Environmental Transformation and Aquatic Toxicity of Synthetic Dyes: Parent Compounds, Transformation Products, and Treatment Implications

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ABSTRACT

Synthetic dyes are chemically diverse contaminants whose effects on aquatic organisms depend on molecular structure, exposure conditions, environmental transformation and biological susceptibility. This narrative review examines azo, anthraquinone and triarylmethane dyes, distinguishing the hazards of parent compounds from those of their transformation products. Reductive cleavage of an azo linkage can produce aromatic amines, but the identities and toxicological properties of the products cannot be generalized across the entire dye class. The review distinguishes laboratory toxicity, human carcinogenic hazard and ecological risk, using selected literature published on or before 31 December 2022. An embryo–larval fathead minnow study reported LC_{50} values based on measured aqueous concentrations of 25.4 µg/L for Disperse Yellow 7 and 16.7 µg/L for Sudan Red G in exposures extending to 14 days post-hatch; these laboratory values must not be described as demonstrated adverse concentrations in receiving waters. IARC classifications further demonstrate why malachite green and leucomalachite green require separate treatment. Practical assessment should combine parent and product measurements, relevant biological endpoints and wastewater-specific treatment evaluation. Colour removal alone does not establish mineralization or detoxification.

Keywords: *azo dyes; aromatic amines; transformation products; aquatic ecotoxicology; genotoxicity; malachite green; textile wastewater*.

1. Introduction and review objectives

Industrial dyes enable durable coloration of textiles and other materials, but wastewater can convey unbound dyes, processing auxiliaries and reaction products into receiving environments. Aquatic exposure is therefore not necessarily exposure to a pure compound. The chemical mixture, water quality and discharge history influence biological outcomes, while the visible colour of the water provides only incomplete information about its toxicity. A visually clear effluent may retain colourless contaminants, and a strongly coloured sample need not have the same hazard as a different sample of similar appearance [1].

Carcinogenic potential varies among individual dyes and their transformation products. A shared chromophore does not establish equal potency or a common hazard classification. Chemical class, industrial application and toxicological classification describe different attributes [2, 3, 4].

Four questions organize the discussion. First, which structural features influence environmental fate and transformation? Second, how do exposure routes and metabolism connect external contamination to internal dose?

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Third, what do measured biological endpoints actually demonstrate? Fourth, how can treatment performance be evaluated without equating decolourization with safety? These questions link physical chemistry with ecotoxicology while keeping mechanistic possibilities separate from experimentally demonstrated findings.

The review gives particular attention to two primary research studies: Parrott and colleagues' embryo–larval fish study and Balakrishnan and colleagues' investigation of Disperse Yellow 7 reduction products. These provide specific evidence on delayed toxicity and transformation chemistry. IARC's official assessments provide the appropriate basis for statements about human carcinogenic-hazard categories. Broader reviews support classification and treatment context [1, 2, 3, 4, 5, 6, 7].

This narrative review examines relationships among dye structure, environmental transformation, aquatic exposure and biological effects. No new experiment, field survey or quantitative human-health risk assessment is reported.

2. Evidence selection, verification and limitations

This article is a narrative synthesis of selected literature published on or before 31 December 2022. It is not a systematic review. Exhaustive database searching, formal screening and standardized study-quality assessment were not undertaken; selection bias and incomplete coverage remain possible.

Verification has three distinct levels. Bibliographic verification establishes that a publication exists and that its identifying metadata agree with a traceable record. Claim verification establishes that the accessible source supports the particular statement used in the manuscript. Methodological appraisal evaluates whether sampling, controls, exposure confirmation, endpoints and analysis support the interpretation. Completion of one level does not guarantee completion of the others.

Balakrishnan and colleagues examined the reductive transformation of Disperse Yellow 7. Their findings provide a compound-specific example of the formation of toxicologically important products [6].

A consequential correction concerns environmental relevance. Parrott and colleagues reported that concentrations measured entering the Canadian environment were much lower than their larval-effect concentrations, and all 162 assessed municipal-effluent samples were below detection limits for the investigated dyes. Consequently, the observed LC_{50} values are reported as laboratory hazard findings, not as proof of adverse effects at actual environmental concentrations [5].

The literature cutoff is 31 December 2022. Interpretation is bounded by the selected studies, their experimental conditions and available exposure evidence. Results should not be generalized to every commercial dye or receiving environment.

3. Molecular classification and structure–property relationships

Chemical classification groups dyes by chromophore or molecular framework, whereas application classification groups them by how they interact with a substrate. A dye can belong to both systems simultaneously. For example, "reactive" describes an application-related function and does not identify a single chromophore. Comparing "azo" and "reactive" as mutually exclusive chemical families would therefore be misleading [1].

Azo dyes contain one or more –N=N– linkages within conjugated structures. Their optical properties depend on the entire electronic system, including substituents and molecular environment. Sulfonate, amino and other groups may influence aqueous behaviour and interactions with surfaces, but no single substituent predicts complete environmental fate. Structure should guide hypotheses that are then tested using solubility, speciation, sorption and transformation measurements [1, 2].

Anthraquinone dyes have a quinone-containing aromatic framework; triarylmethane dyes include malachite green and gentian violet. Different frameworks support different redox and transformation behaviour. Nevertheless, neither framework alone establishes environmental safety. A result for a particular anthraquinone dye and fish test cannot be extended automatically to every member of the class or to all chronic endpoints [3, 5, 7].

Commercial formulations add another layer of complexity. A trade name may refer to an active colourant together with dispersants, salts or other auxiliaries. The composition affects both exposure preparation and attribution of toxicity. Reproducible studies should report chemical identity, formulation type, active-dye content or purity, supplier and preparation procedure. The measured sample should match the chemical entity named in the conclusion [1].

Table 1. Working classification of dyes.

Classification	Examples / basis	Interpretation priority
Azo	One or more –N=N– linkages	Identify actual cleavage products
Anthraquinone	Quinone-containing aromatic framework	Avoid class-wide safety claims
Triarylmethane	Malachite green; gentian violet	Distinguish parent and leuco forms
Reactive / direct / disperse	Application categories	Report formulation and exposure conditions

Note: These categories do not rank toxicity or carcinogenicity [1, 2, 3, 5].

4. Reductive cleavage and aromatic-amine formation

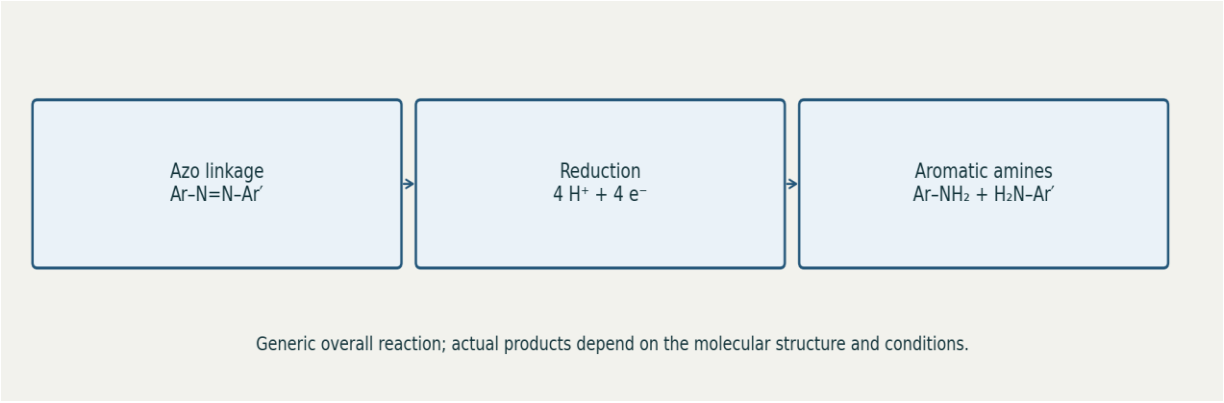
The generic net reaction for complete cleavage of a simple aromatic azo linkage is $Ar–N=N–Ar' + 4\ H^+ + 4\ e^- \rightarrow Ar–NH_2 + H_2N–Ar'$. This equation represents overall stoichiometry rather than an elementary reaction mechanism. Ar and Ar′ denote aryl groups. Protonation state, intermediate formation and subsequent reactions depend on the actual molecule and conditions [2, 6].

Reduction can remove visible colour by interrupting conjugation while leaving substantial organic material in solution. In a molecule with multiple azo groups, partial reduction may generate intermediates that still contain an azo linkage. The time-dependent product mixture is therefore more informative than a single percentage of colour loss. Parent disappearance, product formation and subsequent removal should be measured separately [2, 6].

Balakrishnan and colleagues investigated Disperse Yellow 7 reduction using zero-valent iron and accurate-mass LC–QToF-MS. Confirmed products included p-phenylenediamine, 4-aminoazobenzene and 4-aminobiphenyl. The article also examined a sediment-associated exposure setting. This directly illustrates how a parent-dye-only assessment can omit relevant products. It does not imply that every azo dye produces these three compounds [6].

The toxicological importance of aromatic amines is compound specific. Certain amines may undergo metabolic activation and contribute to genetic injury, but product identity and the relevant biological system need to be established before a detailed mechanism is asserted. A plausible reaction pathway is a hypothesis, not analytical confirmation. Conversely, a colourless product should not be assumed harmless because it no longer absorbs strongly in the visible region [2, 4, 6].

Figure 1. Schematic of generic azo reduction. No experimental rate or yield is implied [2, 6].



5. Environmental fate, compartments and exposure history

Discharged dye-related material can partition among dissolved water, suspended particles and sediment, or undergo biological and chemical transformation. Which processes dominate depends on the substance, formulation and receiving environment. A water-only measurement may characterize an important exposure pathway but cannot represent every compartment relevant to benthic organisms or particle ingestion. Environmental assessment should begin with a conceptual model of sources, pathways and receptors [1, 6].

Operational definitions matter. Filtered concentration, total-water concentration and extractable sediment concentration are not interchangeable. Filtration can remove particle-associated material, while extraction may

measure a fraction that differs from the portion available to organisms. Methods should state sample preparation and the fraction actually quantified. Otherwise apparent differences between studies may reflect analytical definitions rather than environmental variation.

Transformation can alter partitioning. A product may differ from the parent in ionization, solubility and sorption behaviour. Consequently, effective removal of a parent from the water column does not necessarily demonstrate that its products or total hazard have disappeared. Sediment can be a relevant compartment for both persistence and biological contact; its role should be examined rather than assumed from the colourant's application category [6].

Exposure also has a temporal dimension. A short pulse, repeated discharge and continuous release can produce different concentration histories. Laboratory test renewal schedules and observation periods should therefore accompany effect concentrations. Parrott and colleagues renewed solutions daily and observed organisms through 14 days post-hatch, allowing detection of delayed larval mortality. Without those details, the numerical endpoints can be misinterpreted as generic acute thresholds [5].

6. Case study: delayed toxicity in fathead minnow larvae

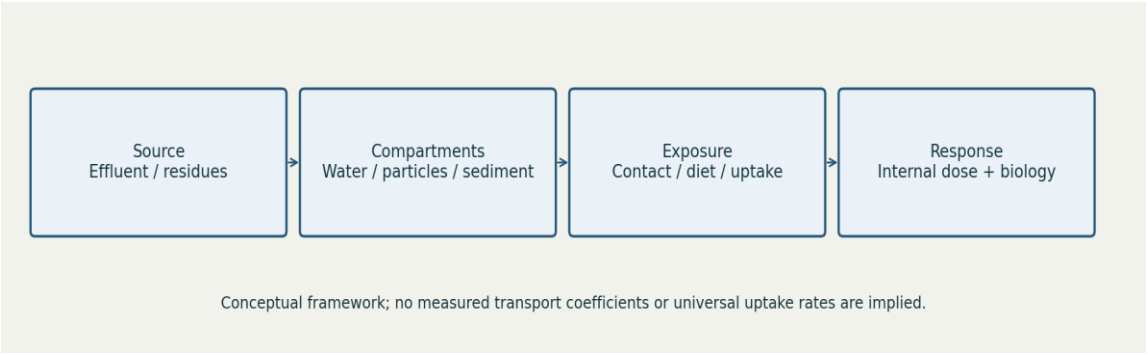
Parrott, Bartlett and Balakrishnan exposed newly fertilized *Pimephales promelas* embryos through hatching and through 14 days post-hatch, renewing dye solutions daily. The study examined two azo dyes and two anthracenedione dyes. The duration is central to interpretation because effects of the azo dyes emerged after the embryo stage [5].

The reported LC_{50} values based on measured aqueous concentrations were 25.4 µg/L for Disperse Yellow 7 and 16.7 µg/L for Sudan Red G. The abstract describes mortality 4–10 days after hatch. These are not 96-hour adult-fish LC_{50} values or universal thresholds for azo dyes. They should be quoted with the life stage, exposure window and measured-concentration basis [5].

Acid Blue 80 and Acid Blue 129 caused no reported effects in larval fish at the highest measured tested concentrations of 7,700 and 6,700 µg/L, respectively. Those upper tested concentrations must not be presented as their LC_{50} values: the study did not establish LC_{50} values at those concentrations. Nor do the findings prove safety for every organism or chronic endpoint [5].

The environmental context is equally important. The authors reported much lower environmental concentrations than those affecting survival and all 162 assessed municipal-effluent samples below detection limits for the investigated dyes. This does not establish zero environmental exposure, but it prevents an accurate summary from claiming that the LC_{50} values were demonstrated environmental concentrations. The study offers specific hazard evidence and a reason to consider delayed effects, not a universal prediction of field harm [5].

Figure 2. Reported LC_{50} values for the two azo dyes. Confidence intervals omitted; anthracenedione upper tested concentrations not plotted as LC_{50} values [5].



7. Malachite green: parent–metabolite distinctions

Malachite green is a triarylmethane dye with applications that have included materials coloration, staining and aquaculture-related use. Srivastava, Sinha and Roy reviewed its toxicological effects and the significance of the reduced compound leucomalachite green. This review remains useful background, but an authoritative cancer-hazard statement should also reflect the later IARC evaluation [3, 7].

IARC classified malachite green as Group 3, not classifiable as to its carcinogenicity to humans. The evidence was inadequate in humans and limited in experimental animals and mechanistic research. Group 3 is not a finding of harmlessness; it reflects the available evidence and the classification rules [3].

Leucomalachite green was classified as Group 2B, possibly carcinogenic to humans, based on sufficient evidence in experimental animals. Human and mechanistic evidence had different limitations. Because the metabolite and parent received different classifications, they should not be combined into one unqualified statement that "malachite green is an established human carcinogen" [3].

Table 2. Compound-specific IARC Volume 129 outcomes.

Compound	IARC classification
Malachite green	Group 3
Leucomalachite green	Group 2B
Gentian violet	Group 2B
Leucogentian violet	Group 3
CI Direct Blue 218	Group 2B

Note: IARC categories concern carcinogenic hazard to humans, not aquatic exposure limits or demonstrated tumour induction in aquatic organisms [3].

8. Species, life stages, mixtures and ecological context

An aquatic assessment should not treat fish as a complete substitute for all ecological functions. Primary producers, planktonic invertebrates and benthic organisms encounter different compartments and have different relevant endpoints. Multi-trophic testing is therefore useful when the assessment question extends beyond one species. The literature includes acute and chronic assays of dye formulations using *Daphnia magna* and *Raphidocelis subcapitata*; results should be interpreted in the context of the test design [1, 8].

For algae and aquatic plants, colour can alter light availability while the chemical mixture can impose direct stress. Experimental interpretation should separate these processes where the research question requires it. A lower optical growth signal can also be complicated by colour interference. In whole-effluent assessment, shading may itself be relevant, whereas an intrinsic-toxicity experiment needs appropriate consideration of optical effects [1].

Sensitivity changes with life stage and endpoint. The fathead minnow study demonstrates that an embryo-only observation period would have underestimated effects of two tested azo dyes. It does not prove that every embryo assay is less sensitive than every larval test. Species, developmental stage, exposure barriers, metabolism and duration must be compared explicitly [5].

Mixtures create attribution problems. A downstream biological response may reflect dyes, transformation products, salts, surfactants or other co-contaminants. Detection of a response near textile activities warrants investigation but does not automatically identify one dye as the cause. Stronger evidence combines source characterization, chemical data, relevant comparison conditions and convergent biological observations [1].

9. Prevention, adsorption, coagulation and membranes

Treatment begins with understanding the waste stream. Process optimization and segregation of chemically different or highly concentrated streams can help match treatment to the actual load. Reuse opportunities and improved dye fixation may reduce the quantity requiring disposal, but feasibility depends on the textile process and product requirements. These are management options rather than a universal plant design [1, 9].

Adsorption lowers aqueous concentration by transferring material to a sorbent. Activated carbon, modified minerals and other materials can differ substantially in affinity, capacity and regeneration behaviour. Batch results using a single dye in clean water cannot establish industrial performance in a saline, mixed effluent. Competing constituents, sorbent dose, contact conditions and spent-material handling belong in the evaluation [10, 11].

Coagulation and flocculation can remove colour-bearing and particulate material by aggregation and separation. Their effectiveness depends on wastewater chemistry and operating conditions, and the resulting sludge requires

management. Better visual appearance does not demonstrate removal of every dissolved product. A proper evaluation includes the residual aqueous phase and the separated solids [12].

Membrane processes separate or concentrate constituents according to membrane characteristics and operating conditions. Retained contaminants are not automatically destroyed. Fouling, cleaning requirements, energy demand and concentrate disposal affect the overall practicality of a process. The membrane work of Akbari and colleagues is a supporting example, not a basis for asserting one universal dye-removal efficiency [13].

The distinction between transfer and destruction is essential. Adsorption, precipitation and filtration can be valuable exposure controls even when they do not mineralize the contaminant. However, a complete assessment must follow the transferred material into spent sorbents, sludge or concentrate. Otherwise pollution can be shifted from one compartment to another while the reported water concentration improves [9, 12, 13].

Biological treatment can complement physicochemical separation, but colour loss and biodegradation are distinct outcomes. Sequential microaerophilic–aerobic treatment and biological treatment of model dyes and textile wastewaters provide relevant examples; performance remains dependent on the dye, microbial system and wastewater composition [14, 15].

Chemical oxidation can transform dyes without complete mineralization. Oxidation pretreatment and potassium permanganate decolourization should therefore be evaluated alongside residual chemical and biological endpoints [16, 17, 18].

Residue analysis should distinguish parent compounds from metabolites. Analytical work on malachite green and its metabolite in fish samples illustrates this distinction, but residue detection does not itself establish an adverse-effect threshold [19].

10. Research priorities, synthesis and conclusions

Priority 1: transformation-product identification. Future work should distinguish confirmed structures from tentative assignments and connect time-resolved product profiles to biological outcomes. The Disperse Yellow 7 example demonstrates the importance of moving beyond parent disappearance [6].

Priority 2: realistic and reproducible exposure. Studies should report formulation composition, measured concentrations, exposure renewal, relevant water chemistry and time course. Field investigations should characterize the compartments and conditions that organisms actually encounter [1, 5].

Priority 3: biological comparability. Life-stage coverage should be justified, and delayed effects should be considered when the evidence suggests they may matter. Multi-trophic assessment can broaden ecological interpretation [5].

Priority 4: treatment endpoints. Real-effluent studies should pair chemical characterization with residual-effect testing and follow contaminants into solids, sorbents and concentrates. Treatment performance should distinguish decolourization, removal, transformation and mineralization [9, 12, 13, 14, 15, 16].

Conclusions. Dye-related aquatic impacts require compound-specific assessment. Certain azo compounds can form hazardous products, but their identity depends on structure and conditions. The verified fathead minnow study establishes delayed larval toxicity for two dyes while also demonstrating why laboratory hazard must be separated from measured environmental exposure. IARC's different outcomes for malachite green and its leuco metabolite reinforce the need for precise terminology. A defensible response combines source control, relevant exposure measurements, fit-for-purpose treatment and confirmation that residual biological hazard decreases [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19].

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