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Health Effects of Chronic Low-Dose Exposure to Pesticide Mixtures: A Systematic Review

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Abstract

Chronic human exposure to pesticide mixtures, even at low doses, is widespread but remains poorly characterized in conventional risk assessment, which largely focuses on single compounds. This systematic review synthesizes current evidence on the health effects of long-term exposure to pesticide mixtures. Bibliographic searches were conducted in PubMed Central, ScienceDirect, and Google Scholar using predefined PICO criteria and structured search equations. After screening 608 records and applying eligibility criteria, 63 experimental and epidemiological studies addressing chronic, low-dose exposure to pesticide mixtures were included. Across diverse designs and biological models, the reviewed studies consistently reported multi-system toxicity. Documented outcomes included metabolic disturbances (glucose and lipid dysregulation, hepatic steatosis, diabetes risk), enzymatic alterations (CYP induction, AChE/BChE inhibition), neurological and neurobehavioral effects, oxidative stress, genotoxicity, epigenetic alterations, endocrine disruption, reproductive toxicity, respiratory impairment, and histopathological damage in hepatic, renal, hematopoietic, and reproductive tissues. Several studies also suggested emerging multidrug resistance phenomena following chronic pesticide-mixture exposure. Mechanistically, oxidative stress, mitochondrial dysfunction, endocrine and immune dysregulation, DNA damage, and persistent epigenetic reprogramming emerged as recurrent pathways, often amplified by additive, synergistic, or non-linear interactions within mixtures. Methodological heterogeneity, incomplete exposure characterization, and limited multi-

generational data remain important constraints, particularly for vulnerable populations such as agricultural workers, children, and pregnant women. Nevertheless, the overall convergence of findings underscores that chronic exposure to pesticide mixtures cannot be considered innocuous, even at doses individually deemed acceptable. These results highlight the urgent need to integrate mixture toxicity and cumulative risk assessment into regulatory frameworks, strengthen environmental and occupational monitoring, and promote safer pest-management strategies to better protect public and environmental health. Despite the overall convergence of findings, the interpretation of results remains constrained by methodological heterogeneity, variable exposure assessment, and limited multigenerational and longitudinal data.

Keywords

Pesticide Mixtures, Chronic Low-Dose Exposure, Genotoxicity, Endocrine Disruption, Oxidative Stress

1. Introduction

1.1. General Context of the Topic

Pesticides, chemical substances designed to prevent, destroy, or control harmful organisms, play a central role in modern agricultural practices. Their use increases yields, secures food production, and ensures crop quality [1]. Beyond agriculture, these products are also applied in public health, forestry, fisheries, and urban areas, particularly for vector control and the management of domestic pests [2].

However, the widespread presence of pesticides raises major concerns regarding their potential impacts on human health and the environment [3]. Intensive and sometimes inappropriate use of these substances leads to their dissemination in soil, air, water, and food, thereby exposing the entire population to low-dose residues [4].

1.2. Scientific and Socio-Economic Importance of the Topic

From a socio-economic perspective, pesticides remain essential tools for global food security and the fight against agricultural losses, especially in developing countries where production systems still heavily rely on their use [5]. Nevertheless, their contribution to sustainable development is increasingly questioned in light of growing scientific evidence of their adverse effects on human health and biodiversity.

Pesticides have been associated with a wide range of harmful effects, including endocrine disruption, respiratory and cancerous diseases, and neurological, immune, and reproductive disorders [6] [7]. However, these effects are often non-specific and difficult to attribute to a single substance, highlighting the complexity of actual chemical exposures. These effects vary according to dose, duration, route of exposure, and individual characteristics such as age, sex, and pre-existing health

conditions. Understanding these mechanisms is essential to developing more protective prevention and regulatory strategies.

1.3. Key Previous Studies

Research on the health impacts of pesticides has historically focused on evaluating the individual effects of isolated molecules. Such approaches have led to the identification of major pesticide classes (organochlorines, organophosphates, carbamates, pyrethroids) and their mechanisms of action, particularly on the nervous and endocrine systems [8] [9].

Epidemiological and toxicological studies have demonstrated associations between chronic pesticide exposure and increased risks of neurodegenerative diseases, cancers, and hormonal disorders [10] [11]. However, most of these studies examine single substances, while mixture exposures are multiple and simultaneous [10] [12] [13].

1.4. Identified Gaps in the Literature

One of the major challenges in environmental toxicology lies in the complexity of pesticide mixtures to which individuals are exposed. In real-world conditions, agricultural and non-agricultural populations encounter cocktails of residues resulting from the combined or successive application of multiple products [14] [15].

These mixtures can interact synergistically or additively, altering overall toxicity and making health outcomes difficult to predict [4]. Yet, most regulatory and scientific assessments continue to rely on single-substance analyses, without integrating the cumulative dimension of exposure.

Furthermore, current regulatory frameworks, whether international (Codex Alimentarius, Stockholm, Rotterdam, and Basel Conventions) or national (chemical management plans, Maximum Residue Limits), are still poorly adapted to assessing the risks related to low-dose multiple exposures [16] [17]. This limitation reduces the ability to anticipate emerging risks and effectively protect public health.

In addition, emerging frameworks such as cumulative risk assessment and exposome-based approaches remain insufficiently integrated into current pesticide risk evaluation strategies.

1.5. Purpose and Objectives of the Review

In light of these observations, the present review aims to synthesize current knowledge on the health impacts of chronic exposure to low doses of pesticide mixtures. Specifically, it seeks to:

- examine exposure pathways and health effects associated with long-term exposure;
- analyze toxic interactions among compounds within mixtures;
- identify existing methodological and regulatory limitations; and
- propose directions for future research and integrated risk management.

This review ultimately aims to contribute to a broader and more critical understanding of the combined effects of pesticides, thereby supporting stronger prevention and risk assessment policies for sustainable public health protection.

2. Methodology

2.1. Objective of the Study

The objective of this review is to conduct a systematic synthesis of the existing literature on the health effects of chronic, low-dose exposure to pesticide mixtures.

The review serves as the central methodological approach, allowing for the rigorous and comprehensive aggregation, evaluation, and analysis of results drawn from a wide range of scientific publications.

This method provides a coherent overview of the available data and highlights emerging trends related to the health impacts of pesticide mixtures.

2.2. Methodological Approach

The reproducibility of this research is ensured through a transparent and systematic approach, documenting each stage of the process, from the literature search to the synthesis of data, so that other researchers can trace and evaluate the methodology with full transparency [18].

Among the various methods available for literature synthesis, a systematic review was selected because of its ability to combine the results of multiple primary studies addressing the same research topic.

This study was conducted following six main stages:

1) Formulation of the research question;

2) Search and identification of relevant literature;

3) Selection of studies according to predefined eligibility criteria;

4) Data extraction;

5) Synthesis and analysis of results;

6) Interpretation and discussion of findings.

This sequence ensures methodological rigor, validity of results, and reproducibility of the overall process. The review was conducted in accordance with established reporting guidelines for systematic reviews, including the PRISMA framework.

Due to substantial heterogeneity in study designs, exposure metrics, and outcome measurements, a formal meta-analysis was not feasible. Therefore, a qualitative synthesis approach was adopted.

2.3. Selection Criteria

The selection of studies included was based on specific eligibility criteria, as follows:

- Observational or experimental studies;
- Research explicitly addressing the health impacts of pesticide mixtures;
- Studies assessing chronic exposure rather than acute exposure;

- Original research articles published in peer-reviewed scientific journals;
- Availability of the full text;
- Presence of sufficient data for extraction and analysis.

Review articles, editorials, short communications, and studies focusing exclusively on individual pesticides without consideration of mixtures were excluded from the analysis.

For the purpose of this review, "low-dose" exposure was strictly defined according to toxicological safety standards. It refers to pesticide concentrations that are:

- Below or equal to established No Observed Adverse Effect Levels (NOAELs) in experimental models;
- Within the range of Acceptable Daily Intakes (ADIs) or Maximum Residue Limits (MRLs) for human populations;
- Reflective of real-world environmental background exposure, this excludes cases of acute poisoning or high-dose accidental spills.

In epidemiological studies, this includes chronic, long-term exposure to residual levels found in diet, water, or ambient air, as well as chronic occupational exposure where health outcomes result from prolonged low-level accumulation rather than acute toxicity.

This definition excludes acute poisoning scenarios and high-dose accidental exposures. However, some occupational studies were retained when they reflected chronic, repeated exposure patterns with biomarker or health outcomes indicative of cumulative low-dose effects rather than acute toxicity.

2.4. Formulation of the Research Question

The research question was developed using the PICO framework (Population - Intervention/Exposure - Comparison - Outcome), a method commonly applied in systematic reviews to structure the research problem and define inclusion parameters.

Based on this conceptual framework, the research question was formulated as follows:

What health risks are associated with chronic, low-dose exposure to pesticide mixtures?

This formulation served as the foundation for constructing the search equation, which was based on carefully selected keywords in both English and French to ensure comprehensive coverage across international databases.

2.5. Information Sources and Search Equations

The bibliographic data were collected from three major electronic databases:

- PubMed Central (PMC)
- ScienceDirect
- Google Scholar

Each database was queried using tailored search equations, combining selected keywords through Boolean operators (AND, OR).

These search strategies were designed to maximize the retrieval of relevant publications while maintaining specificity to studies addressing the health impacts of chronic, low-dose exposure to pesticide mixtures.

The details of structuring the research question according to the PICO method, as well as the search equations applied to each database, are summarised in Table 1.

Table 1. PICO method and search equations.

PICO Concept	Description
Population (P)	Individuals chronically exposed to low doses of pesticide mixtures
Intervention/Exposure (I)	Chronic exposure to low doses of pesticide mixtures
Comparison (C)	Absence or low level of exposure
Outcome (O)	Health effects on humans
Keywords Used	
Category	**Main Terms (in English)**
Exposure	Chronic exposure, long-term exposure, low dose, low level
Pesticide mixtures	Mixture of pesticides, Pesticides mixture, Pesticide mixtures, Pesticide mix, Pesticide cocktail, Combinations of pesticides, Mixture of herbicides, Mixture of fungicides, Mixture of insecticides, Mixture of organochlorines, Mixture of organophosphates, Mixture of carbamates, Mixture of pyrethroids
Health effects	Health impact, health effects
Search Equations Applied	
Database	**Search Equation**
Google Scholar	allintitle: "pesticides mix" OR "mixture of pesticides" OR "cocktail of pesticides" OR "combinations of pesticides" OR "mixture of herbicides" OR "mixture of fungicides" OR "mixture of insecticides" OR "mixture of organochlorines" OR "mixture of organophosphates" OR "mixture of carbamates" OR "mixture of pyrethroids"
PubMed Central (PMC)	("chronic exposure" [All Fields] OR "long-term exposure" [All Fields]) AND ("pesticide mixtures" [All Fields] OR "pesticide mixture" [All Fields]) AND ("low dose" [All Fields] OR "low level" [All Fields])
ScienceDirect	("mixture of pesticides" OR "pesticide cocktail" OR "pesticide mix") AND ("chronic exposure" OR "long-term exposure") AND ("low dose" OR "low level") AND ("health impact" OR "health effects")

2.6. Study Selection Process

The study selection process is illustrated in Figure 1, which presents the PRISMA flow diagram (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). This diagram summarizes the various steps of study identification, screening, and inclusion undertaken in this review.

A total of 608 potential records were identified across the three databases searched:

- Google Scholar (n = 266)
- PubMed Central (PMC) (n = 188)

- ScienceDirect (n = 154)

After removing 42 duplicates, 566 unique articles remained for title and abstract screening. Following this initial screening, 124 studies were selected for full-text evaluation to determine their eligibility according to the predefined inclusion criteria.

At this stage, 63 studies met all eligibility requirements and were retained for qualitative synthesis, while 61 were excluded for reasons such as:

1) single-compound focus,
2) acute rather than chronic exposure,
3) insufficient health outcome data, or
4) non-original publication type (e.g., reviews or editorials).

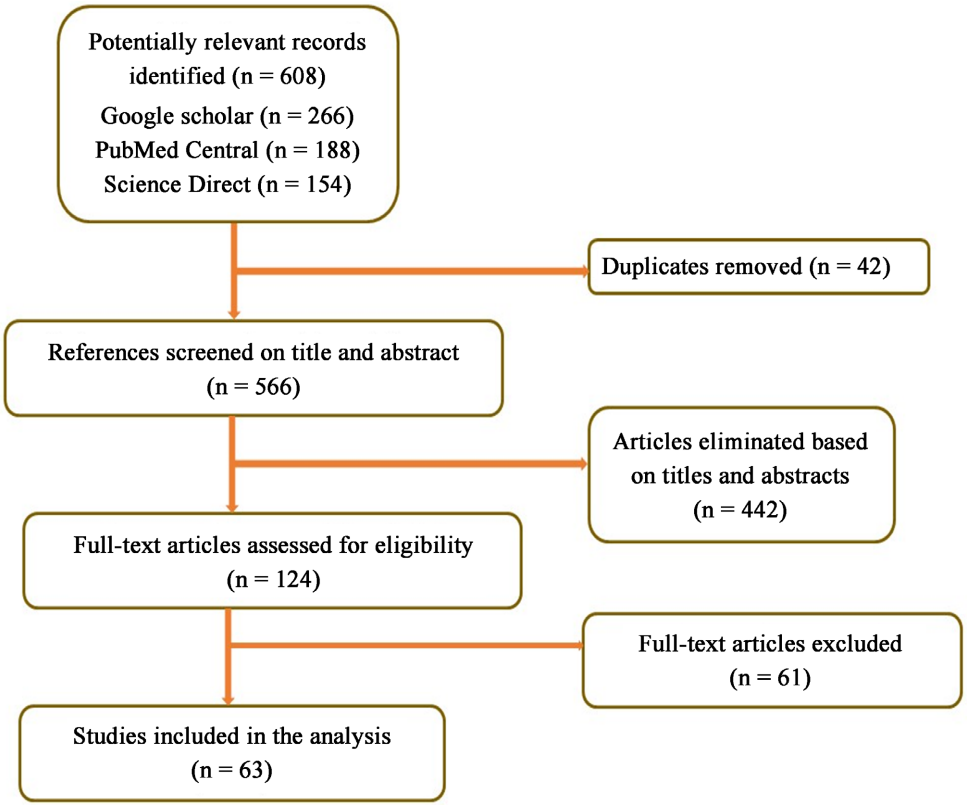

Figure 1. PRISMA flow diagram of the study selection process.

The flowchart depicts the sequential steps of identification, screening, eligibility assessment, and inclusion of the studies considered in the systematic review.

3. Results

3.1. Description of Included Studies

A total of 63 studies met the inclusion criteria and were included in this review. These investigations were conducted across diverse geographical regions and encompass a wide range of methodological designs, reflecting the complexity of assessing chronic low-dose exposure to pesticide mixtures.

Overall, the dataset comprises:

- 29 experimental studies, including 27 *in vivo* models (conducted on mammals, fish, and insects) and 2 *in vitro* assays.
- 34 epidemiological studies, consisting of 17 cross-sectional studies, 5 cohort studies, 4 case-control studies, 3 longitudinal studies, and 5 other observational or field designs.

The selected studies address a broad spectrum of health outcomes, including reproductive toxicity, genotoxicity, metabolic disturbances, oxidative stress, endocrine disruption, respiratory effects, neurobehavioral impairments, and mechanisms of antimicrobial or drug resistance.

This thematic diversity highlights both the complexity of pesticide mixture toxicology and the heterogeneity of research approaches used to investigate their health effects.

A complete list of the 63 included studies, including authors, study titles, and study designs, is provided in Supplementary Table S1 (General Overview of Included Studies).

3.2. Characteristics of the Included Studies

The 63 studies retained for this review encompass a broad range of experimental and epidemiological designs, reflecting the complexity of assessing chronic low-dose exposure to pesticide mixtures. The evidence base includes *in vivo* studies (n = 27) that examined toxicological mechanisms and physiological alterations in animal models, *in vitro* investigations (n = 2) focused on cellular and biochemical responses, and epidemiological studies (n = 34) composed of cross-sectional, cohort, longitudinal, and case-control designs.

Across these studies, a wide spectrum of health outcomes was documented. Overall, most epidemiological studies were rated as having a moderate risk of bias, primarily due to exposure misclassification and reliance on self-reported data, while experimental studies showed variable quality depending on study design and reporting completeness.

Recurrent endpoints include metabolic disorders, neurological and neurobehavioral impairments, genotoxic and oxidative stress responses, endocrine disruption, reproductive and developmental toxicity, respiratory disturbances, and early indications of antimicrobial or multidrug resistance mechanisms. The thematic distribution of results highlights that pesticide mixtures are linked to multisystemic effects, often more pronounced than those observed for individual compounds, suggesting additive, synergistic, or non-linear interactions.

A series of detailed tables (Tables 2-11) synthesizes the findings by major health outcome categories, each presenting the mixtures investigated, the biological models, Methodological approaches, and the main toxicological or clinical effects observed.

Summary of Observed Effects

A summary of the main categories is provided in Table 12.

Table 2. Metabolic effects of chronic low-dose exposure to pesticide mixtures.

Pesticide Mixtures	Biological Models	Methods/Exposure Conditions	Key Metabolic Findings	Reference
Atrazine, Chlorpyrifos, Endosulfan	Female C57BL/6J mice	Dietary exposure from gestation to 11 weeks post-weaning; metabolomic analyses	Hematological disturbances; alterations in cellular metabolism	Demur *et al.*, 2013 [19]
Cypromazine, MCPB, Pirimicarb, Quinoclamine, Thiram, Ziram	Wistar rats	Exposure from gestational day 7 to postnatal day 16; body-weight monitoring; diabetes evaluation; Nmbr, Nby1r, and Lepr gene sequencing	Reduced body weight; decreased insulin and glucagon secretion; altered maternal leptin levels	Svingen *et al.*, 2018 [20]
Acetamiprid + Difenoconazole	Asian honeybee	10-day dietary exposure; gut-microbiota profiling; detoxification/immunity-gene sequencing	Altered gut microbiota composition; dysregulation of detoxification and immune-related genes	Han *et al.*, 2023 [21]
Difenoconazole (DIF) and Tebuconazole (TEB)	Zebrafish embryos	Five-day embryonic exposure; additive interaction modeling	Embryotoxicity; metabolite accumulation; disrupted steroidogenesis; metabolic disturbances	Jiang *et al.*, 2022 [22]
Boscalid, Captan, Chlorpyrifos, Thiofanate, Thiacloprid, Ziram	C57BL/6J mice (normal and CAR-deficient)	52-week dietary exposure; metabolic, hepatic, histological, transcriptomic, and metabolomic analyses	Sex-specific obesity/diabetes effects; stronger effects in males; partial involvement of CAR	Lukowicz *et al.*, 2018 [23]
Acetochlor, Bromoxynil, Carbofuran, Chlormequat, Ethephon, Fenpropimorph, Glyphosate, Imidacloprid	Wistar rats	Gestational day 4 to day 21 exposure; blood, liver, and brain metabolomics (NMR, GC)	Disturbed energy, lipid, and amino acid metabolism; increased oxidative stress; and mitochondrial dysfunction in F0 and F1	Bonvallot *et al.*, 2018 [24]
Malathion + Terbuthylazine	Zebrafish	Low-dose aquatic exposure; ROS, TBARS, LDH, PCR assays	Mitochondrial lesions; metabolic disruption; stronger combined toxicity	Khatib *et al.*, 2023 [25]
Boscalid, Captan, Chlorpyrifos, Thiacloprid, Thiophanate, Ziram	C57BL/6J mice	Perinatal DJT-level dietary exposure during gestation and lactation; metabolomics, biochemistry, and RT-qPCR	No increased susceptibility to diet-induced metabolic disorders; altered urinary and fecal metabolic fingerprints.	Smith *et al.*, 2020 [26]
β-HCH, Chlordane, DDT, HCB, Heptachlor	Zebrafish	12-week exposure; LC-MS/MS residue analysis; proteomics	Non-linear disturbances in metabolic and mitochondrial pathways; type-2-diabetes-like metabolic signatures	Gao *et al.*, 2022 [27]
Chlorpyrifos, Malathion, Parathion	300 human participants	≥6-month chronic exposure; GC-MS blood pesticide quantification	Hypertension, hyperglycemia, obesity, and dyslipidemia—consistent with metabolic syndrome	Leonel Javeres *et al.*, 2021 [28]
Multiple organochlorines (β-HCH, DDT, HCB…)	914 elderly Chinese individuals (≥65 years)	GC-MS/MS pesticide-residue quantification	Increased type-2 diabetes risk, predominantly in women	Chen *et al.* 2024 [29]
Imidacloprid,	Helix aspersa	21-day dietary exposure;	Reduced body weight and shell	Zouaghi *et*

Acetamiprid	snails	physiological, biochemical, and enzymatic measurements	diameter; decreased hepatic/renal carbohydrates; increased total proteins and catalase	*al.*, 2020 [30]
Triazophos + Fenvalerate	Male AB zebrafish	60-day aquatic exposure; 16S rRNA profiling; hepatic transcriptomics	Strong synergistic toxicity; liver damage; gut-liver axis disruption; ↑ apoptotic genes (p53, bax); ↓ anti-apoptotic genes (bcl-2)	An *et al.*, 2024 [31]
Dichlorvos, Dicofol, Dieldrin, Endosulfan, Permethrin	Female Sprague-Dawley, Wistar, and Lewis rats	10-week dietary exposure; body weight, estrous cycle, histopathology	Slowed weight gain; increased liver weight and hepatocytic hypertrophy; disrupted estrous cycle; fewer ovarian follicles	Pascotto *et al.*, 2015 [32]

Table 3. Enzymatic effects associated with chronic exposure to pesticide mixtures.

Pesticide Mixtures	Biological Models	Methods/Exposure Conditions	Key Metabolic Findings	Reference
Cyproconazole, Epoxiconazole, Prochloraz	Adult male Wistar rats	Dietary exposure for 28 days Assessment of liver disease markers Biochemical and histopathological analyses Evaluation of gene expression and enzymatic activity	Greater increase in relative liver weight More pronounced effects on gene expression and enzyme activities were observed for the mixture compared with the individual compounds.	Heise *et al.*, 2018 [33]

Table 4. Neurological and neurobehavioral effects.

Pesticide Mixtures	Biological Models	Methods/Exposure Conditions	Key Findings	Reference
16 insecticides, 7 herbicides, and 4 fungicides	353 participants (290 exposed, 63 controls)	• Exposure >20 years (1 - 9 y: 8.2%; 10 - 30 y: 47.2%; >30 y: 34.4%) • LC-MS/MS for pesticide residues • RP-HPLC for AChE • Assessment of NCDs	• Lower AChE in exposed farmers • Symptoms correlated with exposure (fatigue, dizziness) • Association with obesity and reduced AChE • Links with Alzheimer's, Parkinson's, and diabetes	Kumar *et al.*, 2023 [34]
Diazinon, Dimethoate, Azinphos-ethyl, Chlorpyrifos, Malathion, Acephate, Parathion, Methamidophos, Carbofuran, Methomyl, Oxamyl, Aldrin, Dicofol, Endosulfan, Cyfluthrin	54 agricultural workers	• Mean exposure: 5.36 ± 0.43 y • Comet assay (primary DNA damage) • Micronucleus test (buccal/lymphocytes) • AChE & BChE assays	• Significant DNA damage • Slight decrease in cholinesterase activity	Valencia-Quintana *et al.*, 2021 [35]
Organophosphates, Glyphosate, Paraquat, Urea herbicides, Triazoles, Dithiocarbamates, Carbamates, Organochlorines, Pyrethroids, and Pyrethrins	Agricultural workers	• OP exposure mean: 16.9 ± 9.7 y • Non-OP exposure: 14.9 ± 13.4 y • Urinary OP metabolites • Buccal micronucleus cytome	• Significant AChE inhibition • DNA damage	Silvério *et al.*, 2017 [36]
Organophosphates, Pyrethroids, Organochlorines, Fungicides, Paraquat, and Glyphosate	143 female agricultural workers (77	• Mean exposure: 24.1 ± 10.1 y • AChE measurement • Comet assay	• Significant AChE inhibition • Increased DNA damage	Dhananjayan *et al.*, 2019 [37]

	exposed, 66 controls)			
Two- and three-pesticide combinations: Neonicotinoid + OP; Neonicotinoid + Pyrethroid; Neonicotinoid + Novaluron; OP + Carbamate; OP + OP + Neonicotinoid; OP + OC + Neonicotinoid; OP + Pyrethroid + Neonicotinoid; OP + OC + Glyphosate	283 agricultural workers (183 exposed, 100 controls)	• Exposure: ≤5 y, 5–10 y, ≥10 y • Serum AChE measurement • Occupational spraying	• Significant reduction in serum AChE • Correlated with exposure duration, age, and BMI	Dhalla & Sharma, 2013 [38]
Alachlor, Bendiocarb, β-BHC, Clomazone, Dicrotophos, Dimethenamid, Dimethylvinphos, Diphenamid, Metribuzin, Monocrotophos, Paclobutrazole, Pentachloroaniline, Phosphamidon, Pyraclofos, Pyrimethanil, Terbacil, Myclobutanil, Furalaxyl, Isocarbamide, Methidathion, EPN	299 men aged 25 - 50	• GC-MS/MS quantification of 21 pesticides • Semen-quality evaluation • GLM, WQS, ENR, and Bayesian kernel regression	• Reduced progressive and total sperm motility	Chang *et al.*, 2024 [39]
Diquat, Imazamox, Imazethapyr, Tepraloxydim, Bentazone, Acifluorfen	Wistar rats	• Dietary exposure at 3, 6, and 12 months • Cognitive and anxiety tests	• Neurobehavioral impairment even at low doses • Non-linear responses (improved or worsened depending on dose/time)	Sergievich *et al.*, 2020 [40]
Macrocyclic lactones, neonicotinoids, pyrethroids, carbamates, and fungicides	175 greenhouse workers	• Biomarkers: TBARS, FRAS, SHT, GGT, PON1 • AChE • Low- vs. high-exposure season	• Mild increase in oxidative stress • Decreased AChE	Lozano-Paniagua *et al.*, 2018 [41]
Zineb + Endosulfan	Male C57BL/6 mice	• Postnatal exposure J5–J19 (IP) • Re-exposure at 8 months • Dopamine quantification • AChE measurement	• Altered dopaminergic system • Neurotransmitter imbalance • Increased AChE	Jia & Misra, 2007 [42]
Atrazine + Paraquat	Drosophila melanogaster	- Dietary exposure + gel strips - Climbing test - Lifespan analysis	• Synergistic reduction in female lifespan • Reduced climbing ability	Lovejoy & Fiumera, 2019 [43]
Glyphosate, Chlorpyrifos, and Copper Sulfate	Oncorhynchus mykiss (rainbow trout) embryos	• 3-week embryonic exposure • Comet assay, RT-qPCR • Assessment of mobility, DNA integrity, detoxification & repair gene induction	• Impaired larval mobility at the highest concentration • DNA damage • Reduced detoxification and DNA-repair capacity	Weeks Santos *et al.*, 2021 [44]
Endosulfan, Thiodon, Monocrotophos, Dichlorvos, Malathion, Methyl-parathion, Carbaryl, Cypermethrin	72 human participants (52 sprayers exposed, 20 unexposed	• GC-ECD analysis of OC residues • AChE & BChE activity • Hematological profile (RBC, WBC, monocytes, neutrophils)	• Significant inhibition of AChE and BChE • Hematological abnormalities: increased RBCs, WBCs, monocytes, and neutrophils	Fareed *et al.*, 2010 [45]

	controls)			
Chlorpyrifos, Thiacloprid, Captan, Thiophanate, Boscalid, Ziram	C57BL/6 mice	• 12-month dietary exposure from weaning • Brain & behavioral assessments: GFAP, EEG • Peripheral P450 gene expression (liver, kidney)	- Moderate hippocampal astrogliosis - Male-specific behavioral impairments - Activation of peripheral P450 metabolic pathways	Klement *et al.*, 2020 [46]
Chlorpyrifos + Profenofos	37 workers	• Urinary metabolite quantification • BChE and AChE blood assays • Sampling before, during, and after spraying • Statistical correlation of exposure and inhibition	• Significant inhibition of BChE and AChE	Singleton *et al.*, 2015 [47]
Acetochlor, Bromoxynil, Carbofuran, Chlormequat, Ethephon, Fenpropimorph, Glyphosate, Imidacloprid	Wistar rats	• Dietary exposure during gestation (GD4–GD21) • NMR + GC metabolomics • Oxidative-stress biomarkers • Blood, liver, and brain histology	• Disturbances in energy, lipid, and amino acid metabolism • Increased oxidative stress parameters • Mitochondrial dysfunction	Bonvallot *et al.*, 2018 [24]
Malathion + Terbuthylazine	Danio rerio(zebrafish)	• Simulated 14-day exposure • Oxidative stress biomarkers • Immune response • Apoptosis and detoxification assays	• Increased oxidative stress biomarkers • Mitochondrial structural damage • Metabolic disruptions • Stronger effects under combined exposure	Khatib *et al.*, 2023 [25]
Chlorpyrifos + DDT	Danio rerio(zebrafish)	• Chronic exposure: 5 weeks DDT → 5 weeks chlorpyrifos • Behavioral tests (sensorimotor, anxiety, predation, sociability) at 1 week and 14 months • Cholinesterase activity	• Stronger neurobehavioral alterations than single exposures • Impaired locomotion, anxiety responses, and memory defects	Hawkey *et al.*, 2021 [48]
Chlorpyrifos + Iprodione	Juvenile male Sprague-Dawley rats	• Oral gavage exposure J23–J60 (single vs. mixture) • Locomotion, anxiety, and depression tests • Brain analysis: AChE, noradrenaline, and oxidative stress	• Oxidative damage • Brain histological alterations • Mixture aggravates lesions vs. individual pesticides	Abd-Elhakim *et al.*, 2023 [49]
Chlorpyrifos, Deltamethrin, Acetamiprid, Abamectin, and Kresoxim-methyl	Male Wistar rats	• 90-day dietary exposure • Behavioral assessment • Oxidative stress: MDA, GSH • Hippocampal histology	• Neurobehavioral impairment • Neuronal loss in the hippocampus • Oxidative stress modification • Dose-dependent neurotoxicity	Ghasemnejad-Berenji *et al.*, 2021 [10]
Deltamethrin + Acetamiprid	Male Wistar rats (Rattus rattus)	• 90-day oral gavage • Quercetin treatment adjunct • Neurotransmitter assays • Brain histology • Minitab	• Clear disruption of neurotransmitters (dopamine, serotonin, adrenaline, glutamate) • Quercetin (10	Gasmi, 2020 [50]

		statistical analysis	mg/kg/day) shows a protective effect	
Epoxiconazole, Mancozeb, Prochloraz, Tebuconazole, and Procymidone	Wistar rats	• Oral gavage exposure from gestational day 7 to postnatal day 16 • Examination of reproductive organs and blood • Histopathology, sperm count • Behavioral tests	• Transgenerational effects • Reproductive developmental abnormalities: reduced prostate and epididymis weight, decreased sperm count • Spatial learning impairments	Jacobsen *et al.*, 2012 [51]
Glyphosate + Mancozeb (Ethylene Thiourea, ETU)	384 agricultural workers	• 12-month follow-up • Questionnaires and urinary biomarkers (recent exposure: 7 days; cumulative exposure: 12 months) • Pre- and post-work urine samples • Logistic regression models • Sleep-disorder assessment	• Sleep disturbances: poor sleep quality, inadequacy, increased snoring	Fuhrimann *et al.*, 2023 [52]
Diquat, Imazamox, Imazethapyr, Tepraloxydin, Bentazone, Acifluorfen	Male Wistar rats	• 9-month dietary exposure to increasing doses • Assessment of vitamin balance (deficiency/excess) • Neurobehavioral tests: maze, passive avoidance, open field	• Reduced locomotor activity • Increased anxiety linked to water-soluble vitamin imbalance	Tsatsakis *et al.*, 2019 [15]

Table 5. Oxidative stress effects.

Pesticide Mixtures	Biological Models	Methods/Exposure Conditions	Key Findings	Reference
• Macrocyclic lactones • Neonicotinoids • Pyrethroids • N-methyl carbamates • Various other insecticides • Fungicides	266 individuals (175 greenhouse workers exposed, 91 controls)	• Biological sampling at two periods of the growing season • Measurement of non-specific oxidative stress biomarkers (TBARS, FRAS, SHT, GGT, PON1) • Measurement of cholinesterase activity (AChE)	• Increased oxidative stress biomarkers • Decreased cholinesterase (AChE) activity	Lozano-Paniagua *et al.*, 2018 [41]
Environmental mixture of 46 pesticides detected in natural habitat	127 adult frogs (Leptodactylus latrans and Leptodactylus latinasus)	• Exposure through natural habitat • Pesticide residue quantification by LC-MS and GC-MS • Histological examination of muscle, testis, and kidney tissues	• Increased oxidative stress markers, especially in females • Increased hepatic glutathione • Reduced seminiferous tubule diameter in males	Brodeur *et al.*, 2022 [53]
Acrinathrin, Abamectin, Cyproconazole, Deltamethrin, Metalaxyl-M, Propamocarb, and Thiamethoxam	104 workers (52 exposed agricultural workers and 52 organic farmers as controls)	• Occupational exposure ≈ 3.7 h/day, 5–6 days/week • Blood and urine sampling • Measurement of oxidative stress biomarkers (TBARS, total glutathione) • DNA damage assessment (8-oxodG) via LC-MS/MS	• Increased oxidative stress • Increased DNA damage • Evidence of an adaptive response enhancing antioxidant defenses	Ledda *et al.*, 2021 [54]
Pesticide Mixtures	Biological Subjects	Methods/Exposure Conditions	Main Findings	
Cypermethrin	Male Wistar rats	• Exposure of 4 groups of 8 rats for	• Reduced sperm integrity,	Bouabdallah

(insecticide), Mancozeb (fungicide), and Metalaxyl (fungicide)		8 weeks to increasing doses • Measurement of reproductive parameters and oxidative-stress biomarkers • Post-mortem examination of biological alterations • Assessment of sperm quality	concentration, and motility • Increased oxidative stress • Histopathological alterations in male reproductive organs	*et al.*, 2022 [55]
Difenoconazole (fungicide), glyphosate (herbicide), and imidacloprid (insecticide)	Winter honeybees (Apis mellifera)	• Dietary exposure for 16 days • Measurement of oxidative-stress biomarkers • Assessment of oxidative tissue damage in bee bodies	• Increased mortality at intermediate concentrations • Disruption of antioxidant defenses, leading to systemic oxidative stress	Pal *et al.*, 2022 [56]
Atrazine and Glyphosate	Daphnia magna	• Exposure of two generations (F0 and F1) for 21 days • Measurement of oxidative stress biomarkers (CAT, GST) • Ecotoxicological tests	• Increased organism mortality, particularly in the F1 generation • Increased oxidative stress • Elevated catalase and glutathione-S-transferase activity	Ramsdorf *et al.*, 2021 [57]
Diclofop-methyl (herbicide) + Difenoconazole (fungicide)	Wistar albino rats (Rattus norvegicus)	• Oral exposure for 28 days (5 days/week) at increasing doses • Measurement of biochemical parameters and oxidative-stress biomarkers	• Altered biochemical parameters • Increased oxidative stress	Abd-Alrahman *et al.*, 2014 [58]
Imidacloprid + Acetamiprid (neonicotinoids)	Helix aspersa(land snail)	• Dietary exposure to increasing mixture doses for 21 days • Measurement of physiological, biochemical, and enzymatic parameters	• Physiological, biochemical, and histopathological disturbances • Reduced body weight and shell diameter • Dose-dependent depletion of hepatic and renal carbohydrates (increased energy consumption under stress) • Increased total protein levels • Increased catalase activity	Zouaghi *et al.*, 2020 [30]
Glyphosate + Paraquat + 2,4-D	93 agricultural workers	• Occupational exposure for 20 - 40 years (1–5 h/day) • Measurement of urinary biochemical markers • Assessment of oxidative-stress biomarkers	• Increased urinary creatinine levels • Increased oxidative stress biomarkers	Intayoung *et al.*, 2021 [59]

Table 6. Genotoxic effects.

Pesticide Mixtures	Biological Subjects	Methods/Exposure Conditions	Main Genotoxic Findings	Reference
Organophosphates (OP): Diazinon, Dimethoate, Azinphos-ethyl (Gusathion), Chlorpyrifos (Lorsban), Malathion, Acephate (Orthene), Parathion (Folidol), Methamidophos (Tamaron).	54 agricultural workers and 26 controls	• Occupational exposure for 1 - 10 years • Measurement of cholinesterase activity • DNA damage assessment using the Comet assay and Micronucleus test	• Significant increase in DNA damage • Slight decrease in cholinesterase activity	Valencia-Quintana *et al.*, 2021 [35]

Carbamates: Carbofuran (Furadan), Methomyl (Lannate), Oxamyl (Vydate). Organochlorines (OC): Aldrin, Dicofol (Kelthane), Endosulfan. Pyrethroids: Cyfluthrin (Baytroid).				
Malathion, phosphamidon, carbamates, carbaryl, methyl-parathion, chlordane, and heptachlor.	70 exposed agricultural workers and 70 controls	• Occupational exposure for 3–13 years • DNA damage assessment using the Comet assay in peripheral lymphocytes	• Significant increase in DNA damage among exposed workers	Hazarika & Deka, 2017 [60]
Disulfoton, Chlorpyrifos, Acephate, Dimethoate, Glyphosate, Paraquat, urea-based pesticides, triazoles, dithiocarbamates, carbamates, organochlorines, pyrethroids, and pyrethrins	94 exposed agricultural workers and 94 controls	• Mean occupational exposure: 16.9 ± 9.7 years (exposed) and 14.9 ± 13.4 years (controls) • Measurement of cholinesterase activity • Metabolite quantification (DETP, DEDTP) by GC-MS • Buccal micronucleus cytome assay for DNA damage	• Significant alterations in cholinesterase activity • Increased DNA damage	Silvério *et al.*, 2017 [36]
Dimethoate, Phosalone, Quinalphos, Profenofos, Ethion, Deltamethrin, Fenpropathrin, Dicofol, Paraquat, Glyphosate, and several fungicides	77 exposed women and 66 non-exposed controls	• Mean occupational exposure: 24.1 ± 10.1 years • Cholinesterase activity measurement • DNA damage assessment via Comet assay • Statistical analysis: t-tests and multiple regressions (confounder adjustment)	• Significant decrease in acetylcholinesterase and butyrylcholinesterase activity • Increased DNA damage	Dhananjayan *et al.*, 2019 [37]
Herbicides: 2,4-D, acetochlor, bentazone, dicamba, dichlobenil, clethodim, clomazone, etc. Insecticides: Abamectin, acetamiprid, bifenthrin, chlorpyrifos, cypermethrin, fenitrothion, etc. Fungicides: Copper oxychloride, carbendazim, chlorothalonil, difenoconazole, fluazinam, etc.	26 pesticide-exposed factory workers and 32 controls	• Mean occupational exposure: 11.56 years • Measurement of cholinesterase activity and transaminases • Chromosomal aberration assays	- Cytogenetic abnormalities, including premature centromeric division - Significant increases in chromosomal and chromatid breaks among exposed workers	Jovičić *et al.*, 2013 [61]
Atrazine, alachlor, cyanazine, 2,4-D (2,4-dichlorophenoxyacetic acid), and malathion	20 exposed pesticide-factory workers and 20 controls	- Occupational exposure for 4 - 30 years - First sampling after 8 months of exposure, second after 8 months without exposure - DNA damage and repair assessment using the Comet assay	• Significant increase in chromosomal aberrations: sister chromatid exchanges, micronuclei, and DNA damage • Partial recovery after 8 months without exposure	Garaj-Vrhovac & Zeljezic, 2001 [62]
Cypermethrin, Cyhalothrin, Deltamethrin, Endosulfan	47 exposed agricultural workers and 50 controls	• Occupational exposure for 3–20 years • Blood sampling • DNA damage evaluation using the Comet assay • Pesticide residues measured in serum via HPLC	• Significant increase in DNA damage in exposed workers	Bhalli *et al.*, 2009 [63]
Glyphosate (GLY), chlorpyrifos (CPF), and copper sulfate (Cu)	Rainbow trout	• Exposure through water for 3 weeks • Assessment of lethal and sublethal	• Increased DNA damage • Impaired larval mobility	Weeks Santos *et al.*, 2021

	(Oncorhynchus mykiss)	effects, DNA damage, locomotor behavior, and gene expression (RT-qPCR) • Lipid peroxidation assays for oxidative stress	• Reduced viability	[44]
N,N-Diethyl-meta-toluamide (DEET) and permethrin	Sprague–Dawley rats	• Gestational exposure from GD8 to GD14 via intraperitoneal injections • Transgenerational monitoring (F0-F3) • Epigenetic biomarkers measured through EWAS and MeDIP-Seq	• Specific sperm epimutations associated with transgenerational diseases affecting the kidneys, prostate, and testes	Thorson *et al.*, 2020 [64]
2,4-D, Acetochlor, Aldrin, Atrazine, Chlordane, DDT, Dicamba, Dieldrin, Glyphosate, Heptachlor, Lindane, Malathion, Mesotrione, Metolachlor, Picloram, Toxaphene	1,170 farmers	• Exposure estimated from self-reported pesticide use over the previous 12 months • DNA damage evaluation via methylation profiling (Illumina EPIC array) • Robust linear regression models	• Specific alterations in DNA methylation are associated with pesticide mixture exposure.	Hoang *et al.*, 2021 [65]
2,4-D (2,4-dichlorophenoxyacetic acid), atrazine, and glyphosate	758 women (exposed and unexposed)	- Long-term exposure to agricultural pesticides - Pesticide residues screened using immunoassay kits (Abraxis) - Urine samples collected from 30 women - Evaluation of pesticide exposure in relation to breast cancer risk	• Increased breast cancer risk • Increased lymph node metastasis	Panis *et al.*, 2024 [66]
Acetamiprid (neonicotinoid) and difenoconazole (triazole fungicide)	2,000 Asian honeybees (Apis cerana)	• Dietary exposure for 10 days • 16S rRNA sequencing • Gene-expression analysis via qPCR	• Altered gut microbiota composition • Stronger changes in detoxification- and immunity-related gene expression under combined exposure	Han *et al.*, 2023 [21]
Cyproconazole, Epoxiconazole, Prochloraz	Male Wistar rats (6–7 weeks old)	• Dietary exposure for 28 days • Biochemical parameters measured • Assessment of liver pathology • Organ-weight measurements • Histopathology • Molecular analyses: gene expression, enzyme activity	• Additive and synergistic hepatotoxicity • Increased relative liver weight • Stronger effects on gene expression and enzymatic activity under mixture exposure	Heise *et al.*, 2018 [33]
2,4-D, MCPA, Chlorpyrifos, Bifenthrin, Cyfluthrin, Cypermethrin, Permethrin, Tebuconazole, Thiabendazole, Pyrimethanil	297 farmers	• Mean occupational exposure: 8 years • Urinary pesticide metabolites measured by LC-MS/MS • DNA damage evaluated via Comet assay and micronucleus test	• Significant DNA strand breaks • Increased frequency of micronuclei and genotoxic lesions	Cuenca *et al.*, 2019 [67]
Dichlorvos, Dimethoate, Malathion	Sprague–Dawley rats	• Gavage exposure from gestational day 15 to day 28 of lactation • Examination of reproductive organs in F1 offspring • Measurement of sex hormones • Neonatal developmental assessment (reflexes) • Behavioral and cognitive testing	• Reproductive dysfunction in exposed dams (F0) • Developmental delays and hormonal abnormalities in F1 • Endometrial hyperplasia and uterine	Yu *et al.*, 2013 [68]

			thickening (F0) • Altered reflexes and impaired cognition (F1) • Decreased progesterone (F1 males & females) • Reduced testosterone/LH and increased estradiol in F1 males	
Bifenthrin, Chlorpyrifos	Cyprinus carpio (180-day-old fish)	• Dietary exposure for 70 days • DNA damage assessed using the Comet assay	• Significant increase in DNA damage	Ambreen & Javed, 2019 [69]
Acrinathrin, Abamectin, Cyproconazole, Deltamethrin, Metalaxyl-M, Propamocarb, and Thiamethoxam	52 exposed agricultural workers and 52 organic farmers (controls)	• Professional exposure: ~3.7 h/day, 5–6 days/week • Blood and urine sampling • Oxidative stress biomarkers (TBARS, total glutathione) • DNA damage measured via 8-oxodG (LC-MS/MS)	• Increased oxidative stress • Increased DNA damage with adaptive upregulation of antioxidant defenses	Ledda *et al.*, 2021 [54]
Epoxiconazole, Mancozeb, Prochloraz, Tebuconazole, and Procymidone	Wistar rats	- Oral gavage exposure from gestational day 7 to postnatal day 16 - Examination of reproductive organs - Behavioral assessments - Histological examination	• Transgenerational effects • Reproductive impairment: reduced prostate and epididymis weight, decreased sperm count • Neurobehavioral effects: impaired spatial learning	Jacobsen *et al.*, 2012 [51]
HCB, β-HCH, α-endosulfan, pp'DDE, endrin, β-endosulfan, pp'DDT, endosulfan sulfate, and mirex	47 exposed pesticide applicators and 53 controls	• Exposure duration recorded • Pesticide residues measured by GC-MS • Cholinesterase activity measured • Genotoxicity evaluated	• Higher prevalence of genotoxicity • Increased symptomatology in exposed workers • Strong association between occupational exposure and adverse health indicators	Filippi *et al.*, 2021 [70]

Table 7. Endocrine effects.

Pesticide Mixtures	Biological Subjects	Methods/Exposure Conditions	Key Endocrine Findings	Reference
Glyphosate, Dicamba, 2,4-D	Pregnant Wistar rats (F0) and their offspring (F1)	• Dietary exposure from gestational day 6 to postnatal day 28 • Assessment of renal and thyroid function • Neonatal evaluation: anogenital index, body weight • Dams sacrificed after weaning • Biochemical analyses	• Anti-androgenic effects in male offspring • Maternal renal dysfunction (glomerular + tubular) • Dose-dependent increase in maternal thyroid hormones	Docea *et al.*, 2023 [71]
Cyromazine, MCPB, Pirimicarb, Quinoclamine, Thiram, Ziram	Wistar rats	• Oral gavage from gestational day 7 to postpartum day 16 • Monitoring of offspring body weight • Measurement of insulin, glucagon, and glucose tolerance • Gene expression analysis in adipose tissue	• Lower birth weight • Altered leptin levels in adult females • No significant effects on insulin or glucagon regulation	Svingen *et al.*, 2018 [20]

Difenoconazole (DIF) and tebuconazole (TEB)	Zebrafish embryos (Danio rerio)	• Embryonic exposure for 120 hours with daily renewal • Morphological monitoring • Histology of yolk sac • RNA sequencing • Metabolomic and biochemical profiling • Additivity index analysis	• Altered embryonic development • Accumulation of metabolic intermediates • Disruption of steroid hormone biosynthesis • Broad metabolic disturbances	Jiang *et al.*, 2022 [22]
Captan, Chlorpyrifos, Boscalid, Thiacloprid, Thiophanate, Ziram	Female C57Bl/6J mice	• Dietary exposure from the fetal stage to postnatal week 8 • Assessment of ovarian follicles and hormone levels • Histological and biochemical analyses	• Disrupted folliculogenesis with decreased corpora lutea • Structural ovarian alterations • Reduced progesterone levels	Dopavogui *et al.*, 2022 [72]
Alachlor, Bentazone, Dicamba, Dimethenamid, Glyphosate, Mesotrione, and Terbuthylazine	Podarcis bocagei (wild lizards)	• Chronic environmental exposure (>30 years) • Histology of thyroid and testes • Immunohistochemical and biochemical assays (thyroid receptors, testosterone)	• Thyroid dysfunction • Enlarged thyroid follicles • Increased seminiferous tubule diameter	Bicho *et al.*, 2013 [73]

Table 8. Reproductive effects.

Pesticide Mixtures	Biological Models	Methods/Exposure Conditions	Key Reproductive Findings	Reference
Glyphosate, 2,4-D	Male C57BL/6J mice	• Oral gavage for 6 months • MS, LC-MS, and GC-MS analyses • Evaluation of reproductive parameters: sperm, epididymis, and serum testosterone • Genotoxicity assays	• Severe male reproductive toxicity: sperm abnormalities • Reduced Leydig cell volume and surface • Decreased proliferation of epididymal epithelial cells • Lower serum testosterone	Valente *et al.*, 2024 [74]
Dichlorvos, Dicofol, Dieldrin, Endosulfan, Permethrin	Female adult rats (Sprague-Dawley, Wistar, Lewis)	• Dietary exposure for 10 weeks at increasing doses • HPLC-UV, RIA • Immunohistochemistry • Body and organ weight measurements • Histopathology of reproductive organs	• Reduced body-weight gain • Increased relative liver weight • Estrous cycle disruptions • Decrease in primordial and primary ovarian follicles	Pascotto *et al.*, 2015 [32]
DEET + Permethrin	Sprague-Dawley rats	• Exposure from gestational day 8 to 14 • Follow-up from F0 to F3 generations • Epigenome-wide association studies (EWAS), MeDIP-Seq • Intraperitoneal injections • Identification of transgenerational epigenetic biomarkers	• Sperm-specific epimutations • Transgenerational disease risks involving the kidney, prostate, and testes	Thorson *et al.*, 2020 [64]
Dichlorvos, Dimethoate, Malathion	Sprague-Dawley rats	• Oral gavage from gestational day 15 to day 28 of lactation • Examination of reproductive organs in offspring • Sex hormone assays • Neonatal development tests (reflexes) • Cognitive testing	• Reproductive dysfunction in exposed mothers • Developmental delays and hormonal abnormalities in F1 • Endometrial hyperplasia and uterine thickening in F0 • Altered reflexes and cognitive deficits in F1 • ↓ Progesterone (F1); ↓ Testosterone/LH and ↑ Estradiol in males	Yu *et al.*, 2013 [68]
Captan,	Female	• Dietary exposure from the prenatal	• Disrupted folliculogenesis with	Dopavogui *et*

Chlorpyrifos, Boscalid, Thiacloprid, Thiophanate, Ziram	C57Bl/6J mice	period to week 8 • Ovarian follicle evaluation • Histological & biochemical analysis • Assessment of hormonal alterations	fewer corpora lutea • Structural ovarian alterations • Decreased progesterone levels	*al.*, 2022 [72]
Alachlor, Bentazone, Dicamba, Dimethenamid, Glyphosate, Mesotrione, and Terbuthylazine	Podarcis bocagei (lizards)	• Natural environmental exposure for >30 years • Histology of thyroid and testes • Immunohistochemistry & biochemical assays: thyroid receptors, testosterone	• Thyroid dysfunction • Enlarged thyroid follicles • Increased seminiferous tubule diameter	Bicho *et al.*, 2013 [73]
Cypermethrin, Mancozeb, Metalaxyl	Male Wistar rats	• Exposure for 8 weeks at increasing doses • Evaluation of reproductive parameters • Measurement of oxidative-stress biomarkers • Sex hormone quantification • Histology of testis	• Reduced sperm integrity, concentration, motility, and viability • Increased oxidative stress biomarkers • Histopathological lesions in male reproductive organs	Bouabdallah *et al.*, 2021 [55]

Table 9. Respiratory disorders.

Pesticide Mixtures	Biological Models/Study Population	Methods/Exposure Conditions	Key Respiratory Findings	Reference
Paraquat, Profenofos, Methamidophos, Glyphosate, Methomyl, Chlorpyrifos, Mancozeb, Chlorothalonil	217 agricultural workers	• Mean occupational exposure: 21 years • Assessment of respiratory symptoms and spirometry • Urinary pesticide quantification (SPE-HPLC) • Identification of frequently co-occurring pesticide mixtures (truth table) • Poisson regression analysis	• Weak but statistically significant association with asthma, influenza-like symptoms, chest pain, and allergic rhinitis • Spirometric evidence of obstructive patterns	Díaz-Criollo *et al.*, 2020 [75]

Table 10. Histological effects.

Pesticide Mixtures	Biological Models	Methods/Exposure Conditions	Key Histological Findings	Reference
Organochlorines (Endosulfan, Thiodon), Organophosphates (Monocrotophos, Dichlorvos, Malathion, Methyl parathion), Carbamates (Carbaryl), and Pyrethroids (Cypermethrin)	52 exposed sprayers and 20 unexposed controls	• Occupational exposure: >5 years vs <5 years • Residue quantification of OCs (GC-ECD) • Measurement of cholinesterase activity (AChE, BChE) • Hematological parameters analysis	• Significant reduction in AChE and BChE activity • Hematological abnormalities: increased RBCs, WBCs, monocytes, and neutrophils	Fareed *et al.*, 2010 [45]
Atrazine, Chlorpyrifos, Endosulfan	C57BL/6J mice	• Dietary exposure during gestation through 11 weeks post-weaning • Plasma metabonomic profiling (^{1}H-NMR)	• Specific metabolic alterations • Disruption of hematopoiesis and blood parameters • Proliferation of stem cells • Changes in signaling proteins in bone marrow progenitors	Demur *et al.*, 2013 [19]

Imidacloprid, Acetamiprid (Neonicotinoids)	Helix aspersa(land snail)	• Dietary exposure to increasing mixture doses for 21 days • Measurement of physiological, biochemical, and enzymatic parameters	• Decreased body weight and shell diameter • Dose-dependent reduction of carbohydrates in the liver and kidney • Increased total protein levels • Increased catalase activity	Zouaghi *et al.*, 2020 [30]

Table 11. Drug resistance effects.

Pesticide Mixtures	Biological Models	Methods/Exposure Conditions	Main Findings (Drug Resistance)	Reference
Insecticides (chlorpyrifos-ethyl, deltamethrin), a fungicide (metiram), and a herbicide (glyphosate)	Human glioblastoma cells (U87 cell line)	- 40-day exposure with repeated treatments every 4 days - Cell viability assessed with the MTT assay - Analysis of MDR (multidrug-resistance) gene expression - Apoptosis assay by flow cytometry - Cellular imaging under treated conditions	• Development of resistance to multiple chemotherapeutic agents (cisplatin, 5-fluorouracil, temozolomide) • Upregulation of multidrug-resistance transporters (ABCB1, ABCG2) • Increased detoxification enzyme activity (GST)	Doğanlar *et al.*, 2020 [76]

Table 12. Summary of health effects reported across included studies.

Category of Effects	**Key Findings Reported**	**Types of Evidence Supporting the Effect**	**Consistency Across Studies**
Metabolic Effects	Altered glucose and lipid metabolism Hepatic steatosis Mitochondrial dysfunction Gut microbiota disruption Endocrine-metabolic imbalance (leptin, insulin–glucagon)	Rodent studies, zebrafish, mollusks, and human cohorts	High
Enzymatic Effects	Induction of CYP450 enzymes and inhibition of AChE/BChE Altered hepatic detoxification enzyme profiles	*In vivo* rodent studies and human biomonitoring	High
Neurological and Neurobehavioral Effects	Decreased AChE activity Motor and cognitive deficits Neurotransmitter imbalance (dopamine, serotonin, glutamate) Anxiety-like or depressive behaviors	Human agricultural cohorts, rodent studies, and zebrafish	High
Oxidative Stress	Increased lipid peroxidation (MDA, TBARS) Elevated oxidative DNA damage (8-oxo-dG) Reduced antioxidant defenses (GSH, CAT, PON1)	Human worker studies, rodents, and aquatic species	Very high
Genotoxicity	DNA strand breaks Micronuclei formation Chromosomal abnormalities Impaired DNA repair	Human biomonitoring, rodent models, fish, and *in vitro*	Very high
Epigenetic Alterations	Altered DNA methylation Histone modifications	Rodent multigenerational studies	Moderate–High

	miRNA deregulation Transgenerational effects (F1–F3)		
Endocrine Disruption	Thyroid hormone disruption Gonadal hormone dysregulation Nuclear receptor modulation (PXR) Anti-androgenic effects	Rodents, reptiles, fish	High
Reproductive Effects	Reduced sperm quality Altered ovarian folliculogenesis Estrous cycle disruption Developmental toxicity in offspring	Rodents, wildlife models	High
Respiratory Effects	Asthma-like symptoms Chronic bronchitis Obstructive spirometry patterns	Human epidemiological cohorts	Moderate
Histological Effects	Hepatic, renal, and intestinal lesions Hematopoietic alterations Structural damage to reproductive organs.	Rodent studies, mollusks, and exposed workers	High
Drug Resistance (MDR)	Overexpression of ABCB1/ABCG2 Increased GST activity Reduced apoptosis Increased resistance to chemotherapy	*In vitro* human cell models	Low (Emerging evidence)

4. Discussion

The issue of pesticide exposure has been extensively investigated, yet it remains a critical public health and environmental concern in the contemporary context [77]. Pesticides today play a major role across multiple sectors: industrial production [78] [79], agriculture, public health interventions [80], and even domestic settings. Their widespread use leads to diffuse contamination of ecosystems and food resources [81], resulting in chronic and often unnoticed human exposure to complex mixtures of pesticides [82]. The health effects arising from such exposures are particularly difficult to assess because they involve cumulative, interactive, and potentially synergistic mechanisms rather than the toxicity of individual compounds alone [83].

This systematic review aims to synthesize the available scientific evidence to improve understanding of the potential health risks associated with chronic low-dose exposure to pesticide mixtures. By analyzing studies across different designs and methodological approaches, it offers a comprehensive perspective on the wide range of adverse outcomes reported in various biological systems and human populations. While several studies included in this review (see **Table S1**) conclude that synergistic interactions occur between pesticides, these findings should be interpreted with caution. In many cases, the term "synergy" is used by authors to describe a potentiation of effect. From a strict toxicological perspective, most of these combined actions likely follow the Concentration Addition (CA) model, where the total effect is the sum of individual toxicities. Few studies applied formal mathematical interaction models to rigorously distinguish between additive and

synergistic effects. True synergy, which implies an effect significantly greater than the predicted additive sum, remains sparingly demonstrated through formal mathematical modeling in the current literature.

The comparison between experimental and epidemiological findings should be interpreted with caution. While experimental studies provide mechanistic insights under controlled conditions, epidemiological studies are subject to multiple confounding factors, including co-exposure to other environmental toxicants, variability in personal protective equipment use, lifestyle factors, and socioeconomic conditions.

Despite these terminological nuances, the present review reveals a strong convergence of evidence. The data collectively show that chronic exposure to pesticide mixtures can induce a broad spectrum of adverse health effects, including metabolic disturbances, enzymatic alterations, oxidative stress, genetic and transgenerational abnormalities, epigenetic modifications, neurological and neurobehavioral impairments, endocrine disruption, reproductive toxicity, respiratory dysfunction, and histopathological damage. This diversity of outcomes reflects the multi-systemic and multifactorial nature of pesticide mixture toxicity, which is frequently more pronounced than the effects attributed to individual compounds.

4.1. Metabolic Disturbances

Recent studies demonstrate that chronic low-dose exposure to pesticide mixtures is associated with substantial metabolic disturbances in humans and experimental models. Epidemiological evidence from Pakistan, Cameroon, and China links long-term exposure to organophosphates and mixed organochlorine-herbicide formulations with dyslipidemia, hyperglycemia, hepatic dysfunction, and an increased risk of type 2 diabetes, particularly through insulin resistance and oxidative stress pathways [28] [29]. Experimental findings corroborate these associations. In rodents, chronic or perinatal exposure to pesticide mixtures produces hepatic steatosis, glucose intolerance, altered lipid metabolism, and sex-dependent obesogenic and diabetogenic effects, largely driven by mitochondrial impairment, oxidative stress, and endocrine disruption [23] [24].

Alterations of the gut-liver axis and microbiota dysbiosis emerge as central mechanisms. Studies in zebrafish and other models reveal increased intestinal inflammation, disruption of tight-junction integrity, modulation of apoptotic pathways, and significant shifts in bacterial communities that affect bile acid turnover and lipid processing [21] [30] [31]. Synergistic effects within pesticide mixtures further disrupt hormone biosynthesis and fatty-acid metabolism [19] [22].

Collectively, these findings show that pesticide mixtures, even at low environmental doses, produce coherent metabolic toxicity patterns potentially linked to cardiometabolic diseases such as atherosclerosis [59].

Beyond a qualitative description, the magnitude of these metabolic disruptions is noteworthy. Across the included studies, chronic exposure to pesticide mixtures frequently led to measurable reductions in body weight gain and significant alterations in insulin secretion [20] [32]. In human cohorts, these biological shifts

translated into clinical relevance, with reported increases in odds ratios for metabolic syndrome and type-2 diabetes [28] [29]. This confirms that the impact of these mixtures is not only present but possesses substantial clinical potency even at doses individually deemed safe.

4.2. Enzymatic Effects

Chronic exposure to pesticide mixtures causes significant disruption of enzymatic function, affecting key pathways involved in metabolism and detoxification. *In vivo* findings from [33] indicate strong induction of hepatic cytochrome P450 isoenzymes (CYP1A1, CYP2B1, CYP3A1) in rats exposed to triazole fungicide mixtures, reflecting enhanced biotransformation activity and increased metabolic burden. Similarly, [32] observed elevated hepatic enzyme levels in rats treated with insecticide mixtures, suggesting cumulative activation of detoxification pathways under combined exposures.

Conversely, inhibitory effects on essential enzymes have also been documented. Dopavogui *et al.* [72], in a longitudinal study, reported significant reductions in blood cholinesterase activity following chronic exposure to mixed pesticides, consistent with the synergistic neurotoxic actions of organophosphates, carbamates, and related compounds.

Overall, pesticide mixtures induce both up- and down-regulation of phase I and phase II enzymes, highlighting their cumulative impact on metabolic homeostasis, xenobiotic processing, and biochemical signaling, even at environmentally relevant doses.

4.3. Neurological and Neurobehavioral Effects

Chronic exposure to pesticide mixtures consistently leads to inhibition of acetylcholinesterase (AChE), a central biomarker of neurotoxicity, and produces widespread neurological and neurobehavioral disturbances. Experimental studies demonstrate that combined exposures disrupt neurotransmitter balance, promote oxidative stress, and trigger neuroinflammatory responses. Gasmi (2020) [50] reported that a deltamethrin-acetamiprid mixture reduced dopamine and serotonin while increasing glutamate, resulting in anxiety-like behavior and cognitive deficits. Sergievich *et al.* (2020) [40] further described dose-dependent behavioral impairments under chronic exposure to complex mixtures. Tsatsakis *et al.* [13] showed that exposure to a six-pesticide mixture at ADI-equivalent doses impaired memory, locomotion, and anxiety regulation, with greater severity under vitamin-deficient conditions.

Human studies corroborate these effects. Kumar *et al.* [34] and Dhalla and Sharma (2013) [38] observed AChE inhibition and associated neurobehavioral symptoms among agricultural workers. Cytogenetic alterations reported by Valencia-Quintana *et al.* (2021) [35] and oxidative imbalance described by Silvério *et al.* (2017) [36] further support multifactorial neurotoxicity driven by combined pesticide exposures.

A major limitation across studies lies in the complexity and variability of real-life exposure scenarios. Human exposure to pesticide mixtures is dynamic, involving fluctuating combinations of compounds over time, which are rarely captured by single-point measurements. In addition, the frequent reliance on self-reported exposure data or indirect environmental indicators introduces uncertainty and potential misclassification bias.

4.4. Oxidative Stress

Chronic exposure to pesticide mixtures is consistently linked to oxidative stress, reflecting a disruption of redox homeostasis through excessive reactive oxygen species (ROS) generation and depletion of antioxidant defenses. Among Spanish agricultural workers, Lozano-Paniagua *et al.* [41] reported increased TBARS levels, indicating lipid peroxidation, accompanied by compensatory activation of antioxidant systems such as FRAS and paraoxonase-1 (PON1). Reduced acetylcholinesterase (AChE) activity was also observed, likely mediated by oxidative mechanisms. In Thailand, Intayoung *et al.* [59] found decreased total antioxidant capacity and elevated urinary 8-isoprostane among farmers exposed to glyphosate–paraquat mixtures. Experimental studies corroborate these findings: Bouabdallah *et al.* [55] showed reduced glutathione (GSH) and increased malondialdehyde (MDA) in rats exposed to cypermethrin, mancozeb, and metalaxyl.

These alterations have genotoxic implications. Ledda *et al.* [54] reported elevated 8-oxo-dG, a key biomarker of oxidative DNA damage, among exposed workers. Overall, oxidative stress emerges as a central mechanism linking pesticide mixtures to DNA instability, mutagenesis, and long-term disease risks.

These findings are consistent with the concept of non-monotonic dose–response relationships, frequently observed with endocrine-disrupting chemicals, where low-dose effects cannot be predicted from high-dose toxicity data.

4.5. Genetic Abnormalities

Genetic abnormalities linked to chronic exposure to pesticide mixtures are well documented in both experimental and epidemiological studies. In Mexico, Valencia-Quintana *et al.* [35] reported significant DNA damage among agricultural workers exposed to mixtures of organophosphates, carbamates, organochlorines, and pyrethroids. Notably, these genotoxic effects occurred despite the absence of acetylcholinesterase (AChE) inhibition, suggesting a distinct dose-response relationship for genotoxicity or the involvement of additional chemical classes. Similarly, Brazilian workers exposed to comparable mixtures exhibited cholinesterase inhibition, chromatin condensation, budding cell abnormalities, and increased karyolysis, indicating broad genomic instability [36].

Unsafe agricultural practices exacerbate these effects. Increased chromosomal breaks and micronuclei were observed in Pakistani agricultural workers, who showed a nearly threefold increase in comet tail length after pesticide spraying compared with controls [63]. Serbian pesticide-factory workers exposed to com-

plex mixtures likewise demonstrated chromosomal aberrations [61]. Numerous studies confirm that prolonged exposure and a lack of personal protective equipment amplify genotoxic risk [37] [67], with biomarkers such as micronuclei and chromosomal breaks serving as reliable indicators [70].

Evidence from Hazarika and Deka [60] and Ledda *et al.* [54] further shows that mixtures containing organophosphates, carbamates, and fungicides significantly increase DNA strand breaks and oxidative lesions, including 8-oxo-2'-deoxyguanosine (8-oxo-dG). Importantly, pesticide-related genetic damage is not limited to occupational settings: Panis *et al.* [66] reported an increased cancer risk in women exposed exclusively to domestic pesticides.

Experimental studies parallel these findings. Garaj-Vrhovac and Zeljezic [62] observed persistent chromosomal abnormalities in exposed workers, while Ambreen and [69] and Weeks Santos *et al.* [44] documented cumulative DNA damage and upregulation of repair mechanisms in fish models exposed to binary or ternary mixtures. In bees, acetamiprid-difenoconazole disrupted detoxification and immune genes, with effects exceeding those of single pesticides [21]. Crépet *et al.* [84] confirmed mixture-specific genotoxicity in human hepatocytes, showing that some combinations induced comet-assay DNA damage or γ-H2AX phosphorylation, while others had no detectable effect.

Finally, many pesticide mixtures are associated with stable epigenetic modifications, including altered DNA methylation, histone changes, and microRNA dysregulation, which may persist across generations (F1 - F3), contributing to long-term disease susceptibility and developmental abnormalities.

4.6. Epigenetic Effects

Transgenerational evidence shows that chronic exposure to pesticide mixtures can induce heritable epigenetic alterations affecting DNA methylation, histone structure, and microRNA profiles without modifying the underlying DNA sequence. *In vivo* work by Yu *et al.* [68] demonstrated that gestational exposure to organophosphate mixtures caused reproductive abnormalities and reduced fertility in F1 offspring, indicating persistent endocrine-related epimutations. Similarly, Jacobsen *et al.* [51] found that mixtures of endocrine-disrupting pesticides altered sexual and neurological development from early life to maturity, consistent with synergistic, mixture-driven reprogramming during sensitive developmental windows. Strong evidence of true epigenetic inheritance was provided by Thorson *et al.* [64], who observed stable sperm-DNA methylation changes and transgenerational testicular and renal diseases across F0 - F3 generations following permethrin-DEET exposure.

4.7. Endocrine Disorders and Reproductive Health

A substantial body of experimental and epidemiological evidence shows that chronic exposure to pesticide mixtures disrupts endocrine regulation, particularly affecting thyroid and gonadal pathways. These disruptions often translate into

significant reproductive impairments and appear more pronounced than the effects induced by individual compounds.

Field observations by Bicho *et al.* [73] in lizards exposed to environmental mixtures revealed enlarged thyroid follicles and dysregulated hormone-receptor expression, directly impairing reproductive and thyroid function. In mammals, Dopavogui *et al.* [72] reported disrupted ovarian folliculogenesis and reduced progesterone levels in female mice exposed to multi-class mixtures, indicating impaired steroidogenesis. Similarly, Docea *et al.* [71] found that perinatal exposure to a mixture including glyphosate, dicamba, and 2,4-D produced dose-dependent thyroid-hormone alterations and renal dysfunction in dams and offspring.

Reproductive toxicity is consistently documented. Pascotto *et al.* [32] showed that combined dichlorvos-permethrin exposure disturbed estrous cyclicity, reduced estradiol, and induced ovarian abnormalities through hypothalamic-pituitary-ovarian axis disruption. In males, Valente *et al.* [74] reported exacerbated sperm defects and hormonal imbalance following glyphosate-2,4-D exposure, including germ-cell apoptosis and impaired blood-testis barrier integrity. Bouabdallah *et al.* [55] observed reduced sperm quality, low testosterone, oxidative stress, and testicular lesions in rats exposed to cypermethrin-mancozeb-metalaxyl mixtures.

At the molecular level, Crépet *et al.* [84] demonstrated that several mixtures strongly activated the human pregnane X receptor (PXR), inducing CYP3A4 expression and altering steroid and xenobiotic metabolism.

Collectively, these findings show that pesticide mixtures profoundly disturb endocrine signaling and reproductive function, highlighting the heightened vulnerability of hormonal systems to combined low-dose exposures.

4.8. Respiratory Disorders

Chronic exposure to pesticide mixtures, including highly toxic compounds such as paraquat, profenofos, and glyphosate, has been consistently linked to adverse respiratory outcomes in agricultural populations. In a cross-sectional study among Colombian farmers, Díaz-Criollo *et al.* [75] reported an increased prevalence of asthma, chronic bronchitis, allergic rhinitis, and chest pain in workers chronically exposed to these mixtures. Spirometry revealed reduced forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1), indicating both obstructive and restrictive patterns.

Mechanistically, these effects are attributed to oxidative injury of alveolar membranes, excess reactive oxygen species, and chronic inflammatory responses. Paraquat, in particular, accumulates in lung tissue and generates superoxide radicals, promoting inflammation and fibrosis. Combined exposures may therefore exacerbate airway dysfunction, underscoring the elevated respiratory risks posed by pesticide mixtures.

4.9. Histological Effects

Histopathological and hematological alterations induced by pesticide mixtures are consistently documented in both experimental and epidemiological research. In a cross-sectional study of pesticide sprayers, Fareed *et al.* [45] reported significant hematological abnormalities, including reduced hemoglobin levels, decreased red blood cell counts, and pronounced cholinesterase inhibition—indicating subclinical yet biologically relevant impacts on hematopoietic and detoxification systems.

Experimental models reinforce these findings. In Helix aspersa, Zouaghi *et al.* [30] observed severe hepatic and renal lesions, cellular necrosis, hypertrophy, and metabolic disruption following subchronic exposure to neonicotinoid mixtures. Demur *et al.* [19] similarly reported altered signaling pathways and impaired hematopoiesis in bone-marrow progenitors of mice exposed to endosulfan, atrazine, and chlorpyrifos.

In vitro analyses reveal mixture-specific interactions: combinations such as M1 and M5 induced strong cellular responses exceeding additive expectations, whereas M2 and M6 showed minimal effects [84]. Overall, chronic exposure to pesticide mixtures can provoke substantial histological injury across hepatic, renal, and hematopoietic systems.

4.10. Drug Resistance Effects

In vitro evidence indicates that chronic exposure to pesticide mixtures can promote multidrug resistance (MDR) in human cells. Doğanlar *et al.* [76] showed that repeated exposure to a mixture containing chlorpyrifos-ethyl, deltamethrin, metiram, and glyphosate induced strong and persistent resistance to chemotherapeutic agents—including cisplatin, 5-fluorouracil, and temozolomide—in U87 glioblastoma cells. This durable resistance was driven by marked overexpression of efflux transporters (P-gp/ABCB1, BCRP/ABCG2) and elevated glutathione-S-transferase (GST/M1) activity, enhancing xenobiotic clearance. These mechanisms impair drug accumulation and efficacy, raising concerns that environmental pesticide mixtures may contribute to reduced cancer treatment responsiveness.

5. Scope and Limitations of the Study

It should be noted that the distinction between chronic low-dose exposure and intermittent higher exposure peaks, particularly in occupational settings, remains a methodological challenge in environmental health research. This is particularly relevant for occupational settings, where intermittent high-exposure peaks may coexist with chronic low-level exposure, complicating the strict classification of such exposures as “low-dose”.

This review demonstrates the breadth and severity of health effects associated with chronic, low-dose exposure to pesticide mixtures. Across more than sixty studies, including *in vivo*, *in vitro*, and epidemiological evidence, consistent alterations were identified in metabolic, neurological, reproductive, endocrine, immunological, and genetic systems. Key mechanistic pathways such as oxidative stress,

mitochondrial dysfunction, endocrine disruption, and DNA damage emerged repeatedly, underscoring the capacity of mixtures to elicit multi-system toxicity that often exceeds the effects of individual compounds. Frequent reports of synergistic or supra-additive effects, although often not confirmed by formal modeling, are noted. The observation of synergistic and cumulative interactions further challenges the long-standing hypothesis that low doses of isolated pesticides are biologically insignificant.

However, several limitations must be acknowledged. First, the substantial methodological heterogeneity across studies complicates direct comparison. Variations in mixture composition, exposure duration, administered doses, biological matrices, and analytical protocols introduce uncertainty in establishing consistent dose–response relationships (Table 2). Second, some studies do not report precise exposure levels, relying instead on estimated or self-reported data, which reduces toxicological accuracy. Third, many investigations focus on highly exposed populations, such as agricultural workers, which limits the generalizability of findings to the broader population. Finally, the mechanistic interactions between pesticides within mixtures, particularly concerning endocrine, epigenetic, mitochondrial, and immunological pathways, remain only partially understood, making it difficult to conduct a complete cumulative risk assessment.

Additional limitations include potential publication bias, language restrictions, and the absence of a quantitative meta-analysis, which may limit the generalizability and strength of the conclusions.

6. Conclusions

The analysis also highlights methodological and regulatory limitations. Most toxicological assessments still rely on single-compound testing, failing to reflect real-world exposure scenarios in which humans encounter complex and variable chemical mixtures. Significant gaps persist regarding long-term, multigenerational, and environmentally relevant exposure conditions, especially for vulnerable populations such as agricultural workers, children, and pregnant women.

These findings underscore the urgent need for updated regulatory frameworks that explicitly incorporate mixture toxicity, cumulative risk assessment, and mechanistic biomarkers of early effect. Strengthening environmental monitoring, improving occupational protection, and promoting integrated pest-management strategies are essential steps to reducing health risks. At the same time, research efforts must prioritize multidisciplinary approaches capable of capturing the complexity of mixture-driven toxicity, including systems biology, exposome-focused methods, and advanced computational modeling.

Overall, this review provides robust scientific evidence to support more protective public-health policies and to guide future research on the health risks associated with pesticide mixtures. These findings call for a paradigm shift in regulatory toxicology, moving from single-compound assessments toward an integrated evaluation of cumulative and mixture effects.

Addressing these challenges is critical for ensuring safer agricultural practices, preventing chronic diseases, and preserving long-term environmental and population health. Failing to account for mixture effects may lead to a systematic underestimation of real-world health risks.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Supplementary

Table S1. General overview of included studies.

Authors	Study Title	Study Type
Valente *et al.*, 2024	A mixture of glyphosate and 2, 4-D herbicides enhances the deleterious reproductive outcomes induced by Western diet in obese male mice	*In vivo* study
Kumar *et al.*, 2023	Assessing farmer's exposure to pesticides and the risk for non-communicable diseases: A biomonitoring study	Cross-sectional study
Valencia-Quintana *et al.*, 2021	Assessment of Cytogenetic Damage and Cholinesterases' Activity in Workers Occupationally Exposed to Pesticides in Zamora-Jacona, Michoacan, Mexico	Cohort study
Hazarika et Deka., 2017	Assessment of DNA damage in agricultural workers exposed to mixture of pesticides in Assam (India)	Case-control study
Silvério *et al.*, 2017	Assessment of exposure to pesticides in rural workers in southern of Minas Gerais, Brazil	Cohort study
Dhananjayan *et al.*, 2019	Assessment of genotoxicity and cholinesterase activity among women workers occupationally exposed to pesticides in tea garden	Cross-sectional study
Dhalla and Sharma., 2013	Assessment of Serum Cholinesterase in Rural Punjabi Sprayers Exposed to a Mixture of Pesticides	Comparative observational study
Chang *et al.*, 2024	Associations between exposure to pesticides mixture and semen quality among the non-occupationally exposed males: Four statistical models	Cross-sectional study
Chen *et al.*, 2024	Associations of chronic exposure to a mixture of pesticides and type 2 diabetes mellitus in a Chinese elderly population	Cross-sectional study
Sergievich *et al.*, 2020	Behavioral impacts of a mixture of six pesticides on rats	*In vivo* study
Lozano-Paniagua *et al.*, 2018	Biomarkers of oxidative stress in blood of workers exposed to non-cholinesterase inhibiting pesticides	Longitudinal cohort study
Doğanlar *et al.*, 2020	Chronic exposure of human glioblastoma tumors to low concentrations of a pesticide mixture induced multidrug resistance against chemotherapy agents	*In vitro* study
Leonel Javeres *et al.*, 2021	Chronic Exposure to Organophosphates Pesticides and Risk of Metabolic Disorder in Cohort from Pakistan and Cameroon	Cross-sectional study
DÍAZ-CRIOLLO *et al.*, 2020	Chronic pesticide mixture exposure including paraquat and respiratory outcomes among Colombian farmers	Cross-sectional study
Brodeur *et al.*, 2022	Concentration of current-use pesticides in frogs from the Pampa region and correlation of a mixture toxicity index with biological effects	*In vivo* study
Jovičić *et al.*, 2013	Cytogenetic biomonitoring in a Serbian population occupationally exposed to a complex mixture of pesticides	Cross-sectional study
Garaj-Vrhovac et Zeljezic., 2001	Cytogenetic monitoring of Croatian population occupationally exposed to a complex mixture of pesticides	Longitudinal study

Intayoung *et al.*, 2021	Decreased Antioxidant Capacity in Corn Farmers Occupationally Exposed to the Mixture of Herbicides	Cross-sectional study
Jia et Misra., 2007	Developmental exposure to pesticides zineb and/or endosulfan renders the nigrostriatal dopamine system more susceptible to these environmental chemicals later in life	*In vivo* study
Demur *et al.*, 2013	Dietary exposure to a low dose of pesticides alone or as a mixture: The biological metabolic fingerprint and impact on hematopoiesis	*In vivo* study
Bhalli *et al.*, 2009	DNA damage in Pakistani agricultural workers exposed to mixture of pesticides	Cross-sectional study
Docea *et al.*, 2023	Effect of perinatal exposure to glyphosate and its mixture with 2,4-D and dicamba on rat dam kidney and thyroid function and offspring's health	*In vivo* study
Pascotto *et al.*, 2015	Effects of a Mixture of Pesticides on the Adult Female Reproductive System of Sprague-Dawley, Wistar, and Lewis Rats	*In vivo* study
Lovejoy and Fiumera, 2019	Effects of Dual Exposure to the Herbicides Atrazine and Paraquat on Adult Climbing Ability and Longevity in Drosophila melanogaster	*In vivo* study
Svingen *et al.*, 2018	Effects on metabolic parameters in young rats born with low birth weight after exposure to a mixture of pesticides	*In vivo* study
Weeks Santos *et al.*, 2021	Environmentally relevant mixture of pesticides affect mobility and DNA integrity of early life stages of rainbow trout (Oncorhynchus mykiss)	*In vivo* study
Thorson *et al.*, 2020	Epigenome-wide association study for pesticide (Permethrin and DEET) induced DNA methylation epimutation biomarkers for specific transgenerational disease	*In vivo* and EWAS study
Hoang *et al.*, 2021	Epigenome-Wide DNA Methylation and Pesticide Use in the Agricultural Lung Health Study	Case-control study
Zouaghi *et al.*, 2020	EVALUATION OF THE TOXICITY OF A MIXTURE INSECTICIDES USED ON A BIOLOGICAL MODEL: THE SNAIL HELIX ASPERSA	*In vivo* study
Abd-Alrahman *et al.*, 2014	Exposure to difenoconazole, diclofop-methyl alone and combination alters oxidative stress and biochemical parameters in albino rats	*In vivo* study
Panis *et al.*, 2024	Exposure to Pesticides and Breast Cancer in an Agricultural Region in Brazil	Case-control study
Neta *et al.*, 2011	Fetal exposure to chlordane and permethrin mixtures in relation to inflammatory cytokines and birth outcomes	Cross-sectional study
Han *et al.*, 2023	Gut microbiota composition and gene expression changes induced in the Apis cerana exposed to acetamiprid and difenoconazole at environmentally realistic concentrations alone or combined	*In vivo* study
Fareed *et al.*, 2010	Hematological and biochemical alterations in sprayers occupationally exposed to mixture of pesticides at a mango plantation in Lucknow, India	Cross-sectional study
Heise *et al.*, 2018	Hepatotoxic combination effects of three azole fungicides in a broad	*In vivo* study

	dose range	
Barrón Cuenca *et al.*, 2019	Increased levels of genotoxic damage in a Bolivian agricultural population exposed to mixtures of pesticides	Cross-sectional study
Jiang *et al.*, 2022	Insights into the combined effects of environmental concentration of difenoconazole and tebuconazole on zebrafish early life stage	*In vivo* study
Klement *et al.*,2020	Life-long Dietary Pesticide Cocktail Induces Astrogliosis Along with Behavioral Adaptations and Activates p450 Metabolic Pathways	*In vivo* study
Singleton *et al.*, 2015	Longitudinal assessment of occupational exposures to the organophosphorous insecticides chlorpyrifos and profenofos in Egyptian cotton field workers	Longitudinal study
Yu *et al.*, 2013	Maternal exposure to the mixture of organophosphorus pesticides induces reproductive dysfunction in the offspring: Organophosphorus Pesticides Induces Reproductive Dysfunction in the Offspring	*In vivo* study
Lukowicz *et al.*, 2018	Metabolic Effects of a Chronic Dietary Exposure to a Low-Dose Pesticide Cocktail in Mice: Sexual Dimorphism and Role of the Constitutive Androstane Receptor	*In vivo* study
Bonvallot *et al.*, 2018	Metabolome disruption of pregnant rats and their offspring resulting from repeated exposure to a pesticide mixture representative of environmental contamination in Brittany	*In vivo* study
Khatib *et al.*, 2023	Molecular and Biochemical Evidence of the Toxic Effects of Terbuthylazine and Malathion in Zebrafish	*In vivo* study
Khatib *et al.*, 2021	Neurobehavioral anomalies in zebrafish after sequential exposures to DDT and chlorpyrifos in adulthood: Do multiple exposures interact?	*In vivo* study
HAWKEY Smith Khatib *et al.*, 2023	Neurobehavioral Responses and Toxic Brain Reactions of Juvenile Rats Exposed to Iprodione and Chlorpyrifos, Alone and in a Mixture	*In vivo* study
Ghasemnejad-Berenji *et al.*, 2021	Neurological effects of long-term exposure to low doses of pesticides mixtures in male rats: Biochemical, histological, and neurobehavioral evaluations	*In vivo* study
Gasmi., 2020	Neurotransmission dysfunction by mixture of pesticides and preventive effects of quercetin on brain, hippocampus and striatum in rats	*In vivo* study
Ambreen et Javed., 2019	NUCLEAR DAMAGE IN PERIPHERAL ERYTHROCYTES OF CYPRINUS CARPIO EXPOSED TO BINARY MIXTURE OF PESTICIDES	*In vivo* study
Ledda *et al.*, 2021	Oxidative stress and DNA damage in agricultural workers after exposure to pesticides	Case-control study
Smith *et al.*, 2020	Perinatal exposure to a dietary pesticide cocktail does not increase susceptibility to high-fat diet-induced metabolic perturbations at adulthood but modifies urinary and fecal metabolic fingerprints in C57Bl6/J mice	*In vivo* study
Jacobsen *et al.*, 2012	Persistent developmental toxicity in rat offspring after low dose exposure	*In vivo* study

	to a mixture of endocrine disrupting pesticides	
Dopavogui *et al.*, 2022	Pre- and Postnatal Dietary Exposure to a Pesticide Cocktail Disrupts Ovarian Functions in 8-Week-Old Female Mice	*In vivo* study
Fuhrimann *et al.*, 2023	Self-reported and urinary biomarker-based measures of exposure to glyphosate and mancozeb and sleep problems among smallholder farmers in Uganda	Cross-sectional study
An *et al.*, 2024	Synergistic risk in the gut and liver: Insights into the toxic mechanisms and molecular interactions of combined exposure to triazophos and fenvalerate in zebrafish	*In vivo* study
Tsatsakis *et al.*, 2019	The effect of chronic vitamin deficiency and long term very low dose exposure to 6 pesticides mixture on neurological outcomes–A real-life risk simulation approach	*In vivo* study
Bicho *et al.*, 2013	Thyroid disruption in the lizard Podarcis bocagei exposed to a mixture of herbicides: a field study	*In vivo* study
Bouabdallah *et al.*, 2022	Toxic impacts of a mixture of three pesticides on the reproduction and oxidative stress in male rats	*In vivo* study
Pal *et al.*, 2022	Toxicity of the Pesticides Imidacloprid, Difenoconazole and Glyphosate Alone and in Binary and Ternary Mixtures to Winter Honey Bees: Effects on Survival and Antioxidative Defenses	*In vivo* study
Ramsdorf *et al.*, 2021	Transgenerational Effects of Environmentally Relevant Concentrations of Atrazine and Glyphosate Herbicides, Isolated and in Mixture, to Freshwater Microcrustacean Daphnia Magna	*In vivo* study
Gao *et al.*, 2022	Type 2 Diabetes Induced by Changes in Proteomic Profiling of Zebrafish Chronically Exposed to a Mixture of Organochlorine Pesticides at Low Concentrations	*In vivo* study
Filippi *et al.*, 2021	Validation of exposure indexes to pesticides through the analysis of exposure and effect biomarkers in ground pesticide applicators from Argentina	Cross-sectional study