Review Article

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TOXICITY OF DYES AND DYE INTERMEDIATES

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Dyes and pigments are mostly colored substances used for coloration.The chemicals used for their synthesis are hazardous for human life. The metabolism occurs primarily in liver and the metabolites formed are transported in the blood where they can form protein adduct or undergo renal filtration in urinary bladder lumens where at acidic pH, they can react covalently with DNA and the carcinogen DNA adducts formed cause disorders in the whole metabolic reactions. Many carcinogenic/ mutagenic hazards, which occur in the body, have been summarized for public awareness.

Key words**:** Toxicity of dyes, Coloration, Dye intermediates, Pigments.

Introduction

Dyes are intensely colored substances that can be used to produce a significant degree of coloration when dispersed in or reacted with other materials by a process which at least temporarily destroys the crystal structure of the substrate. They are retained in the substrate by adsorption solution and mechanical retention or by ionic or covalent chemical bonds (Anon 1974).

Organic pigments are finely divided crystalline solids. They are insoluble in the systems in which they are used, like inks, surface coatings, plastics and artificial fibres, must be dispersed in them with the expenditure of mechanical energy (Schewaebel and Nordmyer 1971).

Dye Intermediates are the chemicals or substances which are used in the synthesis of dyes and pigments. For example, intermediates used in the production of dyes and pigments are naphthols, naphylaminophenols and many others, having different substituents on aromatic rings and having structure as required according to their use in any class of dyes (Anon 1955).

Purpose of this review is to create awareness about the toxicity and carcinogenicity of these synthetic dyes and intermediates among who are manufacturing these dyes or are using them in their goods. This review covers the literature from 1956 to 2002 with all the hazardous/carcinogenic / mutagenic effects of dyes and dye intermediates on humans and animals. Toxicity of many intermediate chemicals are presented in Table 1 and 2. Various reports are already can be seen in the literature (Clontero *et al* 1990, Anliker 1979) however, these reports lack mechanism of toxicity induced by these synthetic dyes.

Prohibition of certain dyestuffs in developed countries began with the discovery of that handling benzidine-based dyes that could cause cancer of the bladder for over 25 years (Melick *et al* 1955, Huang *et al* 1979). Studies comparing the combined effect of chemical carcinogens on laboratory rats revealed that concurrent, sequential or mixed quantities of compounds targeted at liver tissue resulted in syncarcinogenic effects. Current test of relevance to human tissue involved change in morphology in respect of stiochiometric adducts, haemoglobin or DNA.

Carcinogenic aromatic amine metabolism and DNA adduct detection in humans. Initial metabolism of an aromatic amine occurs primarily in the liver and involves N-hydroxylation by cytochrome P - 450 IA2 and N-acetylation by acetyltransferase which serve as competing activation and detoxification reactions, respectively. Both the enzymes are polymorphic in humans and can be readily assessed using caffeine ingested in coffee followed by urinary metabolite analysis. N-hydroxyarylamines can then be transported in the blood where they can form protein adducts with haemoglobin (Hb) or undergo renal filtration into the urinary bladder lumen where, at acidic pH, they can react covalently with urothelial DNA. Carcinogen-DNA adduct levels in human bladder are smoking-related and the C_8-substituted deoxyguanosine derivatives of 4-aminobiphenyl are the major product formed.

Alternatively, N-hydroxyarylamines can be conjugated in the liver by glucuronyl transferases which provides a mechanism for biliary transport of the colon lumen where α-glucuronidases can regenerate the aglycon. In the colon mucosa, acetyltranferases can further activate the N-hydroxy metabolite by *O*-acetylation, and persons with rapid acetylation are known to be at higher risk for colorectal cancer. Detection of specific arylamine - DNA adducts in human colon should provide direct evidence for the role of these carcinogens in the etiology of this disese (Kadlubar 1991, Pei *et al* 1993).

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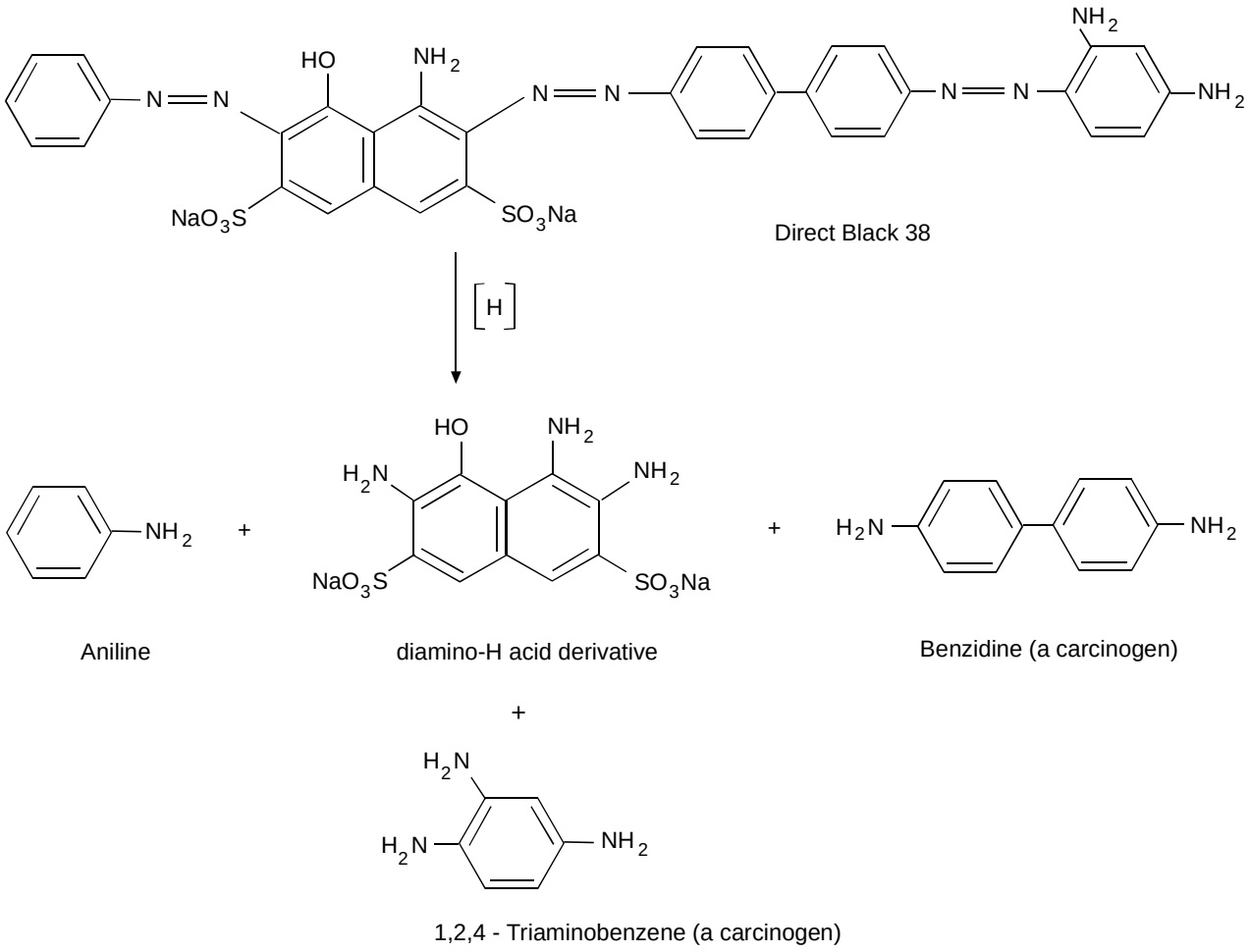

Scheme 1. The metabolism of an azo dye via a reductive- cleavage reaction.

Table 1
Dyes Intermediates

Compound/Class	Area of application
Monochloroanilines	Intermediate for herbicides, pharmaceuticals and azo dyes
Dichloroanilines	Dyes, azo dyes, isocyanates, plant protection agents
Trichloranilines	Pigment intermidiates
Toluidines	Herbicides, Pharmaceuticals, dye Intermediates
Benzidines and substituted benzidines	Intermediates for azo dyes and pigments, cross linking agents for polyurethanes and polyamides

The azo dyes have ability to undergo reductive cleavage of the azo linkage. The metabolism *via* a reductive - cleavage reactions has been shown in Scheme - 1.

Toxicity of Dyes and dye Intermediates. Certain dye intermediates e.g., substituted aromatic amines (anilines and benzidines) are known to possess high toxicological potential. They are classical environmental pollutants by virtue of their high degree of water solubility and the large amounts produced annually in the chemical industry for the synthesis of dyes and other compounds. As a consequence of their common usage, certain members of aromatic amines and benzidines are commonly found not only in waste water but also in surface water. For this reason, their accumulation in free or bound form is also possible in sediments or suspended matter (Scholz *et al* 1988). Some examples given in Table 1.

*Dyes.*Azo and anthraquinone dyes are the major chemical classes of commercial dyes. Among azo dyes, the direct dyes and pigments are the major classes which are derived from mono and disubstituted anilines. Much of the research on the ecological aspects of nitro, azo and anthraquinone dyes has shown the potential carcinogenic risk to humans and animals.

The International Agency for Research on Cancer (IARC) has also reported an evaluation of the Carcinogenic and Mutage-

Table 2
Toxicity of some colorants

C.I. Generic name & No.	CAS No.	CAS Name	Active ingredient in test sample (%)	Toxicological			Symbol
					Irritation		
				LD50 (mg/kg rat)	Skin rabbit	Eye rabbit	
C.I. Acid Orange 156 C.I. 26501	68555-86-2	Benzenesulphonic acid, 4-[(5-methoxy-4-[(methoxyphenyl)azo]-2-methylphenyl]-azo]-, sodium salt	95	120200	-	-	T
C.I. Acid Orange 165 C.I. 28682	85030-26-8	Benzenesulphonic acid, 3-[(4-[(3,5-dimethyl-IH-pyrazol-4-yl)azo]2,5-dimethoxyphenyl)azo]-, sodium salt	100	60	-	+	T
C.I. Basic Blue 3 C.I. 51004 (chlorozinoate)	63589-47-9	Phenoxazin-5-ium, 3,7-bis(diethylamine)-, (T-4)- 95 100 - + T(chlorozincate) tetrachlorozincate (2-1) (2:1)	95	100	-	+	T
C.I. Basic Blue 7 C.I. 42595	2390-60-5	Ethanaminium, N-[4-((4-(diethylamino)-phenyl] [4-(ethylamino)-1-napthalenyl]methylene]-2, 5-cyclohexadien-1-ylidine]-N-ethylchloride	>98	100	-	+	T
C.I. Basic Blue 81 C.I. 42598	73309-46-3	Ethanaminium, N-[4-(diethylamino)-phenyl] [(4-ethoxyphenyl)amino]-1-naphthalenyl]methylene]2,5-cyclohexadien-1-ylidine]-N-ethyl-, chloride	>98	205	-	+	T
C.I. Basic Red 12 C.I. 48070	6320-14-5	3H-Indolium, 2-[3-(dihydro-1,3,3,-trimethyl-2H-indol-2-ylidine)-1-propenyl]-1,3,3,-trimethyl-, chloride	>98	25+ 310	-	+	T
C.I. Basic Violet 16 C.I. 48013	6359-45-1	3-H-Indolium, 2-(2-[4-(diethylamino)phenyl]-ethenyl]-1,3,3-trimethyl-, chloride	94	90	-	+	T
C.I. Basic Yellow 21 C.I. 48060	6359-50-8	3H-Indolium, 2-[2-[2,3-dihydro-2-methyl-1H-indol-1-yl]ethenyl]1-3-trimethyl-, chloride	>98	171	-	+	T
C.I. Direct Orange 62 C.I. not available	70304-37-9	Benzensulphonic acid, 2,2″-(1,2-ethenediyl)-bis(5-[(2-methoxy-5-methyl-4-[(4-sulphophenyl)azo]phenyl]-azoxy]-tetrasodium salt	96	150	-	+	T
C.I. Basic Blue 81 C.I. 42598	2390-60-5	Ethanaminium, N-[(4-diethylamino)-phenyl][(4-ethoxyphenyl)amino)-1-napththalenyl)]methylene]-2,5-cyclohexadien-l-ylidine]-N-ethyl-, chloride	>98	205	-	+	T
C.I. Azoic Diazo Component 20 C.I. 87175	120-00-3	Benzamide, N-(4-amino-2,5-diethoxyphenyl)-	90	49	-	-	T
C.I. Azoic Diazo Component 24 C.l. 37155	6268-05-09	Benzamide, N-(4-amino-2,5-dimethyoxyphenyl)-	90	70	-	-	T
C.I. Azoic Diazo Component 41 C.l. 37165	99-21-8	Benzamide, N-(4-amino-5-methoxy-2-methylphenyl)-	90	115	-	-	T

Symbol T=Toxic

nic risk of some aromatic amines and related nitro compounds and azo dyes to man. It seems clear from these reports that, as a general rule, azo dyes which are based on these aromatic amines and which also lack at least one sodium sulfonate (-SO_3Na) group pose a risk to man and this point is also supported by more recent work. On the other hand, those dyes which contain two or more hydrophilic - SO_3Na groups are non-mutagenic and non-carcinogenic (Anon 1982).

For a number of colorants e.g, the benzidine - based dyes for which the metabolism in man and animals has been shown to involve reductive cleavage of the azo bonds. The use of such dyes in high-exposure applications e.g. hair dyes, children's wear, finger paints, do-it-yourself dyeing or under poor working conditions in processing plants, poses an elevated risk.

Toxicity of dye intermediates and their biochemistry. Health Hazard Information. The ETDA members have completed the project which involved the testing of over 5000 commercial dyes during 5 - 7years and reported the acute oral toxicity on the rats and rabbits. The analysis of short - term health effects showed skin and eye irritation which may occur immediately or shortly after exposure to these aromatic amine (Hunger and Jung 1991, Sewekow 1997).

Chronic Health Effects. At present, the possible chronic effects that attracting most of the attention are carcinogenicity and to a lesser extent sensitization. Technical dyes (excluding food, hair, cosmetics and drug dyes), properly handled and used, are only taken up in small traces, if at all. The available evidence indicates that these trace quantities do not present any unreasonable risk. However, a risk situation could arise due to improper handling or use. This is the main reason for ETDA's continuing efforts to identify any such product. The chronic (long - term) health effects can occur at some time after exposure to these aromatic amines and can last for months or years (Slawomir *et al* 1998).

Cancer Hazard. The aromatic amines are carcinogens in humans and cause liver, breast, bladder intestine and skin cancer not only in humans but in animals also (Helmes *et al* 1986).

Many scientists believe that there is no safe level of exposure to a carcinogen. Such substances may also have the potential for causing reproductive damage in humans. These compounds caused cancer in the offspring of animals exposed during their pregnancy. Other long - term effects may cause a skin allergy. If allergy develops, very low future exposure can cause itching and a skin rash (Rockeville 1984, Hillier and Rome 1986).

Acute (short-term) ecological effects. Acute toxic effects may include the death of animals, birds or fish and death or low growth rate in plants. Acute effects are seen after two or four days when animals or plants come in contact with a toxic chemical substance (Jaskot and Costa 1994).

Chronic (Long-term) Ecological effects. Chronic toxic effects may include shortened lifespan, reproductive problems, lower fertility and changes in appearance or behavior. Chronic effects can be seen long after first exposure to a toxic chemical (Sun *et al* 1998).

Water solubility. The solubilities of these aromatic amines is between 1 to 1,000 mg in a liter of water.

Distribution and persistence in the environment. These compounds are moderately persistant in water, with a half - life of between 20 to 200 days. The half life of a pollutant is the amount of time it takes for one - half of the chemical to be degraded. About 60 - 80% of these aromatic amines will eventually end up in water, the rest will be divided about equally between terrestrial soils and aquatic sediments.

Bioaccumulation in aquatic organisms. Some substances increase in concentration or bioccumulate, in living organisms as they breath contaminated air, drink contaminated water, or eat contaminated food. These chemicals can become concentrated in the tissues and internal organs of animals and humans.

Biochemistry of dyes/dye intermediates and their carcinogenicity. There is sufficient evidence for the carcinogenicity in experimental of these aromatic amines animals in when administered in the diet, these aromatic amines induced transitional cell carcinomas of the urinary bladder in hamsters and female dogs and hepato-cellular carcinomas in female dogs (Lakshmi *et al* 1995).

When administered by transplacental exposure, the compound increased the incidences of lymphoid leukemia in mice (Kennelly *et al* 1982).

The dye intermediates have ability to undergo reductive cleavage of the azo linkage. The metabolism by a reductive cleavage reaction has been shown in Scheme - 1.

Binding of benzidine / derivatives to rat and mouse tissue DNA. Heamoglobin binding of benzidine and some benzidine congeners (Beland *et al* 1997).

Hydroperoxidase I catalyzed peroxidative activation of benzidine / derivatives to a mutagen in *Salmonella tuphimurium* (Jung *et al* 1985).

Comparative activation of benzidines to mutagens by hepatic S9 and microsomes from rat pretreated with different inducers of Cytochrome P - 450 (Parkinson *et al* 1983).

Table 3
Mutagenicity data of some dye intermediates

Test	Mutagencity (Revertants μmol^{-1})	
	Standard Assay S9	Prival Modification
Aniline	Negative	Negative
3-Aminoquinoline	Negative	-
5-Aminoquinoline	42	-
3-Aminopyridine	0.7	-
4-Amino-N,N-bis-(2-hydroxyethyl)aline	1007	689
4-Aminodiphenylamine	Negative	154
4-Amino-N,N-dimethylaniline	Negative (toxic)	-
2-Aminothiazole	4.1	-
3-Aminophenol	1.7	-
Chromatropic acid	Negative	Negative
Broenner's acid	4.9	7.7
Cleve's acid	4.9	12.5
S-acid	13.8	24.4
Gamma acid	15.0	32.6
H-acid	Negative	Negative
J-acid	5.2	41
4-Amino-1-naphthol	1413	Negative
4-Amino-5-hydroxy-8-phenylazo-2,7-naphthalenedisulfonic acid disodium salt	Negative	Negative
4-Amino-8-(4-carboxyphenylazo)-5-hydroxy-2,7-naphthalenedisulfonic acid trisodium salt	Negative	Negative
7-Amino-4-hydroxy-1-phenylazo-2-naphthalenesulfonic acid monosodium salt	16.1	67.8

Benzidine and derivatives induce micronuclei in the bone marrow and the fetal liver of mice after gavage (Przybjewska *et al* 1985).

Mutagenicity of some benzidine congeners and their acetylated and diacetylated derivatives in different strains (Hatcher and Swaminatham 1992).

Induction of hepaticmicrosomal cytochrome P - 448 - mediated oxidases by benzidine derivatives in the rat (Parkinson *et al* 1983). Binding of benzidine derivatives to DNA and polyribonucleotides (Osborne and Monogr 1984).

Mutagenicity of some congeners of benzidine in the *Salmonella typhimurium* assay system (Kranen *et al* 1997).

Covalent interaction of benzidine/derivative with hepatic lipids. Enzymic basis and stability of the adducts (Mule and Lomte 1994).

Activation of benzindine/derivatives in rat liver microsomes to mutagens with the involvement of Cytochrome P - 450 (Manus 1989).

Structure - toxicity relationship. The toxicity and carcinogenic activity also depend on the structure and substitution on biphenyls. For dyes that are carcinogenic and are resistant to chemical attack, such as true azo dyes, the dye itself is likely to be procarcinogen. In contrast, azo dyes which exist in the hydrazone form are more likely to be broken down e.g. reduced. In this case, the procarcinogen is likey to be an amine breakdown product of the dye and the ultimate carcinogenic potential can then be deduced from the availabitlity of a suitable active site on the metabolite. Azo pigments because of their extreme insolubilities are likely to be broken down, even if they exist in the hydrazone form (Gregory 1986).

A relationship also exists between structure and carcinogenic activity of substituted benzidines. Proton accepting substituents which are capable of forming intramolecular hydrogen bonds in ortho - position in relation to amino groups, decrease the carcinogenicity of the title intermediates of dye synthesis. Lipophilicity of the compounds and electronic effects of the substitents are found to be the main parameters of carcinogenicity. Substitution at both *ortho* positions 2, 6-disubstitued aniline and 2,4,5-trimethyl aniline could prevent genotoxicity due to steric hindrance of an enzymic activation to electrophilic intermediates (Belogoro *et al* 1981).

Direct Dyes. It is now well established that the ability of azo dyes to undergo reductive cleavage of the azo linkage could lead to an indirect route of exposure to an established carcinogen. The absorbance potency of these dyes, however was the most potent of the dyes tested and their inducing activity was much greater than that of its azo reduction products (Kornburst and Barfknecht 1984). The literature surveyed contains the following reports which described work published on the toxicity of direct dyes.

A report on the toxicology and carcinogensis properties of C.I. Direct blue 14 and 15 was published under the national toxicology programe. The rats were fed these dyes in drinking water and the experimental results showed a clear evidence of carcinogenic activity, as indicated by benigu and malignant neoplasms of the skin, Zymbal's gland, preputial gland, liver, oral cavity and small and large intestine. Increased incidences of mononuclear cell leukemia and neoplasms of the brain may have been related to chemical administration (Anon 1992, 1993).

A thirteen-week subchronic toxicity studies were conducted on direct blue 6 (Anon 1974), direct black 38 and direct brown 95 by administering the test chemicals in feed to rats and mice. Deaths occurred among rats but not among mice during the test period. In rats, neoplastic lesions of the liver, hepatocellular carcinomas and neoplastic nodules, occurred only in the treated groups (Roben *et al* 1980).

In an investigation, the textile dyes, Navy blue M3R(I) and direct brown 2G(2), not only reduced the percentage of seed germination in *Vigna radiata* (L.) but also suppressed various morphological, biochemical and physiological parameters. I affected the various parameters more adversely than 2. The adverse effects of both dyes were higher in black soil than in red soils (Wilczek *et al* 1992). Toxicological and carcinogenesis studies were conducted by adiministering direct blue 218 in feed to groups of male and female rats in *Salmonella typhimurium*, cultured Chinese hamster ovary cells and *Dorsophila melanogaster* and their carcinogenic and toxic effects are reported (Anon 1994).

The azo reductase activity of a cell - free extract of *Fusobacterium* sp.2 was characterized using trypan blue (I) as a substrate. Either chemical reduction of this dye with sodium hydrosulfite or reduction by the cell-free extract produces a mutagenic product, *o* - tolidine. The *o* - tolidine is mutagenic in the Ames *Salmonella* / mammalian - microsome mutagenicity test when activited by a rat liver S9 preparation (Hartman *et al* 1978).

The effects of prenatal administration of the benzidine-based dyes which are Congo red, Diamine blue and Chlorazil black E, whereas, the dimethylbenzidine-based dyes include: Trypan blue, Evans blue and Benzopurpurin 4B and dimethoxybenzidine-based were investigated, whereas, Chicago sky blue, Azoic diazo component 48, a dimethoxybenzidine congener, two diazo dyes , Naphthol blue black and Sudan III were tested for developmental testicular toxicity. In mice and rats, prenatal exposure to the dye, Congo red permanently reduces the number of germ cell in male and female offspring. In this study, the structural component of the dyes responsible for the parental induction of germ cell aplasia was identified. Only benzidine-based dyes altered testicular development and caused hypsopermatogenesis in mice during adulthood. Dimethyl-and dimethoxybenzidine based dyes were without effect (Gray *et al* 1993).

β -Amyloid peptides are neurotoxic when applied to primary cultures of hippocampal neurons from the embryonic rat. This neurotoxic effect can be inhibited completely by certain diazo dyestuffs. The most potent of these are Congo black(I) and Congo rubin, while direct garnet and sodium-4-aminonaphthalene-1- sulfonate are inactive. I also inhibits the neurotoxic effects of the human pancreatic amyloidogenic peptide amylin. It is postulated that these dyes, by interacting with the (- pleated sheet structure of amyloidogenic peptide, prevent aggregation and hence neurotoxicity (Bargevin *et al* 1994). In a similar study the gonadal effects of fetal exposure to the azo dye Congo red (I) in mice was investigated. This study describes the relationship between gonadal agenesis and fertility in male and female mice exposed to the diazo dye (I). Maternal (I) treatment inhibited testicular and ovarian function in the offspring after oral administration. It was found that prenatal exposure to the dye (I) affects the gonads of both male and female offspring, but only the female offspring display reduced fertility (Gray *et al* 1992).

Food colors. The toxic effects of nine natural food dyes on *Paramecium caudatum* showed the inhibitory effects on leucine aminopeptidase acid phosphatase and esterase *in vitro* and were proportional to the toxic effects of the dyes expressed in terms of the survival time of *P. caudatum* (Chughtai *et al* 1993). In a similar studies, the toxic effect of Xanthene dyes containing halogen atoms were found to be more toxic than other types of food dyes. Phloxin and Rose Bengal (containing chlorine) were particularly toxic. The inhibitory effect of food dyes on leucine aminopeptidase were not consistent with their toxic on *A.salina*. On the other hand, the inhibitory effects of food dyes on lactate dehydrogenase *in vitro* were consistent with the toxic effects of the dyes on *A.salina* (Sako *et al* 1979).

The carcinogenic effect of Metanil Yellow on albino mice have developed hemangioendotheliosarcoma in 80% of the female albino mice and none in males after feeding them food colour additive for 1 year at a dose of 3.0 g / kg body weight. Degenerative changes and metastates were also observed in other important vital organs of the body such as stomach, ileum, rectum, liver, spleen, kidney and ovary (Prasad and Rastogi 1982).

A skin painting studies in mice was conducted with 14 Food, C, D and C colours: FD and C blue No. 9, red No.10, red No.19 & red No. 21, red No. 27 and red No. 31, red No. 36, orange No. 5, orange No. 10 and orange No. 17. These 14 cosmetic colour were submitted to dermal toxicity testing. Dosage levels were based on lipstick use determination made in a group of human female volunteers. The groups of lipstick colors were divided into 3 treatment series and painted on twice weekly to an area 6 cm. A total of 1400 mice were used comprising groups of 100 mice (50/sex) plus an additional pos. control group of the same size and a vehicle control group of 300 mice (150/sex). All colours were prepared at 1.0% suspensions in water. The pos. control received benzo[a] pyrene(1) dissolved in acetone.

Survival was approximated equivalent in all experimental groups except the pos. controls who died earlier which was consistent with survival recorded by others for I-treated mice. Extramedullary hematopoiesis was found in all treated groups, equivalent to the findings in the controls. The repeated application of 0.1 ml containing 1.0% dye did not increase the incidence of neoplasia when compared to controls in any of the groups receiving application of the 14 days (Carson *et al* 1984).

The mutagenicity of food colors in the Ames/*Salmonella* of 14 food dyes tested, including Methylene green 203A, Strawberry red 1023, Purple red 0048, Raspberry red 0059 and Black 0051 was determined but none was mutagenic. In another investigation, the effect of food colors on the induction of sex-linked recessive lethal mutation with *Dorsophila melanogaster* (Rapic *et al* 1985) was determined of 13 food dyes tested, inclusive Citronine rose 200A, Methylene green 203A, Black 0051, Purple red 0048, Strawberry red 1023 and Raspberry red 0059, none were mutagenic in a sex-linked recessive lethal mutation test with *D. melanogaster* (Vijosevic *et al* 1985). In a similar report, the results of *in vivo* study on mammalian chromosomes of genotoxicity of 16 food dyes tasted, including Raspberry red 0059, Orange color 963, Strawberry red 1023, Citron rosy 200 A and Chocolate brown 1022, none was genotoxic to bone marrow cell chromosomes when given to mice as a single dose of a 1% aqueous solution (Jankovic *et al* 1985).

The mutagenic evaluation of Orange color 963, Vanilla red 0050, May green 1025, Black 0051 and Methylene green 203A by the micronucleus test (no of polychromatic and normochromatic erythrocytes containing micronuclei) with rats only 1 (Black / 0051) increased the percentage of erythrocytes with micronuclei indicating mutagenic activity by this dye (Savkovic *et al* 1985). In another investigation, the influence of 16 food colors on the induction of dominant lethal mutation and spermatozoid abnormality in mammals was also determined. None of 16 tested food dyes reduced fertility or caused spermatozoid abnormalities. No significant changes in dominant lethal mutations were induced. The dyes tested included Orange color 963, Vanilla red 0050, May green 1025, Black 0051 and Methylene green 203A (Konishi *et al* 1992, Dillion *et al* 1994).

The carcinogenicity testing of Food red No.106 (acid red) (I) in male and female rats were determined. Body and organ weights, hematology, urinalysis and histopathological evaluations do not reveal any evidence of adverse effects associated with the compound relative to the untreated controls. The spectrum, incidence and histology of tumors developing in both treated and control animals were consistent with spontaneous incidences reported in this strain of rat. This study thus indicates that (I) is not carcinogenic to F 344 rats after 2 year of dietary administration at a maximal level of 5.0% in the basal diet by weight (Osman *et al* 1984).

The effect of feeding synthetic food and drug colorants belonging to four different chemical classes i.e. Brilliant black (BK), Brilliant blue (B), Erythrosine (Er) and Indigo carmine (In) were studied. Groups of male and female mice were fed-libitum diets mixed with the synthetic food colorants. The activities of liver and heart tissues were inhibited in male and female mice relative to control and males were more affected than females. The blood hemoglobin (Hb) content was increased under the effect of the 4 synthetic food colorants in the order: Er > Bk > In > Bl as well as the red blood cells while white blood cell count were increased relative to control in male and female mice. Pathalogical and histopathological changes were observed either in female or male organs especially in the liver and stomach. Some changes were found in the liver tissues and cell nucleus (polynucleus) in addition, enlargement of the stomach size was also evident (Nishiuchi 1984).

The toxicity of 25 dyes on freshwater organisms was tested. These synthetic dyes used in food was tested on carp (*Cayprinus cariop*), *Dephnia carinata*, *Indoplanorbis exustus*, larvae of *Sympetrum frequens* and tadpoles of toad (*Bufo bufo*) and frog (*Rana brevipoda porosa*). With the exception of the effect of Food Black No. 105 (Rose Bengal) on carp, all the tested dyes showed very low toxicity to all tested fresh water organisms with median lethal concentration value (LC50) of > 40 ppm (Wagner *et al* 1995).

Basic and Solvent Dyes. The potential for methylene blue genotoxicity was investigated in two mammalian test systems. Different concentrations of Methylene blue were prepared in plasma (heat-treated at 56°C for 1h to reduce cytotoxicity) and used, without illumination, in an *in vitro* mouse lymphoma cell assay designed to detect forward mutations in the gene encoding thymidine kinase. The assay was performed in the presence and absence of rat liver microsomal fraction. Methylene blue did not increase the frequency of micronuclei in polychromatic red cells harvested from bone marrow. It is mutagenic in cultured mammalian cells (Michaels *et al* 1985).

The toxicity and sorption of 5 azo and triphenylmethane dyes named as Basic violet 1(I), Basic violet 2, Basic violet 3, Basic green 4 and Tropaeolin O were established by determining the percent to fresh water microbiota and determined the risks, that these dyes may pose to the aquatic environment. Basic violet 3 was the most toxic, with a mean survival rate of 20.7% at a dye concentration of 5.0 mg/l. Tropaeolin O was the least toxic, with a survival rate of 92.0%. Survival increased with

decreasing dye concentration equilibrium. Partition coefficients were higher for viable cells than for heat-killed cells, suggesting that a metabolic process may be involved in sorption of these dyes or that autoclaving the cells reduces the organisms' cation exchange capacities (Milanova *et al* 1997).

Aquatic toxicity effects of dyes used in the manufacture of news print and telephone directory-grade papers were studied on luminescent bacteria, rainbow trout and activated sludges. The results showed that triphenyl methane dyes, used in making telephone directory-grade papers inhibited respiration of flora in activated sludge at higher concentrations not likely to exist in mill effluents. Data from microtox tests agreed with fish toxicity data (Ahmed *et al* 1985).

The toxicity of Aniline blue, Methylene blue, Xanthene, Eosin and Fuchsin dyes against the cotton leaf worm (*Spodoptera littorialis*) was investigated for their possible use in combination with pyrethroids. Deltamethrin was the most effective against (*S. littoralis*) 4th instar larvae followed by flucythrinate and cypermethrin, whereas, Aniline blue and Methylene blue showed higher toxic index than Xanthine followed by Eosin and Fuchsin. The joint action studies demonstrated strong synergism in mixtures of deltamethrin with Fuchsin, Methylene blue or in the mixture of Flucythrinate with Xanthine or Eosin. The other combinations indicated only additive joint action (Vachalkova 1996).

The reduction of some triphenylmethane dyes and their potential carcinogenic activity was examined in strictly anhydrous solutions in the absence and presence of α-lipoic acid. The highest potential carcinogenicity values determined for Crystal violet and Methyl violet are found to be 0.420 and 0.440, respectively (Selyuzhitskii *et al* 1982).

A study was conducted to find out the blastomogenic properties of Basic blue K (1) on animals. I induced formation of 2 tumors in 2 males of 10 and 10 tumors in 7 females of 13; no tumors were developed in control animals (Zimina and Pavlenko 1991).

The toxic and mutagenic effects of arylmethane dyes Victory blue (C.I. 44040), Methyl violet (C.I. 42535) and Brilliant green (C.I. 42040) and the carcinogenic aminoazo dye Chrysoidin (C.I. 14270) were evaluated. All the dyes exhibited high toxicity (as cell killing and growth inhibition) and increased the frequency of nuclear point mutations and cytoplasmic mutations of respiratory deficiency (Serova *et al* 1992).

The frequency of neoplasms and chromosomal aberrations (CA) in the liver and bone marrow were investigated after administration of carcinogenic *O*-aminoazotoluene (OAT) and non-carcinogenic analog - 4 - aminoazo-toluene (AB) to male and female rats in the early postnatal period. AB increase of CA in the liver was small (although substantial in males), but the increase was high in the bone marrow of both sexes. In contrast, OAT increase of CA was higher in the liver than that in the bone marrow and it was higher in the males. In addition, OAT induced greater number of hepatic neoplasm in the males. Both these effects of OAT in males suggest a relation between its mutagenic and carcinogenic properties (Kaledin *et al* 1994; Ashby *et al* 1994).

The evaluation of Butter yellow (4-dimethylaminoazobenzene) (I) and 12 of its structural analog were made in a cell transformation assay. The *in vitro* results agreed with long-term animal data for 8 compounds, but disagreed in finding I-4 sulfonic acid and sodium salts, 4-trifluoromethyl-I and 4-diethylaminoazobenzene pos. 9-Phenylazotoluidine and N-methyl-5-phenylazoindoline may have carcinogenic potential and 3,5-dimethyl-4-aminozobenzene-4-sulfonic acid may be noncarcinogenic. Addition of azobenzene to the *in vitro* assay medium increased the transforming potency of I 25-fold. This assay cannot be relied upon to predict the *in vivo* potency of a carcinogen (Sandhu and Chipman 1991).

The role of oxidation and azo reduction in the activation of Chrysoidine (I) dyes to genotoxic products. The enzymes involved in metabolic activation of (I) azo dye to genotoxic products were investigated. The mutagenicity of the component 2,4-diamino-3-methylazobenzene in *Salmonella typhimurium* strain TA 100 potentiated > 4-fold. The results indicate a role for cytochrome P - 450 particularly, cytochrome P - 448 in metabolic activation of these dyes and indicate that azo reduction was a detoxification process. Neverthless, the oral and injected dosing of Chrysoidine to rats led to unscheduled DNA synthesis in hepatocytes (Collier *et al* 1993).

The mechanism of azoreduction of *p*-dimethylamino-azobenzene (I) dye carcinogens was conducted by rat liver microsomal cytochrome P - 450. To elucidate the mechanisms involved, the reduction of structurally related azobenzenes by hepatic microsomes was investigated. High substrate reactivity was observed for I, its corresponding secondary (II) and primary (III) amines and *p*-hydroxyazobenzene (IV). In contrast, only negligible rates were obtained for unsubstituted azobenzene (V), hydroazobenzene (VI), *p*-isopropylazobenzene (VII) and (VIII), the benzoylamide derivatives of III. These results clearly indicate that electrons-donating groups, such as hydroxyl or primary, secondary and tertiary amines, are essential for binding of azo dye carcinogens to liver microsomal cytochrome P - 450 and by implication, their enzyme reduction. No inhibition of azoreduction of I and IV was obtained by

addition of VII, V or IV to the reaction mixture. In contrast, very weak binding was observed for the unreactive compounds VII, VIII, V, VI. Thus, there is good correlation between binding and substrate reactivity. The apparent lack of binding may explain the inability of the non-reactive compounds to inhibit azoreduction. The difference in the reduction rate observed for V *vs*. IV suggested that hydroxylation would facilitate the reduction of an otherwise non-reactive azo dyes (Zbaida *et al* 1989, Anon 1992).

Pigment and vat dyes. Under National Toxicology Program (NTP), a technical report on the toxicology and carcinogenesis studies of C.I. Pigments red 3(I) in rats and mice is prepared. Under the condition of 2-year feeding studies, there was some evidence of carcinogenic activity of (I) in male rats as exhibited by increased incidences of benign pheochromocytomas of the adrenal gland. The marginal increase in the incidences of squamous cell papillomas of the skin and Zymbal's gland carcinomas may have been related to (I) administration. There was some evidence of carcinogenic activity of (I) in female rats as indicated by the increased incidence of hepatocellular adenomas. There was some evidence of carcinogenic activity of (I) in male mice as exhibited by the increased incidences of tubuler adenoma of the renal cortex and follicular cell adenomas of the thyroid glands. There was no evidence of carcinogenic activity of (I) in female mice that received 12,500, 25,000 or 50,000 ppm. The incidences of mononuclear cell leukemia and preputial gland tumors in male rats and mononuclear cell leukemia, mammary gland fibroadenoma and clitoral gland tumors in female rats were lower in the exposed groups. The incidences of liver foci were markedly increased in exposed male and female rats. The severity of nephropathy was observed in male and female mice, cytomegaly (karyomegaly) of renal tubule epithelium was observed in male mice. Thyroid follicular cell hyperplasia occurred with an increased incidence in male and female mice receiving (I) (Anon 1994).

In a similar study, toxicology and carcinogenesis of C.I. Pigment Red 23(I) was determined in rats and mice. Under the conditions of the 2-year feed studies, there was equivocal evidence of carcinogenic activity of (I) in male rats as evidenced by a marginally increased incidence of renal tubule cell neoplasms. There was no evidence of carcinogenic activity of (I) in female rats fed diets containing 10,000, 25,000 or 50,000 ppm. The severity of kidney nephropathy was increased in exposed male rats. In mice, (I) caused an increased in hyperkeratosis and epithelial hyperlasia of the forestomach (Hofman and Schmidt 1993).

The possible metabolism of Pigment Yellow 17(I), a 3,3´-dichlorobenzidine based pigment was investigated after inhalation exposure in rats. Rats were exposed by inhalation to the technically highest administrable concentration of 230 mg (I) / m^3 air for 4 h. Inhalability of the dust was guaranteed by a mass - median aerodynamic diameter of 1.0 - 1.1 m. For 14 days after exposure, urine and serum samples were analyzed for 3,3´-dichlorobenzidine, the parent carcinogenic amine of the test compound. No 3,3´-dichlorobenzidine could be detected either in urine of blood, the detection limit being 5 mg/ml for both media. Based on the results of this study there is no evidence for metabolic cleavage of (I) to 3,3´-dichlorobenzidine in the rat (Kurlyanskii *et al* 1988).

The water-soluble phthalocyanine dyes are found to be low toxic and are not very harmful. The copper phthalocyanine derivatives-disulfonic acid, trisulfonic acid reactive turquoise 2 Z and reactive turquoise K affected hepatic mitochondrial electron transport chain. Toxicological properties of dyes are also described (Rannung *et al* 1992).

The presence of genotoxic and bioactive components in indigo-dyed fabric was examined. Extractions of pure cotton and jean fabrics were tested for mutagenicity in *Salmonella typhimurium* strains TA 98 and TA 100. Synthetic indigo, indirubin and isatin were tested in competition expermients *in vitro*. The mutagenicity of the indigo dyed fabric was dependent on type and treatment of the fabric. Extracts of both bleached and nonbleached jeans gave mutagenic effects on TA 98 (S9 and TA 100 S9). The greatest effects were seen in the presence of S9. Indigo can, therefore, still be a potential health risk either by eliciting toxic effects of other compounds or by being a non - genotoxic carcinogen. The world wide use of jeans with a possible exposure of a large population to genotoxic and biological active components emphasizes the need for a more thorough characterization of these effects (Conde *et al* 1984).

The contact dermatitis was determined in 3 patients induced by the use of dyed textiles or by occupational exposure to azo dyes *p*-aminoazobenzene (I), disperse yellow 3, disperse orange 3, diazodiethylaniline and diazodimethylaniline (Kosaka *et al* 1991).

Miscellaneous. The genotoxic components of commercially available synthetic dyes were examined. Dyes which showed toxicity were separated on silica gel coated plates and the genotoxicity of each component was examined. Among the dyes examined, the main component of Disperse red 73 showed genotoxicity. In the case of 5 other dyes, Acid black 26, Acid black 50, Acid brown 2, Disperse red 145 and Disperse red 157, a minor component of Disperse violet 52 was not determined because of the insolubility of its main component (Wedzisz and Grzelka *et al* 1988).

Table 4
Characterization of 1,5-dihydroxy napthalene formaldehyde (1,5-DHNF) Oligomer

1,5-dihydroxy Napthalene Formaldehyde oligomer (1,5-DHNF)	Color	Softning point (°C)	Elemental analysis(%) C Calcd	C Found	H Calcd	H Found	Mn estimated by VPO
	Brown	> 230	77.41	77.30	5.37	5.30	744

Where VPO=Vapour Pressure Osmometry.

LD50 (96 h) values of Mordant black 11, Basic brown 1 and Disperse yellow 1 in guppies (*L. reticulatus*) were found to be 4.02, 3.06 and 3.74 mg / l, respectively; thus the dyes have moderate toxicity to fish. LC50 of Mordant black 15, Acid violet 7, Acid black 26, Acid yellow 44, Acid violet 49, Acid blue 7 and Direct black 32 and 151 were > 400mg/l; these dyes can be considered non-toxic (Kour *et al* 1993).

The mutagenicity testing of textile (azo) dyes with *Salmonella* / microsome assay. Five textile azo dyes-Acid violet 17, Acid green 16, Acid red 85, Acid red 114 and Direct green 6 were tested for bacterial mutagenicity with *S.typhimurium* TA98 and TA100 strains using a plate incorporation assay. After testing over the concentration range 1 - 500 mg with and without metabolic activation these dyes showed no mutagenicity or toxicity (Flammang *et al* 1992).

Dyes intermediate toxicity. The DNA adduct levels were determined in congenic rapid and slow acetylator mouse strains following chronic administration of 4-aminobiphenyl. The levels of the hepatic DNA adducts were 2-fold higher in the liver of the female as compared to the male animals. The DNA adducts also increased with the dose in bladder of the male mice, but in contrast to the liver, the adduct levels were 2-fold lower in the bladder DNA of the female mice (Hughes *et al* 1992).

New thiophene analogs of benzidine and 4-aminobiphenyl have been tested for their carcinogenicity in the *Salmonella* revere-mutation assay Ames and the cell-transformation assay of styles. Their activity profiles *in vitro* were consistent with their known potential carcinogenicity. The possible carcinogenicity of the analogs *in vivo* is discussed (Ashby *et al* 1978).

3,3′-Dimethylbenzidine dihydrochloride (I) is one of five chemicals being evaluated in 2-years carcinogenicity and toxicity studies as part of NTP's Benzidine Dye initiative. Toxicology and carcinogenesis studies were conducted by administering (I) (approximately 99% pure) in drinking water to groups of rats of each sex, whereas, Genetic toxicology studies were conducted in *Salmonella typhimurium*, Chinese hamster ovary (CHO) cells and *Drosophila melanogaster*. Hematology and serum chemical analyses and thyroid hormone determinations were conducted and the results indicated a rapid declining of animal survival (Anon 1991).

It has been reported that the carcinogen 3,3′-dichlorobenzidine is bioactivated in the liver *in vivo* and *in vitro* to mutagenic and lipid - binding metabolites. To characterize the metabolites involved, adult male rats with treated with a single dose of either DBC, the spin trap 1-phenyl-N-tert - butylnitrone (PBN) and both chemicals. It is concluded that in the rat aryl radicals may be the predominant stable radicals from arylamines in the liver, whereas, radicals derived from N-oxygenation may predominate in the blood. The tissue distribution of the two may predominate in the blood. The tissue distribution of the two radical species may reflect the major site of their formation from some arylamines in the rat (Iba *et al* 1991).

The non-genotoxic rodents of the potent bladder carcinogens, *o*-anisidine and *p*-cresidine has been reported which indicated that the genotoxicity of *o*-anisidine was acknowledged and misquoted data cited (Bolcsfoldi *et al* 1992). In another studies, the mutagenicity of *o*-anisidine to the bladder of lacl transgenic mice was also conducted. A single oral administration of the maximum tolerated dose level (750 mg/kg) of *o*-anisidine to mice yielded negative results in 32P - post - labelling assays of bladder and liver DNA (24 h after dosing). The possibility that *o*-anisidine is mutagenic and carcinogenic to the rodent bladder via formation of radical species is suggested (Sasaki *et al* 1994).

The toxicities of chloroanilines to *Photobacterium phosphoreum* was determined and their correlations with effects on other organisms and structural parameters using the Microtox assay was presented. The values obtained correlated well with the toxic effects of these compounds to 4 different species of yeast and with the effects of the octanol / water partition coefficient (Ashby 1992).

The toxicity, DNA binding and adduct formation of chloroaniline and 4,4′-methylene - bis (2 - chloroaniline) has been found and the data showed DNA damage in cultured explants

of human and dog bladder urothelium. The level of DNA damage in human bladder was higher than that in the dog. 33 *P*-postlabeling analyses indicated that Methylene-bis (2-chloroaniline) forms several DNA adducts in both human and dog bladder tissues. These results suggest caution in the occupational exposure of humans to Methylene-bis (2-chloroaniline) (Ribo and Kaiser 1983).

The aromatic amine metabolism catalyzed by prostaglandin H synthase (PHS) was investigated as an enzyme system responsible for the conversion of carcinogens to reactive metabolite(s) in extrahepatic tissues. The bladder carcinogens benzidine 2-aminofluorene, and 2-naphthylamine are oxidized by PHS to free radicals. Metabolic activation of carcinogenic aromatic amines catalyzed by hepatic cytochrome P - 450 proceeds via an N-hydroxylation pathway analogous to other well - studied aromatic amine carcinogens. PHS - catalyzed metabolism of these compounds results in the formation of reactive species which bind covalently to cellular macromols (Stoner *et al* 1988).

The metabolism of naphthylamines in isolated rat hepatocytes has been studied and compared in freshly isolated hepatocytes from 3-methylcholanthrene (MC) - treated and untreated rats. At 10 (M, 2-naphthylamine was mainly N-acetylated and N-glucuronidated. Minor pathways led to C-oxidation and N-oxidation. In hepatocytes, from MC-treated rats total metabolism was slightly affected (1.5-fold increase). Similar experiments were carried out with 1-naphthylamine. Its N-glucuronide was the predominant metabolite (68%) followed by the N-acetylated compound (15%) while C-oxide was low and N-oxidized metabolites could not be detected, even after induction. Thus, MC treatment markedly shifted 2-naphthylamine metabolism from N-acetylation and N-glucuronidation to N-and C-oxidation. In the case of 1-naphthylamine metabolism, extensive N-glucuronidation together with the lack of N-oxidation may prevent carcinogenesis (Eling *et al* 1987). In a similar study, rat peritoneal macrophages were incubated with 2-naphthylamine (I), a well known carcinogen and respiratory burst was studied. (I) induced a time and dose-dependent stimulation of superoxide anion production which was suppressed by superoxide dismutase treatment. Other observations were as follow (i) the simultaneous presence of polymyxin B and stauros-porine inhibitors of protein kinase C, inhibited (I)-dependent O_2 -production; (ii) NADPH-oxidase contained in postnuclear fraction from (I)-incubated macrophages showed a greater activity than control fractions; (iii) the stimulation of O_2 -production elicited by (I) was several-fold enhanced in activated macrophages compared to resident cells. These data suggest that (I) produces the activation of NADPH-oxidase through protein kinase C (Orzechowski *et al* 1992).

The toxicity of toluidine isomers have been determined. It has been reported that *p*-toluidine was 2-fold more toxic than the *ortho* and *meta* isomers according to LD50 in rats, mice and rabbits. The *m*-toluidine had the most damaging effect on blood composition, followed *o*-toluidine and *p*-toluidine. The last also showed some hepatotoxicity. The *meta* isomer somewhat impaired the N excretion by the kidney. Skin resorption followed the order *ortho* > *meta* > *para*. The most local irritating action in rabbit eye was shown by *m*-toluidine (Chiara and Sobrino 1992). In similar work, the author have re-evaluated the carcinogenicity of *o*-toluidine and presented the evidence of carcinogenicity/genotoxicity of *o*-toluidine (Vashenko *et al* 1977). The preventions of occupational urinary bladder tumours in the manufacture of toluidines were investigated. The results of experimental, hygienic and occupational - pathology investigations indicate the carcinogenic nature of *o*-toluidine. A most effective measure for radically improving working conditions is the use of the catalytic method. The maximum perimissible concentration of I should be revised, taking into account its carcinogenic activity.

Among the aromatic amines examined, the mutagenicity was in the order: 3,3′-dichlorobenzidine > toludine > benzidine> 2-naphthylamine. Dichlorobenzidine was extremely mutagenic in the Ames assay with *Salmonella typhimurium* TA 98 with S9 microsomal activation. The last 2 aromatic amines were moderate mutagenicity. Tobias acid and 2,2′, 4,4′-tetra-aminobiphenyl gave negative results with *S. typhimurium* TA 98 and 100 stains and with or without S9 microsomal activation. The carcinogenicity of these compounds was in the same order as their mutagenicity of these compounds. Plus tobias acid and tetra-aminobiphenyl were not carcinogenes.

Conclusion

The primary motivation for the design of novel, marketable dyes and pigments have been the need for colorants having improved technical performance. In more recent years, consumers are getting awareness of the hazards of synthetic dyes and pigments. It has therefore, become clear that the toxicological properties of dyes and their precursors must also be factored into the dye design equation. This means that the development of environmentally friendly colorants must embrace all aspects of the life cycle of synthetic dyes, from manufacture through dye application and handling of residual dye bath color. In this regard, it is also clear that dye chemists must continue to work closely with genetic toxicologists and environmental toxicologists to assure the viability of our industry without compromising human health or the

environment. It is the role of dye chemists to work especially closely with genetic toxicologists to enhance our understanding of the nature of the binding site(s) with which azo dyes interact *en route* to eliciting a genotoxic response. The ability to define these sites and develop working models would shed light on the reason that closely related dye structures can differ significantly in genotoxicity. Many efforts have been made to meet this challenge and synthesize intermediates to develop non-genotoxic dyes. It should also be excersied to handle these intermediates properly to prevent/reduce the health risks.

References

Ahmed Y M, Mustafa A M A, Eleiva M A 1985 Toxicity of certain dyes as insecticides and their joint action with some pyrethroids. *J Environ Sci Health Part-B* **20** 689 - 699.

Anliker R 1979 Ecotoxicology of dyestuffs - a joint effort by industry. *JSDC* **95** 317 - 326.

Anonymous 1955 *The Chemistry of Synthetic Dyes and Pigments*. Edited by H A Lubs, Organic Chemical Department, E I Du Pont de Nemours and Company, p 65.

Anonymous 1971 Schwaebel Nordmeyer, *Text Chem Colorist* **3** 133.

Anonymous 1974 *Riegles Hand Book of Industrial Chemistry, published by Von Nostrand Reinhold Company*, 7th ed. pp 667.

Anonymous 1982 Monographs on the evaluation of the carcinogenic risk of chemicals to man. lyon IARC, Vol. **27**.

Anonymous 1991 *Carcinogenic aromatic amine metabolism and DNA adduct detection in humans*. F F Kadlubar, *Proc Int Symp Princess Takamatsu Cancer Res Fund* **21** 329 - 338.

Anonymous 1992 NTP technical report on the toxicology and carcinogenesis studies of CI direct blue 15 (desalted industrial grade) in F344/N rats (Drinking water studies). *Natl Toxicol Program Tech Res Ser* **397** pp 249.

Anonymous 1993 Toxicology and carcinogenesis studies of CI direct blue 15 (CAS No.2429-74-5) in F 344/N rats (drinking water studies). *National Toxicology Program, Gov Rep Announce Index* **93**.

Anonymous 1994 NTP technical report on the toxicology and carcinogenesis studies of C.I. direct blue 218 (Cas No. 28407-37-(6) in F 344/N rats and B6 C3F1 mice (Feed studies). *Natl Toxicol Program Tech Rep Ser* **430** pp 284.

Anonymous NAT 1991 Toxicology and carcinogenesis studies of 3,3´-dimethylbenzidine dihydrochloride (CAS No.612-82-8) in F 344/N rats (drinking water studies). *National Toxicology Program Report*, NTP-TR-390, NIH/BUB-91-2845; order No.PB 92-103779, pp 237.

Anonymous NTP 1994 NTP technical report on the toxicology and carcinogenesis studies of C.I. Pigment Red 3(CAS No. 2425-85-6) in F344/N rats and B6C3F1 mice (Feed studies). *Natl Toxicol Program Tech Rep Ser* **407** pp 292.

Ashby J 1992 The non-genotoxicity of *ortho* - anisidine. *Mutat Res* **279** 255 - 256.

Ashby J, Short J M 1994 Mutagenicity of *ortho* - anisidine to the bladder of lac I transgenic B6 C3 F1 mice: Absence of 14C or 32P bladder DNA adduction. *Carcinogenesis* **15** 2291 - 2296.

Ashby J, Styles J A, Paton D 1978 Thiophene analogs of the carcinogens benzidine and 4 - aminobiphenyl:evaluation *in vitro*. *Br J Cancer* **38** 34 - 50.

Bargevin M C, Passat M, Daniel N, Capet M, Doble A 1994 Congo Red protects against toxicity of α-amyloid peptides on rat hippocampal neurons. *Neuro Report* **5** 2429 - 2432.

Beland F A, Melchior W B J, Mourato L L G, Santos L G, Amelia M, Marques M M 1997 Arylamine - DNA adduct confirmation in relation to mutagenesis. *Mutat Res* **376** 131 - 219.

Belogoro V V, Muller N R, Ivin B A, Filov V A 1981 Quantitative relation of antitumour activity and toxicity with the structure of some para-substituted N,N-di(2-choloroethyl) aniline. *Eks P Onkol* **3** 59 - 62.

Bolcsfoldi G, Hellmer L 1992 Comments on the paper, The non-genotoxic rodents of the potent bladder carcinogens *ortho*-anisidine and *para*-cresidine. *Mutat Res* **279** 223 - 224.

Carson S 1984 Skin painting studies in mice-11 colors. *J Toxicol Cutaneous Ocul Toxicol* **3** 357 - 370.

Chaira M D, Sobrino F 1992 2-Naphthylamine induces anion superoxide production in rat perioteneal macrophages. *Biochem Pharmacol* **43** 1841 - 1845.

Chughtai S R, Mohammad A S, Siddiqui B A 1993 Genotoxicity testing of some dyes in diploid yeast *Saccharomyces cerevisiae*. *Pak J Sci Ind Res* **35** 283 - 287.

Clonfero E, Venier P, Granella M, Levis A 1990 Azo dyes for leather: mutagenic and carcinogenic hazards *Med Cav* **81** 222 - 229.

Collier S W, Storm J E, Bronaugh R L 1993 Reduction of azo dyes during *in vitro* percutaneous absorption. *Toxicol Appl Pharmacol* **118** 73 - 79.

Conde-Salazt L, Guimeraens A, Romero L, Harto A 1984 Contact dermatitis from azo dyes. *Med Seg Trab* **31** 29 - 33.

Dillion D, Combes R, Zeiger F 1994 Activation by cecal reduction of the azo dye D and C black No.9 to a bacterial mutagen. *Mutagenesis* **9** 295 - 299.

Eling T E, Petry T W, Hughes M F, Krauss R S 1988 Aromatic amine metabolism catalyzed by prostaglandin H synthase. *Carcinogen mutagen responses aromatic amines and nitroarenes Proc Int Conf Carcinog Mutagen.N-substi-*

tuted aryl compd **3** 161 - 172.

Flammang T J, Couch L H, Levy G N, Weber W W, Wise C K 1992 DNA adduct levels in Congenic rapid and slow acetylator mouse strains following chronic administration of 4-aminobiphenyl. *Carcinogenesis* **13** 1887 - 1891.

Gray (Jr.) L E, Ostby J S, Kavlock R J, Marshall R 1992 Gonadal effects of fetal exposure to the azo dye Congo black in mice: infertility in female but not male offspring. *Fundam Appl Toxicol* **19** 411 - 422.

Gray L E, Ostby J S 1993 The effect of prenatal administration of azo dyes on testicular development in the mouse. A structure activity profile of dyes derived from benzidiene, dimethylbenzidine, or dimethoxybenzidine. *Fundam Appl Toxicol* **20** 177 - 183.

Gregory P 1986 Azo dyes: Structure-Carcinogenicity relationship. *Dyes Pigments* **7** 45 - 56.

Hartman C P, Fulk G E, Andrews A W 1978 Azo reduction of Trypan blue to a known carcinogen by a cell-free extract of a human intestinal anaeroba. *Mutat Res* **58** 125 - 132.

Hatcher J F, Swaminathan S M 1992 Microsome-mediated transacetylation and binding of N-hydroxy-4-amino-biphenyl to nucleic acids by hepatic and bladder tissue from dog. *Carcinogenesis* **13** 1705 - 1711.

Helmes C T, Sigma C L, Fung V A 1986 A study of azo and nitro dyes for the selection of candidates for carcinogen bioassay. *J Environ Sci Health* **A19**, Hillier R A *International Congress on Industrial Hygiene*, Rome, Italy.

Hillier R A 1986 Effects of using liver fractions from different mammals, including man, on results of mutagenicity assay in *Salmonella typhimurium. International Congress on Industrial Hygiene*, Rome, Italy.

Hofmann T, Schmidt D 1993 Investigation of possible metabolism of Pigment yellow 17, a 3,3´-dichlorobenzidine-based pigments after inhalation exposure in rats. *Arch Toxicol* **67** 141 - 144.

Huang M T, Miwa G T, Lu A Y H 1979 Rat liver cytosolic azo reductase. Purification and characterization. *J Biol Chem* **254** 3930.

Hughes M F, Smith B J, Eling T E 1992 The oxidation of 4-aminobiphenyl by horseradish. *Chem Res Toxicol* **5** 340 - 355.

Hunger K, Jung R 1991 *Chimia* **45** 297 - 300.

Iba M M, Ghosal A, Poyer J L, Downs P, Massion W H 1991 *Prog Pharmacol Clin Pharmacol* **8** 255 - 266.

Jankovic S V, Jelic V 1985 Mutagenic evaluation of food colors by the micronucleus test. *Hrana Ishrana* **26** 27 - 28.

Jaskot R H, Costa D L 1994 Study on the genotoxic activity of selected dyes by a micronucleus test. *Fundam Appl Toxicol* **22** 103 - 112.

Kaledin V L, Ronishevskaya G M, Zvereva L N 1994 Apparent discrepancy between carcinogenic and mutagenicity effects of aminoazo dyes in suckling mice. *Dokl Akad Nauk* **335** 810 - 812.

Kaur A, Singh R, Grover I S 1993 Mutagenicity testing of textile (azo) dyes with *Salmonella* / microsome assay. *J Environ Biol* **14** 327 - 333.

Kennelly J C, Hertzog P J, Martin C N 1982 The release of 4,4´-diaminobiphenyls from azo dyes in the rat. *Martin Carcinogenesis* **5** 947-951.

Konishi N, Kitahori Y, Nishioka H, Yane K, Hiasa Y 1992 Carcinogenicity testing of food red 106 (acid black) in Fischer 344 rats. *J Toxicol Pathol* **5** 157 - 165.

Kornburst D J, Barfknecht T R 1984 Comparison of 7 azo dyes and their azo reduction products in the rat and hamster hepatocyte primary culture / DNA repair assays. *Mutat Res* **136** 255 - 266.

Kosaka H, Nakamura S 1991 Comments on the paper. The non-genotoxic rodents of the potent bladder carcinogens *ortho*-anisidine. *Chem Express* **6** 257 - 260.

Kranen M, Donk J N M, Laires A, Rueff J, Vermuelen N P E 1997 Characterization of enzyme activities and co-factors involved in bioactivation and bioinactivation of chemical carcinogens in the tester strain *Escherichia coli* K12 M x 100 and *Salmonella typhimurium* LT2 TA100. *Mutagenens* **12** 245 - 254.

Kurlyanskii B A, Torshina N L, Balabanova E L, Nevzorova N I, Kibrik V B E 1988 Some characteristics of the toxic effect of water soluble phthalocyanine dyes. *Gig Tr Prof Zabol* **9** 47 - 48.

Lakshmi V M, Zenser T V, Goldman H, Spencer G G, Gupta R C, Hsu F F, Davis B B 1995 The role of acetylation in benzidine metabolism and DNA adduct formation in dog and rat liver. *Chem Res Toxicol* **8** 711 - 720.

Le J, Jung R, Kramer M 1985 Effects of using liver fractions from different mammals, including man, on results of mutagenicity assays in *Salmonella typhimurium*. *Food Chem Toxicol* **23** 695 - 700.

Mc Manus M E 1989 The role of cytochromes P - 450 and N-acetyl transferase in the carcinogenicity of aromatic amines and amides. *Clin Exp Pharmacol Physiol* **16** 491 - 495.

Melick W F, Escue H M, Naryka J J, Mezera R A, Wheller E P 1955 Bladder tumors due to a carcinogen Xenylamine. *J Urol* **74** 760.

Michaels G B, Lewis D L 1985 Sorption and Toxicity of azo and triphenylmethane dyes to aquatic microbial populations. *Environ Toxicol Chem* **4** 45 - 50.

Milanova F, Wood S, Sithole B B 1997 Toxicity of news prints dyes to rainbow trout and activated sludge. *Tappi J* **80** 113 - 118.

Mule M B, Lomte V S 1994 *Cyperkill* (cypermethrin) toxicity to lipid metabolism in *Thiora tuberculate*. *Proc Acad Environ*

Biol **3** 131 - 134.

Nishiuchi Y 1984 Toxicity of agrochemicals to fresh water organism: 2. Dyes. *Suisan Zoshoku* **32** 173 - 175.

NTP 1992 Toxicology and carcinogenesis studies of CI Pigment Red 23 (CAS No. 6471-49-4)in F 344 rats and B6 C3F1 mice (Feed studies). *Natl Toxicol Program Tech Rep Ser* **411** pp 287.

Orzechowski A, Schrenk D, Bock K W 1992 Metabolism of 1 and 2-naphthylamines in isolated rat hepatocytes. *Carcinogenesis* **13** 2227 - 2232.

Osborne M R 1984 DNA Interactions of reactive intermediates derived from carcinogenes. *ACS Monogr* **182** 485-524.

Osman M A, Hussien R M, Adel S A, Abdel-Halim M, Sherifa H S 1984 Biochemical effect of some synthetic food and drug colorants on liver functions, some hormones and hemotological parameters in male and female mice. *Bull Fac Pharm* **33** 173 - 175.

Parkinson A, Safe S H, Robertson L W, Thomas P E, Ryan O E, Reik L M, Levin W 1983 Immunochemical quantitation of cytochrome P - 450 isozymes and epoxide hydrolase in liver microsomes from polychlorinated or polybrominated biphenyl-treated rats. A study of structure-activity relationships. *J Biol Chem* **258** 5967 - 5976.

Parkinson A, Stephen H S, Robertson L W, Thomas P E, Ryan D E, Reik L M, Levin W 1983 Immunochemical quantitation of cytochrome P - 450 isozymes and epoxide hydrolase in liver microsomes from polychlorinated or polybrominated biphenyl-treated rat. *J Biol Chem* **258** 5967 - 5976.

Pei S, Zhu X, Xiang M, Yang L, Dai O 1993 *Recent Adv Chem Mol Biol Cancer Res Int Symp*, pp 111 - 119.

Prasad O., Rastogi P B 1982 Orange II-induced cytogenetic changes in albino mice. *Natl Acad Sci Lett* **5** 205 - 207.

Przybojewska B, Dziubaltowska E 1985 Study on the genotoxic activity of selected dyes by a micronucleus test. *Bromatol Chem Toksykol* **18** 280 - 283.

Rannung U, Bramstedt H, Nilsson U 1992 The presence of genotoxic and bioactive components in indigo - dyed fabrics - a possible health risk. *Mutat Res* **282** 219 - 225.

Rapic V, Latinovic M 1985 Mutagenicity of food colors in the Ames / *Salmonella* assay. *Hrana Ishrana* **26** 19 - 21.

Ribo J M, Kaiser K L F 1983 Toxicities of chloroaniline to photobacterium phosphorium and their correlations with effects on other organisms and structural parameters. *QSAR Environ Toxicol* 3136.

Roben J F, Dill G S, Ward J M 1980 Thirteen-week subchronic toxicity studies of direct blue 6, direct black 38 and direct brown 95 dyes. *Toxicol Appl Pharmacol* **54** 431 - 442.

Rockeville M D 1984 Process of Seminar, Risk management of existing chemicals (Chemical Manufacturers Association), Government Institute Inc., USA.

Sako F, Kobayashi N, Taniguchi N 1979 Toxicity test for food dyes using *Artemia salina* and its enzyme activity. *Shokuhin Eiseigaku Zasshi* **20** 233 - 238.

Sandhu P, Chipman J K 1991 *Prog Pharmacol Clin Pharmacol* **8** 319 - 325.

Sasaki Yu F, Sakaguchi M, Yamada H 1994 Evidence for nonclastogenicity to mice of possible human carcinogen (IARC) para-cresidine. *Mamm Mutagen Study Group Commun* **2** 83 - 85.

Savkovic, Pecevski J, Alavantic D, Sunjarevic I, Radivojevic D 1985 Influence of some food colors on the induction of dominant lethal mutation and spermatozoid abnormality in mammals. *Hrana Ishrana* **26** 29 - 31.

Scholz B, Palanschek N 1988 The determination of substituted aromatic amines in water and sediment samples. *Fresenius Z Anal Chem* **331** 282 - 289.

Selyuzhitskii G V, Malysheva V V, Zus B A 1982 Experimental study of the blastomogenic properties of basic blue K. *Gig Saint* **4** 30 - 32.

Serova IA, Kaledin V I, Semenova L A 1992 Inheritance of susceptibility to induction of hepatic tumors and stimulations of α-fetoproteins synthesis at early stages of hepatocarcinogenesis induced by *ortho*-amino-azotoluene in DD/He and CC 57 BR/MV mice. *Eksp Onkol* **14** 31 - 35.

Sewekow U 1997 Leather articles under the aspect of the German legislation for consumer protection. *Leder* **48** 187 - 192.

Slawomir K, WlokPrzegl 1998 Dyes, the environment and man, Part V, a. Effect of dyes on mammals and man. *Tech Wolk* **4** 23 - 25.

Specht K, Platzek T 1995 Textile dyes and finishes, Remarks to toxicological and analytical aspects. *Dtsch Lebensm Rundsch* **91** 352 - 358.

Stoner G D, Shivaparker M N, Schut H A J, Lehman T A 1988 DNA binding and adduct formation of 4,4´-methylene-bis (2-chloroaniline) (MOCA) in explant cultures of human and dog bladder. *Carcinogen Mutagen Responses Aromatic Amines and Nitroarenes Proc Int Conf Carcinog Mutagen. N-Substituted Aryl Compd* **3** 237 - 240.

Sun H, Huang G, Wang C, Song W 1998 Toxic effect of azo dyes to *Scenedesmus obliquus* and relationship between structure and activity. *Huanjing Kexue* **19** 22 - 25.

Vachalkova A, Novotny L, Blesova M 1996 Polarographic reduction of some triphenylmethane dyes and their potential carcinogenic activity. *Neoplasma* **43** 113 - 117.

Vashenko N M, Yaremenko V D, Baturina T S, Nedbailo E P 1977 Comparative toxicity of toluidine isomers. *Deposited Doc VINITI* 4035 - 4077.

Vujosevic M, Zivkovic S, Mikalacki J 1985 *In vivo* study on mammalian chromosomes of genotoxicity of food colors. *Hrana Ishrana* **26** 25 - 26.

Wagner S J, Cifone M A, Dodd R Y, Myhr B 1995 Mammalian genotoxicity assessment of methylene blue in plasma: implications for virus inactivation. *Transfusion* **35** 407 - 413.

Wedzisz A, Grzelka M 1988 Determination of some toxicity parameters of dyes produced by dyestuff factory *Boruta* Part II. Toxicity studies in mammals. *Bromatol Chem Toksykol* **21** 61 - 64.

Wilczek S R, Krishnasamy R, Subburam V 1992 Toxicity of selected dyes to *Vigna radiata* (L.). *Geobios* **19** 81 - 87.

Zbaida S, Stoddart A M, Levine W G 1989 Role of electronic factors in binding and reduction of azo dyes by hepatic microsomes. *Chem Biol Interact* **69** 61 - 71.

Zimina T A, Pavlenko V V 1991 *Genetika* **26** 2246 - 2249.