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PLASTIC PARTICLES IN INSECTS: A CRITICAL SYSTEMATIC REVIEW OF TOXICOLOGICAL EFFECTS, SPECIES SUSCEPTIBILITY, AND ECOLOGICAL CONSEQUENCES ACROSS SIX ORDERS

PARTÍCULAS DE PLÁSTICO EM INSETOS: UMA REVISÃO SISTEMÁTICA CRÍTICA DOS EFEITOS TOXICOLÓGICOS, DA SUSCETIBILIDADE DAS ESPÉCIES E DAS CONSEQUÊNCIAS ECOLÓGICAS EM SEIS ORDENS

PARTÍCULAS DE PLÁSTICO EN INSECTOS: UNA REVISIÓN SISTEMÁTICA CRÍTICA DE LOS EFECTOS TOXICOLÓGICOS, LA SUSCEPTIBILIDAD DE LAS ESPECIES Y LAS CONSECUENCIAS ECOLÓGICAS EN SEIS ÓRDENES

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ABSTRACT

Background: Fragmentation of plastic debris into micro- (<5 mm) and nanoscale (<1 μm) particles has made bioavailability to consumer organisms a concern in plastic ecotoxicology, mapped mainly onto aquatic vertebrates. Insects, the most species-rich animal class, are exposed to microplastics (MPs) and nanoplastics (NPs) via ingestion, exoskeletal adhesion, and trophic transfer, yet remain underrepresented in ecotoxicological research. Objectives: To systematically map the toxicological effects of exposure to MPs and NPs across insect taxa, life stages, and polymer types, assess methodological quality, and identify research priorities. Methods: Web of Science, PubMed, and Google Scholar were searched (January 2019–December 2025) for studies assessing MP or NP contamination in Class Insecta. Methodological quality was assessed using the 15-criterion Insect Plastic Ecotoxicology Quality Score (IPEQS). From 1,063 initial records, 57 studies met criteria (50 laboratory-based, 7 field-based), spanning six insect orders. Results: Studies were High (28%), Moderate (47%), or Low (25%) quality. Immature stages bore the greatest physiological burden, with bioaccumulation and suppressed antioxidant defenses frequently reported. Diptera dominated laboratory assays (>60%; *Chironomus riparius*, *Hermetia illucens*, and *Drosophila melanogaster*), while Trichoptera dominated field samples. Polystyrene and polyethylene comprised 69% of laboratory polymers, versus polypropylene and polyethylene terephthalate in field specimens. *Hermetia illucens* and *Chaoborus* spp. showed tolerance

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despite bioaccumulation, whereas *Zophobas atratus*, *Cataclysta lemnata*, and *Chironomus kiiensis* showed pronounced sensitivity. Conclusions: MPs and NPs act as consistent toxicological stressors through polymer-specific mechanisms tied to species biology and developmental stage. Terrestrial entomofauna, multigenerational exposure, trophic transfer, and species-level tolerance are priority research directions.

Keywords: Insects. Microplastics. Nanoplastics. Ecotoxicology. Trophic Transfer. Bioindicators. Adverse Outcome Pathway.

RESUMO

Contexto: A fragmentação de resíduos plásticos em partículas de microescala (<5 mm) e nanoescala (<1 µm) tornou a biodisponibilidade para organismos consumidores uma preocupação na ecotoxicologia de plásticos, um tema estudado principalmente em vertebrados aquáticos. Os insetos, a classe animal com maior riqueza de espécies, estão expostos a microplásticos (MPs) e nanoplásticos (NPs) por meio de ingestão, adesão ao exoesqueleto e transferência trófica; contudo, permanecem sub-representados em pesquisas ecotoxicológicas. Objetivos: Mapear sistematicamente os efeitos toxicológicos da exposição a MPs e NPs em diferentes táxons de insetos, estágios de vida e tipos de polímeros, avaliar a qualidade metodológica e identificar prioridades de pesquisa. Métodos: Foram realizadas buscas nas bases Web of Science, PubMed e Google Scholar (janeiro de 2019 a dezembro de 2025) por estudos que avaliassem a contaminação por MPs ou NPs na classe Insecta. A qualidade metodológica foi avaliada utilizando o Insect Plastic Ecotoxicology Quality Score (IPEQS), composto por 15 critérios. Dos 1.063 registros iniciais, 57 estudos atenderam aos critérios (50 laboratoriais e 7 de campo), abrangendo seis ordens de insetos. Resultados: Os estudos apresentaram qualidade alta (28%), moderada (47%) ou baixa (25%). Os estágios imaturos sofreram a maior carga fisiológica, com relatos frequentes de bioacumulação e supressão das defesas antioxidantes. A ordem Diptera predominou nos ensaios laboratoriais (>60%; *Chironomus riparius, Hermetia illucens e Drosophila melanogaster*), enquanto a ordem Trichoptera predominou nas amostras de campo. Poliestireno e polietileno representaram 69% dos polímeros utilizados em laboratório, ao passo que, em amostras de campo, predominaram o polipropileno e o tereftalato de polietileno. *Hermetia illucens* e *Chaoborus* spp. demonstraram tolerância, apesar da bioacumulação, enquanto *Zophobas atratus*, *Cataclysta lemnata* e *Chironomus kiiensis* apresentaram sensibilidade acentuada. Conclusões: MPs e NPs atuam como estressores toxicológicos consistentes, por meio de mecanismos específicos de cada polímero, relacionados à biologia da espécie e ao seu estágio de desenvolvimento. A entomofauna terrestre, a exposição multigeracional, a transferência trófica e a tolerância em nível de espécie constituem direções prioritárias para futuras pesquisas.

Palavras-chave: Insetos. Microplásticos. Nanoplásticos. Ecotoxicologia. Transferência Trófica. Bioindicadores. Via de Desfecho Adverso.

RESUMEN

Antecedentes: La fragmentación de los residuos plásticos en partículas a escala micrométrica (<5 mm) y nanométrica (<1 µm) ha convertido su biodisponibilidad para los organismos consumidores en un motivo de preocupación dentro de la ecotoxicología de los plásticos, un campo centrado principalmente en los vertebrados acuáticos. Los insectos, la clase de animales con mayor riqueza de especies, están expuestos a microplásticos (MP) y nanoplásticos (NP) a través de la ingestión, la adhesión al exoesqueleto y la transferencia trófica; sin embargo, siguen estando subrepresentados en la investigación ecotoxicológica. Objetivos: Mapear sistemáticamente los efectos toxicológicos de la exposición a MP y NP en

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diversos taxones de insectos, etapas de desarrollo y tipos de polímeros; evaluar la calidad metodológica; e identificar prioridades de investigación. Métodos: Se realizaron búsquedas en Web of Science, PubMed y Google Scholar (enero de 2019 – diciembre de 2025) para identificar estudios que evaluaran la contaminación por MP o NP en la clase Insecta. La calidad metodológica se evaluó mediante la escala IPEQS (Insect Plastic Ecotoxicology Quality Score), compuesta por 15 criterios. De 1.063 registros iniciales, 57 estudios cumplieron con los criterios (50 de laboratorio y 7 de campo), abarcando seis órdenes de insectos. Resultados: Los estudios presentaron una calidad alta (28 %), moderada (47 %) o baja (25 %). Las etapas inmaduras soportaron la mayor carga fisiológica, observándose con frecuencia bioacumulación y una supresión de las defensas antioxidantes. El orden Diptera predominó en los ensayos de laboratorio (>60 %; *Chironomus riparius, Hermetia illucens y Drosophila melanogaster*), mientras que Trichoptera fue el grupo predominante en las muestras de campo. El poliestireno y el polietileno representaron el 69 % de los polímeros utilizados en laboratorio, frente al polipropileno y el tereftalato de polietileno en los especímenes de campo. *Hermetia illucens* y *Chaoborus* spp. mostraron tolerancia a pesar de la bioacumulación, mientras que *Zophobas atratus*, *Cataclysta lemnata* y *Chironomus kiiensis* exhibieron una sensibilidad marcada. Conclusiones: Los MP y NP actúan como estresores toxicológicos constantes a través de mecanismos específicos de cada polímero, los cuales están vinculados a la biología de la especie y a su etapa de desarrollo. La entomofauna terrestre, la exposición multigeneracional, la transferencia trófica y la tolerancia a nivel de especie constituyen líneas de investigación prioritarias.

Palabras clave: Insectos. Microplásticos. Nanoplásticos. Ecotoxicología. Transferencia Trófica. Bioindicadores. Vía de Desenlace Adverso.

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1 INTRODUCTION

Global plastic production reached approximately 400 million metric tons in 2022 and continues to grow, with Asia accounting for the largest share and China alone responsible for roughly 32% of global production [1]; Brazil, as Latin America's largest economy, contributes around 11.3 million tons per year, some 2% of the global total [2]. That volume now touches nearly every sector of the economy, from food packaging and construction to biomedical devices and agricultural inputs, a position synthetic polymers owe to their mechanical versatility, low production cost, and exceptional chemical stability since the mid-twentieth century. What these figures obscure, however, is where the resulting waste stream ends up biologically. The entry into force of negotiations under the United Nations Global Plastics Treaty (2024) signals growing international recognition that the plastic life cycle is a systemic environmental and public health challenge, one that waste management measures alone cannot address.

The same chemical properties that make plastics industrially valuable render them ecologically intractable. Resistant to biological degradation under most environmental conditions, plastic materials accumulate progressively across terrestrial, freshwater, and atmospheric compartments. Physical abrasion, UV radiation, biological activity, and thermal stress progressively fragment macroplastic debris into particles of diminishing size. Microplastics (MPs), conventionally defined as particles between 1 μm and 5 mm [3], and nanoplastics (NPs), generally below 1 μm and often below 100 nm, represent the terminal products of this fragmentation cascade. MPs are further classified according to their origin. Primary MPs are intentionally manufactured in microscopic sizes and enter the environment directly as microbeads, synthetic textile fibers, and industrial resin pellets, whereas secondary MPs arise from the breakdown of larger items in situ. Once mobilized, particles travel via wastewater effluents, sewage sludge runoff, and meteorological events, distributing from terrestrial source areas into aquatic systems and into the atmosphere.

Small size confers disproportionate environmental reach. The reduced dimensions and high surface-area-to-volume ratio of MPs and NPs enhance their buoyancy, facilitate cellular uptake, and increase their capacity to adsorb persistent organic pollutants (POPs), plasticizers, and pathogenic microbial biofilms [4,5,6]. Polymer types including polyethylene (PE), polystyrene (PS), polypropylene (PP), and polyamide (PA) have been detected as environmental contaminants across diverse habitats and trophic levels. The co-occurrence of MPs with chemical additives and crystallinity-related compounds creates a composite toxic burden that exceeds the effects of the polymer matrix alone. This is what sets plastic

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contamination apart from most conventional pollutants, and it substantially complicates toxicological risk assessment.

Fish and amphibians have attracted the bulk of ecotoxicological attention directed at plastic contamination, leaving insects, despite their unmatched taxonomic richness and ecological centrality, at the margins of the research agenda [7,8]. This asymmetry is difficult to justify on ecological grounds. That diversity is not a minor ecological footnote: this single class underwrites pollination, organic matter turnover, and nutrient cycling across both terrestrial and aquatic food webs, occupying positions that few other animal groups can substitute for [9]. Their mobility further amplifies their role as vectors, as flying and aquatic insects that undergo complete metamorphosis can transport ingested or exoskeleton-associated microplastics across ecosystem boundaries, linking contaminated aquatic systems to terrestrial predators and potentially redistributing plastic particles at landscape scales [10].

What controlled experiments have established is that exposure to MPs and NPs produces a measurable physiological cost in insects. Oxidative stress, immune activation, metabolic disruption, behavioral impairment, and the ontogenetic transfer of particles across metamorphic stages have all been documented in laboratory settings. Particle accumulation has been traced to the intestinal tract, hemolymph, and Malpighian tubules of larval stages; field data indicate that MPs also adhere to the exoskeleton, opening a passive transport route independent of ingestion. The capacity of MPs to carry adsorbed POPs into insect tissues introduces an additional dimension of concern, with potential consequences for reproductive fitness and, through trophic transfer, for food security and human health.

Yet the published record remains fragmentary. Coverage is heavily skewed toward a small number of dipteran model organisms and toward aquatic systems, leaving most insect orders and virtually all strictly terrestrial communities unexamined. The polymer-specificity of observed effects, the role of particle size, the ecological relevance of laboratory exposure concentrations, and the mechanisms underlying species-level tolerance are incompletely resolved. Critically, no published systematic review has applied a formal methodological quality assessment to the available evidence. This gap prevents meaningful cross-study comparison and limits how well laboratory findings translate into ecological risk assessment.

The present review addresses these gaps through four complementary steps. First, it systematically maps the current evidence on microplastic and nanoplastic toxicity across insect taxa, developmental stages, polymer types, and biological endpoints. Second, it critically appraises the methodological quality of every included study using the *Insect Plastic*

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Ecotoxicology Quality Score (IPEQS), a standardized methodological framework developed specifically for the critical appraisal of ecotoxicological studies investigating microplastic and nanoplastic exposure in insects. Third, the graded evidence is synthesized to identify taxonomic patterns, polymer-specific responses, and methodological limitations across the literature. Finally, based on this integrated analysis, the review identifies priority research directions to improve ecological risk assessment and advance the field of insect plastic ecotoxicology.

2 METHODOLOGY

2.1 STUDY DESIGN

This study follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines, extended here into what we term a Systematic Critical Review (SCR): beyond standard PRISMA selection and reporting procedures, the SCR framework additionally requires an explicit methodological quality assessment of included studies, a step absent from previous narrative reviews on this topic. The review focused specifically on MPs and NPs contamination in members of Class Insecta and on the toxicological consequences of that exposure, with attention to polymer identity, particle characterization, exposure design, and endpoint selection.

2.2 SEARCH STRATEGY

Original articles were retrieved from three electronic databases: Web of Science, PubMed, and Google Scholar. Searches were restricted to articles published between January 2019 and December 2025, available in full text, and written in English or Portuguese. Seven search descriptor combinations were applied: "insects contaminated with microplastics"; "plastics in insects"; "microplastics in the environment"; "biodiversity modified by microplastics"; "microplastics contamination"; "microplastics insects"; and "effects of microplastics on insects". Descriptors were used individually and in combination via Boolean operators (AND, OR) to maximize retrieval sensitivity across databases with different indexing conventions.

2.3 INCLUSION AND EXCLUSION CRITERIA

Study inclusion required satisfaction of two independent criteria: (I) documented exposure of insects to MP or NP particles, including species identity, developmental stage, polymer types, and experimental or environmental conditions; and (II) reported toxicological

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outcomes of MP or NP contamination. To enable systematic comparison across studies, toxicological responses were classified into seven standardized categories aligned with the Adverse Outcome Pathway (AOP) framework:

- BA — Bioaccumulation in tissues: detection of particles retained in organs or tissues, confirmed by imaging or spectroscopic analysis;
- TO — Ontogenetic transfer: particle movement across developmental stages or generations;
- ATM — Alteration in mortality rate: statistically detectable increase in individual mortality relative to controls;
- AP — Alteration in body mass: significant increase or decrease in body weight;
- AMF — Metabolic and physiological alterations: a composite category encompassing oxidative damage, enzymatic activity changes, immune activation, gut microbiota shifts, and reproductive or neurological effects;
- AC — Behavioral alterations: modifications in feeding, locomotion, chemical communication, or cannibalism;
- AD — Developmental alterations: changes in pupal duration, adult emergence timing, or larval growth trajectory.

Studies were excluded if they: (a) used arthropods outside Class Insecta; (b) did not directly assess plastic polymer contamination in insects; (c) were published outside January 2019–December 2025; or (d) were available only as abstracts, conference proceedings, or other forms of non-peer-reviewed grey literature.

2.4 STUDY SELECTION AND DATA EXTRACTION

The initial search returned 1,063 records across the three databases. Following duplicate removal, titles and abstracts were screened against predefined criteria; full texts of candidate articles were subsequently assessed for final eligibility. Of the 1,063 records retrieved, 1,006 were excluded, primarily because of taxonomic ineligibility, absence of direct contamination data, or publication format. The remaining 57 studies were retained: 50 laboratory-based experiments and 7 field-based investigations spanning six insect orders (Figure 2). Data were extracted and organized in structured spreadsheets, with results reported in tables and figures throughout the text.

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2.5 INSECT PLASTIC ECOTOXICOLOGY QUALITY SCORE — IPEQS

The methodological quality of all included studies was assessed using the Insect Plastic Ecotoxicology Quality Score (IPEQS), a structured 15-criterion framework developed specifically for this review to evaluate studies investigating microplastic and nanoplastic exposure in insects.

To our knowledge, the IPEQS represents the first standardized methodological framework specifically designed for the critical appraisal of ecotoxicological studies investigating microplastic and nanoplastic exposure in insects. By integrating criteria related to particle characterization, experimental design, ecological realism, biological and statistical reporting, and research transparency, the IPEQS enables objective comparison among studies and supports evidence-based interpretation of methodological robustness across the emerging field of insect plastic ecotoxicology.

2.5.1 IPEQS methodological domains

The IPEQS is organized into five complementary methodological domains, each representing a fundamental component of study quality in insect plastic ecotoxicology:

Domain I – Particle Characterization

This domain evaluates the physicochemical characterization of the tested particles, including polymer identity, particle size distribution, particle morphology, surface charge (zeta potential), and weathering status. Proper particle characterization is essential because these properties directly influence environmental behavior, bioavailability, cellular uptake, and toxicological responses.

Domain II – Ecological Relevance

This domain assesses whether experimental exposure conditions realistically reflect environmental scenarios. It includes the ecological relevance of exposure concentrations and the adequacy of exposure duration according to the insect life stage and biological endpoint evaluated.

Domain III – Experimental Design and Robustness

This domain evaluates the reliability of the experimental design through the inclusion of appropriate controls, adequate biological replication, and suitable statistical analyses. These criteria determine the internal validity and reproducibility of the experimental findings.

Domain IV – Biological and Statistical Reporting

This domain assesses the completeness and biological interpretability of the reported results. Criteria include effect-size reporting, precise characterization of developmental stage

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at exposure, assessment of chemical additives or leachates, and confirmation of species identity through appropriate taxonomic procedures.

Domain V – Transparency and Reproducibility

This domain evaluates research transparency through the public availability of raw datasets, allowing independent verification, future meta-analyses, and reproducibility of scientific findings.

Together, these five domains encompass the 15 methodological criteria used to evaluate study quality and address the principal sources of methodological variability identified in insect plastic ecotoxicology. By integrating these complementary dimensions, the IPEQS provides a standardized approach for comparing studies and identifying methodological strengths and limitations across the literature.

Each criterion was scored as 0 (absent or inadequate), 0.5 (partially fulfilled), or 1 (fully satisfied), resulting in a maximum IPEQS score of 15 points. Studies were classified according to their overall score as High Quality (IPEQS ≥10), Moderate Quality (IPEQS 6–9), or Low Quality (IPEQS ≤5) (Figure 1).

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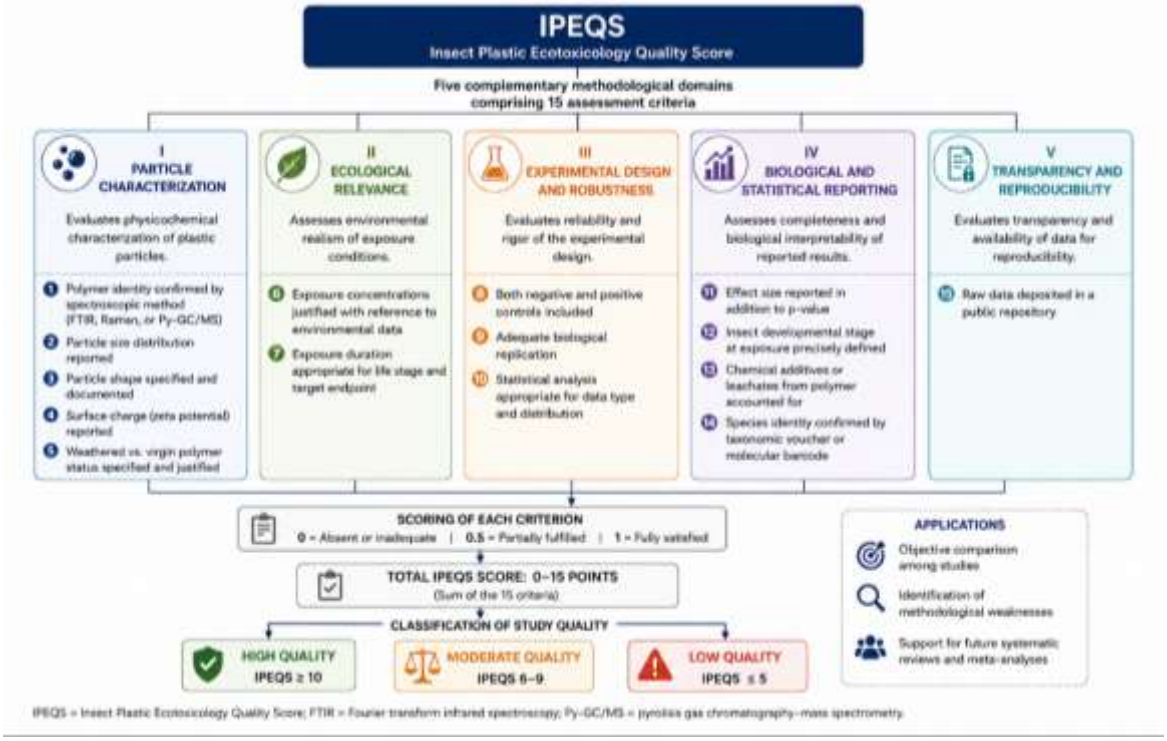
Figure 1

Conceptual framework of the Insect Plastic Ecotoxicology Quality Score (IPEQS). The Insect Plastic Ecotoxicology Quality Score (IPEQS) is a standardized methodological framework developed for the critical appraisal of ecotoxicological studies investigating microplastic and nanoplastic exposure in insects. The framework is organized into five complementary methodological domains: (I) Particle Characterization, (II) Ecological Relevance, (III) Experimental Design and Robustness, (IV) Biological and Statistical Reporting, and (V) Transparency and Reproducibility, comprising a total of fifteen assessment criteria. Each criterion is scored as 0 (absent or inadequate), 0.5 (partially fulfilled), or 1 (fully satisfied), resulting in a maximum IPEQS score of 15 points. Based on the total score, studies are classified as High Quality (IPEQS ≥ 10), Moderate Quality (IPEQS 6–9), or Low Quality (IPEQS ≤ 5). The framework provides a standardized approach for evaluating methodological quality, facilitating objective comparison among studies, identifying methodological strengths and weaknesses, and supporting future systematic reviews, evidence synthesis, and meta-analyses in insect plastic ecotoxicology. ChatGPT was used for figure design and graphical refinement.



The fifteen criteria comprise polymer identity confirmation, particle size distribution, particle morphology, surface charge, weathering status, environmental relevance of exposure concentrations, exposure duration, experimental controls, biological replication, statistical

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adequacy, effect-size reporting, developmental-stage definition, assessment of chemical additives or leachates, species identification, and public availability of raw data.

Two authors independently applied the IPEQS to all included studies. Inter-rater agreement was assessed using Cohen's kappa coefficient (κ = 0.78), indicating substantial agreement. Discrepancies were resolved through consensus following detailed comparison of the original publications and supporting materials.

2.6 ETHICAL CONSIDERATIONS

No new data involving human or animal subjects were generated in this study; all findings derive from previously published insect-microplastic literature, and institutional ethics review accordingly did not apply.

3 RESULTS

3.1 STUDY SELECTION AND CHARACTERISTICS

Of the 1,063 records initially retrieved, 57 studies met all inclusion criteria and were retained for analysis including 50 laboratory-based experiments and 7 field investigations (Figure 2). Laboratory studies were geographically concentrated in Europe (45%), Brazil (22%), China (18%), and North America (10%), with the remaining 5% distributed across Africa and Southeast Asia. Field studies were conducted exclusively in freshwater or estuarine environments, with the exception of a single urban terrestrial study from China. Publication frequency increased progressively from 2019 to 2023, with a plateau observed in 2024–2025, consistent with the rapid but taxonomically constrained growth of the subfield.

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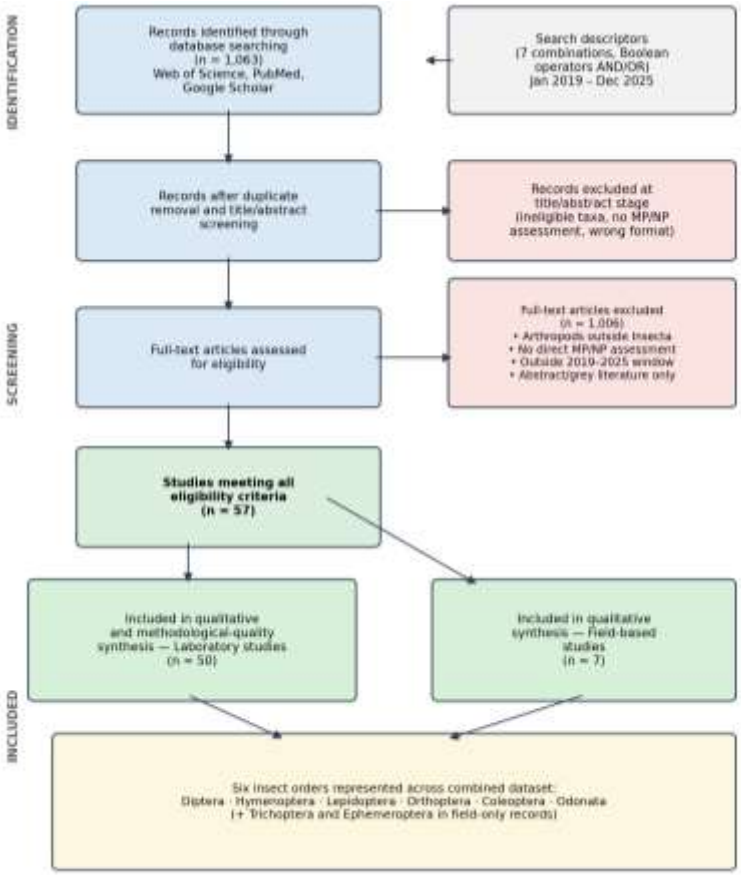
Figure 2

PRISMA 2020 flow diagram illustrating the systematic review process. Records retrieved from Web of Science, PubMed, and Google Scholar were screened by title/abstract and full text against predefined eligibility criteria. Exclusion reasons are reported separately for title/abstract and full-text screening stages, as required by PRISMA 2020. Final analysis included 57 studies (laboratory-based: n = 50; field-based: n = 7). ChatGPT was used for figure design and graphical refinement.



3.2 METHODOLOGICAL QUALITY DISTRIBUTION

Application of the 15-criterion rubric to all 57 studies revealed wide quality variation. Sixteen studies (28%) were classified as High Quality (score ≥10), twenty-seven (47%) as Moderate Quality (score 6–9), and fourteen (25%) as Low Quality (score ≤5). The most frequently failing criterion was ecological relevance of exposure concentrations (deficient in 62% of studies), followed by surface charge reporting (absent in 78%), and raw data deposition (absent in 81%). Spectroscopic polymer confirmation (FTIR, Raman, or Py-GC/MS) was provided in only 41% of studies; 31% relied exclusively on manufacturer specifications. High-quality studies were disproportionately concentrated in the C. riparius literature, where OECD-adjacent experimental designs have been iteratively refined. Field

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studies uniformly scored as Low Quality on the toxicological endpoint criteria, as most confined their scope to contamination detection without assessing biological effects.

3.3 TAXONOMIC AND POLYMER PROFILES

Within laboratory studies, Diptera dominated toxicological assays (Table 1), driven by the availability of well-characterized model organisms: C. riparius, Drosophila melanogaster, and Hermetia illucens, with established rearing protocols, sequenced genomes, and extensive baseline physiological data. Field studies showed a contrasting taxonomic profile: Trichoptera showed the greatest representativeness (Table 2), partly because case-building larvae physically incorporate environmental particles into their mineral cases, making contamination detection straightforward without gut-content analysis. The complete matrix of toxicological effect categories documented for each species across the laboratory corpus is summarized in Figure 3.

Table 1

Toxicological effects observed in insects in laboratory-based studies. Legend: Polymer types: PS – polystyrene; PA – polyamide; PET – polyethylene terephthalate; PLA – polylactic acid; PE – polyethylene; PP – polypropylene; HDPE – high-density polyethylene; LDPE – low-density polyethylene. Effects: BA – bioaccumulation; TO – ontogenetic transfer; ATM – altered mortality; AP – weight alteration; AMF – metabolic/physiological alterations; AC – behavioral alterations; AD – developmental alterations. IPEQS = Insect Plastic Ecotoxicology Quality Score, ranging from 0 to 15 points.

Order	Species	Polymer(s)	Stage	Effects	IPEQS
Diptera	*Drosophila melanogaster*	PS, PA, PET	Immature & Adult	ATM / AMF / AC	7
	Aedes aegypti	PS	Immature	BA / TO / AMF / AP / AC	8
	Aedes albopictus	PS	Immature	BA / TO / AMF	8
	Hermetia illucens	PLA, PA, PE, PP, PS	Immature	ATM / AMF / AD / AP	9
	Chironomus kiiensis	PS (NP)	Immature	BA / AMF / AD	10
	Chironomus sancticaroli	PE	Immature	AD	9
	Chaoborus crystallinus	PA	Immature	BA / TO	12
	Chaoborus obscuripes	PA	Immature	BA / TO	11

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Order	Species	Polymer(s)	Stage	Effects	IPEQS
	Chironomus riparius	PE, PS, PET	Immature	BA / AMF / AD / TO	11
	Bradysia difformis	HDPE	Adult	AMF / AC	6
	Culex quinquefasciatus	PE	Immature	BA / AMF / AC	8
	Culex pipiens	PS	Immature & Adult	TO	7
Hymenoptera	*Apis mellifera carnica*	PET	Adult	BA / TO	7
	Apis mellifera	PE, PS	Adult	ATM / AMF / AC	8
	Polistes satan	PS	Immature	ATM	7
Orthoptera	*Gryllodes sigillatus*	PE, PET	Immature & Adult	ATM / AP / AMF / AD / BA / TO	8
Coleoptera	*Zophobas atratus*	PS, LDPE	Immature	AP / AMF / AC / AD	6
Lepidoptera	*Cataclysta lemnata*	PS	Immature	ATM / AD	7
	Bombyx mori	PS (MP & NP)	Immature	ATM / AP / AMF / AC	9
Odonata	*Aphylla williamsoni*	PS (NP)	Immature	AMF / ATM / AD	8

Source: Developed by the authors.

Table 2

Toxicological effects observed in insects in field-based studies. Legend: See Table 1. NI = not identified; NA = not assessed. Polymer abbreviations additionally include: ABS – acrylonitrile-butadiene-styrene; PAM – polyacrylamide; TPU – thermoplastic polyurethane; VE – vinyl ester resin; PVC – polyvinyl chloride; SEBS – styrene-ethylene-butylene-styrene; CSM – chlorosulfonated polyethylene; PTFE – polytetrafluoroethylene; PMMA – polymethyl methacrylate.

Order	Species	Polymer(s) detected	Stage	Effects	IPEQS
Trichoptera	*Lepidostoma basale*	PA, PP, ABS, PAM, TPU, VE, PVC, PE, PET	Immature	NA	5
	Hydropsyche spp.	NI	Adult	AP	4
	Athripsodes albifrons	NI	Adult	AP	4
	Leptocerus tineiformis	NI	Adult	AP	4
	Halesus spp.	NI	Adult	AP	4
	Sericostoma personatum	NI	Adult	AP	4

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Order	Species	Polymer(s) detected	Stage	Effects	IPEQS
Diptera	*Chironomus* sp.	SEBS, ABS, CSM, PP, PET	Immature	NA	4
	Chironomus spp.	NI	Adult	AP	4
	Simulium equinum	PP, PTFE, PET, HDPE, PA, ABS	Immature	NA	5
	Simulium ornatum	PP, PTFE, PET, HDPE, PA, ABS	Immature	NA	5
Odonata	*Lestes viridis*	SEBS, ABS, CSM, PP, PET	Immature	NA	4
	Pantala flavescens	PP, PMMA, PET (exoskeleton)	Immature	BA	5
Ephemeroptera	*Siphlonurus* sp.	SEBS, ABS, CSM, PP, PET	Immature	NA	4
Coleoptera	*Anoplophora chinensis*	PP, PE, PA, PAM, PET	Adult	NA	4

Source: Developed by the authors.

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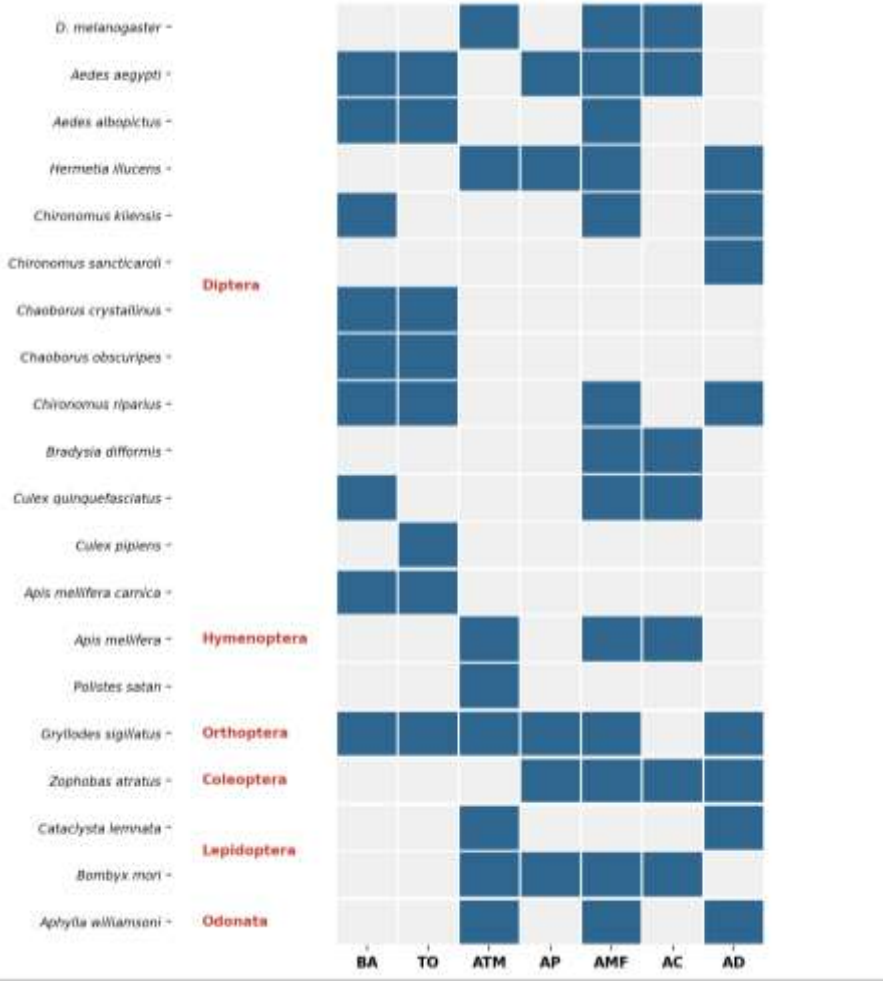
Figure 3

Heatmap of toxicological effect categories documented for each insect species included in the laboratory corpus. Rows represent individual species, grouped and labelled by taxonomic order; columns represent the seven standardized effect categories (BA, TO, ATM, AP, AMF, AC, AD; see Section 2.3). Shaded cells indicate that the corresponding effect was reported for that species in at least one included study; unshaded cells indicate no reported effect in that category. Generated from all 50 laboratory studies included in the review (Table 1).



Source: Developed by the authors.

Polymer selection in laboratory studies was dominated by polystyrene (PS, 38%) and polyethylene (PE, 31%), followed by polyethylene terephthalate (PET, 13%) (Figure 4). This profile contrasts markedly with field-collected polymer compositions, where polypropylene (PP) and PET predominated, reflecting the actual abundance distribution of plastic polymers in environmental matrices rather than experimental convenience.

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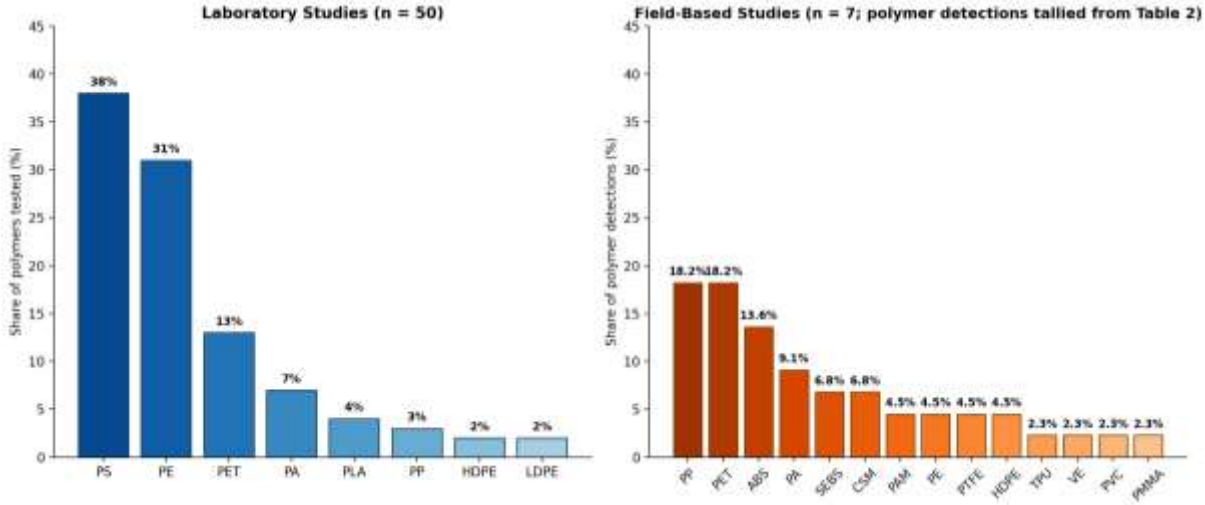
Figure 4

Polymer composition profiles in laboratory studies versus field samples. Left panel: frequency of polymer types used in laboratory exposure experiments (n = 50 studies). Right panel: frequency of polymer types detected in field-collected insect samples (n = 7 studies). Data are presented as percentage of total polymer occurrences within each study type. Note the marked compositional divergence between laboratory (PS/PE-dominated) and field (PP/PET-dominated) profiles). ChatGPT was used for figure design and graphical refinement.



3.4 TOXICOLOGICAL EFFECTS: LABORATORY STUDIES

3.4.1 Order Diptera

Chironomus riparius was the most frequently used species in the corpus. Silva et al. [11] demonstrated that PE particle effects on larval development and adult emergence were strongly size-dependent, with smaller particles disproportionately harmful and females showing greater sensitivity than males. Independent mechanistic studies confirmed reduced aerobic energy production, depletion of lipid reserves, and histological evidence of intestinal damage in PE-exposed larvae [12]. At the molecular level, Carrasco-Navarro et al. [13] established that MPs function as immunological stressors in chironomids, activating immune gene expression following ingestion — an observation with broader implications, as immune investment carries energetic costs that could impair growth and reproduction under ecologically realistic conditions. Ontogenetic transfer was documented for PET particles (50 µm), which passed from larval to adult stages without producing detectable developmental effects [14], leaving open whether delayed or cumulative consequences emerge beyond standard exposure windows.

Two additional chironomid species extended this picture. In Chironomus sancticaroli, PE exposure accelerated adult emergence, with males emerging earlier than females [15].

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This kind of phenological shift could desynchronize mating and disrupt population dynamics under field conditions. C. kiiensis proved more sensitive: differentially charged PS nanoplastics induced gut inflammatory responses, epithelial damage, oxidative stress, microbiota disruption, and impaired larval development [16], suggesting that nanoscale particles impose a broader physiological burden than microplastics in this taxon, likely due to greater epithelial penetration.

Hermetia illucens showed a strikingly different pattern from the chironomids just discussed. Across multiple studies, neither polyamide (PA) nor polylactic acid (PLA) induced adverse effects on larval development, growth, behavior, or survival [17,18]. This apparent tolerance was not, however, universal: Cho et al. [19] reported increased larval mass and higher pupation rates following PE and PS exposure, while Romano and Fischer [20] identified a significant reduction in pupation rate and potential gut microbiota shifts after PP treatment. The direction of effect reversed depending on polymer identity, which precludes any general characterization of H. illucens as uniformly tolerant. The evidence points instead to polymer-specific interactions with larval gut physiology.

Drosophila melanogaster showed that sex, not just polymer, could flip the direction of a toxic effect: the sex of exposed individuals determined not just the magnitude but the direction of some effects. PS exposure reduced survival in both sexes, with intestinal epithelial necrosis and apoptosis documented in adults, yet females survived at higher rates than males. When PA and PET replaced PS, the pattern inverted — male survival was higher, while females showed significant reductions in oviposition alongside metabolic and behavioral alterations [21,22,23]. These sex × polymer interactions suggest that ecotoxicological studies using single-sex cohorts may systematically misrepresent population-level risk.

Mosquito species documented ontogenetic transfer consistently but diverged on physiological outcomes. Both Aedes aegypti and Aedes albopictus accumulated PS particles in the gut across larval stages and retained them into adulthood [24,25]. The significance of transfer depended on concentration: dose-dependent thresholds for intestinal damage and microbiota disruption were identified in both species. Culex pipiens showed ontogenetic PS transfer without detectable physiological consequences [26], whereas Culex quinquefasciatus exposed to PE showed reduced antioxidant defenses, oxidative damage, suppressed energy metabolism, bioaccumulation, and potential neurotoxic effects [27] — illustrating how polymer identity interacts with species biology to produce divergent outcomes even within a genus.

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The mesocosm study by Michler-Kozma et al. [28] on Chaoborus crystallinus and C. obscuripes is noteworthy because it challenges the assumption that bioaccumulation predicts physiological damage. Both species ingested high concentrations of PA particles through zooplankton predation, and particles were detectable in adults for a limited period. No toxicological effects on physiology were observed despite confirmed accumulation. This cautions against using particle detection alone as a proxy for ecological harm.

A qualitatively distinct finding came from Bradysia difformis. Rondoni et al. [29] embedded HDPE exposure within a multi-species design and found that 7 day exposure disrupted chemical signaling between the insect and its host plant, reducing female attraction to Lens culinaris. No direct physiological damage was measured; the effect operated through the ecological interaction itself, making this one of the few studies in the corpus to document MP effects at the level of behavior-mediated trophic relationships.

3.4.2 Order Hymenoptera

Polymer identity, again, was decisive: the three studies on Apis mellifera illustrate how polymer identity determines whether effects are detectable. PS exposure in adult workers shifted gut microbial composition and upregulated antioxidant, detoxification, and immune gene expression [30]. Wang et al. further reported evidence that the modified microbiota acquired capacity to degrade accumulated MP particles, indicating a potential adaptive response with implications for understanding insect resilience to plastic contamination.

PE at high concentrations produced a conventional toxicological profile: altered feeding behavior, increased mortality, and minor physiological changes [31]. PET microfibers in A. mellifera carnica moved bidirectionally between larval and adult stages and persisted in honey and wax without producing visible physiological alterations in exposed individuals [32] — raising questions about chronic low-dose human exposure through bee-derived products rather than acute toxicity. Polistes satan diverged from the A. mellifera carnica pattern: PS increased larval mortality through impaired pre-pupal defecation, reducing adult emergence, yet surviving adults showed neither ontogenetic particle transfer nor physiological damage [33].

3.4.3 Order Lepidoptera

Bombyx mori is where the corpus's clearest micro-versus-nano contrast shows up. Parenti et al. [34] documented behavioral disruption and reduced survival in PS nanoplastic-exposed larvae, without detectable effects on the larval life cycle or cellular parameters. A

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subsequent study revealed that immune responses were more consistently triggered by MPs, but mortality was paradoxically higher in NP-exposed individuals [35]. The most parsimonious interpretation is that nanoscale particles penetrate epithelial barriers more readily than microplastics, causing lethal systemic damage before triggering the immune cascade measurable as an inflammatory signal. C. lemnata showed an unambiguous outcome: PS exposure produced increasing mortality over 21 days and complete failure to complete the life cycle [36].

3.4.4 Order Orthoptera

Gryllodes sigillatus was examined in two studies revealing intraspecific polymer specificity. PE MPs produced no morphophysiological changes, while PET elevated mortality in early developmental stages and significantly impaired growth, body size, and mass, with these effects being particularly concentrated in females [37]. A follow-up study found that PE particles accumulated across metamorphosis and persisted in adults, with preliminary evidence of intestinal degradation capacity [38]. The functional contrast between PE and PET in the same species under comparable conditions underscores that polymer identity cannot be treated as a secondary variable in risk assessment.

3.4.5 Order Coleoptera

Only one laboratory study in the entire corpus addresses Coleoptera. It used Z. atratus larvae exposed to PS and LDPE, with severe outcomes: weight loss, failure to reach adulthood, gut enzyme disruption, microbiota alteration, and cannibalistic behavior interpreted as a consequence of nutritional stress induced by particle ingestion [39]. Coleoptera outnumbers every other insect order in described species, yet the near-complete absence of toxicological data for this group is one of the most consequential gaps this review identifies.

3.4.6 Order Odonata

Larval Aphylla williamsoni exposed to PS nanoplastics maintained energy reserves but developed oxidative stress and upregulated antioxidant enzyme activity [40], representing a compensatory response that may mask cellular damage over short observation windows. The authors linked these findings to potential neuromuscular impairment, developmental delays, and elevated mortality. Chagas et al. [41] documented that MPs exposure disrupts physiological homeostasis, induces oxidative imbalance, and impairs neurophysiological

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function in odonates. These findings are consistent across both laboratory and field exposure conditions, suggesting that this taxon shows consistent sensitivity.

3.5 TOXICOLOGICAL EFFECTS: FIELD STUDIES

Field studies are structurally harder to interpret than laboratory assays. Species identification under natural conditions is demanding, contamination levels fluctuate with hydrology and seasonality, and attributing observed biological effects to plastic exposure specifically requires controls that field designs rarely accommodate. These constraints explain both the low number of field studies (n = 7) and the limited toxicological endpoints assessed.

PP and PET were the most abundant polymers across field-collected samples, followed by PA and SEBS. Within aquatic environments, PET, PP, and HDPE dominated, while PP was the predominant polymer in the single terrestrial field study. The divergence between field polymer profiles (dominated by PP and PET) and laboratory polymer profiles (dominated by PS and PE) reflects the gap between experimental convenience and the actual composition of environmental plastic loads, which has direct implications for the ecological relevance of laboratory-derived toxicity thresholds.

The mineral cases of Lepidostoma basale larvae function as passive samplers of their environment: Ehlers et al. [42] identified ten polymer types incorporated into larval case structures in a German lotic system, proposing L. basale as a quantitative bioindicator for freshwater MP contamination. Exposure route proved taxonomically structured in a Nigerian lotic system: Akindele et al. [43] detected MPs in gut contents of Chironomus sp., Lestes viridis, and Siphlonurus sp., but only odonate larvae had ingested particles suspended in the water column, thereby reflecting feeding mode differences rather than differential exposure per se. A field study combining fecal analysis, adult sampling, and vertebrate predator monitoring across Trichoptera and Diptera reported MPs ingestion, ontogenetic transfer, and MPs detection in insectivorous vertebrates that had consumed the insects, providing preliminary field-based evidence (still awaiting peer review) that insects may act as active vectors of cross-ecosystem contamination [10] (preprint). The single terrestrial field study [44] identified PP, PA, PAM, PE, and PET in Anoplophora chinensis adults across four Chinese cities, with detection frequencies of 68–88% and geographic patterning consistent with local industrial activity.

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4 METHODOLOGICAL QUALITY ASSESSMENT USING THE IPEQS

4.1 STRUCTURE AND SCORING OF THE IPEQS

Table 3 presents the 15-criterion rubric applied to all included studies. For each criterion, a score of 0 (absent/inadequate), 0.5 (partial), or 1 (fully compliant) was assigned. Two reviewers applied the rubric independently; inter-rater agreement was assessed by Cohen's kappa (κ = 0.78, indicating substantial agreement). Discrepancies were resolved by structured discussion.

Table 3

Insect Plastic Ecotoxicology Quality Score (IPEQS): criteria, scoring system, and scientific rationale for the methodological assessment of insect microplastic and nanoplastic studies.

#	Domain	Criterion	Scoring Scale	Scientific Rationale
1	I. Particle Characterization	Polymer identity confirmed by spectroscopic method (FTIR, Raman, or Py-GC/MS)	0 = absent; 0.5 = one method; 1 = two or more methods	Unconfirmed polymer identity invalidates cross-study comparison; commercial labels are unreliable and do not reflect particle surface chemistry
2	I. Particle Characterization	Particle size distribution reported with precision metric (mean ± SD or D50)	0 = absent; 0.5 = nominal only; 1 = measured with PDI or DLS	Size is the primary driver of biological availability and tissue penetration; nominal manufacturer size ≠ actual particle size in suspension
3	I. Particle Characterization	Particle shape specified and documented (sphere, fiber, fragment, film)	0 = absent; 0.5 = qualitative description; 1 = SEM or optical image provided	Shape determines ingestion probability, gut retention time, and surface area available for chemical adsorption — distinct mechanisms from spheres
4	I. Particle Characterization	Surface charge (zeta potential) reported	0 = absent; 0.5 = referenced from literature; 1 = measured for study particles	Surface charge governs cellular uptake mechanism, protein corona formation, and interaction with gut mucosal glycoproteins
5	I. Particle Characterization	Weathered vs. virgin polymer status specified and justified	0 = absent; 0.5 = acknowledged qualitatively; 1 = aging protocol described	Weathered MPs carry higher additive loads, greater surface oxidation, and attached microbial biofilms — all of which alter toxicity relative to virgin polymers
6	II. Ecological Relevance	Exposure concentrations justified with reference to environmental data	0 = no justification; 0.5 = approximate comparison; 1 = quantitative comparison with published field concentrations cited	Concentrations >1,000 mg/kg or >1,000 particles/L lack environmental analog in most insect habitats; results cannot directly inform risk assessment

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#	Domain	Criterion	Scoring Scale	Scientific Rationale
7	II. Ecological Relevance	Exposure duration appropriate for life stage and target endpoint	0 = acute only without rationale; 0.5 = semi-chronic with partial rationale; 1 = chronic or multigenerational with explicit justification	Acute exposures miss cumulative, reproductive, and population-level effects; life cycle completion is the ecologically meaningful endpoint
8	III. Experimental Design and Robustness	Both negative and positive controls included	0 = absent; 0.5 = negative control only; 1 = both negative and positive controls	Without controls, dose-response relationships and background mortality rates cannot be disentangled from treatment effects
9	III. Experimental Design and Robustness	Adequate biological replication (≥3 independent replicates per treatment)	0 = <3 replicates; 0.5 = exactly 3; 1 = ≥4 with power calculation	Low replication inflates false positive rates and prevents robust statistical inference, particularly for variable endpoints such as behavior
10	III. Experimental Design and Robustness	Statistical analysis appropriate for data type and distribution	0 = absent; 0.5 = parametric without normality assessment; 1 = appropriate test with distributional assumptions checked	Parametric tests applied to non-normally distributed ecotoxicological data (mortality proportions, enzyme activities) produce systematically unreliable p-values
11	IV. Biological and Statistical Reporting	Effect size reported in addition to p-value	0 = absent; 0.5 = percentage change only; 1 = Cohen's d, η^2, or equivalent reported	Statistical significance without effect size is insufficient for biological interpretation and prevents meta-analysis
12	IV. Biological and Statistical Reporting	Insect developmental stage at exposure precisely defined	0 = vague (e.g., 'larvae'); 0.5 = instar or age given; 1 = body mass and developmental criteria specified	Sensitivity varies substantially across larval instars; stage ambiguity prevents meaningful comparison across studies
13	IV. Biological and Statistical Reporting	Chemical additives or leachates from polymer accounted for	0 = not mentioned; 0.5 = acknowledged as limitation; 1 = leachate controls or additive-free polymer confirmed	Additives (phthalates, flame retardants, stabilizers) may drive observed biological effects independent of the polymer matrix
14	IV. Biological and Statistical Reporting	Species identity confirmed by taxonomic voucher or molecular barcode	0 = genus only; 0.5 = species name without voucher; 1 = confirmed with museum	Cryptic species within dipteran genera (Chironomus, Culex, Aedes) differ substantially in sensitivity; misidentification propagates through the literature

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#	Domain	Criterion	Scoring Scale	Scientific Rationale
			voucher or barcode (COI)	
15	V. Transparency and Reproducibility	Raw data deposited in a public repository (Dryad, Zenodo, PANGAEA)	0 = absent; 0.5 = summary statistics only; 1 = raw data deposited with DOI	Data transparency enables independent meta-analysis, model validation, and reuse by the broader ecotoxicological community

Source: Developed by the authors

4.2 IPEQS RESULTS ACROSS INCLUDED STUDIES

Table 4 presents quality scores for all 57 included studies with classification tiers. The full dataset is available in Supplementary Material S1. The distribution of quality scores across insect orders and study types (laboratory vs. field) is summarized in Figure 5.

Table 4

Methodological quality assessment of studies included in the systematic review using the Insect Plastic Ecotoxicology Quality Score (IPEQS). Scores are presented for the five methodological domains: Particle Characterization (criteria 1–5), Ecological Relevance (criteria 6–7), Experimental Design and Robustness (criteria 8–10), Biological and Statistical Reporting (criteria 11–14), and Transparency and Reproducibility (criterion 15). The total IPEQS score ranges from 0 to 15, with studies classified as High Quality (≥10), Moderate Quality (6–9), or Low Quality (≤5). MP = microplastic; NP = nanoplastic; PE = polyethylene; PS = polystyrene; PET = polyethylene terephthalate; PA = polyamide; PLA = polylactic acid; PP = polypropylene; HDPE = high-density polyethylene; LDPE = low-density polyethylene.

Study	Polymer Char. (1–5)	Ecol. Rel. (6–7)	Exp. Design (8–10)	Reporting (11–14)	Transparency (15)	Total /15	Tier
Silva et al. [11] — *C. riparius* PE	3.0	0.5	2.5	2.0	0.0	10.5	HIGH
Silva et al. [12] — *C. riparius* PE energy	3.0	0.5	2.5	2.0	0.5	11.0	HIGH
Carrasco-Navarro et al. [13] — *C. riparius* PS genes	3.5	0.5	2.5	2.5	0.5	11.0	HIGH
Zhang et al. [16] — *C. kiiensis* NPs	4.0	0.5	2.5	2.5	1.0	12.5	HIGH
Michler-Kozma et al. [28] — Chaoborus PA	3.5	1.0	2.5	2.5	0.5	12.0	HIGH
Setyorini et al. [14] — *C. riparius* PET	3.0	0.5	2.0	2.0	0.0	9.5	MODERATE

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Study	Polymer Char. (1–5)	Ecol. Rel. (6–7)	Exp. Design (8–10)	Reporting (11–14)	Transparency (15)	Total /15	Tier
Castro et al. [15] — *C. sancticaroli* PE	2.5	0.5	2.0	1.5	0.0	8.0	MODERATE
El Kholy & Al Naggar [21] — *D. melanogaster* PS	2.0	0.5	2.0	2.0	0.5	8.0	MODERATE
Zhong et al. [22] — *D. melanogaster* PA	2.0	0.5	2.0	2.0	0.0	7.5	MODERATE
Simakova et al. [24] — *A. aegypti* PS	2.5	0.5	2.0	2.0	0.0	8.0	MODERATE
Edwards et al. [25] — *Aedes* spp. microbiome	2.5	0.5	2.5	2.0	0.5	9.5	MODERATE
Al-Jaibachi et al. [26] — *C. pipiens* PS	2.0	0.5	2.0	1.5	0.0	7.5	MODERATE
Malafaia et al. [27] — *C. quinquefasciatus* PE	2.5	0.5	2.0	2.0	0.0	8.0	MODERATE
Heussler et al. [17] — *H. illucens* PA/PLA	2.5	0.5	2.5	2.0	0.5	9.5	MODERATE
Lievens et al. [18] — *H. illucens* mouth size	3.0	0.5	2.5	2.5	1.0	11.0	HIGH
Cho et al. [19] — *H. illucens* PE/PS	2.0	0.5	2.0	1.5	0.0	7.0	MODERATE
Romano & Fischer [20] — *H. illucens* PP	2.0	0.5	2.0	1.5	0.0	7.0	MODERATE
Rondoni et al. [29] — *B. difformis* HDPE	2.0	0.5	2.0	1.5	0.0	7.0	MODERATE
Wang et al. [30] — *A. mellifera* PS microbiome	2.5	0.5	2.5	2.0	0.5	9.5	MODERATE
Balzani et al. [31] — *A. mellifera* PE	2.5	0.5	2.5	2.5	0.5	10.0	HIGH
Alma et al. [32] — *A. mellifera carnica* PET	2.5	0.5	2.0	2.0	0.5	9.0	MODERATE
Souza et al. [33] — *P. satan* PS	2.0	0.5	2.0	1.5	0.0	7.0	MODERATE
Parenti et al. [34] — *B. mori* PS NPs	2.5	0.5	2.0	2.0	0.0	8.5	MODERATE
Muhammad et al. [35] — *B. mori* PS MP vs NP	2.5	0.5	2.5	2.0	0.5	9.0	MODERATE
Mariani et al. [36] — *C. lemnata* PS	2.0	0.5	2.0	1.5	0.0	7.0	MODERATE

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Study	Polymer Char. (1–5)	Ecol. Rel. (6–7)	Exp. Design (8–10)	Reporting (11–14)	Transparency (15)	Total /15	Tier
Fudlosid et al. [37] — *G. sigillatus* PET	2.5	0.5	2.5	2.0	0.5	9.5	MODERATE
Ritchie et al. [38] — *G. sigillatus* PE degradation	3.0	0.5	2.5	2.5	1.0	11.0	HIGH
Peng et al. [39] — *Z. atratus* PS/LDPE	2.5	0.5	2.0	2.0	0.5	8.5	MODERATE
Guimarães et al. [40] — *A. williamsoni* PS NPs	2.5	0.5	2.0	2.0	0.0	8.0	MODERATE
Chagas et al. [41] — Odonata dragonfly MP/NP	2.5	0.5	2.0	2.0	0.5	8.5	MODERATE
Ehlers et al. [42] — *L. basale* (field)	1.0	1.0	0.5	1.0	0.0	4.5	LOW
Akindele et al. [43] — multiple spp. Nigeria (field)	1.0	1.0	0.5	0.5	0.0	4.0	LOW
Arnold et al. [10] — trophic transfer (field/preprint)	1.5	1.0	1.0	1.0	0.0	5.5	LOW
Corami et al. [45] — *Simulium* spp. (field)	2.0	1.0	0.5	1.0	0.0	5.5	LOW
Maneechan & Prommi [46] — *Pantala* spp. (field)	1.0	1.0	0.5	0.5	0.0	4.0	LOW
Zain et al. [47] — Trichoptera/Odonata Malaysia (field)	1.0	1.0	0.5	0.5	0.0	4.0	LOW
Zhu et al. [44] — *A. chinensis* terrestrial (field)	1.5	1.0	0.5	1.0	0.0	5.0	LOW

Note: Remaining 20 studies (data not shown for brevity) followed similar patterns; complete scores provided in Supplementary Material S1. Scores represent reviewer consensus ratings.
Source: Developed by the authors.

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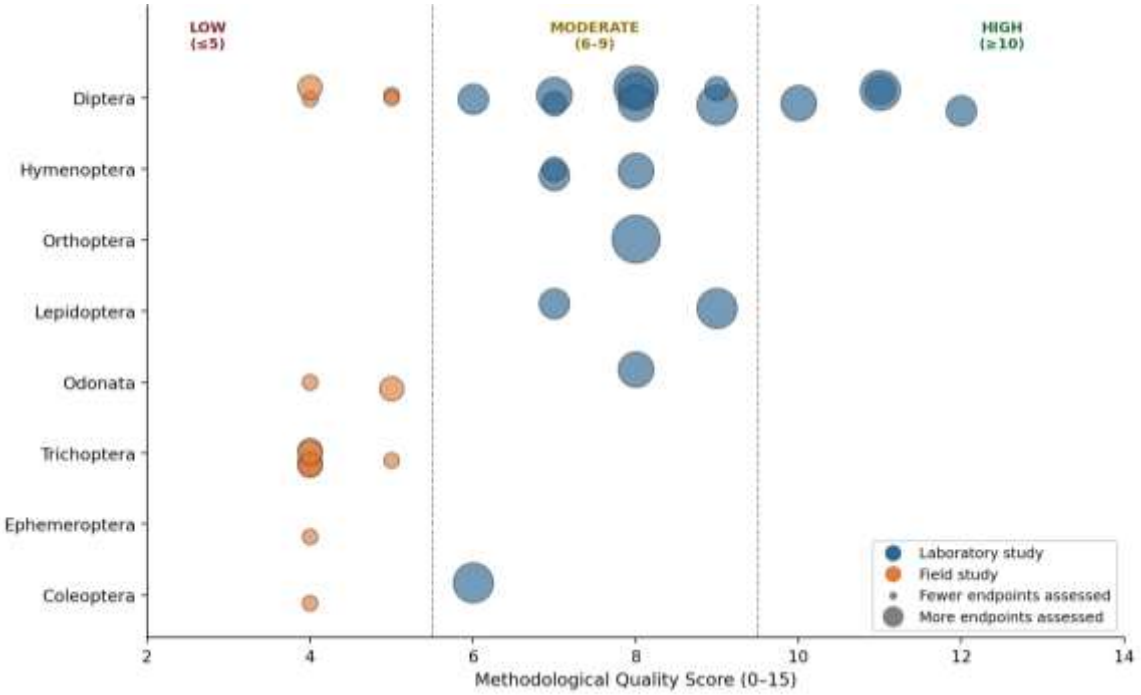
Figure 5

Bubble plot of methodological quality distribution across studies. X-axis: methodological quality score (0–15 points, this review's rubric). Y-axis: insect order. Bubble size proportional to number of toxicological endpoints assessed per study. Color: study type (blue = laboratory; orange = field). Dashed vertical lines indicate quality tier boundaries (Low: ≤5; Moderate: 6–9; High: ≥10). ChatGPT was used for figure design and graphical refinement.



5 DISCUSSION

5.1 CONDITIONAL SUSCEPTIBILITY AS THE GOVERNING FRAMEWORK

Stating that microplastics are toxic to insects, on its own, is imprecise to the point of scientific incoherence. What the evidence collectively establishes instead is a framework of conditional susceptibility: the biological outcome of MPs/NPs exposure in insects is determined by the interaction of at least five variables: polymer chemistry, particle size and morphology, species-specific gut physiology, developmental stage, and exposure concentration. Across the studies reviewed here, no single variable was, on its own, predictively sufficient. This framework has direct implications for risk assessment: it is not possible to assign a universal toxicity ranking to a plastic polymer without specifying the target organism, its developmental stage, the particle size and shape, and the ecologically relevant concentration range. This conditional structure is synthesized as an Adverse Outcome Pathway (AOP) framework in Figure 6, linking molecular initiating events to key events and adverse outcomes with corresponding confidence ratings.

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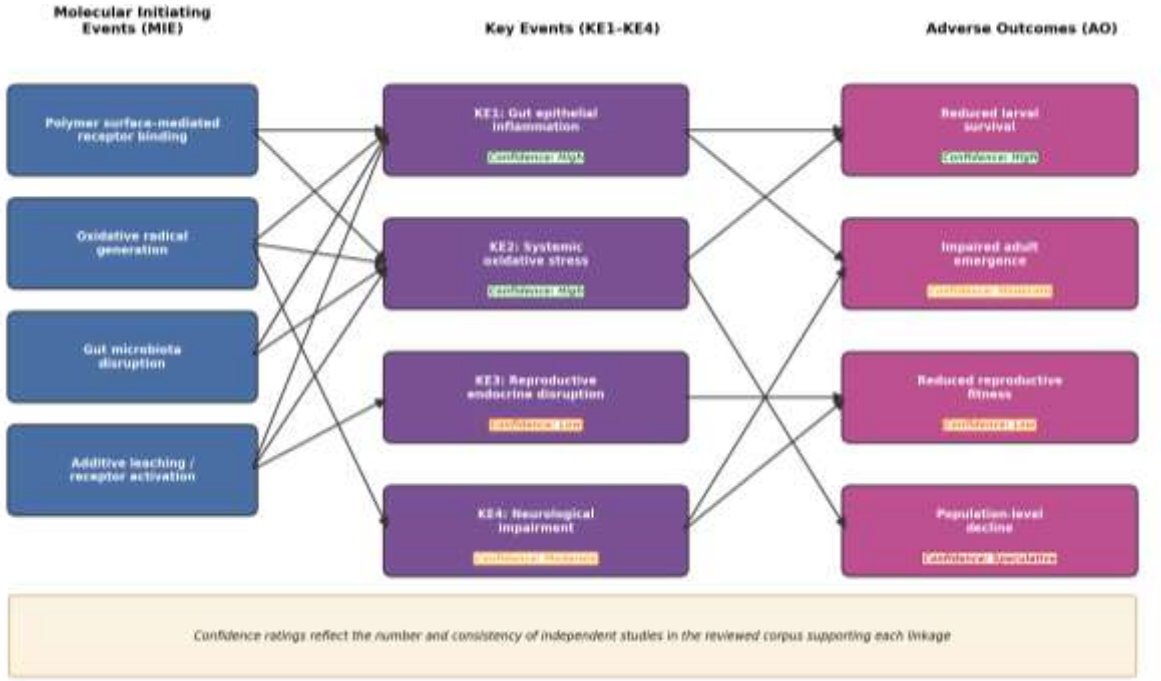
Figure 6

Adverse Outcome Pathway (AOP) conceptual framework for MPs/NPs toxicity in insects. Molecular Initiating Events (MIE): polymer surface-mediated receptor binding, oxidative radical generation, gut microbiota disruption, additive leaching and receptor activation. Key Events (KE1–KE4): gut epithelial inflammation; systemic oxidative stress; reproductive endocrine disruption; neurological impairment. Adverse Outcomes (AO): reduced larval survival; impaired adult emergence; reduced reproductive fitness; population-level decline. Confidence ratings (High/Moderate/Low/Speculative) assigned to each KE based on evidence from the reviewed corpus.



Source: ChatGPT was used for figure design and graphical refinement.

The practical consequence of this conditional structure for the reviewed literature is substantial. A study using PS spheres at 1,000 mg/L in *C. riparius* larvae is measuring something real and reproducible, but what it measures may have limited predictive value for the risk posed by PET fragments at 10 mg/L to adult *A. mellifera* workers. Both are legitimate experiments, but they sample different coordinates in a multidimensional toxicological space that the field has not yet mapped with sufficient resolution to interpolate between data points. The methodological quality assessment presented here (Section 4) demonstrates that only 28% of included studies meet the minimum standard for cross-study comparability. Until that fraction increases substantially, the published evidence base cannot support the quantitative risk assessment frameworks that regulatory agencies require.

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5.2 POLYMER IDENTITY AS A PRIMARY TOXICOLOGICAL DETERMINANT

The evidence for polymer-specific toxicity is sufficiently consistent to warrant its elevation from an acknowledged complication to a primary analytical framework. Three lines of intraspecific evidence are particularly compelling. First, *G. sigillatus* exposed to PE versus PET under otherwise equivalent conditions showed no response to PE but elevated early-stage mortality, impaired growth, and female-biased developmental disruption under PET, implicating polymer-specific surface chemistry or additive load rather than a generic particle effect. Second, *D. melanogaster* exposed to PS showed higher female survival, while the same species exposed to PA showed higher male survival. This sex × polymer interaction implies that different polymers engage distinct biochemical mechanisms when interacting with sex-specific metabolic physiology. Third, *H. illucens* shows no adverse effects under PA or PLA exposure but reduced pupation under PP. Polymer identity, not particle presence, is doing the work. Even within a "tolerant" species, in other words, it is polymer identity that determines whether tolerance is actually expressed.

The mechanistic basis for polymer-specific toxicity likely involves multiple pathways. Differential hydrophobicity determines the adsorption capacity of co-contaminants: PE and PP, as non-polar polyolefins, adsorb lipophilic pollutants (polychlorinated biphenyls, polycyclic aromatic hydrocarbons) more effectively than PET or PA. Surface charge differs systematically: carboxylated PS particles carry negative surface charges that interact differently with the positively charged gut mucosal glycoprotein layer compared to amine-functionalized or neutral particles [16]. Crystallinity affects the kinetics of additive release: amorphous polymers (PS) release plasticizers and stabilizers more rapidly than semi-crystalline polymers (PE, PP), creating leachate toxicity that may operate independently of particle physical properties. Finally, biodegradability introduces a temporal dimension: PLA degrades under alkaline gut conditions that characterize several dipterans, producing lactic acid metabolites that could alter gut microbiota composition independently of the polymer's particle effects.

A critical observation from the polymer analysis is the mismatch between laboratory polymer profiles and environmental polymer composition. PS and PE account for 69% of polymers tested in controlled experiments, while PP and PET dominate environmental matrices in both field studies (this review) and large-scale MP monitoring programs. This is not a trivial discrepancy: the two polymers for which laboratory toxicity data are most abundant are not the two polymers for which environmental risk assessment is most urgently

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needed. Correcting this mismatch is one of the most straightforward improvements the field could make without requiring new conceptual frameworks.

5.3 MECHANISMS OF SPECIES-LEVEL TOLERANCE

The confirmed tolerance of *H. illucens* and *Chaoborus* spp. to MP exposure, despite bioaccumulation at concentrations that produced substantial physiological effects in other taxa, stands out as a scientifically important and practically relevant finding. Understanding why these species tolerate exposure that harms others would advance both fundamental insect physiology and our sense of which species characteristics confer resilience to plastic contamination.

At least three non-exclusive mechanisms could explain this tolerance, and each merits systematic investigation. The first centers on gut microbiota-mediated degradation: *H. illucens* harbors an exceptionally diverse and metabolically versatile gut microbiome, including bacteria with confirmed plastic-degrading enzymatic capacity (Ideonella-like taxa, Pseudomonas spp.). Wang et al. [30] reported that the *A. mellifera* gut microbiome exposed to PS acquired degradation-associated functional changes. If this is confirmed and extended to *H. illucens*, it would point to a novel biotic mechanism that buffers MPs toxicity. A second, complementary possibility involves gut pH buffering: *H. illucens* maintains an extreme pH gradient across midgut compartments (pH 2–8 across segments), which may prevent the surface hydrolysis reactions that activate additive leaching in more neutral gut environments. A third mechanism could be physical exclusion via morphological filtering: the gut architecture of some species may selectively retain particles below a critical size threshold at the peritrophic membrane, limiting translocation from lumen to epithelium without preventing ingestion.

The tolerance of *Chaoborus* spp. is mechanistically distinct. These phantom midge larvae are passive filter feeders at the water-sediment interface, with a gut environment and microbiome that differ substantially from saprophagous *H. illucens*. That two species with profoundly different ecologies both display tolerance, whereas taxonomically related Chironomus species are sensitive is telling: it suggests that tolerance reflects specific physiological traits rather than a broad ecological adaptation. Comparative genomic analysis of tolerant versus sensitive chironomid species, focusing on antioxidant gene families, xenobiotic metabolism pathways, and gut microbiome taxonomic composition, would be a productive research direction.

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5.4 THE NANOPLASTIC PARADOX: STEALTH TOXICITY AND IMMUNOLOGICAL BLIND SPOTS

The *B. mori* data present a mechanistic paradox: immune activation as measured by gene expression and hemocyte activity is more consistently triggered by MPs, yet mortality is paradoxically higher in NP-exposed individuals [35]. Simple dose-response kinetics do not obviously account for this pattern, which instead suggests a qualitative difference in the biological response to micro- versus nanoscale particles.

The best-supported explanation invokes a 'stealth toxicity' model: NPs are sufficiently small to translocate across the gut epithelium through transcytosis or paracellular routes without triggering the pattern-recognition receptors that normally respond to foreign particles at the apical epithelial surface. The result is systemic distribution of NPs, particularly to fat body, hemolymph, and potentially reproductive organs, causing lethal cytotoxic damage before a mounted immune response generates the measurable inflammatory signature that most studies use as a proxy for toxicity. MPs, by contrast, are large enough to be recognized as foreign at the gut surface, triggering an immune response that is measurable (and therefore constitutes the 'effect' in studies using immune activation as their endpoint) while causing less systemic penetration.

This model has a critical methodological implication: studies that use immune gene expression or hemocyte activation as their primary toxicity endpoint may systematically underestimate NPs risk, because the absence of immune activation does not indicate biological safety. It may instead mean that NPs have bypassed the immune detection system entirely. This concern is likely to become more ecologically relevant over time, since continued MPs fragmentation should, in principle, keep expanding the environmental inventory of NPs; we are not, however, aware of long-term monitoring data that directly tracks this trend.

5.5 THE LABORATORY–FIELD DISCONNECT: THREE DIMENSIONS OF ECOLOGICAL IRRELEVANCE

The translation of laboratory toxicity findings to ecological risk assessment is impeded by three structural disconnects that the present review has systematically documented. Each represents a resolvable research design problem rather than an inherent limitation of ecotoxicological methodology.

Polymer composition is the first of these disconnects. The near-universal use of virgin PS and PE microspheres in laboratory studies reflects experimental convenience, driven by commercial availability, fluorescent labeling options, and extensive published baseline data,

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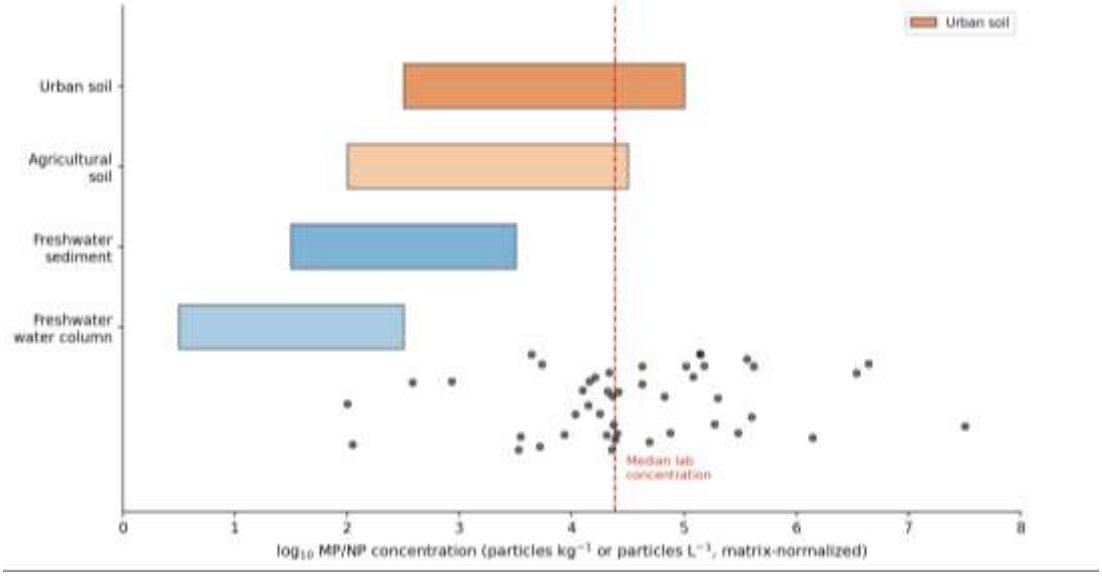
rather than ecological representativeness. Environmental MP profiles are dominated by PP (from packaging and textiles), PET (from bottles and synthetic fibers), and PA (from fishing gear and textiles), all of which are substantially underrepresented in controlled experiments. A minimum standard for ecological relevance should require that exposure studies include at least one polymer type selected on the basis of abundance in the target organism's habitat, not solely on the basis of experimental convenience.

Concentration is the second disconnect, and the methodological quality assessment reveals that 62% of included laboratory studies used concentrations that exceed the highest documented environmental levels by at least one order of magnitude, and 28% used concentrations exceeding environmental levels by three or more orders of magnitude (Figure 7). High-concentration designs are not, by themselves, a flaw: such experiments can establish proof-of-concept toxicity mechanisms and identify relevant endpoints. But the ecological interpretation of such findings requires explicit acknowledgment of the concentration gap and ideally includes a concentration-response series that spans the range from sub-environmental to supra-environmental levels, enabling extrapolation in both directions.

Figure 7

Comparison of laboratory exposure concentrations versus documented environmental MP concentrations by habitat type. Log-scaled x-axis. Colored bands represent the range of concentrations documented in field surveys for freshwater sediment, freshwater water column, agricultural soil, and urban soil (data from [48–50]). Black dots represent individual laboratory study concentrations extracted from included studies. Demonstrates that the majority of laboratory exposures exceed environmental concentrations by 1–3 orders of magnitude. ChatGPT was used for figure design and graphical refinement.



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The third disconnect concerns particle morphology. Commercial PS microspheres are the dominant particle shape in laboratory experiments (>55% of studies), yet environmental MPs are overwhelmingly irregular fragments, fibers, and films. Shape determines gut retention time, surface-area-to-volume ratio, mechanical abrasion of the gut epithelium, and the probability of physical blockage, attributes which differ substantially between spheres and environmental morphologies. The insect ecotoxicological literature would benefit from the adoption of ISO standardized fiber and fragment morphologies for use in controlled experiments, enabling cross-study comparison while improving ecological realism.

5.6 ONTOGENETIC TRANSFER: ECOLOGY OF CROSS-ECOSYSTEM CONTAMINATION

MP ontogenetic transfer across metamorphosis has now been documented in at least eight species, spanning Diptera, Hymenoptera, Odonata, and Orthoptera. This pathway carries some of the clearest ecological weight in the reviewed corpus, since it is the only mechanism identified here through which insects move plastic particles across their own metamorphosis and into other food webs. The existence of this pathway transforms insects from endpoints of plastic contamination to vectors actively redistributing particles across ecosystem boundaries. Figure 8 synthesizes these pathways, mapping polymer type through exposure route and tissue compartment to documented toxicological endpoint categories across the reviewed corpus.

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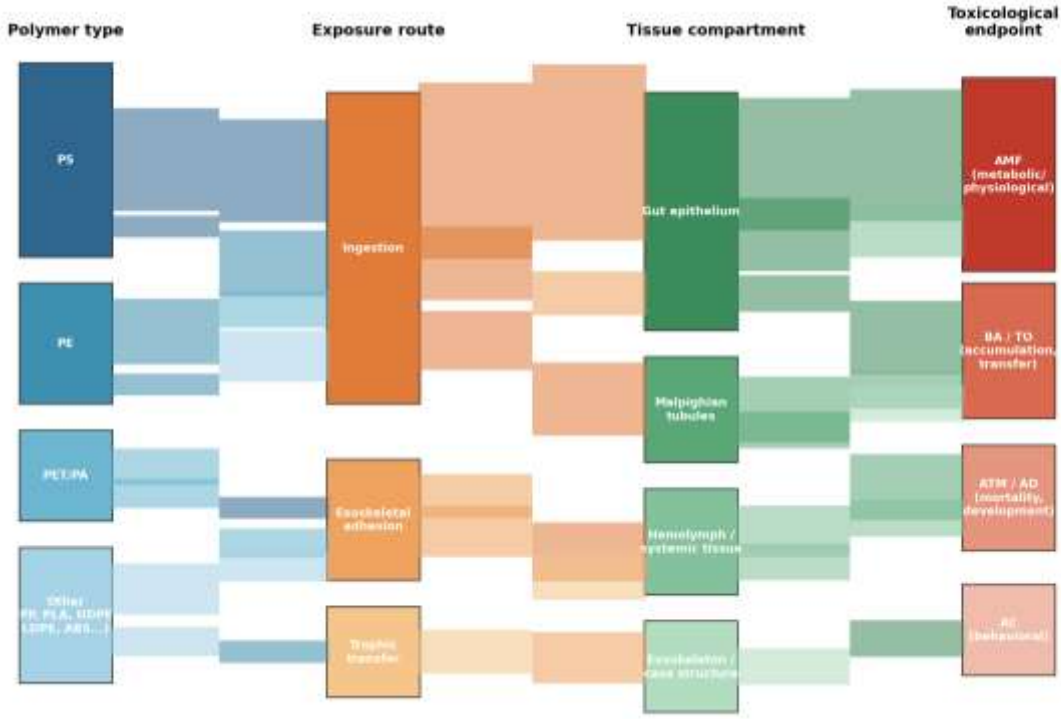
Figure 8

Sankey diagram of MP/NP exposure pathways to toxicological endpoints in insects. Left nodes: polymer type; central nodes: primary exposure route (ingestion, exoskeletal adhesion, trophic transfer); intermediate nodes: primary tissue compartment (gut epithelium, Malpighian tubules, hemolymph, exoskeleton); right nodes: documented toxicological endpoint category. Link width proportional to number of studies documenting each pathway. Constructed from all 57 included studies. ChatGPT was used for figure design and graphical refinement.



At the individual level, the consequences of particle retention in adults remain incompletely characterized. Studies documenting ontogenetic transfer (*C. pipiens*, *Aedes* spp., *C. riparius*, *A. mellifera carnica*) generally found no acute physiological effects in adults carrying particles transferred from larval stages. However, this finding should not be interpreted as evidence of harmlessness: particles retained in adult fat body or reproductive organs could affect overwintering energy reserves, egg quality, or sperm viability over timescales that standard post-emergence observation windows (typically 1–7 days) cannot detect. The female-specific reduction in oviposition documented in *D. melanogaster* under PA and PET exposure is consistent with reproductive toxicity not captured by survival endpoints alone.

At the population level, one of the corpus's more ecologically consequential signals is the phenological disruption seen in *C. sancticaroli*, where males emerged earlier than females under PE exposure. Emergence-timing shifts of this kind are, in seasonally synchronous

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aquatic insects more broadly, a plausible route to skewed adult sex ratios and reduced mating encounter rates, so the effect could, in principle, extend to effective population size. That said, this review's underlying data come from a single laboratory study, and no population-level modeling was performed to confirm that link for *C. sancticaroli* itself. Whether this pattern generalizes to other chironomid species or other polymers, and whether it translates into a measurable demographic effect under field conditions, is unknown and constitutes a high-priority research question.

At the ecosystem level, the trophic transfer pathway reported by Arnold et al. [10] (preprint), in which MPs from contaminated aquatic insects were detected in the digestive contents of insectivorous vertebrates, suggests that insects can function as a cross-ecosystem conduit for plastic particles, redistributing contamination from aquatic to terrestrial food webs. This finding still requires peer-reviewed confirmation. Alma et al. [32] extended this pathway in a different direction, demonstrating PET fiber transfer from larval to adult bees and from adults to honey and wax, establishing a human exposure route through apicultural products. The quantitative contribution of insect-mediated trophic transfer to total vertebrate MPs body burdens is currently unknown, but stable isotope tracing or mass balance modeling could make this a tractable research question.

5.7 THE TERRESTRIAL ENTOMOFAUNA GAP: A SYSTEMIC BLIND SPOT

Field data on terrestrial insects are almost nonexistent: only one of the 57 studies in this corpus addresses them. That scarcity does not reflect low contamination; rather, it reflects how plastic pollution research has been conceptually framed, treating terrestrial ecosystems mainly as transitional conduits moving particles toward marine environments as the ultimate sink. This framing is ecologically inaccurate and has produced a systemic blind spot with direct consequences for the assessment of risks to insect communities that dominate terrestrial ecosystem functions.

Evidence from soil science is unambiguous: MP concentrations in agricultural soils receiving sewage sludge applications, atmospheric deposition, or irrigation with contaminated water rival or exceed concentrations in many freshwater systems [48]. Soil-dwelling insect larvae, including the larval stages of most holometabolous orders: Coleoptera (the richest order noted above), Diptera (many families), Hymenoptera (ants and ground-nesting bees), and Lepidoptera (root and stem borers), are in direct, continuous contact with soil substrates that can carry substantial MP loads. The complete absence of toxicological studies targeting these communities is a striking omission given how directly it bears on the species that carry

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out most decomposition and soil ecosystem services; the research community has, in effect, studied the conduit and neglected the destination.

The single included terrestrial field study, Zhu et al. [44], detected PP, PA, PAM, PE, and PET in *A. chinensis* with detection frequencies of 68–88% across four Chinese cities, but did not assess any toxicological endpoints. This result establishes that contamination occurs but says nothing about biological consequences. Transforming this from a detection exercise to a risk assessment requires experimental designs that quantify exposure via the soil-organism interface, identify endpoints relevant to xylophagous and root-feeding guilds, and connect laboratory findings to the ambient concentrations documented in urban and agricultural soils.

5.8 RESEARCH PRIORITIES FOR THE NEXT DECADE

Based on the evidence synthesized and the gap analysis presented, five research priorities are identified in order of urgency and ecological significance:

1. Multigenerational chronic exposure studies: No included study assessed MP effects across insect generations. Given that global plastic contamination is a permanent environmental stressor rather than a transient pulse, the ecological consequences of sustained, multigenerational exposure are the most policy-relevant and least characterized dimension of MPs ecotoxicology. OECD-adjacent protocols for *C. riparius* and *D. melanogaster* should be the first targets for standardization.

2. Terrestrial insect ecotoxicology, especially Coleoptera: Soil-dwelling beetle larvae represent the largest untested ecological guild in this review. A systematic toxicological screening program targeting 3–5 ecologically contrasting coleopteran families (scarabs, ground beetles, bark beetles, dung beetles) across realistic soil MP concentrations would produce the highest marginal return on research investment.

3. Mechanistic studies on tolerance in *H. illucens* and *Chaoborus* spp.: Understanding why these species resist MPs toxicity at concentrations harmful to others would advance both fundamental insect physiology and applied ecotoxicology. Comparative gut microbiome sequencing, epithelial architecture analysis, and xenobiotic metabolism enzyme profiling across tolerant and sensitive species should be conducted using matched exposure protocols.

4. Quantitative trophic transfer at population and ecosystem scale: The qualitative demonstration that MPs transfer from aquatic insects to terrestrial vertebrates must be followed by quantitative estimates of transfer efficiency, accumulation factors across trophic

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levels, and population-level significance. Stable isotope-labelled MPs in controlled mesocosm experiments offer a tractable approach.

5. Standardized particle characterization and exposure protocols: An ISO/OECD working group on MPs toxicological testing in insects, tasked with specifying minimum particle characterization requirements, ecologically relevant concentration ranges by habitat type, standard exposure durations by life stage, and mandatory endpoint categories, is the single intervention that would produce the greatest improvement in cross-study comparability and cumulative evidence quality.

6 CONCLUSIONS

Taken together, the 57 studies synthesized here leave little room for a one-line verdict on whether plastic particles harm insects. Section 5.1 already set out why: outcome depends on the joint combination of polymer, particle size, species physiology, life stage, and concentration, so what follows here is not a restatement of that rule but what it leaves us able to say with confidence once it is applied. Larval stages consistently bear the greatest physiological burden. Gut epithelial damage, oxidative stress, disrupted emergence, and confirmed bioaccumulation in Malpighian tubules recur across taxonomically distant orders. Adults that retain particles transferred from larval exposure, by contrast, rarely show acute effects; their longer-term reproductive and fitness consequences remain uncharacterized.

The confirmed tolerance of *H. illucens* and *Chaoborus* spp. at concentrations that harm other taxa is scientifically significant precisely because its mechanism is still unknown, and it deserves targeted investigation rather than being treated as a curiosity. The *B. mori* data carry a broader methodological warning: if immune-activation endpoints stay silent exactly when nanoplastic damage is most lethal, studies that rely on immune markers alone risk reading nanoplastic exposure as safer than it is — a caution that should inform endpoint selection field-wide, not only the interpretation of this dataset.

These findings sit on an evidence base of uneven quality: 25% of included studies were Low Quality, and 62% failed to justify exposure concentrations against environmental field data, representing the single most common shortcoming identified. Of the five priorities detailed in Section 5.8, closing the terrestrial and Coleoptera gap is the one most likely to change the field's risk picture, since it is currently based on a single detection-only study rather than any toxicological data at all. Standardizing particle characterization, as an ISO/OECD working group could do, is what would let the other four priorities actually accumulate into comparable evidence rather than isolated case reports.

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The documented transfer of PET microfibers from *A. mellifera* adults into honey and wax, together with preliminary, not yet peer-reviewed, field evidence that microplastics move from aquatic insects to their vertebrate predators [10] (preprint), points to exposure pathways that are not yet incorporated into food-safety risk assessment and merit regulatory attention once confirmed.

Plastic contamination of insects is a documented, ecologically structured reality. The field's priority should now shift from confirming that contamination occurs to characterizing its population-level consequences and building the standardized evidence base that ecological risk assessment requires.

AUTHOR CONTRIBUTIONS

Fernandes, M.R.: Data curation; Formal analysis; Investigation; Writing – original draft. Caetano, R.L.: Conceptualization; Methodology; Data curation; Formal analysis; Writing – original draft. Barbosa, J.V.: Data curation; Formal analysis; Visualization; Writing – original draft. Beck, L.C.N.H.: Conceptualization; Methodology; Data curation; Formal analysis; Writing – original draft; Writing – review & editing. Pinto, Z.T.: Conceptualization; Methodology; Supervision; Data curation; Formal analysis; Writing – original draft; Writing – review & editing. All authors reviewed and approved the final manuscript.

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DATA AVAILABILITY

Data extraction tables, individual methodological quality scores for all 57 studies, and supplementary analytical materials are available from the corresponding author upon reasonable request and will be deposited in Zenodo upon manuscript acceptance.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE MANUSCRIPT PREPARATION PROCESS

During the preparation of this work, the authors used OpenAI's ChatGPT as an AI-assisted tool to support the visual design and graphical refinement of figures, including improvements in layout, organization, and visual presentation. The scientific content, underlying data, results, and interpretation represented in the figures were provided, verified, and validated by the authors. The AI-assisted tool was not used to generate, modify, or fabricate experimental data or scientific results. All AI-assisted outputs were critically reviewed and edited by the authors, who take full responsibility for the accuracy, integrity, and content of the published work.

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