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Advancing Forensic Investigations: Biomarker Identification through Entomology and Chemical Fingerprinting

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Abstract

Evolving practices in forensic science are shifting toward the use of biological and chemical evidence to enhance precision, reliability, and interpretive depth in criminal investigations. Integration of entomology with chemical fingerprinting presents a powerful strategy for identifying biomarkers that remain stable across varied postmortem and environmental conditions. Insects associated with decomposing remains serve as sensitive biological matrices capable of recording temporal exposure to drugs, toxins, and pollutants. Their tissues, exoskeletons, and byproducts such as secretions and pupal casings act as chemical archives that retain forensic relevance when traditional samples degrade. This review explores the underlying principles of chemical fingerprinting applied to entomological evidence, addressing methods for biomarker discovery, analytical validation, and forensic deployment. Emphasis is placed on the expanding role of aquatic and semi-aquatic insects, enabling forensic investigations in challenging environments. Advantages, limitations, and the evidentiary significance of insect-based biomarkers are critically examined. Furthermore, the discussion highlights future research directions involving integration of omics technologies, predictive modeling, and cross-disciplinary training to bridge laboratory innovation with applied forensic practice. Consolidation of these emerging approaches reveals the potential for insect-derived chemical biomarkers to contribute meaningfully to forensic toxicology, postmortem interval estimation, and environmental crime investigation.

Keywords: Forensic entomology, Biomarker discovery, Chemical fingerprinting, Toxicological analysis, Insect-derived evidence

1. Introduction

Forensic science has undergone a transformative evolution, moving from the reliance on morphological assessments and physical evidence to embracing molecular-level analyses. Historically, crime scene investigations depended heavily on eyewitness testimonies, visual examinations, and gross anatomical assessments. These methods often suffered from subjectivity and limited reproducibility. The growing demand for objectivity, accuracy, and standardization has shifted the forensic paradigm toward biochemical, molecular, and chemical markers that can offer sensitive and reliable insights [1].

This transformation is particularly evident in sub-disciplines such as forensic entomology, where technological innovations now enable a deeper exploration of insect-derived evidence. The requirement for tools that can withstand decomposition, environmental interference, and long postmortem intervals has further emphasized the need for robust and reproducible molecular techniques [2]. These trends reflect a broader scientific consensus advocating for forensic methodologies that can provide high evidentiary value under judicial scrutiny.

Forensic entomology the use of insect biology in legal contexts has emerged as an invaluable resource in postmortem investigations. Insects colonizing decomposing remains do so in a predictable manner, forming a biological timeline that can aid in estimating the postmortem interval (PMI) and detecting possible toxicological influences [3]. These insects act as dynamic biological recorders, accumulating environmental toxins, drugs, and degradation products that reflect the biochemical condition of the corpse at the time of colonization.

The interactions between insects and the decomposition substrate, including tissues and body fluids, are central to their forensic significance. Through ingestion or surface contact, insects assimilate biochemical constituents, which can later be retrieved and analyzed to reconstruct environmental exposure or poisoning scenarios. Advances in endotoxicology and analytical biology have further enabled the detection of these biochemical traces within various insect developmental stages, opening new dimensions in forensic interpretation [4].

Chemical fingerprinting refers to the comprehensive profiling of chemical substances either endogenous metabolites or exogenous contaminants within a biological matrix. In forensic investigations, this technique is employed to identify specific compounds or compound classes that serve as biomarkers of exposure, pathology, or environmental context. Unlike traditional toxicology, which often targets a single substance, chemical fingerprinting provides a holistic view of the biochemical landscape associated with forensic samples [5].

In entomological applications, chemical fingerprinting enables the extraction of molecular data from insect tissues or by-products, facilitating the identification of drugs, pollutants, or decomposition-related compounds. When combined with biomarker science, this approach enhances the capacity to interpret insect-derived evidence not just for PMI estimation but also for understanding the chemical environment surrounding the cadaver. Analytical platforms such as mass spectrometry, nuclear magnetic resonance (NMR), and infrared spectroscopy have proven essential in supporting such detailed chemical analyses, bridging entomology with advanced forensic chemistry [5].

Given the increasing relevance of insect-based biochemical data in forensic science, this review aims to synthesize recent advancements in biomarker identification through entomological matrices supported by chemical fingerprinting techniques. The field is rapidly progressing from descriptive entomology toward a molecularly informed, data-rich science capable of yielding highresolution forensic interpretations [6].

Furthermore, the inclusion of aquatic and semi-aquatic insects, such as caddisflies, expands the toolkit available to forensic practitioners working in diverse environmental conditions. These nontraditional models, often overlooked in classical forensic entomology, offer new opportunities for biomarker discovery, especially in contexts involving submerged remains or environmental crime scenes.

The review will first explore the foundational principles of forensic entomology and chemical fingerprinting, followed by strategies for biomarker discovery, evidence validation, and forensic interpretation. Case studies, technological innovations, and methodological limitations will be addressed, culminating in a discussion on future research directions and practical applications.

Objectives of the Review

1. To critically evaluate the integration of chemical fingerprinting techniques in identifying forensic biomarkers from entomological evidence
2. To explore the emerging roles of insect-derived biochemical data in enhancing the sensitivity, specificity, and scope of modern forensic investigations

2. Entomology-Derived Biomarkers in Forensic Science

2.1 Insect–Environment and Insect–Toxicant Interactions

Insects serve as biochemical recorders of their environment, exhibiting sensitivity to toxicants present at death scenes. Through direct feeding or contact, insects can bioaccumulate chemical substances such as heavy metals, narcotics, or pesticides, reflecting both spatial and temporal dimensions of exposure. The microbial colonization patterns within insect tissues have also gained attention. For instance, *Wohlfahrtiimonas chitiniclastica* and *Ignatzschineria indica* have been recognized as potential microbial biomarkers during insect succession on decomposing remains, offering insights into cadaver colonization timelines and environmental conditions [7].

Heavy metal detection in insect samples has further enhanced forensic toxicology. Larvae and pupae of necrophagous species have demonstrated the ability to bioaccumulate toxicants like lead (Pb), barium (Ba), and antimony (Sb) after gunshot wounds, with inductively coupled plasma mass spectrometry (ICP-MS) confirming their presence across development stages [8]. These interactions highlight the entomological matrix as a dynamic, integrative platform capable of capturing chemical traces when traditional biological samples have degraded or are unavailable.

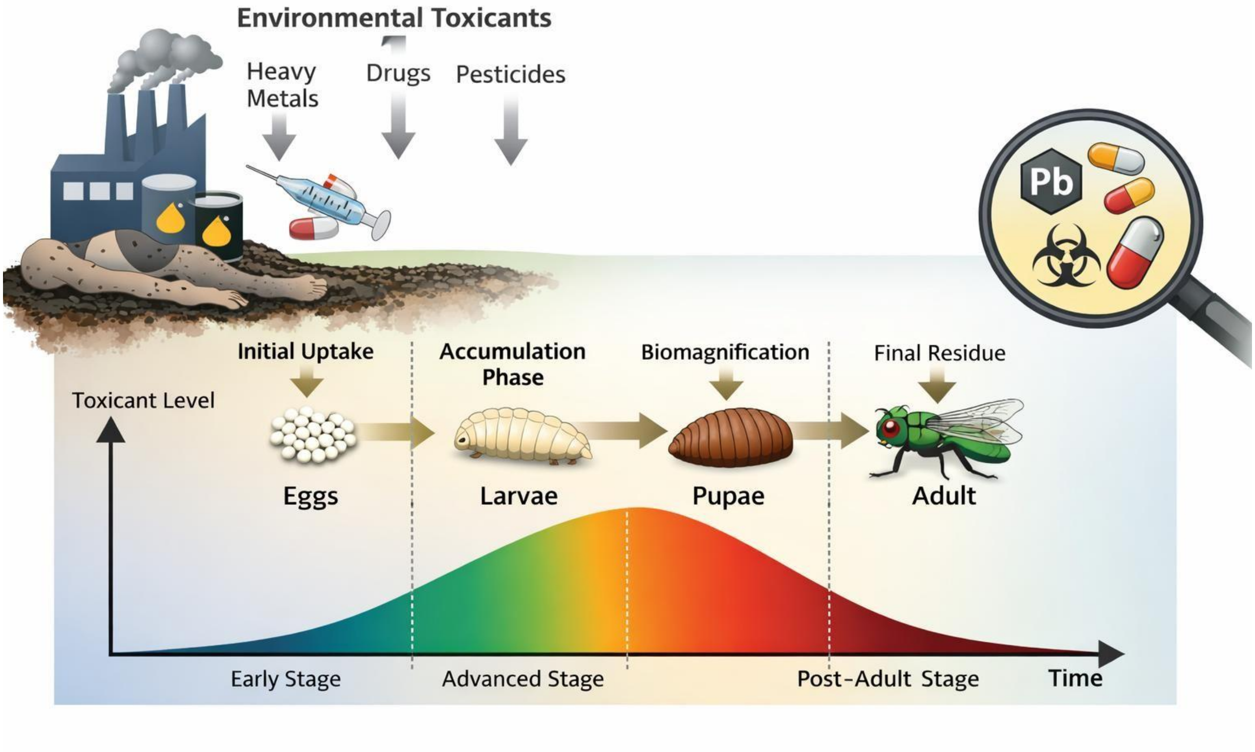

Figure 1. Toxicant accumulation in insect development stages

Figure 1 illustrates the bioaccumulation process and temporal uptake patterns of environmental toxicants within insect development stages, emphasizing the forensic relevance of such data in postmortem analysis [8].

2.2 Insect Tissues and Products as Forensic Matrices

Entomological specimens including larval and adult tissues, exoskeletons, and gut contents serve as viable substrates for chemical fingerprinting. Each matrix offers a unique chemical profile influenced by developmental stage, feeding behavior, and environmental exposure. Beyond tissues, other structures like silk cocoons or larval secretions have potential as chemical repositories. Such matrices resist postmortem degradation and can retain meaningful chemical signals even in advanced decomposition.

Entomotoxicology, a subfield dedicated to studying toxins in entomological specimens, has evolved to include a broad spectrum of analytes. Originally focused on poisons and drugs, its scope now encompasses pharmacokinetics, metabolite transformation, and dose-response relationships within insect tissues [9]. Standardized analytical approaches including GC–MS, LC–MS/MS, and high-resolution time-of-flight mass spectrometry have enabled precise detection of target molecules and their derivatives [10].

In comparative studies, blowfly larvae have demonstrated equivalence or superiority to traditional matrices (e.g., blood or liver) in detecting drugs and poisons, especially in decomposed cases. In instances where soft tissues are degraded beyond analysis, larvae preserved critical toxicological evidence [11].
A tabular comparison of conventional versus insect-derived matrices is presented in Table 1, outlining strengths, limitations, and key forensic use cases [10], [11].

Table 1. Comparison Between Conventional Biological Matrices and Insect-Derived Matrices in Forensic Toxicology [10], [11]

Feature	Conventional Matrices	Insect-Derived Matrices
Availability in decomposition	Limited	Readily available
Toxicant stability	Often degraded	Generally preserved
Sample volume required	High	Low
Bioaccumulation potential	No	Yes
PMI estimation capability	Limited	Strong, via development & chemistry
Analytical challenges	Lower	Requires matrix-specific protocols

2.3 Advantages of Entomological Biomarkers over Conventional Matrices
Entomological biomarkers offer significant advantages over traditional forensic substrates. Their persistence under extreme decomposition makes them invaluable in late postmortem intervals. Unlike blood or soft tissues, insect tissues remain viable for toxicological and chemical analysis even weeks after death.
Additionally, these biomarkers are less affected by postmortem redistribution a confounding factor in interpreting drug concentrations from internal organs. Because insects assimilate compounds through direct feeding, they provide a more localized and temporally anchored chemical signature. Amino acid profiles from necrophagous insect eggs, identified via high-resolution mass spectrometry, further demonstrate their potential in species discrimination and biological trace analysis [12].
Together, these findings underscore the forensic reliability and adaptability of insect-derived biomarkers in modern investigations, particularly when conventional evidence is compromised.

3. Principles of Chemical Fingerprinting in Forensic Investigations

3.1 Definition, Characteristics, and Forensic Relevance
Chemical fingerprinting refers to the systematic profiling of chemical compounds within a biological or environmental sample to generate a distinct and identifiable chemical signature. In forensic science, its value lies in its ability to decode the molecular traces left behind on or within biological materials, including insects, that were associated with a crime scene. These signatures can be used to determine exposure to drugs, poisons, pollutants, or decomposition volatiles, with high levels of specificity and sensitivity [13].
The technique can be either targeted focusing on known compounds or untargeted, enabling the discovery of unknown or emerging substances. Targeted approaches are often used when specific toxins or drugs are suspected, whereas untargeted methods are advantageous for postmortem environments where a wide array of unknown compounds may exist. The reproducibility of chemical fingerprints under controlled protocols makes them legally and scientifically robust tools in modern forensic analysis [14].

3.2 Analytical Techniques for Chemical Fingerprinting
Several analytical platforms are employed in chemical fingerprinting depending on the chemical nature of the analyte and the forensic context. Among these, gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–tandem mass spectrometry (LC–MS/MS) are the most frequently used due to their ability to identify trace-level organic and semi-volatile compounds with high sensitivity [15].
Spectroscopic methods, including Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), and Raman spectroscopy, have also gained prominence. These techniques enable non-destructive analysis and are particularly useful in rapidly screening samples or determining functional group profiles. The integration of these techniques into insect-based forensic models enables researchers to correlate the chemical environment with insect development stages and toxicant accumulation.

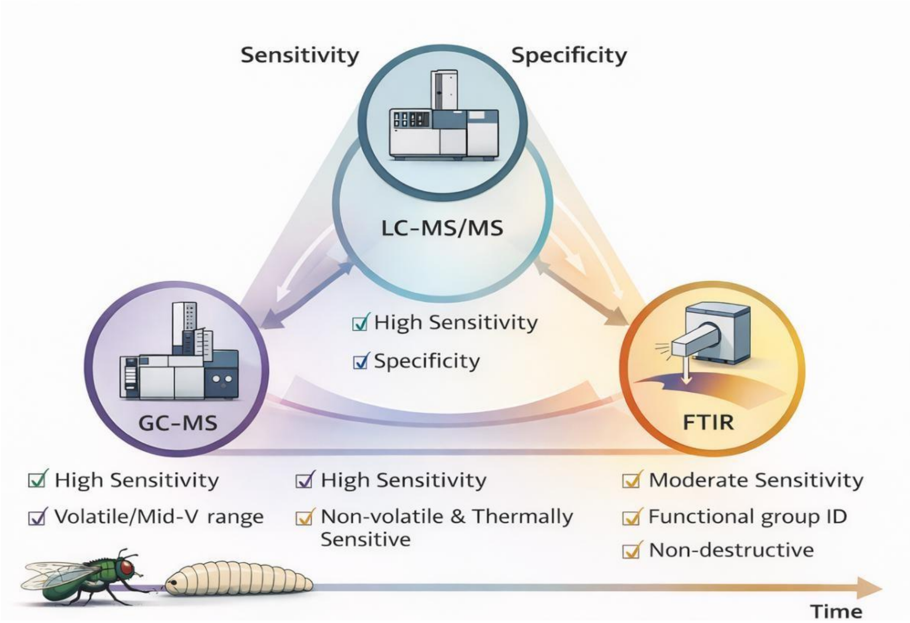

Figure 2. Comparison of GC–MS, LC–MS/MS, and FTIR for insect-based forensic chemical analysis

Figure 2 illustrates a comparative schematic of these techniques in terms of sensitivity, specificity, and applicability to insect-based forensic toxicology [15].

3.3 Data Processing and Chemometric Approaches

Raw analytical outputs from these techniques are complex and multidimensional, necessitating advanced data processing tools. Chemometrics the application of mathematical and statistical techniques to chemical data plays a crucial role in deciphering meaningful patterns from fingerprint data. Common strategies include multivariate analysis, principal component analysis (PCA), partial least squares (PLS), and machine learning classifiers [16].

These techniques allow forensic analysts to identify patterns or clusters that distinguish between types of exposure, degrees of decomposition, or time-since-death. Additionally, classification models trained on known datasets can be used to predict the source or identity of unknown forensic samples with high accuracy. When applied to insect-based matrices, chemometric tools not only enhance identification but also reduce noise from environmental variability.

Stable isotope analysis, particularly carbon and nitrogen isotope ratios, further augments fingerprinting by revealing dietary or geographical histories of insects. This isotopic fingerprinting enables origin tracking and ecological context integration in forensic investigations [17].

Table 2 summarizes the analytical techniques alongside their operational principles, key applications, and advantages in forensic entomology.

Table 2. Summary of Analytical and Data Processing Techniques in Forensic Chemical Fingerprinting [14]–[17].

Technique	Operating Principle	Forensic Use	Advantages
GC–MS	Separation and ion fragmentation	Detection of volatile organic compounds	High sensitivity and specificity
LC–MS/MS	Liquid separation + MS/M fragmentation	Non-volatile and thermally labile compounds	Broad analyte scope, excellent sensitivity
FTIR	Absorption of IR radiation by molecules	Functional group identification in residues	Non-destructive, rapid
Chemometrics (PCA/PLS)	Statistical modeling of chemical data	Pattern recognition, exposure classification	Multivariate insight, prediction accuracy
Stable Isotope Analysis	Ratio of stable isotopes (C, N)	Geographic/dietary tracking in insects	Long-term ecological data retention

This growing suite of analytical and data tools underscores the power of chemical fingerprinting in forensic investigations especially when combined with entomological evidence. These approaches transform insects from simple biological timers to chemically informative forensic recorders.

4. Strategies for Biomarker Identification and Validation

4.1 Biomarker Discovery Approaches

The discovery of biomarkers in forensic entomology has evolved significantly, driven by the need for higher specificity, stability, and postmortem applicability. Two primary strategies dominate this field: targeted and untargeted screening. Targeted biomarker discovery involves the identification and quantification of pre-defined molecules, such as known drugs, poisons, or decomposition products. Untargeted approaches, by contrast, involve global screening through advanced analytical techniques, often coupled with omics technologies, to uncover unexpected or novel markers [18].

Multi-omics integration including metabolomics, proteomics, and transcriptomics has enabled high-throughput screening of insect samples in relation to the postmortem interval (PMI). Such strategies reveal complex biochemical changes within the insect body that correlate with environmental exposure and time since death [18].

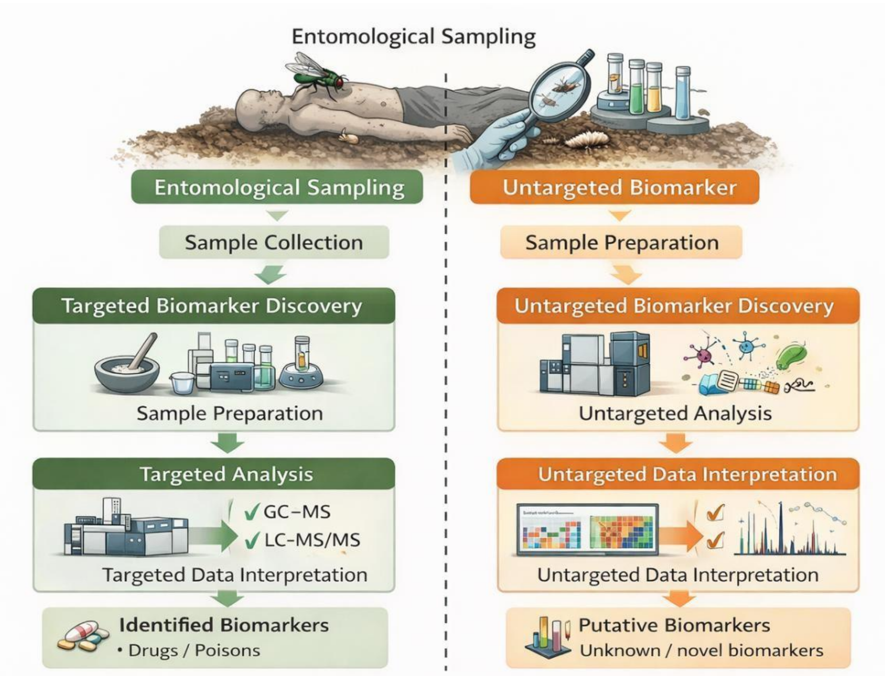

Figure 3. Workflow comparison of targeted and untargeted biomarker discovery approaches in forensic entomology

Figure 3 presents a simplified schematic comparing the workflow of targeted and untargeted biomarker discovery in entomological evidence, from sampling to data interpretation.

4.2 Criteria for Forensically Relevant Biomarkers

Biomarkers intended for forensic use must meet specific criteria to ensure their interpretive reliability. These include specificity to exposure type or timing, resistance to degradation, and detectable persistence under varied environmental conditions. Several field validation studies have demonstrated the increased accuracy in PMI estimation when insect evidence is reared under controlled laboratory settings prior to analysis [19].

Additionally, the relevance of a biomarker can also be influenced by biological origin. For instance, human DNA detected in mosquito blood meals has been successfully profiled using STR analysis for identification purposes [20], underscoring the potential of insect-derived matrices in human-specific forensic evidence.

Environmental variability plays a significant role in biomarker behavior. Comparison studies between accumulated degree-days (ADD) and chemical biomarker trends have shown convergence in PMI estimation outcomes, particularly when thermal conditions are wellaccounted for [21]. This supports the development of integrative biomarker frameworks that combine entomological and environmental data.

4.3 Validation and Reproducibility Considerations

Validation frameworks in biomarker-based forensic entomology must address both analytical precision and biological reproducibility. Analytical validation includes linearity, limit of detection, specificity, and stability of the biomarker in the chosen matrix. Biological validation, on the other hand, accounts for inter-species variation, larval feeding habits, and decomposition stage at collection time.

In recent experimental models using minipigs, toxicological substances administered pre-mortem were successfully recovered from insect tissues using entomotoxicological analysis, supporting their real-world forensic applicability [22]. These studies help refine reproducibility protocols and set benchmarks for standard operating procedures.

Machine learning has also entered the field as a validation-enhancing tool. Deep learning models applied to intra-puparial age estimation of *Sarcophaga peregrina* have achieved high classification accuracy, suggesting the potential for automated biomarker interpretation and model-driven age prediction [23]. Table 3 summarizes key validation criteria, associated challenges, and example solutions based on recent studies [19]–[23].

Table 3. Validation Criteria and Challenges in Biomarker-Based Forensic Entomology [19]–[23]

ValidationDomain	Key Criteria	Challenges	Example Solutions
Analytical Validation	Sensitivity, stability, specificity	Matrix complexity, degradation	Omics tools, sample prep standardization
Biological Validation	Inter-species consistency, environmental resilience	Species feeding variation, climate effects	Controlled lab rearing, ADD correction
Temporal Relevance	Time-correlated expression	Degradation with decomposition	ADD modeling, isotope tagging
Automation and Scaling	Machine-readability, model training	Data annotation, algorithm bias	Deep learning models, image recognition

By aligning biomarker discovery with rigorous validation, forensic entomology is poised to contribute chemically resolute and biologically reliable evidence to death investigations.

5. Expanding the Scope of Forensic Entomology

5.1 Limitations of Traditional Terrestrial Insect Models

Forensic entomology has historically focused on terrestrial Diptera particularly blowflies (Calliphoridae) and flesh flies (Sarcophagidae) as primary indicators of postmortem interval and environmental conditions. While these models have proven reliable in many contexts, they are not universally applicable, especially in aquatic or highly polluted environments. In such settings, conventional colonizers may be absent, delayed, or altered in their succession patterns, reducing their forensic reliability [24].The biochemical diversity of decomposition, particularly the emission of volatile organic compounds (VOCs), may also be underrepresented when relying solely on Diptera. For example, odorant-binding proteins identified in *Hermetia illucens* show selective binding affinity to aldehydes associated with early-stage decomposition, suggesting the untapped potential of nondipteran insect species in chemical sensing applications [24].

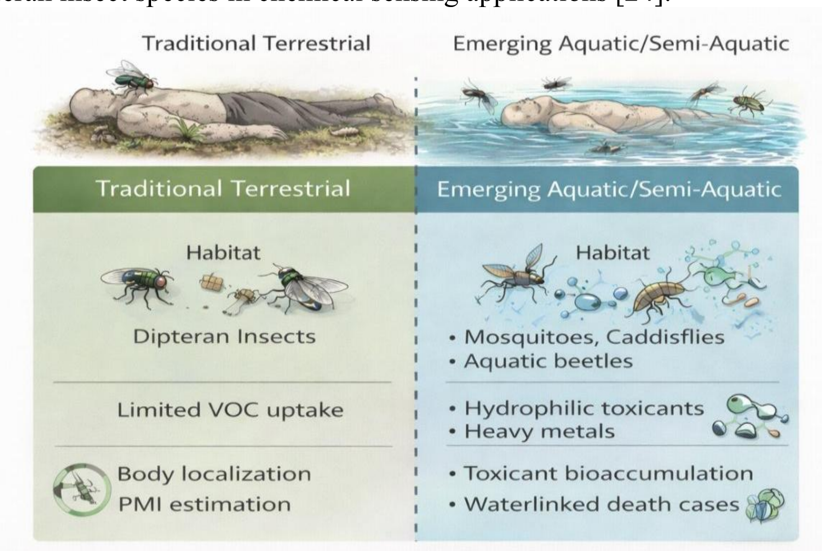

Figure 4. Comparative chart of terrestrial vs. aquatic/semi-aquatic insect models used in forensic entomology

Figure 4 compares traditional terrestrial models with emerging aquatic and semi-aquatic alternatives in terms of habitat, chemical resilience, and forensic applications.

5.2 Emerging Role of Aquatic and Semi-Aquatic Insects

Aquatic and semi-aquatic insects such as mosquitoes, caddisflies, and aquatic beetles offer a unique forensic advantage due to their direct and sustained exposure to waterborne contaminants. In polluted or aquatic death scenes, these insects not only colonize submerged remains but also accumulate hydrophilic toxicants, pesticides, and heavy metals over time.

A study investigating the effect of diazinon on insect succession demonstrated how aquatic systems influence species composition and toxicant bioavailability. The altered colonization behavior in such settings reflects the toxicological burden of the aquatic environment, which is precisely what aquatic insects record within their bodies [25].

The utility of aquatic insects extends beyond PMI estimation. For example, mosquitoes feeding on human hosts can yield forensic STR profiles from blood meals, offering an additional biological trace for victim identification [26]. Such integrations of environmental chemistry with biological matrices broaden the applicability of entomological evidence across a variety of forensic scenarios.

5.3 Contribution of Non-Traditional Insect Models to Biomarker Research

Non-traditional insect models those not routinely considered in forensic protocols offer ecological and biochemical diversity that enriches forensic evidence collection and interpretation. Urbanization, climate change, and habitat disruption have contributed to shifting insect populations, with emerging species now colonizing cadavers in previously unrecorded environments. A long-term comparative study revealed dramatic changes in filth fly populations over a 78-year period, reflecting the evolving ecological landscape [27].

Furthermore, forensic capabilities vary by region. A recent assessment in New Zealand highlighted significant gaps in insect reference databases and species documentation, underlining the importance of region-specific model development [28].

Stable isotope analysis represents another frontier in non-traditional model use. Blowflies analyzed for carbon and nitrogen isotopic ratios have shown potential in determining both feeding origins and ecological history. These isotopic fingerprints allow researchers to backtrack the environmental or dietary context of insect colonizers, enhancing forensic inference [29].

Table 4 outlines key characteristics of non-traditional insect models and their emerging roles in biomarker research and forensic casework [26]–[29].

Table 4. Forensic Contributions of Non-Traditional Insect Models

Insect Type	Forensic Role	Unique Features	Application Area
Mosquitoes (Culicidae)	Human identification via blood meals	Recoverable STR profiles	Victim tracing
Aquatic insects (e.g., Trichoptera)	Bioaccumulation of waterborne toxicants	Continuous submersion, silk case analysis	Aquatic death investigations
Urban filth flies	Environmental exposure history	Responsive to climate and waste shifts	Urban crime scene profiling
Blowflies (Stable Isotopes)	Origin and feeding site identification	Carbon and nitrogen isotope ratios	Ecological context reconstruction

The expansion of forensic entomology into aquatic, semi-aquatic, and non-traditional species introduces new biochemical dimensions and ecological markers, making entomological evidence more versatile, environmentally integrative, and forensically powerful.

6. Chemical Fingerprinting of Entomological Evidence

6.1 Uptake, Metabolism, and Retention of Xenobiotics

Insects associated with decomposing remains are frequently exposed to a broad spectrum of xenobiotics, including drugs, pesticides, and industrial pollutants. These substances can accumulate through trophic interactions or direct contact with substrates. Once inside the insect, xenobiotics may undergo metabolic transformations that alter their chemical structure while preserving their forensic traceability [30].

Recent studies highlight cuticular hydrocarbons (CHCs) as stable indicators of xenobiotic uptake. CHCs not only reflect the insect's developmental state but can also register chemical weathering signatures that result from environmental exposure, such as differences between indoor and outdoor decomposition conditions [31].

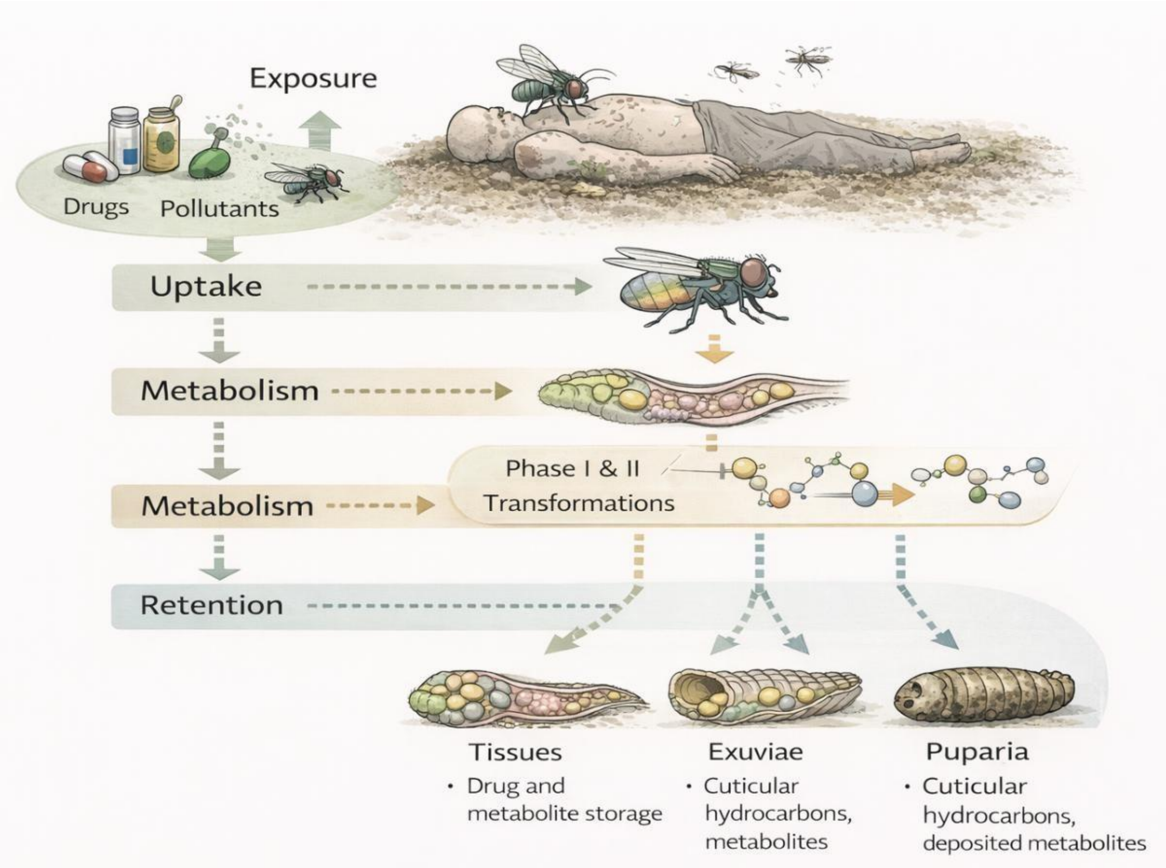

Figure 5. Xenobiotic uptake and retention pathways in insect-derived materials used for forensic chemical fingerprinting

Figure 5 visually outlines the pathways through which xenobiotics are absorbed, metabolized, and retained in various insect structures including tissues, exuviae, and puparia [30]–[32].

6.2 Chemical Signatures in Insect-Derived Materials

Tissue-based chemical fingerprints can reveal time-dependent biochemical changes and compound-specific accumulations. This is particularly evident in direct infusion mass spectrometry (DIMS) studies, which enable high-throughput profiling of developmental stages in Dipteran species [33]. In addition to soft tissues, structural materials such as larval exuviae and empty puparia have emerged as chemical archives. These materials retain metabolite profiles and weathering information long after soft tissues degrade.

Innovations in vibrational spectroscopy, particularly FTIR and ATR-FTIR, have enhanced the resolution of such analyses. For example, the weathering time of *Thanatophilus sinuatus* exuviae and *Sarcophaga peregrina* puparia has been accurately estimated using micro-FTIR combined with machine learning, thereby extending the utility of these matrices for PMI estimation [34], [35].

6.3 Comparative Evaluation of Fingerprinting Studies

The increasing use of spectroscopy and chemometric approaches has brought significant insights into the reliability and variability of insect-based fingerprinting. For instance, temperature fluctuations and developmental stage affect chemical degradation rates and compound persistence, requiring species-specific models for accurate interpretation [36].

Table 5 compares key analytical techniques used in recent studies on chemical fingerprinting of entomological materials, highlighting their spectral range, interpretive strength, and optimal sample type [37]..

Table 5. Comparative Attributes of Analytical Techniques for Insect-Based Chemical Fingerprinting

Technique	Target Matrix	Key Applications	Strengths
Direct Infusion MS	Larvae, pupae	PMI estimation via metabolite profiling	High throughput, rapid detection
Micro-FTIR	Larval exuviae	Weathering estimation	Minimal sample prep, spectral clarity
ATR-FTIR	Puparia	Age and toxicant retention analysis	Surface-sensitive, repeatable
Spectral + ML Models	Multiple stages	Predictive modeling of age/toxicant exposure	Data-driven pattern recognition

Overall, chemical fingerprinting of entomological evidence continues to gain forensic relevance as methodological precision improves and biological matrices diversify. By integrating spectral data with machine learning, forensic scientists can now extract both chronological and chemical information from insect remains under diverse environmental conditions.

7. Forensic Applications of Entomology-Based Biomarkers

7.1 Post-Mortem Interval (PMI) Estimation

Forensic entomology remains integral to PMI estimation, as insect colonization provides a chronological biological record. When supported with entomology-based chemical biomarkers, especially those preserved in insect tissues and puparia, estimations become significantly more accurate under complex conditions such as enclosure or concealment [38].

Studies have shown that drugs and toxicants can modify both the rate of decomposition and insect development, necessitating compound-adjusted PMI models [39], [40]. The chemical signatures retrieved from insect matrices help detect such xenobiotic influences, thereby correcting or supporting morphological succession-based estimates [41].

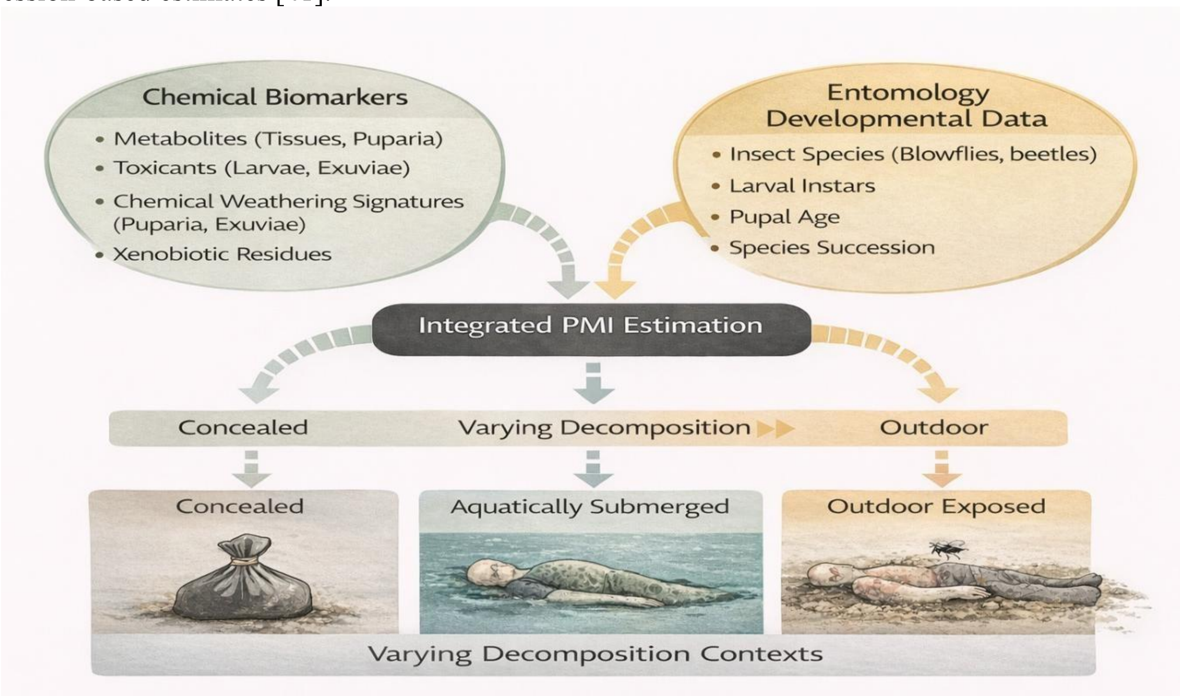

Figure 6. Integration of entomology-derived chemical biomarkers and development data for enhanced PMI estimation

Figure 6 schematically presents how biomarker-based PMI estimation integrates chemical and entomological parameters to improve accuracy across varying decomposition contexts.

7.2 Forensic Toxicology and Poisoning Investigations

In cases where traditional tissues are degraded or unavailable, insect larvae serve as alternative matrices for detecting drugs, pesticides, and toxic compounds. In particular, larvae feeding on drug-laden tissues often reflect temporal exposure trends that can differentiate acute from chronic exposure [39], [42].

Such entomotoxicological evidence not only informs on the cause of death but also reconstructs pre-mortem drug history. In several forensic cases, unexpected toxicological findings in larvae helped redirect investigations and identify substances missed in conventional matrices [39].

7.3 Environmental and Wildlife Forensic Investigations

Beyond human forensics, insect-based biomarkers are gaining traction in environmental crime investigations. Larval assemblages in polluted habitats such as chemically contaminated soils or water bodies record bioavailable toxicants over time, making them valuable for identifying illegal discharges or persistent pollutants [43], [44].

Forensic entomology has supported investigations into animal poaching and abuse, and has even been applied to identify offender DNA from blowfly larvae in sexual assault cases involving decomposed remains [43].

Table 6 summarizes key forensic domains, the role of insect-based biomarkers, and select case types where these methods have been successfully employed [44].

Table 6. Applications of Entomological Biomarkers Across Forensic Contexts

Forensic Domain	Biomarker Utility	Case Type Example
PMI Estimation	Metabolite changes over time	Concealed or enclosed decomposition
Forensic Toxicology	Detection of drugs, pesticides	Poisoning and overdose cases
Environmental Forensics	Exposure profiling via larvae	Industrial discharge and pollution
Wildlife Investigations	Toxicant detection in scavengers	Animal abuse and illegal baiting
Sexual Assault (Postmortem)	Trace DNA recovery from blowfly larvae	Offender identification in decomposed remains

8. Methodological Challenges and Legal Implications

8.1 Sampling, Preservation, and Analytical Challenges

One of the core methodological challenges in forensic entomology lies in ensuring proper sampling, storage, and analytical handling of insect-derived specimens. Environmental contamination, such as soil leachates or ambient pollutants, can interfere with chemical fingerprinting and DNA integrity [45]. Degradation of biomolecules during delayed collection or inappropriate preservation methods may result in loss of analyte fidelity or misrepresentation of postmortem intervals [46].

8.2 Standardization and Reference Database Limitations

A significant gap in entomological forensic practice is the lack of harmonized protocols for both field and laboratory analyses. Inconsistencies in developmental datasets, toxicological baselines, and molecular identification frameworks hinder reproducibility and cross-laboratory data comparisons [47]. The development of centralized, curated reference databases and validated metadata structures would greatly enhance data interoperability.

Table 6 below summarizes major analytical and sampling limitations across PMI estimation, toxicological analysis, and insect DNA profiling, with citation of studies that demonstrate the implications of such gaps.

Application Area	Key Limitation	Impact on Forensic Analysis
PMI Estimation	Inconsistent rearing protocols	Inaccurate age estimation
Toxicological Analysis	Matrix degradation during storage	Reduced analyte detectability
Insect DNA Profiling	DNA fragmentation from poor handling	Species misidentification
Scene Sampling	Environmental contamination	Spurious results in chemical analysis

Reporting Standards	Lack of standardized case reports	Reduced admissibility in court

8.3 Evidentiary Reliability and Legal Admissibility

For entomology-based evidence to be admissible in legal proceedings, it must conform to established forensic standards regarding chain of custody, reproducibility, and scientific validity. The variability in protocols and lack of consensus on biomarker thresholds create barriers to courtroom acceptance [48]. There is a growing call within the forensic community for unified case reporting formats and peer-reviewed benchmarks to assess evidentiary robustness [49].

9. Future Perspectives and Research Directions

The integration of advanced molecular and computational techniques promises to transform the field of forensic entomology and biomarker analysis. Among the most significant developments is the application of omics technologies such as metabolomics, lipidomics, and proteomics which enable the high-resolution characterization of biochemical changes in insect tissues and secretions. These approaches allow for the detection of subtle, time-dependent chemical shifts, offering new possibilities for postmortem interval (PMI) estimation and toxicological screening.

Multi-omics strategies, which combine data from genomics, proteomics, and metabolomics, are especially promising in identifying unique biomarker signatures associated with different environmental exposures, decomposition stages, and species-specific responses. As these datasets grow in complexity and size, artificial intelligence (AI) and machine learning are becoming essential tools. These technologies enhance pattern recognition and support the development of predictive models that can automate the interpretation of chemical fingerprints across varying forensic scenarios.

The use of advanced chemometric techniques is enabling more robust classification models, reducing analyst bias, and improving reproducibility. AI-powered platforms also show potential in intra-puparial age estimation and in differentiating between acute and chronic toxic exposures with greater precision than conventional methods.

Despite these innovations, translational application in routine forensic practice remains limited. To bridge this gap, it is crucial to foster interdisciplinary collaboration between forensic scientists, entomologists, chemists, and data scientists. Comprehensive training programs and standardized protocols are needed to ensure that these tools are accessible, interpretable, and legally defensible. Moving forward, the alignment of research outputs with real-world forensic needs will be essential to establish biomarker-based entomological evidence as a routine and reliable component of modern forensic investigations.

10. Conclusions

The advancement of forensic science has increasingly embraced biological and chemical specificity, positioning entomology-derived biomarkers as a transformative tool in postmortem investigations. This review has outlined the growing significance of insects not merely as temporal indicators of decomposition but as dynamic biological matrices capable of recording chemical exposures and environmental interactions. Through their tissues, secretions, and exuviae, insects offer resilient reservoirs of toxicological and ecological information, especially when traditional human tissues are degraded. Central to leveraging this potential is the application of chemical fingerprinting an analytical framework that combines high-sensitivity instrumentation with robust data modeling to decode xenobiotic uptake, metabolite transformation, and temporal chemical patterns within entomological evidence. The synergy of forensic entomology with advanced techniques such as GC–MS, LC–MS/MS, and FTIR has established a new frontier for detecting poisons, drugs, and pollutants under a range of postmortem and environmental conditions. Moreover, biomarker validation efforts and standardization are gradually reinforcing the evidentiary credibility of insect-based forensic interpretations, although methodological and legal challenges persist. The incorporation of non-traditional insect models, particularly aquatic and semi-aquatic species, has further broadened the ecological and investigative scope of forensic entomology, revealing new opportunities for biomonitoring in complex or polluted environments. As the field moves forward, the integration of omics platforms and artificial intelligence will be vital in refining biomarker discovery and enhancing automation, reproducibility, and interpretive clarity. Ultimately, the continued convergence of biology, analytical chemistry, and data science promises to elevate insect-based biomarkers from experimental promise to mainstream forensic utility. These developments not only enhance the precision and reliability of forensic reconstructions but also expand the toolkit for investigating complex crime scenes, environmental violations, and wildlife offenses. The horizon for entomological biomarkers is thus not only scientifically rich but also forensically indispensable, heralding a new era in evidentiary science.

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