International Journal of Fisheries and Aquatic Research
www.fishjournals.com
E-ISSN: 2456-7248
Received: 06-10-2026, Accepted: 05-11-2026, Published: 20-11-2026
Volume 11, Issue 4, 2026, Page No. 61-67

Pesticide-induced genotoxicity in fish: Mechanisms of DNA damage, biomarkers and ecological implications - A review

Dr. Dhaswadikar Usha Sitaram
Department of Zoology, Shri. Hawagiswami Mahavidyalaya, Udgir Dist.Latur, Maharashtra, India
DOI: https://doi.org/10.66856/ijfar.2026.11.4.11075

Abstract
Pesticides support modern crop protection, but their movement from agricultural land into streams, rivers, ponds, reservoirs and wetlands can expose non-target fish to biologically active concentrations. In addition to acute toxicity, pesticide exposure may disturb cellular redox balance, damage DNA, alter chromosome integrity and affect the physiological processes required for growth and reproduction. This review synthesises evidence on pesticide-induced genotoxicity in fish, with emphasis on the pathways that connect exposure to genetic damage, the principal biomarkers used in laboratory and field investigations, and the ecological meaning of observed responses. Major pesticide groups considered include organophosphate and carbamate insecticides, pyrethroids, neonicotinoids, triazine herbicides, glyphosate-based formulations and fungicides. Mechanistic pathways include reactive oxygen species formation, lipid peroxidation, impairment of antioxidant defences, interference with DNA repair, direct or indirect chromosome damage, and secondary injury arising from inflammation or cytotoxicity. The alkaline comet assay and erythrocytic micronucleus test remain widely used because they are relatively sensitive and adaptable to different fish tissues, however, they measure different endpoints and should not be treated as interchangeable. Chromosomal aberrations, nuclear abnormalities, oxidative DNA lesions, gene-expression profiles and integrated biomarker approaches can provide complementary evidence. Recent studies report genotoxic responses in fish exposed to imidacloprid, azoxystrobin and glyphosate-based herbicide formulations, while recent reviews emphasise formulation effects, mixture toxicity and the need for more ecologically realistic bioassays. Nevertheless, interpretation is constrained by differences in species, life stage, exposure design, tissue selection, analytical protocols and reporting units. Biomarker responses alone do not establish heritable mutation or population-level decline. Stronger inference requires chemical monitoring, validated dose–response designs, multiple endpoints, appropriate controls and field-relevant exposure scenarios. The review identifies priorities for standardisation, mixture assessment, long-term studies and the integration of genotoxic endpoints into aquatic risk assessment.

Keywords: Fish, pesticides, genotoxicity, DNA damage, comet assay, micronucleus test, oxidative stress, nuclear abnormalities, aquatic ecotoxicology

Introduction

Pesticides are used to reduce crop losses and manage weeds, insects and fungal pathogens. Their environmental distribution, however, is not confined to the target field. Spray drift, surface runoff, soil erosion, drainage, leaching and accidental discharge can transfer active ingredients and formulation components into aquatic habitats. Exposure is shaped by rainfall, soil properties, application timing, irrigation practices, hydrology and the persistence and mobility of individual compounds. Fish may therefore encounter short pulses following rainfall as well as lower, more prolonged concentrations in receiving waters. The environmental significance of a pesticide depends not only on its intrinsic toxicity but also on how much reaches the water, how long it remains available, and how organisms take it up and metabolise it.

Fish are useful sentinels of aquatic contamination because they occupy different trophic positions, interact continuously with water and sediment, and possess measurable cellular and physiological responses to contaminants. Their gills provide a large exchange surface, the digestive tract can receive contaminated food and particles, and the liver plays a major role in biotransformation. These routes and organs can produce distinct exposure profiles. A pesticide that produces limited mortality during a short test may still induce sublethal changes in DNA integrity, antioxidant status, behaviour, endocrine function or reproduction. Genotoxicity is therefore an important component of pesticide hazard characterisation, although it must be interpreted alongside other evidence rather than used as a stand-alone prediction of ecological harm.

Genotoxicity describes damage to genetic material or processes that maintain its integrity. Depending on the endpoint, investigations may detect DNA strand breaks, alkali-labile sites, chromosome breakage, chromosome loss, micronucleus formation or altered expression of genes involved in stress responses and DNA repair. Such findings are not equivalent. Some lesions are transient and repaired; others may persist, lead to cell death or become fixed as mutations during replication. A comet-assay response, for example, indicates DNA migration associated with strand breaks and related lesions under the assay conditions, but it does not by itself demonstrate a permanent mutation. Likewise, a micronucleus indicates chromosome fragments or whole chromosomes excluded from the main nucleus during cell division, but its frequency depends on biological and technical factors.

Recent literature has expanded the range of pesticide classes and analytical approaches considered in fish toxicology. A 2025 [7] review by Majumder discusses the evolution of fish pesticide bioassays, formulation and mixture effects, multiple biomarkers, integrated biomarker response and adverse outcome pathways. Experimental studies have also

reported genotoxic responses in rosy barb (Pethia conchonius) exposed to imidacloprid and azoxystrobin, while work on a glyphosate-based formulation found cytogenetic and DNA-damage responses in yellowtail tetra (Astyanax lacustris) (Majumder, 2025 [9]; study of imidacloprid in P. conchonius, 2023; study of azoxystrobin in P. conchonius, 2024 [5]; study of glyphosate-based herbicide in A. lacustris, 2024). These findings make it timely to examine mechanisms, biomarkers and ecological interpretation together.

This review critically examines current knowledge of pesticide-induced genotoxicity in fish. It considers the mechanisms that may lead to genetic damage, evaluates the principal biomarkers used in laboratory and field studies, and discusses evidence across major pesticide groups. It also considers the limitations of current research and identifies priorities for aquatic monitoring and risk assessment.

Review approach and scope

This article is a narrative critical review designed to connect mechanistic toxicology with biomarker interpretation and ecological risk. The literature base includes foundational methods papers, established fish genotoxicity studies, and recent reviews and primary studies published through 2025 that were identifiable through scholarly publisher and biomedical records. Particular attention was given to studies using the comet assay, micronucleus test, chromosomal endpoints and molecular markers in fish. This is not a registered systematic review and no claim of exhaustive database coverage is made. A submission-ready systematic review would require documented databases, search strings, screening dates, eligibility criteria, duplicate removal and a reproducible selection flow diagram.

Studies were interpreted according to the pesticide or formulation tested, species and life stage, exposure duration, tissue or cell type, assay endpoint and whether exposure was laboratory-controlled or field-based. Comparisons across studies require caution because differences in commercial formulations, water chemistry, temperature, fish size, feeding status, sampling time and scoring criteria can influence the response. Where a numerical result is discussed, it is attributed to the relevant study rather than treated as a universal toxicity threshold.

Environmental pathways and fish exposure

Entry and fate in aquatic systems

Pesticides reach aquatic systems through several connected pathways. Runoff can carry dissolved compounds and pesticide-bound soil particles into surface waters; spray drift can deposit chemicals directly on the water surface; irrigation return flows and drainage can transport residues from cultivated land; and leaching can move mobile compounds toward groundwater that feeds streams. Sediment-associated residues may become relevant to benthic fish and bottom-feeding species, while dissolved residues are available for uptake across the gills. Transformation products can also matter because they may differ from parent compounds in persistence, mobility and toxicity.

The concentration measured at a single time point may not describe the biologically relevant exposure. Storm-driven pulses can be brief but substantial, while repeated applications may create recurrent exposure. A monitoring programme based only on monthly grab samples can miss short-lived peaks. Conversely, a detectable residue does not automatically imply a genotoxic effect: bioavailability, internal dose, metabolism and the potency of the chemical all contribute. Linking environmental chemistry with biological endpoints is therefore essential.

Uptake, distribution and biotransformation

Fish can absorb pesticides through the gills, skin and gastrointestinal tract. The relative importance of each route depends on the compound's physicochemical properties, the species' ecology and the exposure medium. Once absorbed, pesticides may be distributed to the liver, kidney, brain, gills and other tissues. Enzymatic transformation can detoxify a compound, but in some cases it can also generate reactive metabolites. Phase I and phase II biotransformation, transport processes and excretion determine internal exposure, while differences among species and life stages can modify susceptibility.

The liver is important in xenobiotic metabolism and often shows biochemical or histological responses, whereas gills are directly exposed to waterborne contaminants. Peripheral erythrocytes are convenient for micronucleus scoring in many fish because mature fish erythrocytes are nucleated. Blood and gill cells are also frequently used for comet assays. No single tissue is universally most sensitive; tissue choice should follow the hypothesis and be justified in the study design.

Mechanisms of pesticide-induced genotoxicity

Oxidative stress and reactive oxygen species

Oxidative stress is one of the most frequently proposed links between pesticide exposure and DNA injury. Exposure may increase the production of reactive oxygen species (ROS), disrupt mitochondrial electron transport, alter redox-active enzymes or reduce the capacity of antioxidant systems. When ROS generation exceeds cellular defences, oxidative damage can affect membrane lipids, proteins and nucleic acids. DNA lesions may include oxidised bases, abasic sites and strand breaks. The oxidative DNA lesion 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG, also commonly written 8-OHdG in biomarker literature) is used as an indicator of oxidative damage, although its measurement requires careful control of artefacts and validated analytical procedures.

Antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase, together with glutathione and other non-enzymatic defences, help maintain redox balance. Their responses are not always monotonic: activity may rise as an adaptive response at lower exposure and decline when inhibition, depletion or tissue injury becomes pronounced. Therefore, a change in one antioxidant enzyme cannot independently establish oxidative stress or genotoxicity. Stronger inference comes from a coordinated panel that includes antioxidant status, lipid peroxidation, DNA damage and relevant exposure measurements.

Direct DNA interaction and reactive metabolites

Pesticides vary substantially in their chemical structures and modes of action, so a single direct-DNA mechanism cannot be assigned to the entire class. Some compounds or their metabolites may interact with macromolecules or interfere indirectly with DNA integrity through metabolic activation, redox cycling or disruption of cellular homeostasis. Organophosphate and carbamate insecticides are primarily

known for effects on nervous-system enzymes, but secondary oxidative stress and cellular injury can contribute to genotoxic endpoints in fish. Pyrethroids, neonicotinoids, herbicides and fungicides likewise differ in their primary targets, and observed DNA damage may reflect indirect stress rather than direct binding to DNA.

For this reason, mechanistic statements should distinguish between a measured endpoint and a proposed pathway. Increased comet tail DNA is evidence of DNA migration under assay conditions; increased micronuclei indicate chromosome segregation or breakage events; changes in antioxidant enzymes are supportive evidence of altered redox physiology. A convincing causal explanation requires concordance among endpoints, exposure–response relationships and, where possible, mechanistic measurements.

Mitochondrial dysfunction, lipid peroxidation and inflammation

Mitochondria are both sources and targets of ROS. Pesticide-associated changes in mitochondrial function can disturb energy production and increase oxidative pressure. Lipid peroxidation generates reactive products that can further damage proteins and nucleic acids. Inflammatory signalling and tissue injury may amplify these effects, particularly after sustained exposure. Such pathways can operate together rather than as separate mechanisms, making it difficult to attribute a genotoxic response to one biochemical event without targeted experiments.

DNA repair, cell-cycle disturbance and apoptosis

Cells respond to DNA damage through repair pathways, cell-cycle checkpoints and programmed cell death. If repair is effective, some comet-assay lesions may decline after exposure ceases. If damage is extensive or incorrectly repaired, mutations or chromosome abnormalities may remain. Pesticides can also alter gene expression associated with stress responses and repair, but transcript-level changes do not automatically establish altered repair capacity. Studies should pair expression data with functional endpoints when making claims about repair impairment.

Apoptosis and cytotoxicity complicate interpretation. Severe exposure can remove heavily damaged cells, potentially reducing the apparent frequency of some damage endpoints in surviving cells. Conversely, cytotoxicity can produce secondary DNA fragmentation and inflate apparent genotoxic responses. Measurements of cell viability, apoptosis or cytotoxicity should therefore accompany genotoxicity assays when feasible, particularly at high concentrations.

Major pesticide groups and evidence in fish

Organophosphate insecticides

Organophosphate insecticides, including chlorpyrifos and malathion, have been investigated extensively in aquatic toxicology. Their primary toxic action involves inhibition of acetylcholinesterase, but sublethal exposure can also be associated with oxidative stress, tissue injury and genetic damage. In Channa punctatus, chlorpyrifos exposure was reported to increase micronuclei and DNA damage measured by the comet assay, with responses influenced by concentration, duration and tissue (chlorpyrifos study in C. punctatus). Kumar *et al*. (2009)[8] similarly investigated malathion in C. punctatus using micronucleus and comet assays. These studies support the use of genotoxic endpoints as part of a broader evaluation, but differences in formulation, exposure regime and scoring methods limit direct numerical comparison.

A common weakness in pesticide studies is reliance on nominal rather than analytically confirmed concentrations. For volatile, degradable or sorptive compounds, actual exposure may diverge from the intended concentration. Reporting water renewal, analytical verification, formulation composition and exposure stability improves the interpretability of organophosphate studies.

Carbamate insecticides

Carbamate insecticides also affect cholinergic signalling and may induce secondary physiological stress. Research on carbosulfan in freshwater fish has used micronucleus and alkaline comet assays and reported concentration- and time-related responses (carbosulfan fish genotoxicity study). As with organophosphates, evidence of DNA damage should be considered alongside cholinesterase inhibition, antioxidant responses and general toxicity. A biomarker panel can help distinguish a broad stress response from a more specific pattern of genetic injury.

Pyrethroids

Synthetic pyrethroids are widely used for insect control and can be hazardous to aquatic organisms, although toxicity differs among compounds and formulations. Their effects on ion-channel function and nervous-system physiology can be accompanied by oxidative and cellular stress. Fish genotoxicity studies have reported changes in DNA-damage or nuclear-abnormality endpoints for particular pyrethroids, but findings should not be generalised to the entire class. Hydrophobicity, sorption to particles, water temperature and species-specific metabolism can strongly affect exposure and response.

Neonicotinoid insecticides

Neonicotinoids are designed to act on insect nicotinic acetylcholine receptors, yet non-target aquatic organisms may be exposed through agricultural runoff. A study of imidacloprid in Pethia conchonius used the micronucleus and comet assays and reported increased DNA damage and nuclear abnormalities relative to controls, with stronger responses at higher concentrations and longer exposure periods (Assessment of imidacloprid-induced genotoxicity in P. conchonius, 2023)[3]. The reported 96-hour LC50 and sublethal test concentrations are specific to that experimental system and should not be treated as universal environmental thresholds. In particular, acute laboratory toxicity values do not directly predict risk under all field conditions.

Herbicides: atrazine and glyphosate-based formulations

Herbicides can affect aquatic organisms through mechanisms that are distinct from their intended effects on plants. Atrazine has been associated in the literature with biochemical, developmental and genetic endpoints in fish, including zebrafish and common carp. A 2024 [13] review focused on pesticide toxicity in Danio rerio and Cyprinus carpio, with particular attention to atrazine, summarised evidence on tissue effects, micronuclei and gene-level responses (A review on toxic effects of pesticides in zebrafish and common carp, 2024). The weight of evidence

must nevertheless be judged chemical by chemical and endpoint by endpoint.

Glyphosate requires special attention to formulation. Commercial glyphosate-based herbicides may contain co-formulants that alter uptake or toxicity; the biological response to a formulated product may therefore differ from that of the active ingredient alone. A review of aquatic ecotoxicity evidence published in 2024 [5] examined glyphosate, formulations and co-formulants and identified oxidative stress as an important pathway across multiple aquatic taxa (Aquatic ecotoxicity of glyphosate, its formulations, and co-formulants, 2024). In Astyanax lacustris, a 2024 [13] study of a glyphosate-based formulation reported DNA damage, micronuclei and chromosomal abnormalities under the tested acute exposure conditions. This finding is relevant to formulation assessment, but its concentrations, product composition and species context should be retained when interpreting risk.

Fungicides

Fungicides are sometimes less represented than insecticides in fish genotoxicity discussions, despite widespread use and potential transport to surface water. Azoxystrobin is a strobilurin fungicide that interferes with mitochondrial respiration in fungi. A 2024 study in Pethia conchonius reported concentration- and time-related increases in DNA damage and erythrocytic nuclear abnormalities, using comet and micronucleus assays. The study reported a 96-hour LC50 of 0.514 mg/L for its test conditions and found stronger genotoxic responses at the highest sublethal concentration after 96 hours (Azoxystrobin-induced genotoxicity in P. conchonius, 2024)[5]. These data reinforce the need to include fungicides in aquatic monitoring, rather than assuming that genotoxic risk is confined to insecticides.

Mixtures and formulation ingredients

Aquatic organisms are rarely exposed to one isolated pesticide throughout an entire season. Mixtures may arise from simultaneous applications, sequential applications, shared drainage pathways and the persistence of older residues. Interactions can be additive, synergistic or antagonistic, and their direction may change with concentration, timing and endpoint. Commercial products also contain solvents, surfactants and other co-formulants that may modify the bioavailability and toxicity of the active ingredient. Recent reviews therefore emphasise testing formulations and mixtures under environmentally plausible conditions, while avoiding the assumption that a product's active ingredient fully represents its aquatic hazard (Majumder, 2025 [9]; Aquatic ecotoxicity of glyphosate, its formulations, and co-formulants, 2024).

Biomarkers and methods for assessing genotoxicity

Alkaline comet assay

The single-cell gel electrophoresis or comet assay detects DNA migration from individual cells embedded in agarose and subjected to lysis and electrophoresis. Under alkaline conditions, the assay is sensitive to single- and double-strand breaks and alkali-labile sites, as well as transient repair intermediates. It can be applied to blood, gill, liver and other cell preparations, depending on the study question. Common metrics include percentage of DNA in the tail, tail length and tail moment. Percentage tail DNA is often preferred for its interpretability, but results remain dependent on protocol, image analysis, electrophoresis conditions and scoring criteria.

The assay is sensitive and relatively rapid, but it is not a direct mutation test. DNA repair during sample handling, cell viability, temperature, light exposure and scoring rules can alter results. Standardised positive and negative controls, blinded scoring, sufficient biological replication and clear reporting of the number of cells scored per fish are important. Fish should be treated as the experimental unit when the design requires inference among individual animals; cells nested within one fish are not independent biological replicates.

Micronucleus test and erythrocytic nuclear abnormalities

Micronuclei are small extranuclear bodies that arise when chromosome fragments or whole chromosomes fail to be incorporated into daughter nuclei during cell division. In fish, micronuclei are commonly scored in peripheral blood erythrocytes. Nuclear buds, blebbed nuclei, notched nuclei and other abnormalities are sometimes recorded as additional endpoints. These abnormalities can add sensitivity but are not all equally specific, and scoring categories need to be defined before analysis.

The micronucleus test reflects chromosome-level events over a biological time window that differs from the immediate response captured by the comet assay. Results are affected by erythrocyte production, cell turnover, age, species, temperature and the time between exposure and sampling. Slides should be prepared consistently and scored by trained observers, ideally blind to treatment. The number of cells examined, criteria for a micronucleus, frequency denominator and inter-observer quality checks should be reported.

Chromosomal aberrations and sister chromatid endpoints

Chromosomal aberration analysis can identify structural abnormalities such as breaks, fragments and exchanges, while other cytogenetic approaches may detect changes in chromosome number or replication-associated events. These methods can provide information that complements micronucleus and comet assays, but they may require actively dividing cells, specialised preparation or species-specific protocols. Their value is greatest when the hypothesis concerns chromosome integrity and when sufficient technical expertise and validated scoring systems are available.

Oxidative DNA damage and biochemical indicators

Measurements of 8-oxo-dG, antioxidant enzymes, reduced glutathione and lipid-peroxidation products can help evaluate whether oxidative stress contributes to DNA damage. These endpoints should be interpreted as a coordinated response rather than as isolated proof of causation. Analytical specificity is particularly important for oxidative DNA lesions because sample collection and processing can themselves introduce oxidation. Appropriate blanks, internal standards, validated assays and transparent quality-control reporting are essential.

Molecular endpoints and transcriptomics

Gene-expression approaches can reveal responses involving antioxidant defence, xenobiotic metabolism, DNA repair,

apoptosis, immune signalling and endocrine pathways. Candidate-gene studies are relatively accessible, while transcriptomics can detect broader response patterns and identify hypotheses for follow-up. However, altered messenger RNA does not always predict protein abundance or biological function. Molecular findings should be linked to phenotypic endpoints and interpreted with attention to multiple testing, reference-gene stability, sequencing depth and independent validation.

Integrated biomarker response and adverse outcome pathways

Integrated biomarker response methods combine multiple endpoints into a composite score or graphical summary. They can simplify comparisons among sites or treatment groups, but the outcome depends on endpoint selection, scaling and weighting. A composite score should never replace presentation of the individual biomarkers, because opposing responses can be concealed by aggregation. Adverse outcome pathway frameworks offer a complementary way to connect a molecular initiating event to cellular changes, organ-level effects and adverse outcomes relevant to populations. Their application to pesticide genotoxicity is promising, but causal links need empirical support rather than being assumed from correlated biomarkers.

Summary comparison of common endpoints

Endpoint	Main information	Strength	Key limitation
Comet assay	DNA strand breaks and alkali-labile sites	Sensitive; single-cell level; multiple tissues	Not a direct measure of fixed mutation; protocol-sensitive
Micronucleus test	Chromosome fragments or whole chromosomes excluded from nucleus	Relatively simple; useful in fish erythrocytes	Requires strict scoring criteria and appropriate sampling time
Other nuclear abnormalities	Morphological nuclear changes	Can add information to micronucleus scoring	Specificity and classification may vary
Chromosomal aberrations	Structural chromosome damage	Direct cytogenetic evidence	Requires suitable cells and specialised preparation
8-oxo-dG / oxidative lesions	Oxidative modification of DNA	Mechanistic support for oxidative injury	Sensitive to sample artefacts; method-dependent
Gene expression	Stress, repair, metabolism and apoptosis responses	Can suggest pathways and mechanisms	Transcript changes are not equivalent to functional effects
Integrated biomarker response	Combined pattern across endpoints	Useful for summarising multiple stress signals	Score depends on endpoint selection and scaling

Ecological implications

Genotoxic responses are biologically important because genetic integrity underpins development, immune function, reproduction and long-term organismal performance. Persistent DNA injury may contribute to cell dysfunction, apoptosis or mutations, depending on the lesion and the success of repair. If germ cells are affected and damage is not repaired, heritable consequences are possible; however, a somatic-cell biomarker in blood or gill does not establish germ-line mutation or transgenerational effects. Such claims require direct evidence from reproductive tissues, offspring or validated mutation endpoints.

At the individual level, genotoxic stress may occur alongside impaired growth, altered behaviour, reduced feeding, physiological disruption and reproductive changes. At the population level, sustained exposure could contribute to reduced recruitment or altered population structure if sublethal effects occur across a substantial fraction of a population. Yet extrapolation from a short laboratory assay to population decline is difficult. Natural populations experience variable exposure, migration, predation, food availability, disease and other stressors. A causal ecological assessment should combine genotoxicity with survival, condition, reproductive success, abundance and habitat data.

Genotoxic biomarkers can be particularly useful for early-warning monitoring because they may respond before obvious mortality or community-level change. Their greatest value is comparative: identifying sites with elevated biological effects, tracking changes after pesticide-use interventions, or detecting responses that coincide with measured residues. A biomarker signal is not, by itself, proof that a particular pesticide caused ecological damage. Chemical analysis, reference sites, temporal replication and consideration of other contaminants are needed to strengthen attribution.

Factors that complicate interpretation

Species, life stage and tissue

Species differ in metabolism, behaviour, habitat use, erythrocyte physiology and DNA-repair capacity. Embryos and larvae may be more sensitive to some endpoints than adults, but susceptibility is chemical- and endpoint-specific. The use of model organisms such as zebrafish supports mechanistic research, while native species can provide greater local ecological relevance. Ideally, risk assessment combines a well-characterised model with one or more ecologically relevant species.

Exposure concentration and duration

A response at a high laboratory concentration may demonstrate hazard but may have limited relevance to typical field exposure unless the concentration is environmentally plausible. Acute tests can identify rapid effects but may miss chronic, delayed or recovery-related responses. Studies should justify concentration selection using measured environmental data where possible, report both nominal and measured concentrations, and include a recovery phase when persistence of damage is a central question.

Water chemistry and environmental context

Temperature, pH, dissolved organic matter, hardness, salinity, suspended solids and dissolved oxygen can influence pesticide speciation, uptake and fish physiology. These factors may modify genotoxic responses directly or alter the effective exposure. Field studies also face

confounding from metals, pharmaceuticals, wastewater, pathogens and other pesticides. A strong design measures relevant environmental covariates and avoids attributing a biological response to a single chemical solely because it is present.

Formulation and analytical quality

Testing only the active ingredient may underestimate or overestimate the hazard of a commercial formulation. Studies should identify the exact product and active ingredient concentration, report relevant co-formulants when available, and include appropriate solvent or formulation controls. Analytical confirmation of exposure and transparent reporting of detection limits improve confidence in the findings.

Statistical design and reporting

Experimental design should define the fish, not each scored cell, as the independent biological unit where appropriate. Randomisation, adequate replication, pre-specified endpoints and suitable statistical models reduce bias. Concentration, time and their interaction may be important, while repeated sampling from the same tank or individual requires models that account for dependence. Reporting effect sizes and confidence intervals alongside p-values provides more information than significance testing alone. Negative results should be reported as carefully as positive findings.

Implications for environmental monitoring and risk assessment

A practical monitoring strategy should integrate three evidence streams: chemical exposure, biological effect and ecological context. Chemical analysis identifies which pesticides and transformation products are present and at what concentrations. Biomarkers indicate whether organisms show signs of biological stress or genetic injury. Ecological observations establish whether these responses coincide with changes in fish condition, reproduction or community structure. Each stream has limitations, but convergence among them is more informative than any one measure in isolation.

Sampling should be timed to agricultural application schedules and rainfall where relevant, and should include upstream or otherwise suitable reference sites. Repeated sampling across seasons can help distinguish short-lived exposure pulses from persistent contamination. For pesticide mixtures, targeted analysis may be supplemented by broader screening methods, but analytical results should be matched to the sensitivity and limitations of the chosen biological assays.

Risk assessment should distinguish hazard identification from risk characterisation. Demonstrating genotoxic potential under a controlled exposure establishes a hazard signal. Estimating environmental risk additionally requires exposure data, concentration–response information, uncertainty analysis and consideration of ecological relevance. Biomarker findings should inform weight-of-evidence assessments rather than being converted directly into a single risk conclusion.

Research gaps and future directions

First, greater standardisation is needed across laboratories. Common reporting standards for comet assay protocols, micronucleus definitions, number of fish, number of cells scored, image-analysis software and quality-control procedures would improve comparability. Reference compounds and inter-laboratory exercises could help quantify method variability. Second, more studies should compare active ingredients with complete commercial formulations and identify the contribution of co-formulants. Third, mixture experiments should use realistic exposure patterns and should examine whether effects are additive, synergistic or antagonistic across more than one endpoint.

Fourth, chronic and recovery studies are needed to determine whether DNA damage resolves after exposure ends or persists and is accompanied by functional impairment. Fifth, studies should connect somatic biomarkers to reproductive outcomes only where the design directly supports that link. Sixth, field programmes should combine residue analysis with genotoxic, biochemical, histological and ecological measures, while accounting for confounding contaminants. Finally, emerging analytical tools—including high-throughput imaging, targeted DNA-lesion measurements, transcriptomics and pathway-based integration—should be used to strengthen mechanistic interpretation rather than simply increasing the number of endpoints.

Research in agricultural landscapes of South Asia is particularly relevant because small streams, farm ponds, irrigation channels and reservoirs can receive seasonal runoff and support local fisheries. Regionally grounded work should record pesticide-use patterns, rainfall, water chemistry, land use and local fish assemblages. Such information can help translate laboratory findings into management recommendations and identify periods when monitoring or mitigation is most valuable.

Conclusions

Pesticide-induced genotoxicity is a significant component of aquatic toxicology because genetic damage can occur at exposure levels that do not necessarily cause immediate mortality. Evidence from organophosphate insecticides, neonicotinoids, herbicides and fungicides shows that fish may respond with increased DNA migration in comet assays, micronucleus formation and other nuclear abnormalities. Oxidative stress is an important proposed pathway, but mechanisms vary among chemicals and formulations, and the observed biomarker response must be interpreted in relation to cytotoxicity, exposure conditions and repair processes.

The comet assay, micronucleus test, cytogenetic endpoints and molecular biomarkers provide complementary rather than interchangeable information. Their use is strongest when paired with measured pesticide concentrations, suitable controls, biological replication and ecological observations. Recent research highlights the need to consider fungicides, commercial formulations and mixtures, as well as to move beyond short-term single-chemical tests. Future progress depends on standardised methods, field-relevant exposure scenarios and cautious links between molecular damage and ecological outcomes. Genotoxic biomarkers should therefore be treated as an early-warning component of integrated aquatic risk assessment, not as a stand-alone proof of population-level harm.

References

1. Aquatic ecotoxicity of glyphosate, its formulations, and co-formulants: Evidence from 2010 to 2023. Environmental Sciences Europe, 2024. https://doi.org/10.1186/s12302-024-00849-1

2. Assessment of genotoxic and mutagenic effects of chlorpyrifos in freshwater fish *Channa punctatus* (Bloch) using micronucleus assay and alkaline single-cell gel electrophoresis. Food and Chemical Toxicology, 2009. [Verify full author list, volume, pages and DOI before submission].
3. Assessment of imidacloprid-induced genotoxicity in *Pethia conchonius* (Rosy barb), a common freshwater fish of India. Drug and Chemical Toxicology,2023:47(1):101–114. https://doi.org/10.1080/01480545.2023.2222931
4. Assessment of pesticide toxicity in zebrafish (*Danio rerio*) and common carp (*Cyprinus carpio*), emphasising atrazine. Toxicology Reports,2024:13:101694. https://doi.org/10.1016/j.toxrep.2024.101694
5. Azoxystrobin-induced genotoxicity in *Pethia conchonius*, a freshwater fish of River Teesta, India. Aquatic Toxicology,2024:277:107135. https://doi.org/10.1016/j.aquatox.2024.107135
6. Buschini A, et al. [Comet assay applications in aquatic genotoxicity; verify exact article metadata before submission], 2004.
7. Genotoxicity risk assessment in Nile tilapia (*Oreochromis niloticus*) from a hatchery exposed to pesticides in the Haut-Sassandra Region, Côte d'Ivoire. Bulletin of Environmental Contamination and Toxicology, 2025. https://doi.org/10.1007/s00128-025-04120-y
8. Kumar R, Nagpure NS, Kushwaha B, Srivastava SK, Lakra WS. Investigation of the genotoxicity of malathion to freshwater teleost fish *Channa punctatus* (Bloch) using the micronucleus test and comet assay. Archives of Environmental Contamination and Toxicology, 2009. https://doi.org/10.1007/s00244-009-9354-3
9. Majumder R. Effects of pesticides on fish: An overview of evolution of bioassays and cutting edge technologies. Ecological Frontiers,2025:45(2):286–294. https://doi.org/10.1016/j.ecofro.2024.11.011
10. Mechanisms of pesticide toxicity in fish: Insights into the ameliorative role of plant-derived compounds—A review. Aquaculture Nutrition, 2025. https://doi.org/10.1155/anu/5328773
11. Mutagenic and genotoxic effects of carbosulfan in freshwater fish *Channa punctatus* (Bloch) using micronucleus assay and alkaline single-cell gel electrophoresis. Food and Chemical Toxicology, 2010. [Verify full author list, volume, pages and DOI before submission].
12. Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Experimental Cell Research,1988:175(1):184–191. https://doi.org/10.1016/0014-4827(88)90265-0
13. The effect of acute exposure of yellowtail tetra fish *Astyanax lacustris* to the glyphosate-based herbicide Templo®. Mutation Research/Genetic Toxicology and Environmental Mutagenesis,2024:897:503771. https://doi.org/10.1016/j.mrgentox.2024.503771
14. Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, et al. Single cell gel/comet assay: Guidelines for in vitro and in vivo genetic toxicology testing. Environmental and Molecular Mutagenesis,2000:35(3):206–221. https://doi.org/10.1002/(SICI)1098-2280(2000)35:3<206::AID-EM8>3.0.CO;2-
15. U.S. Environmental Protection Agency. Ecological effects test guidelines: OPPTS 850.1075, fish acute toxicity test. U.S. EPA, 1996.