? Lancet Public Health 9 (2) (2024) https://doi.org/10.1016/s2468-2667(24)00001-x.
[104] OHID. Deaths Linked to Potent Synthetic Opioids. GOV.UK. https://www.gov.uk/government/publications/deaths-linked-to-potent-synthetic-opioids/deaths-linked-to-potent-synthetic-opioids (accessed on 20th March 2026).
[105] OECD/The World Bank (2023), Health at a Glance: Latin America and the Caribbean 2023, OECD Publishing, Paris, doi: 10.1787/532b0e2d-en.
[106] V. Bugelli, C.P. Campobasso, R. Zehner, J. Amendt, How should living entomological samples be stored? Int. J. Legal Med. 133 (6) (Nov. 2019) 1985–1994, https://doi.org/10.1007/s00414-019-02114-0.
[107] J.W. Seo, J.H. Lee, I.S. Son, Y.J. Kim, K. DY, Y. Hwang, Acute oxalate nephropathy caused by ethylene glycol poisoning, Kidney Res. Clin. Pract. (2012) 249–252.