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Review of the Mutagenicity of Textile Dye Products

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Abstract:
A review of the literature on the mutagenicity of textile dye products is presented in this article. This review discusses genetic hazards associated with the production and use of textile dyes throughout the world. Mutagenicity of azo and non azo dyes has been considered due to extensive recent data on the carcinogenicity and mutagenicity of this group of dyes. In addition, a section on *Salmonella* mutagenicity bioassay has been included. The data discussed mainly concerns the activities of these dyes in short-term tests for mutagenicity whilst reference only is made to animal carcinogenicity and non-specific toxicity. Environmental impact of these dyes has been assessed and an attempt has been made to evaluate the data with respect to correlation with tumour-inducing ability and to cause cancer.

Keywords: Mutagenicity, Textile dye products, Textile dyes, *Salmonella* Mutagenicity Test

1.0 Introduction:
Dyes comprise one of the major groups of chemicals besides fertilizers, pharmaceuticals and petrochemicals. During ancient times, people used dyes from natural resources like tesu flowers, to make their clothes bright and colorful. Likewise, indigo, logwood, madder, etc. are natural dyes originating from plants; while Tyrian purple, kermes, cochineal etc. are natural dyes of animal origin. Such dyes were almost biodegradable, therefore were not hazardous. However, these dyes are very expensive and require a lot of labor in their manufacture and use. Therefore, use of preferably cheaper and easily available sources, such as chemicals, as dyes, began. Perkin was the pioneer in producing man made organic dye, mauve, as early as 1856. The first synthetic organic dye was produced in 1871 when Woulfe prepared picric acid by treating the natural dye, indigo with nitric acid. Since then several new chemical dyes have been added to the ever-increasing list of dyes. These dyes, pigments and colors form around 27% of the chemical industries in India. Dyes are coloring pigments that impart colour to the substrate and find application in variety of industries like textiles, leather, paper, printing ink and food technology. Intermediaries are basically compounds that are formed during the preparation of a dye. Dyestuff is a broad term that includes dyes, intermediates and pigments. The dyestuff sector represents an important segment of the chemical industry having forward and backward linkages with a wide range of industries.

In the last years, the dyestuff industry has grown many folds. The world's annual production of dye stuffs accounts to more than 7×10^5 tones (Moosvi *et al.,* 2007). Though the overall growth of dyestuffs industry during the last 5 years has slowed down, the industry is still expected to maintain a growth of about 2% per annum in the next decade. In India, the per capita consumption of dyes is 50 gms, which is very low as compared to a world consumption of 425 gms, which indicates that there is a tremendous potential for growth of this sector in India. India accounts for around 5 percent of the global output. The dyestuffs market is expected to grow at the rate of 2 percent per annum during the next decade.

The textile industry accounts for the largest consumption of dyestuffs at nearly 70 percent. Reactive Dyes, Vat Dyes & Azo Dyes are mainly required for dyeing and printing of cotton fibers. Disperse dyes constitute the largest market with about 21% share followed by direct dyes and reactive dyes with 16% and 11% respectively. Textile dyes are generally classified either in accordance with their chemical constitution or their application to textile fibers and other coloring applications. The

chemical constitution is shown by a Colour Index (CI) number. CI number determines properties of dyes such as suitability for dyeing a specific substrate and the fastness properties of dyed goods. Classification based on chemical composition is useful to chemists and manufacturers of dyestuffs while application or usage based classification is of vital importance to the dyers. Dyes are derived synthetically from raw materials like hydrocarbons, benzene, toluene, naphthalene and anthracene using coal tar obtained from distillation of coal. Dyes are retained in substrates by physical absorption, metal complex formation or by the formulation of covalent chemical bonds. Dyes obtain their colour due to electronic transitions between various molecular orbital. The intensity of the colour is determined by the probability of these transitions.

In the past few years, performance of the dyestuff industry has been dismal. The ban on application of Azo dyes by Germany and other European countries had an adverse impact on the dyestuff exports. Besides the dye stuff industry has increasingly experienced pressure from the Sino-Chem army in the international markets. The problems of the industry were further compounded by cheap imports and recession in the textile sector. The industry has also not kept pace with the developments in the west. Lastly during the last decade, environmental issues associated with dyestuff production and application have grown significantly and are indisputably among the major driving forces affecting the textile dye industry today. The main environmental issues related to the dyestuff industry can be grouped into air, water, land use, health and safety, and waste management issues. It is generally assumed that substances which are used to produce textiles are thoroughly tested regarding their health consequences. However in recent years various researchers have identified mutagenic effects of textile samples and waste water of the textile industry (Knasmüller *et al*., 1993, Deutsches *et al*., 1994 and Jäger, 1998).Further investigations showed that the dyes used for textile finishing are mainly responsible for the mutagenic effects observed. Besides, effluent discharge from textile and dyestuff industries to neighbouring water bodies and wastewater treatment systems is currently causing significant health concerns to environmental regulatory agencies. Colour removal, in particular, has recently become of major scientific interest, as indicated by the multitude of related

research reports on this issue (Banat *et al*., 1996, Jäger *et al*., 2004 and Schneider *et al*., 2004).

1.1 Previous References to the Literature:
A numbers of reviews concerning the genotoxicity of natural and synthetics dyes, especially those used in food, drug, cosmetics and textile, have been published (Combes and Haveland-Smith, 1982,). Recently, Puvneshwari *et al.* (2006) have reviewed the toxicity assessment of azo dyes. Chung (2006) has discussed the mutagenicity of benzidine, benzidine analogues, and benzidine-based dyes. Industrial and environmental aspects have been debated by Anliker (1977) and Houk (1992). Epidemiologic evidence of cancer risk in textile industry workers was reviewed by Mastrangelo (2002).

1.2 Mutagenicity of Benzidine, Benzidine Analogues, and Benzidine-Based Dyes:
Many of the dyes used to color textiles; drugs, cosmetics, paper and food are azo dyes. Benzidine and its congeners are dye intermediates, i.e., precursors of dyes. A common method used to prepare benzidine and congeners involves the reduction of nitro benzenes followed by the acid catalyzed intra molecular rearrangement of the resulting hydrazobenzenes. Subsequently, the dyes are produced by the diazotization of the amino groups on the benzidines and azo coupling to reactive aromatic ring systems (other dye intermediates). Metallizing of dyes sometimes is carried out to improve the stability of the azo groups; however, the mechanism by which the added metal chelates with the dye is not always well understood. Azo-dyes account for the largest proportion of all synthetic dyes in terms of number and amount of production. They include approx. 70 % of all organic dyes, which are currently on the market and are manufactured mainly in China, India, Korea, Taiwan, and Argentina.

Possible genotoxic effects of textile dyes are most often discussed with respect to selected azo dyes (Platzek, 1996). Some of these dyes, which contain an azo group (-N=N-), are able to split off genotoxic and carcinogenic amines (e.g. Acid Red 85, which releases benzidine). An azo compound if ingested orally can be reduced by anaerobic intestinal micro flora and possibly by mammalian azo reductases in the intestinal wall or in the liver, to free aromatic amines. Reduction of orally ingested azo compounds

to aromatic amines occurs in a wide variety of mammalian species, including mice (Tsuda *et al.,* 2001), Rhesus monkeys (Rhinde and Troll, 1975) and humans (Watabe *et al.,* 1980). Since many aromatic amines are known mutagens, a complete evaluation of the safety of these dyes in the human environment must include an evaluation of their genotoxicity or mutagenicity. The use of these dyes has been drastically reduced in Europe due to national regulations (e.g. by the German Food and Commodities Act; similar regulations exist in the Netherlands and France) and textile quality labels but may still be a problem in non-European countries (Claxton *et al.,* 2001). Azo dyes are believed to posses carcinogenic properties. Most carcinogenic benzidine analogues are also mutagenic, and their metabolism to electrophiles that interact with DNA, leading to mutations, plays a central role in their carcinogenesis. Mutagenicity of many benzidine congeners and their *N*-acetylated and *N, N'*-diacetylated derivatives in different strains of *Salmonella typhiimurium* has been reported. Certain azo compounds are known to be mutagenic in bacterial tests (Venitt & Bushell, 1976 and Kaur, 1993a)**.** Many of these dyes have been tested for mutagenicity using *Salmonella* assay (Garner and Nutman, 1977, Venturini and Tamaro, 1979, Prival and Mitchell, 1982, Wuebbles and Felton 1985 and Schneider *et al.*, 2004). Kaur (1993a and 1993b) screened textile azo dyes for mutagenicity with Ames/*Salmonella* assay with and without metabolic activation.

Discussion of genotoxic substances on textiles in recent years focused on azo dyes splitting off carcinogenic amines. And indeed, recent investigations state that these dyes can still be found on fabric (Yassini *et al.,* 1997, Danish EPA, 1998 and Zeilmaker *et al.,* 2000). The participating TFCs banned these hazardous azo dyes already years ago from their production process. But these azo dyes may be a problem especially with textile products imported to Europe (Lokhande and Naik, 1996). Gregory (1981) evaluated the mutagenic activity of a series of diazo compounds derived from benzidine and its congener's o-tolidine, o-dianisidine and 3, 3'-dichlorobenzidine as well as several monoazo compounds. All of the benzidine and o-tolidine dyes tested were clearly mutagenic. The o-dianisidine dyes except for Direct Blue 218 were also mutagenic. Direct Blue 218 is a copper complex of the mutagenic o-dianisidine dye Direct Blue 15. Pigment Yellow 12, which is derived from 3, 3'-

dichlorobenzidine, could not be detected as mutagenic, presumably because of its lack of solubility in the test reaction mixture. Of the monoazo dyes tested, methyl orange was clearly mutagenic, while C.I. Acid Red 26 and Acid Dye (C.I. 16155; often referred to as Ponceau 3R) had marginal to weak mutagenic activity.

Prival and Mitchell (1982) reported that all soluble dyes tested, with the exception of Direct Blue 218 (copper chelated), produced frame shift mutations in *Salmonella* when tested under conditions that fostered reduction of the azo bonds. Only Direct Black 38 and Direct Blue 2 were found to be mutagenic without activation, possibly due to the presence of mutagenic impurities in these dye preparations. Such information supports the results of metabolism studies relative to the mechanisms of genotoxic action, and increases confidence in predicting the carcinogenicity of these chemicals. In a number of instances, however, the mutagenic responses following reduction of the dye and subsequent oxidative metabolism of the reduced products was greater than that obtained following similar treatment of the parent congener amine (Prival *et al*., 1984). This suggests that the chromophore was also mutagenic. Prival and Mitchell (1982) further documented that although benzidine (Bz), 4-aminobiphenyl (ABP), 3,3'-dichlorobenzidine HCl (DCBz), 3,3'-dimethylbenzidine (DMBz), 3,3'-dimethoxybenzidine (DMOBz) and the benzidine congener-based dye trypan blue (TB) produce primarily frame shift mutations in *Salmonella typhimurium,* the base-substitution strain TA100 also responds to these compounds when S9 is present. Performing DNA sequence analysis, other investigators have shown that ABP induces frame shift, base-pair and complex mutations. Mutagenicity studies provided evidence that the benzidine- and benzidine congener-derived dyes require reductive metabolism before they are mutagenic (Reid *et al*., 1984).

A series of direct dyes based on two non-mutagenic benzidine analogs, 2, 2'-dimethyl-5,5'-dipropoxybenzidine and 3,3'-dipropoxybenzidine, were evaluated for mutagenic activity in *Salmonella typhimurium* strains TA98 and TA100 by Bae (2006). While some toxicity was seen with some dyes at high doses, all of the dyes examined were judged non-mutagenic with and without metabolic activation in the standard *Salmonella* plate-incorporation assay. The results in the standard test were consistent with

the properties of the diamines themselves. However, only one of the dyes was non-mutagenic when a reductive-metabolism pre-incubation assay was used. The results of this study suggest that although benzidine analogs are potential replacements for benzidine, there is a need to understand which mutagenic products are produced when reductive metabolism is present. There is also a need to know whether or not metal complexes of these dyes are mutagenic. Such information will allow the development of new non-mutagenic azo dyes

Benzidine and 12 related aromatic amines have been studied by Sinsheimer (1992) for the effects of substituents groups and π orbital conjugation on their genotoxicity as measured by their mutagenicity in vitro with *Salmonella* and by chromosomal aberrations (CA) in vivo in the bone-marrow cells of mice. The in vitro studies indicated increases in mutagenicity with increases in the electron withdrawing ability of *para'* substituents. Mutagenicity also increases with increased conjugation as shown by the degree of planarity of the biphenyl compounds and by comparing the mutagenecities of biphenyl amines to stilbenes as well as to ethylene bridged diphenyl compounds. The relative in vitro mutagenicity results were not predictive of relative in vivo CA results. The 3 most genotoxic compounds in vivo were the conjugated amines without substituents in the *para'* position. These in vivo results indicate increased genotoxicity for benzidine analogs without substitution in the *para'* position.

Bakshi and Sharma (2003) evaluated mutagenicity for 14 commercial textile dyes used extensively in the northern part of India using both the Ames *Salmonella typhimurium* microsome reversion test as well as the recombination-repair (*rec*)-assay. The Ames test revealed that 57.14% of dyes were mutagenic and acting directly. The rec-assay detected 50% of dyes to be mutagenic; of these, 71.43% were direct acting, whereas 28.57% required Aroclor-induced exogenous metabolic activation. Used together, the two tests detected 78.57% of the dyes to be mutagenic, and a 50% correlation was found between these two tests. Groupwise, three out of four azo dyes and all five anthraquinone dyes were found to be mutagenic by the Ames assay; the rec-assay detected methine/polymethine (1 out of 3), an oxazine, and a triphenylmethane dye to be mutagenic, besides the azo (1 out of 4) and the anthraquinone (3 out of 5) dyes. The structure-

activity analysis attributed the mutagenicity of dyes to the structural alerts such as phenylenediamine, amino and nitro- groups, methylation, CH=CH, and chloro groups; whereas deamination, bulkier groups, and sulfonation may be responsible for diminishing mutagenicity.

Wollin and Gorlitz (2004) studied the genotoxic activity of ten selected commercial textile dyestuffs, which are made up of mixtures of azo dyes and azo metal complex dyes as well as two anthraquinone dyestuffs. *Salmonella* mutagenicity assay and cultured human keratinocytes (HaCaT cell line) were used In the *S. typhimurium* strain TA98, with and without S9, eight often dyestuffs investigated, and in strain TA 100, with and without S9, six often dyes caused frame shift mutations and base-pair substitutions in a dose-related manner. All dyes, including those negative in the *Salmonella* mutagenicity assay, induced clastogenic effects in the in vitro micronucleus (MN) test in HaCaT cells as direct-acting mutagens cells. In the single cell gel/comet assay, all ten dyestuffs investigated caused DNA damage in HaCaT keratinocytes.

Chung (2006) reviewed the mutagenicity of benzidine analogues (including benzidine-based dyes), with a primary emphasis on evaluating results of the *Salmonella*/microsome mutagenicity assay. Many of these amines were found to be mutagenic in tester strains TA98 and TA100 but require exogenous mammalian activation (S9) for activity. A few amines with halogen or nitro-groups in the structure were reported to be direct-acting mutagens. The addition of a sulfonic acid moiety to the molecule of benzidine reduced the mutagenicity of benzidine; whereas, methoxy, chloro, or methyl group additions did not. Complexation with a metal ion also decreased the mutagenicity. A substitution of an alkyl group on the *ortho* position next to an amine group also influenced the mutagenicity.

1.3 Mutagenicity of other Non Azo Textile Dyes:

Whereas hazards resulting from azo textile dyes, which give rise to carcinogenic amines, are recognized and controlled, this is not the case for textile dyes in general. Because of the wide spread use and potential carcinogenicity of certain dyes, there has been a growing interest in assessing the hazards associated with dyes available in local markets. Most of such dyes, being openly sold in the

markets have no information regarding their chemical nature, purity or possible mutagenicity. Unlimited and uncontrolled use of such dyes can lead to grave consequences in terms of human health and ecological balance.

In a modified Ames test with fabric tested *in situ* ("Ames spot test"), 9.2% of 196 tested textile samples showed mutagenic effects in TA98 and/or TA100 (Knasmüller *et al.,* 1993). It was concluded that mutagenic effects of poorly investigated textile dyes pose a problem. Moawad (2003) evaluated toxicity of eight textile dyes using two bioassays namely: Ames test and seed germination test. The eight-textile dyes and Eithidium bromide dye (as positive control) were tested with five "*his*" *Salmonella typhimurium* strains: TA 100; TA 98; TA 1535; TA 1537; TA 1538. Most of the dyes were found to be mutagenic for the test strains used in this study. The high concentrations of dye eliminated microbial colonies due to the high frequency of mutation causing lethal effect on the cells. Mutagenic Activity of Some Textile Dyes in Different Test Systems has been discussed by Przybojewska (1998).

Textile dyes used within the European Union (EU) were examined for available published and unpublished mutagenicity data (EC, European Commission, 2001). Fifty-three dye products that had so far not been investigated for mutagenicity were tested in the bacterial reverse mutation assay with *Salmonella typhimurium* (Ames test) according to a modification of the OECD 471 guidelines (instead of five strains, only TA98 and/or TA100 were used with and without metabolic activation (S9-mix)). About 28% (15 out of 53) of the dye samples were positive in the Ames test. Fifteen samples showed positive results with TA98, two were positive in TA100. The mutagenicity of nine Ames-positive textile dye products was further investigated in the mouse lymphoma assay (MLA) (OECD 476). Sixty-seven percent (6 out of 9) induced genotoxic effects in the MLA. The results confirmed previous findings that dye products are marketed that are not sufficiently tested and that show mutagenic effects in in-vitro tests. The evaluation performed indicates that dyes with potential mutagenic effects are in use in the textile finishing industry.

Currently, there are several thousand textile dyes from various chemical classes in use in Europe. 281

textile dye products in use at nine textile finishing companies from eight European countries were assessed for potential mutagenic properties by Schneider (2004). Most of them belonged to the so-called existing substances, which have been placed on the European market before an effective chemical regulation has been installed in 1983. Toxicological data are often scarce for these substances. Four dye stuffs contained in seven dye products in use at the textile finishing companies were judged to be mutagenic, based on published data from the literature. Mutagenicity testing using *Salmonella typhimurium*, strains TA98 and TA100, revealed positive results for about 28% (15 out of 53) of the dye products investigated. Upon further testing with the mouse lymphoma assay (L5178Y/TK (+/-)) 67% (6 out of 9) of Ames-positive dyes proved to be mutagenic in this mammalian cell test. All data sources combined led to an overall assessment of 14 dye products out of 281 being mutagenic. For 16 there is a suspicion of mutagenicity due to positive responses in one test but 71 of the dye products are without any data on mutagenicity.

The genotoxicity of indigo naturalis was assessed recently using micronucleus test by Dominici (2010). This study estimated the genotoxicity of water and DMSO solutions of indigo naturalis (prepared from Indigofera tinctoria leaves) using the cytokinesis-blocked micronucleus (CBMN) assay in the human metabolically active HepG2 cell line. The cytotoxic effects of indigo solutions were first assessed by propidium iodide and fluorescein-diacetate simultaneously staining. The results of this study indicated that indigo naturalis exhibits neither cytotoxicity, nor genotoxicity for all concentrations tested, which may justify excluding indigofera and its components from the list of carcinogenic agents. Rannug (1992) tested Extracts of pure cotton and jeans fabrics for mutagenicity in *Salmonella typhimurium* strains TA98 and TA100. The vat dye indigo, technical grade as well as 98% and greater than 99.5% pure, was also tested for mutagenicity. The mutagenicity of the extracts was associated with the cotton denim and non dyed cotton gave only marginal effects. The mutagenicity of the indigo dyed fabrics was dependent on type and treatment of the fabrics. Extracts of both bleached and non bleached jeans gave mutagenic effects on TA98 +/- S9 and TA100 +/- S9. The greatest effects were seen in the presence of S9. Bleaching gave an additional increase in the mutagenicity in the absence of S9. Normal washing of the fabrics after bleaching

reduced the mutagenicity. Synthetic indigo of technical grade or 98% pure showed mutagenic effects, especially on TA98 + S9. Considering the amount of indigo in the extracts and its low mutagenicity, the genotoxicity of jeans extracts must be caused by other unknown components. Presence of impurities in many of the commercially available dyes has also been reported to contribute to the mutagenicity of these dyes (Prival and Mitchell, 1984).

Most of the dyes, presently being used in textile industry are known only by their trade name like Golden Top, Grey, etc. while their chemical nature, constituents, purity and possible biological hazards are not known. Data on mutagenicity are virtually absent for many of such dyes. A number of dyes from local markets of India tested for their mutagenicity, using strains of *Salmonella typhimurium* have been reported to be mutagenic (Mathur *et al.*, 2005a, Mathur *et al.*, 2005b, Mathur and Bhatnagar, 2005a and Mathur and Bhatnagar, 2007b).

1.4 Mutagenicity bioassay: The *Salmonella* Mutagenicity Test:

Micro-organisms have demonstrated several attributes that make them attractive for use in quick screening of effluents and chemicals for toxicity. More than 200 short-term tests utilizing micro-organisms, insects, plants and animals, have been developed over the last 20-25 years, to aid in the identification of agents that pose genetic hazard to humans (Waters *et al.,* 1988). The use of bioassays is an essential part of the hazard assessment and control procedures of toxic chemicals (Auletta *et al.,* 1993 and Kirkland, 1993).

Developed by B.N. Ames and co-workers in early 1970's (Ames *et al.,* 1973 and 1975) the *Salmonella* assay has been used worldwide for nearly 4 decades, to evaluate the mutagenicity of pure chemicals and complex environmental mixtures. The test detects a wide variety of mutagens, including those activated by mammalian liver enzymes. In assays on more than 300 chemicals, the histidine requiring *Salmonella* tester strains responded positively to 90% of the known carcinogens (Ames *et al.,* 1973, Ashby and Tennant, 1988 and Ashby *et al.,* 1989). The genetic damage expressed by the *Salmonella* assay represents a class of DNA damage called gene or point mutation. The *Salmonella*/microsome assay

is based on the premises that bacterial assay systems provide an efficient way to detect agents, which could interact with DNA and cause mutations. Such agents would probably also be capable of causing mutations in other species, including man. Secondly, there is an association between mutagenicity and carcinogenicity, based on the empirical observation that many mutagens detected in bacterial systems have been shown to be carcinogens.

The Ames test has several advantages over the use of mammals for testing compounds. Mutagenicity assays are relatively cost effective, only a few days are required for testing a compound and the test is performed with microgram quantities of the material. Such assays are performed on approximately 100 million organisms rather than on a limited number of animals. Thus the Ames test is used widely, which accounts for its validity and an extensive database of tested chemicals (Kier *et al.,* 1986, Ashby and Tennant, 1988 and Ashby *et al.,* 1991). Both Kier *et al.* (1986) and Auletta and Ashby (1988) recommend use of *Salmonella* assay in any screening program, to set priorities for further testing by identification of potentially mutagenic and carcinogenic agents. For all these reasons, the *Salmonella* assay is also included in the Office of Pesticide Programs (OPP) and Office of Toxic Substances (OTS) battery (Dearfield *et al.,* 1991, Auletta *et al.,* 1993 and Kirkland, 1993).

The *Salmonella* or histidine reversion assay, is based on the use of several selected, modified strains of *Salmonella typhimurium* that revert from histidine dependence (auxotrophy) to histidine independence (prototrophy) at an increased frequency or rate, in presence of a mutagen. The test uses a number of *Salmonella* strains with pre-existing mutations that leave the bacteria unable to synthesize the required amino acid, histidine, and thereby unable to grow and form colonies in its absence. New mutations at the site of these pre-existing mutations or nearby in the gene can however, restore the gene function and allow the cells to synthesize histidine. The newly mutated cells can grow in the absence of histidine and form colonies. For this reason, the test is often referred to as a reversion assay. Since innumerable dyes are available in local markets, chemical analysis of each and every dye is not possible because of the time and cost involved. Recent evidence showed that textile dye stuffs caused frame shift mutation in *Salmonella* mutagenicity assay (Wollin and Gorlitz,

2004). Base-level testing of textile dye products requires two *in vitro* assays, a bacterial test (usually reverse mutations in *S. typhimurium*) and a mammalian cell genotoxicity test (MCGT) (Table 1). If available*, in vivo* results are regarded as superior to *in vitro* tests (Schneider *et al.,* 2004).

Table 1: Mutagenicity assessment criteria for textile dye products (Schneider et al., 2004)

Category	Definition
Not mutagenic	Base-level requirements fulfilled, bacterial test and/or MCGT negative
Not mutagenic/ incomplete	Base-level requirements not fulfilled, available test(usually bacterial test) negative
One test positive	One test positive, without refutation by other tests (e.g. the bacterial test is the only test available and leads to positive results), includes also some cases with controversial data
Mutagenic	Base-level requirements fulfilled, both tests or MCGT positive (other data, e.g. *in vivo* tests for clastogenicity or 32 P post labeling, are also considered, if available)
Not tested	Mutagenicity data on the dye product or on components are lacking
No data	No data on identity or genotoxicity provided by the producer, other kind of data also lacking

Evidence indicates that there is of the order of ninety percent correlation between bacterial mutagenicity and long-term animal experiments (McCann *et al.,* 1975). For this reason compounds that are bacterial mutagens, but have not been tested in long-term animal carcinogenicity experiments, are viewed with suspicion as being of potential hazard. Results from genetic bioassays are relevant to human health because the toxicological target is DNA, which exists in all cellular life forms. Thus it can be extrapolated that compounds shown to be reactive with DNA in one species have the potential to produce similar effects in other species. In general, perturbations of genetic material are deleterious to the organisms and can lead to severe and irreversible health consequences.

The Ames test has several advantages over the use of mammals for testing compounds. Mutagenicity assays are relatively cost effective, only a few days are required for testing a compound and the test is performed with microgram quantities of the material. Such assays are performed on approximately 100 million organisms rather than on a limited number of animals. Much of the published textile dye products mutagenicity/genotoxicity studies employ *Salmonella* mutagenicity test with strains TA98 and/or TA100 with and/or without metabolic activation. Testing of chemicals for mutagenicity in *Salmonella typhimurium* is based on the knowledge that a substance that is mutagenic in the bacterium is likely to be a carcinogen in laboratory animals, and thus, by extension, present a risk of cancer to humans. Although about three-fourths of chemicals that are positive in the *Salmonella* test are found to be rodent carcinogens, not all substances that cause cancer in laboratory animals are mutagenic in this assay. However, the ease, rapidity (results in 3-4 weeks) and low cost of the test make it an important tool for screening substances for potential carcinogenicity.

Several strains of the *Salmonella typhimurium* bacterium may be used for testing. Each is genetically different, so using several strains in a test increases the opportunity of detecting a mutagenic chemical. The most frequently used strains are TA97, TA98, TA100, TA102, TA104, TA1535, TA1537, and TA1538. All strains of *Salmonella typhimurium* used for mutagenicity testing carry a defective (mutant) gene that prevents them from synthesizing the essential amino acid histidine from the ingredients in standard bacterial culture medium. Therefore, these "tester" strains can only survive and grow on medium that contains excess histidine. However, in the presence of a mutagenic chemical, the defective histidine gene may be mutated back to the functional state, allowing the bacterium to grow on standard medium that does not contain supplemental histidine. These mutations, which lead to a regaining of normal activity or function, are called "back" or "reverse" mutations and the process is referred to as "reversion." The mutant colonies, which can make histidine, are called "revertants." (There are other mutagenicity assays using other cell-types that measure "forward" mutations, that is,

mutations that alter a functional gene in a way that causes a loss, rather than a gain, of function.) .

In the *Salmonella* assay, a test tube containing a suspension of one strain of *Salmonella typhimurium* plus S9 mix or plain buffer without S9, is incubated for 20 minutes at 37º C with the test chemical. Control cultures, with all the same ingredients except the test chemical, are also incubated. In addition, positive control cultures are also prepared; these contain the particular bacterial tester strain under investigation, the various culture ingredients, and a known potent mutagen. After 20 minutes, agar is added to the cultures and the contents of the tubes are thoroughly mixed and poured onto the surface of petri dishes containing standard bacterial culture medium. The plates are incubated, and bacterial colonies that do not require an excess of supplemental histidine appear and grow. These colonies are comprised of *Salmonella* that have undergone reverse mutation to restore function of the histidine-manufacturing gene. The number of colonies is counted after 2 days (Maron and Ames, 1983). Spontaneous mutations (those that occur by chance, not by chemical treatment) will appear as colonies on the control petri dishes. If the test chemical was mutagenic to any particular strain of bacterium, the number of histidine-independent colonies arising on those plates will be significantly greater than the corresponding control plates for that strain of *Salmonella*. The positive control plates are also counted, and the number of mutant colonies appearing on them must be significantly increased over the spontaneous control number for the test to be considered valid. Failure of the positive control chemical to induce mutation is reason to discard the experiment.

Several doses (at least 5) of each test chemical and multiple strains of *Salmonella typhimurium* are used in each experiment. In addition, cultures are set up with and without added S9 liver enzymes at varying concentrations. Therefore, a variety of culture conditions are employed to maximize the opportunity to detect a mutagenic chemical. In analyzing the data, the pattern and the strength of the mutant response are taken into account in determining the mutagenicity of a chemical. All observed responses are verified in repeat tests. If no increase in mutant colonies is seen after testing several strains under several different culture conditions, the test chemical is considered to be non mutagenic in the *Salmonella* test. The most common

method of evaluation of data from the *Salmonella* assay is the "two fold rule" according to which doubling of spontaneous reversion rate at one or two test chemical concentrations constitutes a positive response (Mortelmans and Zeiger, 2000). This rule specifies that if tests compound doubles or more than doubles, the mean spontaneous mutation frequency obtained on the day of testing, then the compound is considered significantly mutagenic.

Using this procedure the following criteria were used to interpret results:

❖ Positive: A compound is considered a mutagen if it produces a reproducible, dose-related increase in the number of revertant colonies in one or more strains of *Salmonella typhimurium*. A compound is considered a weak mutagen if it produces a reproducible dose-related increase in the number of revertant colonies in one or more strains but the number of revertants is not double the background number of colonies.

❖ Negative: A compound is considered a non-mutagen if no dose-related increase in the number of revertant colonies is observed in at least two independent experiments.

❖ Inconclusive: If a compound cannot be identified clearly as a mutagen or a non-mutagen, the results are classified as inconclusive (e.g. if there is one elevated count).

For this analysis the dose related increases in the number of revertant colonies were observed for the test compounds and Mutagenicity ratios are calculated. Mutagenicity ratio is the ratio of average induced revertants on test plates (spontaneous revertants plus induced revertants) to average spontaneous revertants on negative control plates (spontaneous revertants). Mutagenicity ratio of 2.0 or more is regarded as a significant indication of mutagenicity. Many chemicals are not mutagenic (or carcinogenic) in their native forms, but they are converted into mutagenic substances by metabolism in the liver. Since the *Salmonella* bacterium does not have the same metabolic capabilities as mammals, some test protocols utilize extracts of rat or hamster liver enzymes (S9) to promote metabolic conversion of the test chemical. This permits the investigator to determine if a chemical must be metabolized to express mutagenic activity. Some mutagenic chemicals are active with and without metabolism, while others are active only under one condition or

the other. Ames test can thus easily and quickly assess mutagenic potential of these dyes. Besides the genotoxicity of various dyes can also be compared using the Ames test. Thus, this assay should be used as a regular monitoring tool for assessing the dyes.

1.5 Environmental Impact:

Dyes are introduced into the environment through industrial effluents from food, drug, cosmetic, textile and dyestuff factories. The effluents from dyeing and textile industries contain chemicals with intense colors and the release of these effluents to receiving streams may be objectionable for various aesthetic reasons. Excess salts used in the dyeing process, to increase fixation of reactive dyes to fibers, as well as heavy metal components of some dye waste waters, may adversely affect the aquatic biota of the receiving streams (Wells et al., 1994 and Law, 1995). A number of dyes and chemicals used by these textile industries are not degradable. Further, these colored dye wastes contain compounds that are difficult to treat biologically due to their resistance against biodegradation (Ogawa et al., 1981, Seshdari et al., 1994 and Suzuki et al., 2001). These dyestuffs are highly structured polymers and are very difficult to decompose biologically (Neppolian et al., 1999). They are thus, a potent hazard to the natural sources like soil, water, flora, fauna, livestock and human population. Since large quantities of dyes are used, such pollution due to dyes may occur on a significant scale.

Considerable amounts of dyes have been noticed in these textile wastewaters, due to their incomplete use and washing operations. It is stable and fast, difficult to degrade, toxic, rendering the water unfit for its intended use. Further, the color removal is also not adequate by the conventional chemical and biological treatment. Such dyestuffs can reach the aquatic environment, primarily dissolved or suspended in water, since the conventional treatment of wastewaters from textile mills and dyestuff factories are unable to remove most of the azo and other dyes effectively. Ecological and toxicological problems due to the discharge of textile wastewaters, in natural water bodies, have been one of the most important water pollution problems throughout the world. The most obvious impact of the discharge of dye colored effluent is the persisting nature of the color. It is stable and fast, difficult to degrade, toxic, rendering the water unfit for its

intended use. Thus, dye related industries, in particular, appear to produce consistently genotoxic effluents that have been shown by many scientists, to be potent, relative to other industrial discharges. In the comparative potency rankings of mutagenic wastes, reviewed by Houk (1992), the textile and dye industries generated wastes and effluents of moderate mutagenecities. This review further suggests that the genetic damage induced by the effluents, from this industrial category, includes mutations and chromosomal abnormalities. Dyes and heavy metals have been considered to be the possible source of genotoxic activity.

Dyes and dyestuffs find use in a wide range of industries but are of primary importance to textile manufacturing. Wastewater from the textile industry can contain a variety of polluting substances including dyes. Increasingly, environmental legislation is being imposed to control the release of dyes, in particular azo-based compounds, into the environment. Further as shown by Knasmüller et al. (1993) with the Ames spot test, a relevant proportion of dyes (9.2%), which are active in S. typhimurium, are leachable from textile samples. To verify whether dyes emitted within the discharge of a dye processing plant were contributing to the mutagenicity repeatedly found in the Cristais River, Sao Paulo, Brazil. de Aragão et al. (2005) chemically characterized the following mutagenic samples: the treated industrial effluent, raw and treated water, and the sludge produced by a Drinking Water Treatment Plant (DWTP) located approximately 6 km from the industrial discharge. Considering that 20% of the dyes used for coloring activities might be lost to wastewaters and knowing that several dyes have mutagenic activity, it is natural that these dyes contributed to the mutagenic activity found in the Cristais River and were indirectly affecting the quality of the related drinking water. Wastewater effluents from certain textile, dyeing and finishing operations exhibited a low degree of toxicity to freshwater cladoceran, Daphnia pulex, in acute, static, 48 hr testing (Wells et al., 1994). Kurde and Singh (1995) reported adverse effects of textile effluents and dyes on certain hematological parameters of wistar rats. The effluents of textile dyeing and printing industries are complex in nature, rich in dissolved and suspended solids, organic compounds, heavy metals and have high pH. These effluents have been known to pollute rivers (Srivastava et al., 1994), soil characteristics (Raj et al., 1997 and Nema et al., 1984) and have toxic

effects on a number of plants (Khandelwal *et al.,* 1996 and Raj *et al.,* 1997) and animals (Kundu *et al,* 1997, Chhaya *et al.,* 1997 and Smith *et al.,* 1999).

Ecological and toxicological problems due to the discharge of textile wastewaters, in natural water bodies, have been one of the most important water pollution problems (Chan *et al.,* 2003). In a study on industrial effluents, McGeorge *et al.* (1985), found final effluents, from 4 different dye-related industries, to be mutagenic. In fact, of the various different industrial facilities sampled in this study, the dye producing industries generated some of the most mutagenic wastes tested. Further, Sanchez *et al.* (1988) assessed 9 textile/dye industry wastewaters in their evaluation of urban discharges into the rivers of Greater Sao Paulo, Brazil. Results from two microbial mutagenicity assays showed that of the industrial categories tested, the textile industries contributed the highest percentage (67%) of mutagenic effluents. It was speculated that the activity was associated with the use of mutagenic dyes. Chhoakar *et al.* (2000) characterized the effluents emanating from Pali, and reported high salinity, BOD (400-800 mg/l) and COD (900-1500 mg/l); excessive concentration of sodium and carbonate ions; high alkalinity (pH 10.0-11.5); and low concentrations of calcium in the textile effluents. The total chromium and phenolic compounds in the effluents of Pali have been found to be 0.18 mg/l and 0.24 mg/l, respectively, whereas the mixed industrial effluents (mainly from textiles industries) are known to have pH range 9.0 to 10.0. Occurrence of magnesium ions in concentrations less than 21mg/l and excess of carbonate ions (as high as 30 mg/l) have also been observed in these effluents (Gupta, 1992).Khan *et al.* (1995) have reported high levels of various metals (Ni, Zn, Cd, Fe and Pb) in their case study on the effect of textile effluents on physico-chemical characteristics of Amani Shah Nallah. There is still a dearth of studies pertaining to possible mutagenicity of the dyes being used here and the textile industry effluents.

1.5 Epidemiology:

The International Agency for Research on Cancer (IARC) has classified various chemicals as being associated with cancer in humans (IARC, Suppl. 4, 1982). Benzidine, an aromatic amine is one of them (Shelby, 1988 and Shelby and Zeiger, 1990). Experimental studies with rats, dogs and hamsters have shown that animals administered with BN and

BN based dyes excrete potentially carcinogenic aromatic amines and their N-acetyled derivatives in their urine (Powell *et al.,* 1979, Boeniger, 1980 and Nony and Bowmann, 1980). In tests on other laboratory animals, two benzidine dyes, Direct Blue 6 and Direct Black 38, have been reported to be such potent carcinogens that hepatocellular carcinomas and neoplastic liver nodules occurred in rats after only 13 weeks of exposure (Robens *et al.,* 1980). Since benzidine is used as a reactant in dye synthesis, workers could be directly exposed to the carcinogen (Mirkova and Lalchev, 1990). DNA damage induced by textile dyes and their effluents have been demonstrated on tadpoles (Rajaguru *et al.,* 2001) and fishes (Al-Sabti, 2000 and Sumathi *et al.,* 2001).The deleterious effects of many dyes have been studied in a wide variety of mammalian species, including dogs (Lynn *et al.,* 1980), Rhesus monkeys (Rhinde and Troll, 1975) and humans (Watabe *et al.,* 1980 and Clonfero, 1990).

Textile industry has been reported to pose threat of various types of occupational diseases (MacGregor *et al.*1980, Pal and Brijmohan, 1980, Kumar *et al.,* 1992 and Gonzales,1998).The main routes of human exposure to azo dyes identified are a) oral ingestion, mainly referring to the sucking of textiles by babies and young children, b) dermal absorption, the route of primary concern for consumers wearing azo compound-dyed products, as well as for workers in dye production and use plants, and c) inhalation, a route of concern for workers in dye production and use industries as well as those handling newly dyed products. Contact with aromatic amines entering the environment through the whole life-cycle of azo dyes in colored clothes is an additional potential source of human exposure. Textiles are made from synthetic or natural fibers, or both. Generally, the actual fibers are not allergenic; rather, the dyes used to color the fabrics or formalin finishing resins added to make them wrinkle-resistant, shrink-proof, or easily laundered, are the responsible contactants. The most common sensitizers belong to the disperse dye application class, which loosely hold onto the fibers and are easily rubbed off (Hatch and Maibach, 2000 and Pratt and Taraska, 2000). Disperse blue 106 and disperse blue 124 have been reported to cause an allergic contact dermatitis to a variety of garments, which include underwear, blouses, pants, swimming suits, pantyhose, shoulder pads, and the velvet material of leggings and body suits. Exposure to consumers can prove fatal when these chemicals

come in contact with the skin as they might generate incurable diseases (Joe, 2001).

Genotoxic risk of workers from textile dyeing plants has thus been reported by a number of workers (Kumar *et al.,* 1992 and Dönbak, *et al.,* 2006). Yoshida and Miyakawa (1973) reported that occupational exposure to benzidine dyes might have possibly resulted in bladder cancer amongst kimono painters in Japan. Eczema, contact dermatitis, asthma, chronic bronchitis, tuberculosis, haematoma, bladder cancer and irritation to eyes, have been reported amongst the workers of textile industries in Sanganer by Usha (1989). In 42 rotogravure printers exposed to rotogravure printing dyes high incidence of chromosomal aberrations were reported by Pelclova (1990). Occupational Bladder Cancer in Textile Dyeing and Printing Workers and their Significance for Screening Programs has been discussed by Frumin (1990).Triple primary cancers involving kidney, urinary bladder and liver in a dye worker have also been reported (Morikawa *et al.,* 1997).

1.6 Conclusion:

Currently the Indian dyestuff industry is in the midst of major restructuring and consolidation phase. In response to German ban on azo dyes (Bahorsky, 1997) numerous Indian companies are trying to develop herbal dyes, organic cotton and other ecofriendly textiles. An environmental crack down in heart of India's small-scale dyestuff industry has resulted in more than 70 of the listed 100 companies installing primary and secondary treatment plan(Roberts, 1995).Now the shift in emphasis is on product innovation, brand building and environmental friendliness. This industry is increasingly moving towards greater customer orientation. Even though India enjoys an abundant supply of basic raw materials it will have to build up on technical services and marketing capabilities to face global competition and increase its share of exports. Indian companies in future will have to meet customer requirements of quality and delivery on time backed by prompt technical service to compete with the global players

The textile industries use synthetic organic dyes like yarn dye, direct dye, basic dye, vat dye, sulfur dye, nepthol dye, developed dye and reactive dye. The large variety of chemicals used in bleaching and dyeing process render them very complex. These chemicals are used in an attempt to make more attractive popular shades of fabrics for a competitive market (Rajagopalan, 1990). The textile industries are to satisfy the ever growing demands in terms of quality, variety, fastness and other technical requirements, but the use of dye stuffs has become increasingly a subject of environmental concern. Therefore, it is essential to evolve regulations designated to improve the health and safety and the human and natural environment. Research in this area began about three decades ago, and a relatively large and comprehensive body of scientific data has been generated since that time. Many of the dyes and their intermediates have been banned in countries like U.K. and Germany. Their manufacture is now prohibited or closely regulated. Nevertheless, various benzidine derivatives are manufactured for azo dye production and although the manufacture of the starting materials is strictly controlled, the end use of the dyes is not.

Further this review has shown that the mutagenic potential of textile dyes can be reliably and expeditiously measured using short-term genetic bioassays. Although more than 200 short term genetic bioassays are available to aid in the identification of agents that pose a genetic hazards to humans, only a few have been used extensively to analyze industrial waste and effluents. Evaluation of mutagenicity far out numbers any other type of analysis. The *Salmonella* mutagenicity assay is cited more often than any other test system. Other tests that have been used to assess textile dyes for mutagenic potential include the bacterium *E.coli* WP2, the fungi *A. nidulans,* the yeast *S.cerevisiae*, the plant *Arabidopsis*, and mammalian cell culture system such as the mouse lymphoma assay. Test systems that detect a somewhat broader range of genetic damage include the "rec" assay for DNA repair in *B. subtilis* and the prophage induction assay in *E.coli*. Chromosomal damage induced has been evaluated in fungi, plants and cultured mammalian cells. Short term in vivo assay that detect urinary mutagenicity, micronuclei, and DNA adducts have been used to a limited extent on textile dyes due to their complexity and expense. Chemicals analyses are indispensable for identifying and quantitating the hazardous chemical nature of dyes but toxicology assessment establish which compounds or effluents pose the greatest risk. Both approaches can be used to identify the source of the genotoxic constituents, to explain the processes leading to their formation, and to determine the effectiveness

of treatment technologies. Unfortunately, the activities of the environment chemist and toxicologist often remain parallel and distinct, and bilateral investigative studies are uncommon.

The release of azo dyes into the environment is a concern due to coloration of natural waters and due to the toxicity, mutagenicity and carcinogenicity of the dyes and their biotransformation products. They are generally considered as the xenobiotic compounds, which are very recalcitrant to biodegradation. In recent years, use of microbial biomass for decolourization of textile industry wastewater is becoming a promising alternative in which a number of bacteria (Chen *et al*, 2003, Fontenot *et al.,* 2003, Lee *et al.,* 2006, Hsueh and Chen, 2007, Kalme *et al.,* 2007, Moosvi *et al.,* 2007, Dawkar *et al.,* 2008, Hsueh and Chen , 2008 and Khalid *et al.,* 2008), yeast (Jadhav and Govindwar , 2006, Jadhav *et al.,* 2007 and Yu and Wen , 2005) and fungi (Spadaro *et al.,*1992, Chagas *et al.,* 2001, Yesilada *et al.,* 2003, Levin *et al.,* 2004, Kalyani *et al.,* 2008 and Shedbalkar *et al.,* 2008) are used to replace present treatment processes. The dye degrading microbial strains have been isolated from the textile dyes contaminated soils (Jadhav *et al.,* 2008, Ramya *et al.,* 2008 and Kolekar *et al.,* 2008); industrial wastewaters (Meehan *et al.,*2001); sludge samples and mud lakes(Chen *et al.,* 2003). Such strains are likely to have potential application in dye wastewater treatment. Microbial consortiums consisting of a white-rot fungus and a Pseudomonas have also been isolated from wastewater treatment facilities of a local dyeing house and used for rapid decolorization of dyes (He *et al.,* 2004). Among the 27 strains of halophilic and halotolerant bacteria isolated from effluents of textile industries, three showed remarkable ability in decolorizing the widely utilized azo dyes (Asad *et al*., 2007). exploitation of such salt-tolerant bacteria in the bio-treatment system would also be a great improvement of conventional biological treatment systems and the bio-treatment concept. Studies were carried out on the decolorization of textile azo dyes by newly isolated halophilic and halotolerant bacteria (Uddin *et al.,* 2007 and Guo *et al.,* 2008).

Indiscriminate use of synthetic chemical dyes should however be restricted or the workers while handling these dyes should at least take proper precautions. These dyes should be replaced by vegetable dyes, which are eco-friendly. Besides, before using the dyes at large scale, their mutagenic potential should

be assessed by biological assays like Ames test. This bioassay can be used as an initial screening test to analyze various dyes and dye containing effluents, which are causing major damage to the aquatic environment. The mutagenicity of textile dyes is an important consideration for the assurance of consumer protection and work safety. The mutagenicity testing of textile dyestuffs is crucial for accurately predicting health risks for consumers and workers exposed to dyes. Protective measures such as masks and gloves are desirable for preventing or minimizing the occupational exposure (Dönbak *et al.,* 2006).

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