Advances in Bioresearch
Adv. Biores., Vol 16 (2) March 2025: 245-250

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL: http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.16.2.245250

Advances in Bioresearch

REVIEW ARTICLE

Biochemical Study of Carcinogenic Dyes on Human Health

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ABSTRACT
They are special chemicals that pose the risk of health hazards, which may probably include malignancy, by exposing azo dyes to health. Breakdown of azo dye in a human body can lead to almost carcinogenic compounds called aromatic amines. The assimilation and metabolism of these dyes into the body vary with the chemical property of the dye; some are absorbed better while for others it is lower. The detoxification of these dyes also depends on genetic differences of the individuals, age, and health status. We have other dyes, like triphenylmethanes and anthraquinones, which possess a carcinogenic nature; however, the phenomenon by which cancer occurs and is promoted is not well understood. For example, malachite green may be mutagenic to DNA. It is called for a much greater understanding of the health effects of and the effects on the environment by these dyes. The intestinal tract is the primary site for azo dye metabolism and by bacteria in the intestine that produce certain aromatic amines which have been implicated as DNA damaging agents. Some detoxifying systems could also influence a few, very few of them. Apart from cancer, other diseases associated with these dyes include skin diseases, respiratory complications, and several other serious health hazards. Epidemiological studies furnish evidence that can lead to the association of health effects from exposure to dyes. Still, direct and primary proof is always very difficult to ascertain.
Keywords; *Azo dyes, DNA, Carcinogen, Neurotoxic, benzidine, AOPs*

Received 10.02.2025 Revised 01.03.2024 Accepted 14.04.2025

How to cite this article:
Anthati Sreenivasulu. Biochemical Study of Carcinogenic Dyes on Human Health. Adv. Biores. Vol 16 [2] March 2025:245-250

INTRODUCTION

This review deals with certain biochemical mechanisms in which the carcinogenic dyes work against human health. An extensive array of dyes is utilized in the textile, food, and cosmetic industries (1, 2). Many dyes possess azo and other chemical molecular structures that have been linked to mutagenic and carcinogenic properties (3). These dyes are introduced to humans along many routes: through the dermis (with clothing and cosmetics) (3, 4), orally (through the use of food additives) (5, 6), and by inhalation (mostly in occupational settings) (7,8). A general understanding of certain biochemical pathways is essential for devising strategies for intervention and prevention. Some dye classes, their metabolic pathways, and the consequences on health are what this review focuses on. It tries to stress the very complicated biochemical mechanisms involved. The challenge presented by the subject will certainly require factors from various active disciplines such as biochemistry, toxicology, and epidemiology.

CHEMICAL CHARACTERISTICS OF CARCINOGENIC DYES

A. Azo Dyes: Structure and Reactivity

Azo dyes are identifiable dye stuffs, which could be formed due to one or more azo bonds (-N=N-) attached to them. Among significant dye stuffs, these are also implicated in carcinogenicity (3,5,9). The reduction cleavage by bacterial enzymes in the gastrointestinal tract or liver is a crucial factor in their toxicity (10). Cleavage leads to carbocyclic amines (3), many of which are known or suspected to be carcinogenic agents (11,12). The specific formation of aromatic amines is highly structure-dependent concerning the dye (3). A clear example is the azoreduction of the azo dye, carmoisine, in the mouse, which resulted in marked alterations, including organ weights, hematological parameters, and biochemical markers, suggestive of hepatotoxicity and nephrotoxicity (5). Additionally, lipophilicity is

another critical measurable parameter that governs consumption and interaction with metabolic sites. In contrast with the lipophilic spaces, which have a stronger attraction towards fat-soluble milieus, lipophilic dyes present more absorbance as they always get to the sites of metabolic activation (10). Thus, enhanced access would enhance the activation and toxicity that follows in metabolism. Generally, hydrophilic dyes, on the other hand, would tend to be less absorbable, hence limiting access to metabolic enzymes. Therefore, physicochemical properties underline the fate of a dye during metabolism as a top determinant of ultimate toxicity. Furthermore, activation is quite inadequate with reductive cleavage and continues following that event with required oxidative activation to exert mutagenicity effects (10). This multi-step activation process highlights the complexities of azo dye metabolism and the potential for considerable inter-individual variation in susceptibility.

B. Other Dye Classes: Anthraquinones, Triphenylmethanes, etc.

The carcinogenicity of other classes of dyes beyond azo dyes is quite substantial. Anthraquinones, which have been termed to be important toxic agents in textile dyeing, have been associated with unknown biochemical mechanisms of carcinogenicity. These dyes are possibly modulators of intracellular active components affecting several biological processes. The carcinogenicity and mutagenicity of triphenylmethane dyes, malachite green being only one of its members, are certainly popular in aquaculture applications. Malachite green intercalates into DNA, preferably into A: T-rich sequences. Intercalation might block a DNA replication or repair pathway, thus creating further chances for mutations and genomic instability. Malachite green causes lipid peroxidation, another indication of oxidative-stress-associated toxicity of this dye (13). These oxidants can also be produced during lipid peroxidation and injure cellular macromolecules such as nucleic acids and proteins to make them key contributors to mutagenicity and carcinogenicity (14). Thus, these different dye classes have not been thoroughly studied for biochemical effects, but in many instances, intermediates bearing chemical constituents such as biological macromolecules or organelles interfere with the operation of some important cellular functions (13). However, future investigations should strive to make possible even more detailed elucidation of biochemical pathways and molecular targets for these dyes, considering their synergistic or additive action when a mixture of these dyes is present. Persistent environmental pollutants include these dyes, eg, anthraquinones, triphenylmethane (15), raising alarms regarding their long-term impact when discharged into the environment.

METABOLIC ACTIVATION AND DETOXIFICATION PATHWAYS

A. Reductive Cleavage of Azo Dyes

The process of reductive cleavage of the azo bond is the primary metabolic route for many azo dyes (9,10). This key step is primarily performed by bacterial enzymes existing in the gut, particularly under anaerobic conditions typical of the lower intestinal tract (10). The anaerobic conditions in the gut enhance the activities of azoreductases responsible for catalyzing the reductions of the azo bond (10). The produced aromatic amines may then be further processed by oxidation, conjugation reactions, or any of the other metabolites (12). The extent and rate of azo reduction are governed by the chemical structure of the dye in question and the prevailing reducing conditions (10). Other structural features of the dye, such as the presence of electron-withdrawing or -donating groups, affect the susceptibility of the azo bond to reductive cleavage (10). Furthermore, the composition and activity of gut microbiota are vital since changes in the microbial population may influence the rate of azo reduction (10). Nevertheless, some minor contribution to azo dye metabolism occurs in the liver (10). The reductive metabolism of azo dyes is entertained by liver enzymes, which include both microsomal and soluble systems (10). However, certain considerations related to the oxygen sensitivity of azoreduction call for different levels of oxygen to be present in the liver (10). These considerations accentuate the need for a deeper understanding of both gut and liver metabolism about the ultimate toxicity assessment of azo dyes.

B. Oxidative Metabolism of Aromatic Amines

The reduction of azo dyes leads to the production of many aromatic amines that are often subsequently metabolized by cytochrome P450 enzyme(s), which are likely oxidative (10,12,16). Such metabolism is indeed expected to produce very reactive electrophilic intermediates (10,12). The immediate reaction of these highly reactive intermediates might be with the bases of DNA and covalent adduct formation occurs (10,12,17). This event is considered one of the important beginnings of initiation of carcinogenesis (17). Adducts may distort the DNA double helix and thus would interfere with DNA replication and transcription (17). This kind of interference could end in mutations that affect crucial genes, further assist in the uncontrolled growth of cells, and finally develop into cancer (17). Some isoforms of cytochrome P450 (like CYP1A1 and CYP1A2) assume a rather special importance in the aromatic amine activation process (12,16). These isoforms show substantial variability in expression and activity in individuals, and this may direct them to be risk determinants for dye-related cancers. Genetic polymorphisms in the

enzyme gene would be expected to have a strong impact on enzyme activities, as evidenced by variation in metabolic activation and detoxification (16).

C. Detoxification Mechanisms

Humans have developed many detoxification schemes that reduce injuries caused by the dyes and their metabolites. Mechanisms such as conjugation reactions, with glucuronidation and sulfation being two good examples, serve to detoxify the dye and dye-related metabolites. These conjugated metabolites become more water-soluble, thus excreted in urine or bile. The status of effectiveness for these conjugation systems may also be influenced by other factors, including the availability of the conjugating enzymes, the structure of the metabolite, and the health status of the individual in general (18). Another detoxification mechanism that is just as important would be those antioxidant systems, which include glutathione. This is the primary neutralizer of reactive oxygen species (ROS) that originate from the metabolic activation of many dyes. These ROS actively damage cellular macromolecules, including DNA and proteins, in a mechanism of oxidative stress, which becomes associated with many health challenges (14). The action of any such pathway for detoxification is influenced by huge variations in the individual; in these factors, genetic constitution, age, and health status are some prominent ones (18). People who have defective detoxification pathways should be at a significantly increased risk for one or more adverse health effects upon exposure to the dye.

GENOTOXICITY AND DAMAGE TO DNA

A. DNA Adduct Formation

This is the key to carcinogenic processes initiated by dyes, according to 12, 17, and 18. Formation of DNA adducts will ultimately be causes of cancer through covalent binding of reactive electrophilic metabolites to DNA bases (10,17). What specific DNA adducts are formed depends, among others, on the structure of the dye and its metabolic pathways (17). For example, the metabolic activation of benzidine leads to some DNA adducts formed by exposure to benzidine-based dyes (12). They may induce mutations, interfere with the replication process of DNA and repair, and, toward the end, contribute to genomic instability and the onset of cancer (17). The persistence of DNA adducts in the cell is one of the most important factors determining whether it will mutate. The amount of time a given adduct has the potential to produce mutation increases as its stay long in cells: through replication of DNA, in turn, mutation results in increased risk for cancer. Methods used to detect and quantify DNA adducts in different tissues following exposure to dyes include 32 P-postlabeling as well as HPLC-MS (17).

B. The Mutations and Chromosomal Aberrations

Reactive metabolites can damage DNA adducts or other forms of damage, which can lead to mutations and subsequent changes in genetic information within cells. Such mutations might be in genes coding for critical cellular processes such as cell growth differentiation and programmed cell death (apoptosis). Mutations in genes that participate in the control of growth and apoptosis may favor uncontrolled proliferation of cells, leading to tumors. DNA damage induced by dyes can also result in chromosomal aberrations, which are deletions, translocations, and aneuploidy. Chromosomal aberrations further disrupt normal cell function and augment genomic instability, which is risk-augmented for cancers (14,17). All the different types of mutations and chromosomal aberrations are often dye-specific, as it indicates differently reactive metabolites produced, with certain metabolite-DNA interaction types. To detect and characterize chromosomal aberrations, chromosome analyses are carried out; micronucleus assays have also been used, as well as fluorescence in situ hybridization (FISH).

B. Genotoxicity studies *in vitro* and ad *in vivo*

There is a considerable amount of in vitro and in vivo evidence showing that several dyes may be genotoxic (11, 13, 14). The Ames test has been widely used to examine the mutagenic potential of certain dyes and their metabolites (11) while being a bacterial mutagenicity assay. Such strains, with mutations that imply that they can detect many forms of DNA damage, including base-pair substitution mutations and also frame shift mutations (11) are involved in this test. In the Ames test, the results were positive when the substance, which involved the dye or its metabolite, was mutagenic in bacteria; thus, it was presumed to have the potential for genotoxicity in other organisms, including humans, indeed. More conclusive in vivo evidence in animal studies linked some dyes with carcinogenic effects. These tests usually involved prolonged exposure of the dye at several doses to animals and the subsequent examination of the development of tumors (11, 13). All these result from in vitro and in vivo studies, which form a fundamental basis for risk assessment and regulatory decisions about the use of dyes within various industries. According to this work using dyes, such data classify dyes as carcinogenic or possible carcinogen by regulatory bodies such as the International Agency on Cancer Research (IARC). Aspects related to carcinogenic exposures of dyes have been linked with an elevated risk profile for various cancers (11, 12, 15). Specifically, benzidine dyes are associated with bladder cancer. It is mainly

implicated as a human carcinogen and often appears as a parent compound or a metabolite in many azo dyes. Then again, due to occupational exposure to benzidine and associated dye classes, the dye-and-textile industry has seen a markedly increased incidence of bladder cancer (12, 19, 20). Liver cancer has also been attributed to several classes of dyes (5, 17). Mechanistically, liver carcinogenesis triggered by dyes occurs whenever reactive metabolites are formed, which are DNA damaging and disrupt normal biological processes. Some lung and skin cancers can also occur depending on the exposure path and the dye present (4, 18). Certain dyes might induce skin carcinogenesis after dermal exposure from cosmetics or clothes, whereas inhalation of dye dust at work may prime an individual for lung cancer. In any case, research must be intensified on linking the causation of certain cancers to specific dye classes via different exposure routes.

D. Non-Cancerous Health Effects

In addition to the increased risk of cancer, exposure to dyes can cause a plethora of non-cancerous health problems. These comprise allergic contact dermatitis and irritant contact dermatitis (4 , 7, 8). These skin reactions are caused of inflammation and irritating effects on the skin due to direct contact with dyes or their metabolites. Some dyes, especially used in the textile industry, were responsible for respiratory problems such as asthma or allergic rhinitis from occupational exposure (7). Inhalation of dye dust or fumes has been reported as a trigger for these conditions in predisposed individuals. Some dyes are reported to cause liver impairment (5, 7), perhaps by the accumulation of toxic metabolites or by further stimulating oxidative stress. Neurotoxic effects are also reported in some dye exposure cases (4), with the actual bio-mechanism behind these phenomena not always being discerned. The intensity of non-cancerous effects exhibited may vary dramatically due to various factors, including genetic predisposition, concentration, and duration of exposure.

E. Epidemiological Studies: Human Exposure and Disease

Conducted epidemiological studies are very useful in evaluating human health risks related to exposure to dyes (17, 19, 21). These studies deal with the relationship between dye exposure and the incidence of disease in human populations. However, rigorous epidemiology on dye exposure is difficult to set up. It is difficult to accurately quantify exposure levels to more than one dye over time, especially when exposure may come from more than one source and route. Other factors complicate epidemiological interpretation, such as lifestyle, dietary habits, and exposure to other environmental pollutants. The relatively long delays between exposure to carcinogenic dyes and the occurrence of cancer often hinder clarity in establishing causal relationships. Despite these difficulties, ever since studies in epidemiology linked the exposure to some dyes, especially those based on benzidine, to a considerable increase in the risk of bladder cancer, they have been crucial evidence for the regulations on these dyes regarding their use (17), (19). Studies of this kind have proven critical in the decision-making process regarding regulatory criteria for the use of these dyes. Progress in advanced analytical methods for exposure assessment coupled with greater sophistication in statistical techniques for control of confounding factors will allow increased reliability in the results of epidemiological studies on dye exposure.

MITIGATION STRATEGIES AND FUTURE DIRECTIONS

A. Regulatory Measures and Safety Standards

Effective regulations and safety standards are crucial for minimizing human exposure to carcinogenic dyes (4, 15, 18). Many countries have put up regulations restricting the usage of some dyes for food, cosmetics, and textiles. These regulations mostly define maximum allowable concentrations of specific dyes or ban the usage of certain dyes altogether (4, 18). IARC monographs constitute a contemporary resource for classifying the carcinogenic potential of a variety of chemicals, including dyes and for making regulatory choices (18, 20). Nevertheless, challenges remain regarding the enforcement of the regulations and identification and control of all hazardous dyes that need to be entered into regulation (4,18). International cooperation will ensure that consistent regulations and effective monitoring mechanisms are in place at the global level due to the international aspect of the dye industry. Besides, with the introduction of ever-new dyes, the regulatory environment must, therefore, be constantly assessed for safety. These actions will promote the establishment of standard testing protocols and other extraction methods for monitoring dye exposure to ensure compliance with regulatory measures.

B. New technologies that can remove and degrade dyes

Innovation and technology in pollution control will focus on the dye contamination in waste water that is disposed by industries. Many methods have been developed for removing and degrading dye effluents from industrial waste (15, 22, 23). These have included adsorption of many materials such as activated carbon, zeolites, and agricultural wastes (23, 24). They exhibit advanced oxidation processes (AOPs), which act by release of an extremely strong oxidizing agent for breaking down the molecules of dyes, and

promise to advance into total dye degradation (23, 25). Microorganisms can also be used for the degradation of dyes under bioremediation and are a sustainable as well as an inexpensive option (9, 25). The choice of technology is very much dependent on many criteria, including the type of dye present, the concentration of dye in wastewater, the cost of treatment, and the environmental impact of the selected technology (15, 22, 23). Research continues into the development of novel technologies that are more effective, cheap, and environmentally friendly in processing dye stuff removal and degradation. These include innovative materials for adsorption, better AOPs, and optimization of bioremediation processes.

C. Safe Dyes and their Replacements

Developing dyes and alternatives that are safer for human health eliminates hazard risks concerned with dye exposure. It can be well substituted with bio-based dyes that are extracted from natural sources, such as plants or microorganisms. These bio-based dyes are less toxic and more biodegradable than synthetic dyes. Production up-scaling, consistent color fastness, and different color requirements continue to pose some challenges for bio-based dyes (6, 26). Research is dedicated to improving yield and performance in bio-based dyes, searching for new sources of natural pigments, and developing innovative dyeing techniques. This could involve genetically engineering microorganisms to enhance the quantity of dyes in their cells, innovating extraction and purification methods, and designing dyeing processes that will be more sustainable. Going to safer replacements in dyes is the task of a concentrated group of researchers, industry, and regulatory agencies.

CONCLUSION: MERGING BIOCHEMICAL, EPIDEMIOLOGICAL AND TOXICOLOGICAL EVIDENCE

In this paper, I have tried to emphasize different interdependence of biochemical properties of the cacinogenic dyes, their activation and detoxification metabolism, the genotoxic effects of those cacinogens, and finally, their potential outcome in human health. The evidence provided indicates a complete integration of biochemical, toxicological and epidemiological information as a necessity for a thorough appraisal of the risk that exposure to dyes carries. The majority of dyes are even known to have been proven carcinogenic; this is even more so with evidence specifically linked to specific dyes- for example, those based on benzidine, which carries a very high risk for one specific cancer, namely, bladder cancer. Many dyes are carcinogenic, but the exact mechanism behind it is not well known; therefore, a lot of work still needs to be carried out to elucidate the pathways of metabolism, damage done to DNA, cytotoxicity, etc. Protection strategies against carcinogenic dye exposure would include applications of several technologies for removal and degradation, safer alternatives to dyes, and stringent regulations to ensure that blues are safely used all across industries. Further research would then be continued by identifying and recognizing potentially harmful dyes, improving risk assessment protocols, and creating deterrents against injury resulting from misuse or poor application of these dyes on human and environmental health. All such tasks will require the construction of a complete picture of this major public health problem from complementary data gathered and used from almost all research fields.

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