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Review Article

Forensic entomo-toxicology: Xenobiotics effects and AI integration in forensic investigations

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Abstract

Forensic entomo-toxicology is an interdisciplinary field that integrates entomology and toxicology to detect xenobiotics from necrophages insects feeding on decomposed remains especially when traditional matrices are not available or are in highly decomposed stage. The presence of toxicants can alter insect development, physiology, succession rate due to which it could probably hamper the accuracy of post-mortem interval (PMI) calculation. This paper delineates the life cycle of blow fly which primarily colonizers of decomposing carcass and identify prevalent species found in India for interpreting colonization pattern, geographic location of these insects for forensic investigation. It critically evaluates the strengths and limitations of entomo-toxicological approaches within forensic contexts and synthesizes current findings on the impact of various xenobiotics— drugs, pesticides, nanoparticles, antibiotics, and heavy metals on the insect developmental rates which can affect the PMI. Furthermore, the paper explores the recent advancement of integrating it with artificial intelligence (AI), positioning it as a transformative tool for future forensic entomotoxicological applications to aid in scientific investigation. This review offers a systematic and comprehensive overview of insect detoxification mechanisms and xenobiotic interactions, and underscores the potential of AI-enhanced models to advance scientific rigor and operational efficacy in forensic casework.

Keywords: Forensic Entomo-toxicology, Xenobiotics, Artificial Intelligence, Post-mortem Interval, Insects.

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1. Introduction

Forensic entomology is the study of insects and other arthropods in relation to legal investigations, particularly in determining the time of death and other circumstances surrounding a crime.[1] Forensic toxicology is the field that analyzes biological samples to identify toxins and drugs, playing a crucial role in legal investigations related to death, poisoning, and drug use.[2] Traditional toxicological procedures sometimes become impracticable due to tissue disintegration in circumstances of severe decomposition, especially when remains are skeletonised. In these situations, necrophagous insects that live inside decaying remains are useful substitutes for toxicological samples. This method, called forensic entomo-toxicology, has two main goals: first, to identify the toxic substances that have accumulated in necrophagous insects to help identify the cause of death in decomposed remains; second, to examine the effects of these substances on insect developmental biology, which is crucial for increasing the precision of PMI estimation by taking into account xenobiotic-induced variations in insect growth patterns.[3] Usually, insects get xenobiotics by consuming decaying tissues and absorbing bodily fluids from the dead[4]. These chemicals have the ability to drastically change the necrophagous larvae's typical developmental cycle.

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2. Life Cycle of Blow Fly

Multiple kinds of insects feed on the decomposing carcass. However, blowflies and beetles are the most prevalent. Flies, particularly blowflies, may detect the presence of dead stuff within minutes.[4] Most of the body's deterioration is caused by being devoured by fly larvae, often known as maggots. In contrast, beetles eat after the corpse has dried out. For victims who have been dead for a long time, analysing the blowfly life cycle inside the body might be one of the most important pieces of evidence for forensic investigators. Blowflies fall in the Calliphoridae family and often commonly referred to as scavenger flies.[5] They resemble houseflies. Adult blowflies, on the other hand, are slightly larger with a massive head and protruding eyes, and they have a metallic sheen that might be blue, black, or green.[6] These flies eat and reproduce remarkably identically to house flies. Blowfly larvae are known as maggots, and they resemble small whitish worms. Blowflies do not sting humans; instead, they feed on rotting debris, damp rubbish, rotten food, and meat leftovers. They grow well in warm, humid environmental settings.

Female blowflies can lay approximately 150 eggs each batch[7] and **Figure 1** shows the entire life cycle of blowfly. The entire process, from laying eggs to hatching, takes about a day after that the larvae first phase feed on bodily fluids and migrate throughout the body. This stage takes around one day to accomplish. The larvae in second phase move around in worm shape. The shift from the larvae first phase to this takes around one day. In third phase larvae continue to travel in large groups, although they have grown significantly in size at this stage. It is the pre-pupa stage and takes around two days to attain. Pre-pupae migrate away from the body they were eating to a suitable pupation site, usually earth. They are now changed into a pupa, which takes around four days to transition from pre-pupa to pupa form. The pupae remain at the pupation site and develop into adult fly. It takes around 10 days for a pupa to mature into an adult fly. They have not fed since moving to the pupation place.

After emerging from the pupa, adult flies mate, eat on protein-rich bodily fluids, and lay eggs on the dead carcass.

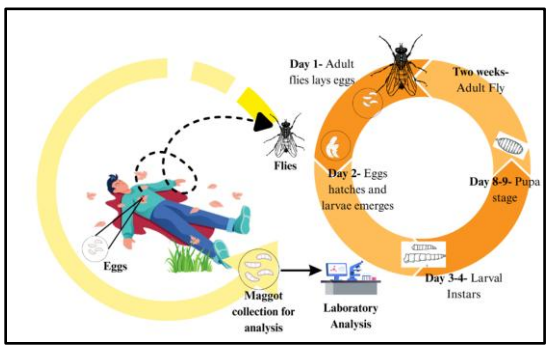

Figure 1: Life cycle of Blow Fly. (original image by the author)

3. Common Species Found in India

The growing database of different insect colonization patterns on buried remains in India contributes and aid in estimating post-burial interval (PBI), detecting body relocation, and interpreting concealed crime scenes in medicolegal investigations.

During putrefaction, corpses undergo considerable physical, chemical, and biological changes. Each decomposition stage is attractive to a group of arthropods, predominantly sarcosaprophagous insects such as Dipterans, Coleopterans, Hemipterans, Hymenopterans, and numerous others. Amongst these, Dipterans are the first ones to arrive on dead and decaying animal or human corpses, followed by Coleopterans, Hymenopterans, and Hemipterans.

Sharif et al. documented five Dipteran species—including *Megaselia scalaris*, *Chrysomya megacephala*, *Calliphora vicina*, *Synthesiomyia nudiseta*, and *Hydrotaea capensis*—alongside three Coleopterans (*Saprinus quadriguttatus*, *Saprinus splendens*, *Onthophagus quadridentatus*), one Hemipteran (*Cydnus* sp.), and one Hymenopteran (*Dorylus* sp.) in an entomofaunal succession experiment on a shallowly buried goat carcass in Aligarh, Uttar Pradesh[8]. These species were associated with distinct decomposition stages, and notably, *M. scalaris* was consistently observed both beneath the soil and on the grave surface.

Verma highlights the forensic relevance of *Chrysomya rufifacies* in estimating time since death, especially in cases involving prolonged decomposition conducted in Noida, Uttar Pradesh.[9] These developmental variations can be used to refine minimum postmortem interval (PMI) estimates, reinforcing the applicability of forensic entomology in the Indian context.

Palavesam et al. highlighted a study on necrophagous fly samples from 24 medico-legal cases in Tamil Nadu, India, spanning 2011 to 2018.[10] Four key species were identified: *Chrysomya megacephala*, *Chrysomya rufifacies*, *Sarcophaga* spp., and *Musca domestica*. Among them, *C. megacephala* was the most prevalent (70.8%) and uniquely colonized both burnt and drowned corpses, as well as indoor and outdoor scenes. *C. rufifacies* was restricted to outdoor cases, while *Sarcophaga* spp. appeared only indoors. The findings align with historical data on necrophagous fly distribution in India and reinforce *C. megacephala* as a dominant forensic indicator species in diverse death scenarios.

Sharma et al. examined necrophagous insect succession on five human corpses (aged 26–52 years) from Northern India (Punjab), involving cases of homicide and suicide [11]. Insects were collected during autopsy to assess their role in postmortem interval (PMI) estimation. Species identified included *Chrysomya megacephala*, *Chrysomya rufifacies*,

Phormia regina, *Chrysomya albiceps*, *Dermestes maculatus*, and *Necrobia rufipes*, across various life stages.

Babu et al. examined the forensic importance of blowflies (*Chrysomya megacephala* and *Chrysomya rufifacies*) for estimating postmortem interval (PMI) of a highly decomposed male corpse found in Bijapur, Chhattisgarh.[12] Using the Accumulated Degree Hours (ADH) method and developmental data of third instar larvae, the estimated PMI was approximately 5.5 days.

4. Forensic Application of Forensic Entomo-toxicology

Forensic entomo-toxicology plays a pivotal role in postmortem investigations, particularly when conventional biological matrices such as blood, urine, or soft tissues are no longer available due to advanced decomposition.

Figure 2 demonstrates the various applications of forensic entomo-toxicology investigations and also demonstrates the potential limitations.

Insects—especially necrophagous larvae like those of blowflies—act as biological reservoirs, capable of bioaccumulating xenobiotics including drugs, poisons, and environmental contaminants from decomposing remains.[13] These larvae serve as alternative matrices for toxicological screening, enabling the detection of substances such as morphine, cocaine, barbiturates, and organophosphates in severely decomposed or skeletonized cases.[14] Importantly, xenobiotics can modulate insect development rates, either accelerating or delaying larval growth, which directly impacts postmortem interval (PMI) estimation.[15] Entomo-toxicological data thus refine PMI models by accounting for biochemical interference, enhancing temporal accuracy in drug-related deaths.[16] Accurate species identification—based on larval spiracles and adult morphology—and local meteorological data were critical for reliable PMI calculation.[12] Beyond toxicological profiling, insect-derived evidence can confirm ante-mortem drug ingestion or poisoning, particularly in cases involving overdose, homicide, or covert toxic exposure. These findings can corroborate or challenge witness statements, reconstruct timelines, and support cause-of-death hypotheses in criminal investigations. Additionally, insect species composition and xenobiotic profiles may reflect regional environmental conditions or local drug usage patterns, offering geolocation insights when remains are relocated post-mortem.[17] Entomo-toxicology also contributes to environmental forensics, where insects collected from contaminated sites help trace industrial toxins or pesticide exposure. Operationally, it informs forensic training and protocol development, guiding standardized procedures for insect collection, preservation, and toxicological analysis across laboratories. As the field evolves, entomo-toxicology continues to bridge critical gaps in forensic science offering biochemical and ecological insight where traditional methods fall short. Despite its growing relevance in postmortem toxicological investigations, forensic entomo-toxicology faces several limitations that hinder its routine application and evidentiary robustness. One of the primary challenges is the absence of standardized protocols for insect collection, preservation, and toxicological analysis, resulting in methodological inconsistencies across laboratories and jurisdictions.[18] The biochemical stability of xenobiotics within insect tissues is another critical concern[19] many drugs undergo rapid metabolic degradation in larvae, making detection highly time-sensitive and potentially unreliable for unstable compounds. Moreover, insect species exhibit distinct metabolic pathways, particularly in the expression of detoxification enzymes such as cytochrome P450s, GSTs, and UGTs, which influence drug absorption, transformation, and excretion.[20] This species-specific metabolism complicates toxicological interpretation unless precise taxonomic identification is achieved. Xenobiotics can also modulate larval development rates, thereby affecting postmortem interval (PMI) estimation. Without controlled developmental baselines, PMI calculations may be skewed, especially in drug-related deaths.[21] Analytical sensitivity poses another barrier; drug concentrations in insect tissues are often low, necessitating advanced instrumentation, which may not be accessible in all forensic settings.[22] Additionally, insects may acquire xenobiotics from environmental sources unrelated to the corpse, introducing contamination risks and false positives. The lack of comprehensive reference databases correlating drug classes, insect species, and developmental effects further restricts comparative analysis and model validation. From a legal standpoint, entomotoxicological evidence remains underutilized due to limited judicial precedent and the absence of standardized admissibility criteria, often leading to skepticism in courtrooms.[23] Sample preservation also plays a crucial role; delayed or improper storage can result in analyte degradation, compromising toxicological integrity. Finally, geographic and seasonal variability in insect availability and species diversity limits the applicability of entomo-toxicological methods in certain climates or timeframes. Collectively, these limitations underscore the need for methodological harmonization, expanded reference datasets, and interdisciplinary collaboration to enhance the reliability and forensic utility of entomo-toxicological evidence.

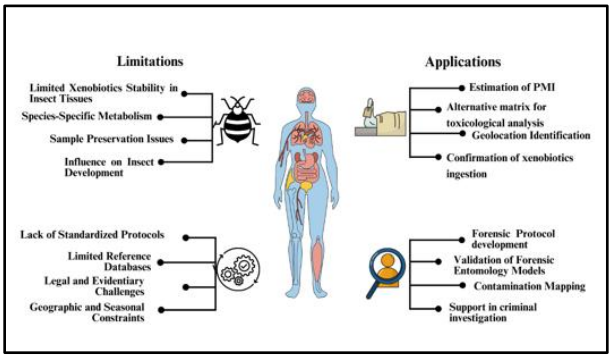

Figure 2: Various Application and potential limitation of Forensic Entomo-toxicology.

4.1. Detoxification mechanism of xenobiotics in insects

The growth, physiology, and forensic significance of insects are significantly impacted by the complicated detoxification response triggered by exposure to xenobiotics, which include pesticides, drugs, and environmental pollutants (**Figure 3**). A complex network of metabolic enzymes and membrane-bound transporters, whose activity is controlled by species-specific gene expression plasticity, orchestrates this response. The cytochrome P450 monooxygenases (CYPs) that catalyse the oxidative biotransformation of lipophilic xenobiotics are essential to Phase I metabolism because they decrease their bioactivity and make processing easier.[19] The ecological adaptability and adaptive resistance significance of these enzymes are highlighted by the fact that they are evolutionarily conserved and can be induced by both synthetic insecticides and phytochemicals. Glutathione S-transferases (GSTs) and UDP-glucuronosyltransferases (UGTs) are involved in phase II metabolism.[24] They conjugate the oxidised metabolites with endogenous molecules like glutathione or glucuronic acid, enhancing their solubility in water and promoting their elimination. ATP-binding cassette (ABC) transporters regulate the final stage of detoxifying by aggressively ejecting conjugated xenobiotics from cells, preserving cellular homeostasis in the midst of toxic stress.[25] Interestingly, these detoxification genes' expression is quite changeable and may be increased in exposure to xenobiotic presence, which can lead to developed resistance and modified developmental paths. The insect fat body, functioning similar to vertebrate liver and adipose tissue, is the primary location for detoxifying enzyme creation and action.[26] Aside from its metabolic function, the fat body combines endocrine and nutritional indicators that influence larval development, immunological responses, and energy balance. Its cellular architecture changes during growth, involving mitosis throughout embryogenesis, endoreplication throughout larval stages, and substantial remodelling during metamorphosis. Recent research indicates that fat body cells play a crucial role in coordinating larval development, lifespan management, and eating behaviour.[27] This makes them a target organ for detoxifying and insect physiology.

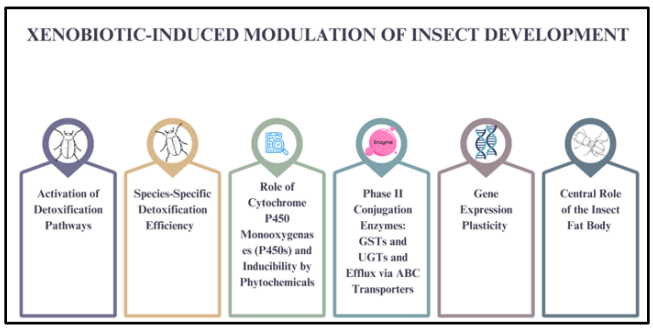

Figure 3: Mechanism of detoxification of xenobiotics in insects

5. Effects of Different Xenobiotics on Necrophagous Insects

Forensic entomo-toxicology investigates how xenobiotics, such as pharmaceuticals and pesticides, influence necrophagous insects, namely blow flies (Calliphoridae) and flesh flies (Sarcophagidae), which are the most common cadaver colonisers. When seen as a whole, the

***Table* 1** depicts different characteristics in how various chemicals influence drug detectability, fly growth, and forensic interpretations of post-mortem intervals (PMI).

Table 1: Effects of xenobiotics on development of necrophagous flies

Aim of study	Family & species	Drug / toxin	Dose	Sample preparation	Analytical method	Developmental rates	Reference
Detect benzodiazepines in carcass tissues and larvae; assess effect on larval development / PMI implications.	Calliphoridae — *Chrysomya albiceps, Lucilia sericata, Lucilia silvarum*	Benzodiazepines (carbamazepine, clobazam)	3271.57/414.08 mg/kg	Carcasses exposed; larvae pooled by stage (800); frozen (−20°C); homogenized in methanol; centrifuged & filtered	UHPLC–QTOF–MS	The development of *L.sericata* and *L.silvarum* larvae slowed and *C.albiceps* larvae fed on drugs developed faster.	28
Test inter- and transgenerational effects of paracetamol on life-history traits	Drosophilidae — *Drosophila melanogaster*	Paracetamol	0–100 mM; experiments at 20–40 mM; ≥50 mM	Drug mixed with instant fly food; eggs/larvae reared at controlled temp; substrate verified by 1H-NMR	1H-NMR;	≥50 mM decreased viability; 20–40 mM caused genotype- and generation-specific changes.	29

Determine malathion levels and effects on Chrysomya megacephala development	Calliphoridae — *Chrysomya megacephala*	Malathion	6.10–137.20 ng/g	Larvae reared on treated tissues; homogenized; extracted with solvents; stored	GC–MS	Delayed oviposition (1–3 days); prolonged pupation.	[30]
Measure methadone residues in puparia and effect on Lucilia sericata	Calliphoridae — *Lucilia sericata*	Methadone	0-4 μg/g	Larvae reared on methadone tissues; puparia collected, cleaned & processed	LC–MS/MS	Altered pupation timing; detectable residues in puparia.	[31]
Validate LC–MS/MS detection of benzodiazepines in single larvae/puparia	Calliphoridae — *Calliphora vicina*	Benzodiazepines	Larvae- 25 to 750 pg/mg Puparia- 50 to 500 pg/mg	Single larvae homogenized; puparia washed, ground, sonicated; extracts reconstituted	LC–MS/MS	Method allows picogram-level detection in single insect/puparium.	[32]
Assess effects of methamphetamine and primary metabolite p-OHMA on development and survival of Calliphora stygia larvae.	Calliphoridae — *Calliphora stygia*	Methamphetamine	10mg/kg	Larvae reared on spiked substrates; developmental monitoring across temperatures; tissue/larvae sampled for analysis.	HPLC (UV)	The pupal stage was prolonged upto78h and larval growth increased.	[33]
Diazepam effect on fly growth	Calliphoridae – Necrophagous flies (Brazil)	Diazepam (spiked tissues)	50 mg/kg	Rabbit tissues dosed with diazepam; larvae reared	Toxicological analysis	Larval growth accelerated; instar transitions faster	[34]
Selective determination of morphine in larvae reared on spiked substrates and measure accumulation	Calliphoridae — *Calliphora stygia*	Morphine		Eggs reared on homogenized meat substrates with known morphine levels; larvae harvested, homogenized; subsamples taken for analysis	HPLC & post-column chemiluminescence / FIA	Larvae reared on ≥2500 ng/g substrates sequestered detectable morphine; lower doses (500–1000 ng/g) not detected in larvae	[35]
Determine drug levels in larvae reared on morphine-containing rabbit carcasses (field model)	Calliphoridae — *Protophormia terraenovae, Calliphora vicina*	Morphine in rabbit carcasses (dose per animal; results reported as ng/g in larvae and tissues)		Larvae collected from carcasses over time, pooled by stage, frozen; homogenized and extracted for analysis	GC–MS / HPLC	Morphine detectable in larvae; stage- and species-dependent accumulation and temporal decline observed	[36]
MDMA (Ecstasy) effect on flies	Sarcophagidae – *Parasarcophaga ruficornis*	MDMA	(11–67 mg)	Rabbit tissues spiked with MDMA; larvae reared	GC–MS	Dose-dependent: low dose = no effect; high dose = accelerated growth, larger larvae	[37]
Effects of antibiotics ceftriaxone and levofloxacin on the growth of *Calliphora vomitoria*	*C.vomitoria*	Ceftriaxone and levofloxacin	28.56/3.56 mg/kg	Minced pork tissues spiked with Ceftriaxone and levofloxacin		Pupation was delayed in both antibiotics, and mortality was reduced. The maggot growth was delayed by levofloxacin but not with ceftriaxone	[38]
Effect of ZnCoS Nanoparticles	*Dermestes maculatus*	ZnCoS nanoparticles		Rat carcass spiked with different	light microscopy	ZnCoS NPs alter both insect	[39]

Toxicity on Beetle (*Dermestes maculatus)*				doses of ZnCoS nanoparticles	, scanning electron microscopy (SEM), and transmissio n electron microscopy (TEM).	growth and the rate of decomposition with structural damages.	
Effect of Different Heavy Metals on the Development of *Lucilia sericata*	*Lucilia sericata*	cadmium, zinc copper	0.25, 0.50, 1, and 2 µg/g	First-instar larvae of *L. sericata* were reared on a diet containing four concentrations of the heavy metals		Larval and pupal survival rate decreased as heavy metal concentrations increased. Pupal weight was significantly different but the adult weight was not significantly different among heavy metal concentration. The larval length was significantly different.	[40]

5.1. Drugs

In an experimental study on *Calliphora vomitoria* Cocaine exposure reduced larval size and prolonged feeding instars but shortened pupation, effectively accelerating adult emergence. Heroin slowed larval growth yet extended pupation, delaying eclosion.[41] When both drugs were combined, their effects were antagonistic and variable. Morphine presence altered larval growth, showing that opioids not only persist but also bias developmental rates in *Chrysomya albiceps*.[42] Parkhideh et al. demonstrated methamphetamine (MA) detection across the life cycle of Lucilia sericata fed on spiked substrates.[43] All instars contained MA, with the highest concentrations in third instars, pupae, and puparia. Crucially, puparia retained drug residues even after tissue decomposition, reinforcing their value in cases where bodies are skeletonized. Boulkenafet et al. examined *Chrysomya albiceps* and *Lucilia* spp. exposed to carbamazepine and clobazam in rabbit carcasses.[28] Results demonstrated species-specific developmental effects: *C. albiceps* developed faster under drug exposure, while Lucilia species slowed, leading to opposite biases in PMI estimation.

5.2. Pesticides

Pesticide ingestion is a leading cause of suicide globally, with organophosphates and carbamates being the most commonly used poisons in both accidental and purposeful cases.

Using *Sarcophaga peregrina*, *S. dux*, and *S. ruficornis*, exposure to dimethoate (1–4 ppm) slowed larval, prepupal, and pupal development. Higher concentrations produced smaller body sizes and increased mortality.[44] Experiments with *Lucilia sericata* and *Phormia regina* under laboratory and field conditions showed that insecticides influence oviposition and mortality [45].

El-Ashram et al. exposed *Chrysomya albiceps* larvae to aluminum phosphide-treated rabbit carcasses and larvae displayed reduced body length, delayed growth, and notable morphological deformities including abnormal spiracles.[46] Saber et al. studied rat carcasses intoxicated with atrazine and it showed the decomposition was prolonged, and insect colonization was delayed compared to controls. *Chrysomya albiceps* and *C. bezziana* dominated initial colonization, but overall Dipteran abundance decreased while Coleopteran proportions rose and this altered succession pattern.[47]

Pesticide residue in corpses can impact the growth of larvae and insect succession, thereby reducing the reliability of PMI estimates.

5.3. Antibiotics

Antibiotics are frequently suggested as first- or second-choice therapy for common illnesses due to their safety and inexpensive cost. Antibiotic resistance has emerged as a result of widespread usage brought on by this growing tendency. Antibiotics have the significant ability to alter the microbial communities present in decaying corpses as well as the necrophagous insects that inhabit there. PreuBer et al. found that maggot development was delayed by levofloxacin in feeding on pork, where a mixture of both antibiotics increased this effect. The maggot growth in the samples with ceftriaxone was not delayed.[38] Pupation was delayed in treatments with a mixture of both antibiotics. The mortality was reduced by separate or combined application of ceftriaxone and levofloxacin. PreuBer et al. used ceftriaxone and levofloxacin antibiotics and found that it did

not significantly influence the maggot weight and length and there was significant delay in the time of pupation.[48] The mortality was significantly increased in all treatments with antibiotics compared to the control, whereby the survivability remained over 80% of all treatments. These results demonstrate how important it is to look at how antibiotics affect necrophagous organisms; otherwise, PMI may be overestimated.

5.4. Nanoparticles

Although their benefits, nanoparticles' possible health risks remain unclear because of their unregulated environmental discharge and their harmful impacts.[49] In order to define the usage of more practical and eco-friendly nanomaterials, nanotoxicology research investigations are crucial.

Pappus et al. examined effect of hydroxyapatite nanoparticles (HApNPs) on *Drosophila melanogaster* and found that it resulted in deformities, such as wing, eye, and thorax, as well as developmental delays, decreased pupae numbers, poor movement, and heightened susceptibility to stress.[50] Compared to larger dosages, lower amounts caused more oxidative stress and reactive oxygen species (ROS), damaging gastrointestinal cells and interfering with the absorption of calcium and phosphorus. Silver nanoparticles (AgNPs) has shown severe morphological damage in midgut epithelium and in disrupted cells, disintegrated organelles and collapsed muscles and it had also depicted external cuticle alteration in 4th instar larvae with DNA damage highlighting the potential risk.[51] These results demonstrate the intricate physiological and biochemical effects of nanoparticle exposure on necrophagous insects, which can drastically change the time it takes for development to occur and result in imprecise PMI calculations and it highlights their environmental risk and hazardous potential.

5.5. Heavy metals

Heavy metals provide very serious toxicological dangers to both plants and animals, making environmental pollution one of the most urgent issues globally. The use of heavy metals has skyrocketed in recent decades due to industrialisation. Heavy metals are not biodegradable and are extremely poisonous. It gets accumulated inside organisms and impact an individual's growth and physiological status. Moophayak et al. showed that in cattle farms in Thailand, necrophagous flies, especially *Chrysomya megacephala* and *Musca domestica*, are useful bioindicators of potentially hazardous elements (PTEs).[52] Heavy metal contaminants can bioaccumulate in insects exposed to them, changing important life- features at the organismal level. Al Momani et al. reported that the study across two generation of *Drosophila melanogaster* showed higher survival rate at pupa stage in second generation compared to the first generation but there was a survival decline in the adult stage indicating heavy metals like Cadmium (Cd), Zinc(Zn), Lead(Pb), Copper(Cu) etc., may disrupt metamorphosis.[53] Furthermore, it has been demonstrated that exposure to heavy metals damages the peritrophic membrane of insects' midgut tissue, causing lesions in epithelial cells and the suppression of vital metabolic enzymes as well as anomalies in development. These hinder nutritional absorption and digestion, interfere with ATP synthesis, limit energy availability, and eventually result in slower growth and poorer survival rates. Singh et al. showed that exposing *Chrysomya megacephala* larvae to varying concentration of $CdCl_2$ has reduced body metrics, showed delay in development and increased mortality across various life stages.[54] Lipid peroxidation and other physiological harm might result from oxidative stress brought on by heavy metals. And if the existence of heavy metals is not handled properly, bbbbbthen using necrophagous insects growth stages for PMI estimation, may result in significant errors.

6. Advances in Forensic Entomo- toxicology

6.1. Application of AI in forensic entomo-toxicology

Forensic entomo-toxicology integrates entomology and toxicology to recover toxicological information from insects feeding on decomposed remains. The field has traditionally relied on chemical analysis and morphological identification, but both methods can be laborious, prone to mistakes, and context-dependent. In order to conquer difficulties in species identification, post-mortem interval (PMI) estimation, and the interpretation of toxicological influences on insect development, artificial intelligence (AI) and machine learning (ML) provide novel opportunities. Through the analysis of carrion insect development and succession, forensic entomo-toxicology (FET) has established itself as a reliable approach for estimating post-mortem interval (PMI). FET used to rely on chemical and morphological approaches to identify toxins and drugs within insect tissues and to link their presence with different insect development. Entomo-toxicology adds complexity by considering how xenobiotics such as drugs or toxins influence insect growth, potentially biasing PMI estimations. Research has shown, for example, that benzodiazepines accelerate development in *Chrysomya albiceps* while slowing *Lucilia sericata.*[55] However, advances in artificial intelligence (AI) and machine learning (ML) now present new opportunities to enhance the accuracy, reproducibility, and speed of PMI estimation and toxicological interpretation. AI methods have already transformed image classification, pattern recognition, and data integration in other biomedical fields, and these developments indicate a future where forensic entomo-toxicology could become more standardized, reproducible, and automated through AI-driven systems.

7. AI for Species Identification

Species identification is the cornerstone of forensic entomology, as different taxa exhibit distinct developmental rates. AI-Powered Species Recognition PMI estimate depends on precisely recognizing necrophagous insects,

however conventional morphological methods are constrained by a lack of prior experience and subtle morphological differences. When dealing with damaged specimens or immature life stages, traditional morphological identification can be difficult and requires taxonomic competence. In this regard, deep learning techniques have shown remarkable effectiveness. In the lab, digital microscopes linked with AI pipelines allow automated spiracle imaging for larval classification. Apasrawirote et al. trained convolutional neural networks (CNNs) to classify maggot species from posterior spiracle images, achieving accuracies up to 97.5%.[56] This represents a major advance since larval spiracle morphology is notoriously difficult for human examiners to differentiate.

Similar to this, Gohe et al. classified adult forensic flies using MobileNetV3 and VGG19 architectures, reporting near-perfect accuracies while highlighting field-deployable, real-time applications where deep learning models have shown >99% accuracy in classifying adult flies from forensic families like Nariidae, Sarcophagidae, and Calliphoridae [57]. By creating a dataset of 2,880 annotated forensic fly photos, Ong and Ahmad et al. made a significant contribution and provided a priceless tool for AI system training.[58] This implies that the issue of recognizing morphologically similar larval instars—like Sarcophagidae species that are visually identical—can be solved by AI.[59] Instrumentation: Drones, microscopes, and high-resolution cameras make up the main instrumentation. For example, camera-equipped drones enable remote surveillance of fly activity at crime scenes, particularly in mass-casualty or disaster settings, where AI can classify fly genera in real time.

8. AI in PMI Estimation

Beyond species recognition, AI can improve PMI estimation by modeling insect growth and succession data under complex environmental conditions. Traditional degree-day calculations and linear regression often fail to capture nonlinear influences such as fluctuating temperature or multi-species interactions.[60] Machine learning models, including random forests, support vector machines (SVMs), and artificial neural networks (ANNs), can learn from noisy, heterogeneous datasets. Deep learning has also been applied to succession data, where convolutional neural networks (CNNs) learned temporal colonization patterns and produced more accurate PMI estimates than classical ecological models.[61]

9. Instrumentation and AI Integration

Integration of Instrumentation and AI typically, entomo-toxicological analysis utilizes molecular or microscopic methods to identify insects and mass spectrometry (MS) or chromatography to quantify toxins. AI can enhance these tools in two ways: (i). MS data pre-processing and peak recognition. UHPLC-QTOF-MS, for example, produces massive, high-dimensional datasets. Compared to manual procedures, deep learning can automate feature extraction, denoise spectra, and identify drug signatures rapidly.[55] (ii) Image-based identification from microscopy. As shown by,[56] CNNs can classify posterior spiracle images or integrated into smart traps and drones that capture insect activity in real time.[59]

Similarly, automating the identification of larval stages with AI-assisted image segmentation could increase the precision of developmental timing. By embedding AI models directly into the software of existing instruments, laboratories can build semi-automated pipelines—from specimen imaging to toxicological quantification—thereby reducing human error and processing time.

10. Species-Specific and Regional AI Models

Due to regional differences in species distribution and development rates, geographic context has significant effects on forensic entomology. *Chrysomya megacephala* and *Chrysomya rufifacies*, for instance, predominate in casework in India[10]. AI models must therefore be tailored to local fauna to ensure accurate PMI predictions. Global datasets are valuable, but regional annotated datasets will improve model generalizability.

Hundebol et al. demonstrated how Drosophila can be used for generational toxicology modeling, showing genotype-specific drug effects that could inspire similar forensic blowfly models.[29] Accurate identification of insect species and life stage is essential for PMI estimation. Deep learning models, particularly convolutional neural networks (CNNs), have achieved >98% accuracy in classifying fly species and stages based on annotated datasets.[58] These models reduce reliance on expert taxonomists and accelerate forensic workflows. Lightweight models such as MobileNet and YOLO also support edge deployment in smart traps and drones.[56]

11. AI in Forensic Toxicology (Toxicant Detection and Spectral Analysis)

Drugs alter insect development in complex ways, making toxicology-aware PMI models essential. High-dimensional spectral data from LC-MS/MS and UHPLC-QTOF-MS are ideally suited for AI-driven classification. According to [28], machine learning makes it feasible to detect xenobiotics and estimate concentration ranges, even in complicated matrices. Interpretability, which is essential for forensic admissibility, is provided by explainable AI tools like SHAP and attention maps [62]. In order predict PMI deviations, AI frameworks may integrate toxicological concentration data (such as ng/g from UHPLC-QTOF-MS) with species and developmental stage variables. Hou et al. noted that inconsistent reporting across studies limits comparability, but AI may overcome this by finding hidden patterns across heterogeneous datasets.[55]

12. Future Directions

Future applications of AI in forensic entomo-toxicology should focus on building global forensic insect AI databases for species ID, toxicology, and growth. Collaborative projects should aim to centralize annotated images, growth curves, and toxicological data into open repositories. Developing hybrid ecological-toxicological AI models and integrating insect succession data with drug concentration profiles into a single predictive AI model could more accurately adjust PMI estimation. Ensuring explainability (XAI) for courtroom admissibility. Black-box models may face skepticism in court. Developing interpretable AI that highlights which features (e.g., spiracle shape, MS peak pattern) influenced predictions will improve admissibility. Deployment of portable AI-enabled apps for field investigators - Smartphone-based AI apps for rapid field identification of larvae could support on-scene PMI estimation by investigators. Modeling generational effects of drugs on insects. As Hundebøl et al. (2024) demonstrated with Drosophila, drugs can produce inter-generational effects.[29] AI can simulate multi-generational outcomes to anticipate colonization dynamics in contaminated remains. Integrating IoT-enabled sensors with Real-time monitoring. Coupling AI with IoT sensors at decomposition research facilities ("body farms") can yield continuous, high-resolution data streams for PMI model training. Cross-disciplinary adoption like adapting lessons from medical AI (radiology, pathology) should be adapted into entomo-toxicology for validation, ethics, and standardization.

Artificial intelligence is poised to revolutionize forensic entomo-toxicology by automating species identification, refining PMI estimation, and accounting for toxicological influences. From CNN-based larval identification to multi-modal models integrating LC-MS spectra, AI can reduce error rates and increase reproducibility. Future research must emphasize explainability, regional dataset development, and interdisciplinary collaboration to ensure admissibility in court and utility in real investigations.

13. Conclusions

Particularly in situations when traditional biological matrices are either unavailable or substantially degraded, forensic entomo-toxicology has become an essential multidisciplinary tool in contemporary forensic research. Through the examination of xenobiotics accumulated in necrophagous insects, this discipline provides toxicological insights that would not otherwise be available, improving the precision of cause-of-death analysis and post-mortem interval (PMI) assessment. Blow flies (Diptera: Calliphoridae), particularly species such as *Chrysomya megacephala*, *Chrysomya rufifacies*, *Lucilia sericata*, and *Calliphora vicina*, are essential to forensic investigations because of their early colonisation of decaying remains and regular life cycle. To ensure accurate PMI estimates in Indian context, it is crucial to comprehend their developmental stages and succession patterns. Insect detoxification systems enable them to metabolise a variety of xenobiotics. Toxicants like drugs, insecticides, heavy metals, antibiotics, and nanoparticles, on the other hand, can drastically change the physiology, growth rates, and death of insects. This might change entomological data and perhaps distort PMI estimations. This discipline has been significantly boosted by recent developments in artificial intelligence (AI), which have made it possible to automate species identification, simulate insect growth under hazardous stress, and improve data integration for PMI estimate. Standardising entomotoxicological tests, increasing accuracy, and expanding forensic capabilities across various investigation settings are all potential benefits of AI-driven technologies. To put it simply the intricacies of xenobiotic-influenced decomposition can be resolved with the help of forensic entomo-toxicology.

14. List of Abbreviation

1. **LC-MS/MS**–Liquid Chromatography coupled with Tandem Mass Spectrometry.
2. **UHPLC-QTOF-MS-** Ultra-high-performance Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry.
3. **1H-NMR**- Proton Nuclear Magnetic Resonance.
4. **GC–MS**- Gas Chromatography–Mass Spectrometry.
5. **HPLC-** High-Performance Liquid Chromatography.

15. Conflict of interest

None.

16. Ethical Approval

None.

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