NIOSH

TECHNICAL REPORT

CARCINOGENICITY AND METABOLISM OF AZO DYES, ESPECIALLY THOSE DERIVED FROM BENZIDINE

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Center for Disease Control
National Institute for Occupational Safety and Health

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Mark Boeniger

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
CENTER FOR DISEASE CONTROL
NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH
ROBERT A. TAFT LABORATORIES

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ABSTRACT

This study presents a comprehensive review of the literature on the sites of carcinogenic action of benzidine in man and experimental animals, on the metabolism of benzidine, on the metabolism of azo compounds, and on the epidemiological experience of industries using azo dyestuffs. It reviews industrial hygiene surveys conducted both to monitor the environment of workers exposed to azo dyes and to monitor those workers' urinary excretion of aromatic amines and benzidine to evaluate the effects of exposure to and metabolism of azo dyes. The study also identifies some jobs that might involve exposure to dyestuffs and other known carcinogenic agents. The results also show that benzidine and its metabolites have been found in the urine of some workers exposed to benzidine-derived azo dyes, and the origin of this urinary benzidine may have resulted from metabolism of the azo dyes. This finding implies that the number of persons previously estimated to be potentially exposed to benzidine may be only a fraction of the total number of workers that may have been indirectly exposed through the use of benzidine-derived dyes. From the results of this study and the existing scientific literature, it is recommended that the manufacture and use of benzidine-derived dyes be handled as if they are potential carcinogens and that safe dyes be substituted when available. It must be recognized, however, that the alternate dyes need to be evaluated thoroughly for carcinogenic activity before being used.

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Chapter 1

BACKGROUND

It has long been recognized that certain aromatic amines can cause cancer in both animals and man. This was first discovered by Rehn when, in 1895, he found a substantially higher incidence of urinary bladder tumors in workers exposed to chemicals used in synthesizing dyes (1). Although called "aniline tumors" by Rehn, subsequent research proved that aniline was not the causative agent; rather it was other aromatic amines used in this process. Many animal studies and epidemiological investigations followed during the next half century, but it was not until the mid 1950's that it was generally agreed that benzidine, 4-aminobiphenyl, and beta(2-)naphthylamine are human carcinogens.

In August, 1973, the recommendation of the Standards Advisory Committee of Carcinogens to the U.S. Department of Labor called for the regulation of 14 chemical compounds, including the aforementioned chemicals (2); and these recommendations became law when published in the Federal Register in January, 1974 (3). It has still not been conclusively proved that 3,3'-dichlorobenzidine, o-tolidine (3,3'-dimethylebenzidine), and o-dianisidine (3,3'-dimethoxybenzidine) are human carcinogens. Nonetheless, substantial evidence collected through animal studies shows a high probability that these compounds are carcinogenic (4,5,6).

The aromatic amines mentioned above are used principally in the manufacture of synthetic dyes and pigments. Two other amines, 2-naphthylamine (2-NA) and 4-aminobiphenyl, were previously used in the manufacture of rubber products to provide added resistance to oxidation, sunlight, and aging of the finished rubber. Since the 1950's, 2-NA has been replaced as an anti-oxidant in rubber with phenyl-beta-naphthylamine (PBNA) and phenyl-alpha-naphthylamine, which were formerly regarded as safe substitutes. It was recently discovered, however, that PNBA reduces metabolically to free 2-NA in both animals and man; and its use is, therefore, being discontinued (7). Thus, this is one instance in which a chemically altered carcinogen was disguised but apparently still remained hazardous.

For at least 50 years 2-naphthylamine has been produced commercially in the U.S. (8), but by 1972 it was mostly discontinued

for dyestuff manufacturing (9). It has been effectively replaced in the dye industry by sulfonated salts of 2-amino-naphthol. The use of 4-aminobiphenyl as an anti-oxidant in rubber has been discontinued, but may be found in trace amounts in some biphenylamine products. Additionally, many of the amino-naphthols have demonstrated carcinogenic potential in animals (10); however, it is possible that several hundred parts per million of the 2-naphthylamine isomer were present in that compound (11-13). Appendix I shows some amino-naphthol containing dyes that have shown carcinogenic activity. Since the ultimate carcinogen of 2-naphthylamine in man has not yet been fully established, it may be that, when metabolized, amino- naphthol containing azo dyes can be hazardous to man. This will require additional research. However, this has not been proven.

Chapter II

PRODUCTION AND USE

Benzidine was first created in 1845 by the reduction of nitrobenzene with zinc and sodium hydroxide; the resulting hydrazobenzene was converted to benzidine after treatment with dilute acid (14). The use of benzidine in dyestuffs today has drastically decreased in comparison to previous decades. In 1948, for instance, some 4 million pounds of benzidine and 31 million pounds of benzidine-based dyes were produced (15); this accounted for 21% of all dyes manufactured and almost all of the direct dyes on the market in that year. Then, some 50 companies in 19 countries manufactured benzidine-based dyes, and about 15 companies had at some time made the dyes in the United States (16). In contrast, the U.S. International Trade Commission reported that in 1971 only 11.4 million pounds of benzidine-based dyes were manufactured. About 3.5 million pounds of o-tolidine- and dianisidine-based dyes were also produced that same year (17). (NIOSH estimates of the number of workers exposed in 1972-74 to benzidine, alpha and beta naphthylamine, and 4-aminophenyl are given in Table I.) Today, only one U.S. company produces benzidine-based dyes, and its current means of production makes the possibility of direct exposure to the chemical almost negligible. Current production figures of both domestically-produced and imported benzidine and related dyes are given in Tables II and III, respectively. In 1973, the Synthetic Organic Chemical Manufacturers Association's (SOCMA) Task Force on Benzidine estimated the proportional use of these dyes by various industries: paper and pulp, 40%; textiles, 25%; leather, 15%; and others, 20% (18).

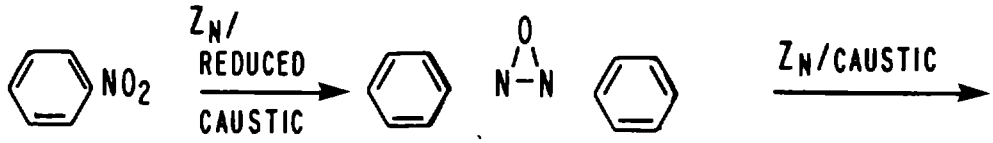
The one remaining U.S. manufacturer of benzidine azo dyes receives hydrazobenzene as an intermediate to benzidine synthesis. Unlike benzidine, hydrazobenzene is not regulated; however, its use may not be appreciably safer than the use of benzidine. (See page 11 for a toxicity review of hydrazobenzene.)

Since 1973 hydrazobenzene has been manufactured by only one U.S. firm, which makes it as an alternative to benzidine or its salts and ships it in drums in a moist flake form. Another U.S. company imports hydrazobenzene from Germany for use in making dyestuffs. Hydrazobenzene is converted at the dye facilities by treatment with

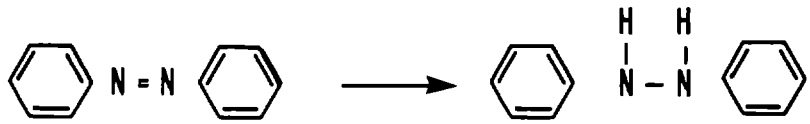
hydrochloric acid, which produces benzidine dihydrochloride. This is subsequently filtered and clarified by a closed delivery plant and frame filter press. The dihydrochloride salt is then tetrazotized in situ to its diazonium derivative and is coupled to other select aromatic compounds to form the dyes.

The general chemical synthesis of hydrazobenzene and benzidine is:

Production of Hydrazobenzene

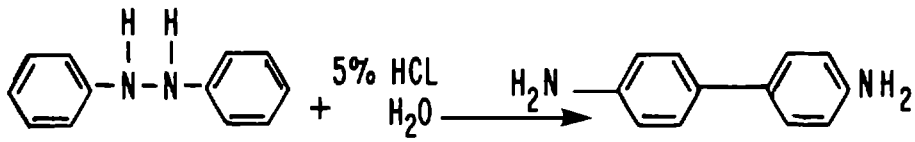


NITROBENZENE AZOXYBENZENE



AZOBENZENE HYDRAZOBENZENE

Benzidine Rearrangement



HYDRAZOBENZENE BENZIDINE

Physical and chemical data for benzidine and its congeners are provided in Appendix II.

Table I. Estimated number of workers exposed to carcinogenic amines, 1972-74.*

SUBSTANCE	PROJECTED NUMBER OF PEOPLE EXPOSED
1-naphthylamine	35,395
2-naphthylamine	503
Benzidine	726
4-aminodiphenyl	83

*These estimates are based on the 1972-74 National Occupational Health Survey.

Table II. Sales of dyes made from benzidine and its derivatives in the U.S. (in thousands of pounds).*

DYE NAME	1971	1973	1975	1976	1978
Direct Orange					
1 bzd†	46	?‡	?	?	0
8 bzd	88	106	83	35	27
Direct Red					
1 bzd	143	188	114	82	26
2 (3,3'-dimethyl)§	231	204	55	57	128
10 bzd	12	8	?	?	0
13 bzd	55	?	?	?	0
28 bzd	136	230	?	?	37
37 bzd	136	96	65	?	0
39 (3,3'-dimethyl)	129	112	32	50	?
Acid Red 85 bzd	208	126	65	75	40
Direct Blue					
1 (3,3'-dimethoxy)ϕ	332	309	174	230	98
2 bzd	1328	1116	243	771	218
6 bzd	334	327	?	327	62
8 (3,3'-dimethoxy)	164	31	20	?	?
14 (3,3'-dimethoxy)	?	?	?	?	?
15 (3,3'-dimethoxy)	172	254	?	?	327
22 (3,3'-dimethoxy)	14	?	?	?	?
25 (3,3'-dimethyl)	48	56	?	?	?
76 (3,3'-dimethoxy)	98	80	53	41	62
98 (3,3'-dimethoxy)	70	311	141	164	195
218 (3,3'-dimethoxy)	1036	1264	1309	1253	856
Direct Green					
1 bzd	229	215	110	216	13
6 bzd	573	389	?	?	109
Direct Brown					
1A bzd	109	?	?	?	0
2 bzd	253	174	118	198	28
6 bzd	?	?	?	?	9
31 bzd	176	?	73	42	37
74 bzd	69	?	?	?	32
95 bzd	549	569	406	532	76
111 bzd	78	38	?	?	0
154 bzd	416	?	?	?	64
Direct Black					
4 bzd	99	136	?	?	26
38 bzd	7330	5330	2168	3923	823
Resin FWP	?	?	?	?	84

* From U.S. International Trade Commission reports on Synthetic Organic Chemicals (1971 to 1978) and through courtesy of Fabricolor, Inc. (E. Angtadt, Written Communication, Jan. 1979).

§ 3,3'-dimethyl (benzidine) is also known as o-tolidine.

ϕ 3,3'-dimethoxy (benzidine) is also known as o-dianisidine.

† bzd = benzidine

‡ Dyes with ? were being produced but sales figures were not available.

Table III. Imports into the U.S. of colors made from benzidine and its derivatives (data given in pounds).*

DYES MADE FROM BENZIDINE	1974	1976	1977	1978
Direct Yellow 20	0	0	3900	0
Direct Red 28	11023	0	0	33069
Direct Orange 8	0	5511	250	0
Acid Red 85	7625	2190	0	1000
Direct Red 1	0	0	4409	0
Direct Brown 1	0	0	0	4409
Direct Brown 2	0	24251	2205	0
Direct Brown 95	0	8205	15962	5512
Direct Green 1	1705	0	0	12660
Direct Green 6	0	0	0	4659
Direct Green 8	0	0	250	0
Direct Blue 2	0	38478	0	30755
Direct Blue 6	0	0	0	4409
Direct Blue 137	1377	0	0	0
Direct Black 38	0	70753	49525	170442
Total	21730	149388	76501	266915
PIGMENTS MADE FROM 3,3'-DICHLOROBENZIDINE				
Pigment Yellow 12	3498	34650	28705	2256
Pigment Yellow 13	18165	19984	4625	9660
Pigment Yellow 14	0	0	110	5592
Pigment Orange 13	9350	2200	9483	3300
Pigment Red 38	8360	3361	0	0
Pigment Yellow 17	12102	0	0	0
Pigment Yellow 83	55628	58386	18992	5781
Total	107103	118581	61915	23289
DYES MADE FROM 3,3'-DIMETHOXYBENZIDINE				
Direct Blue 10	2640	0	0	0
Direct Blue 15	3134	5720	5393	0
Direct Blue 76	0	250	250	0
Direct Blue 91	0	3527	3527	0
Direct Blue 158	2337	3307	14991	13228
Total	8111	12804	24161	13228
DYES MADE FROM 2,2'- DISULFO BENZIDINE				
Mordant Yellow 26	6614	3381	8712	0
Acid Yellow 42	0	0	3215	2864
Acid Red 97	1234	0	0	1000
Total	7848	3381	11927	3864
DYES MADE FROM 3,3'- DIMETHYL BENZIDINE				
Direct Red 2	0	0	1102	0
PIGMENT MADE FROM 3,3'-DIMETHOXYBENZIDINE				
Pigment Red 37	2145	-0	0	0
DYES MADE FROM 2,2'-DISULFO-3,3'-DIMETHYLBENZIDINE				
Acid Red 145	0	7716	4851	0

* Data are from U.S. International Trade Commission Report 762 (released 1976); Report 806 (released 1977); Report 828 (released 1978); Report 900 (released 1978) and Report 990 (released 1979).

Table IV. Types of physiologic changes in various species.*

Species	Administered Amine	Physiologic change
Mouse	Benzidine	Hepatoma, lymphoma, bile duct proliferation
	3,3'-dihydroxybenzidine	Hepatoma, lymphoma, bile duct proliferation, benign bladder papilloma
Rat	Benzidine and its sulfate	Cirrhosis of liver, hepatomas, carcinoma of Zymbal's gland, adenocarcinoma, degeneration of bile ducts, sarcoma, mammary gland carcinoma
	3,3'-dihydroxybenzidine	Hepatoma, adenocarcinoma of colon, carcinoma of fore stomach, Zymbal's gland carcinoma, bladder carcinoma
	Dianisidine	Zymbal's gland carcinoma, ovarian tumor
	3,3'-dimethylbenzidine	Papilloma of stomach, Zymbal's gland carcinoma, mammary tumor leucoses
	3,3'-benzidinedioxyacetic	Papilloma of bladder, hepatic sarcoma
	3,3'-dichlorobenzidine	Extersive cancer
	N,N'-diacetylbenzidine	Chronic giomerulonephritis
Hamster	Benzidine	Hepatoma, liver carcinoma, cholangiomas
	3,3'-dimethylbenzidine	bladder cancer
Rabbit	Benzidine	Proteinuria hematuria, liver cirrhosis, myocardial atrophy, bladder tumor, gall bladder tumor
Dog	Benzidine	Recurrent cystitis, bladder tumor, convalsions, liver cirrhosis, hematuria
Monkey	Benzidine	No pathology
Man	Benzidine	Bladder tumor, papilloma, chroric cystitis, hematuria

*Data taken from: Haley, T.J. Benzidine revisited: a review of the literature and problems associated with the use of benzidene and its congeners. Clin. Toxicol. 1:3-42; 1975.

Chapter III

CARCINOGENESIS OF BENZIDINE IN ANIMALS

Reviews of the experimental induction of cancer by administering benzidine to animals have previously been compiled by Haley and May (19,20). Haley summarized the carcinogenic animal data of benzidine and its congeners according to species and physiological changes (19). (See Table IV.) Because these reviews are available and strong scientific evidence exists positively implicating benzidine as a human carcinogen, only a brief summary of the more interesting animal studies will follow.

Benzidine was not earlier recognized as a human carcinogen primarily because no experimental tumors had been produced with it before 1950 (21). High dose levels that produced acute toxicity and too short observation periods were probably the principal reasons for this failure.

In 1950, Spitz et al. first successfully induced tumors by administering benzidine to rats. Weekly doses of 15 mg of technical grade benzedine in one milliliter of olive oil were subcutaneously injected into one group of 78 male rats, while weekly doses of 15 mg of high purity benzidine were injected in the same manner into a second group of 45 male rats. The injections continued until either grossly obvious tumors appeared or death occurred. A group of control rats was injected with only olive oil. No hepatomas appeared in the control rats; but 9.8% of the 78 animals in the group injected with technical grade benzidine developed hepatomas, as did 11.1% of the 45 rats receiving pure benzidine. Total individual cumulative doses of 0.96 g were estimated for each group injected (22).

A total individual dose of 2.5 g of benzidine sulfate per kilogram of body weight was also fed intermittently to male rats for about 34 weeks. This dosage produced carcinoma of the sebaceous glands of the auditory canal in 5.4% and hepatoma in 2.7% of the rats. Spitz also investigated the carcinogenic action of benzidine and other related compounds in the monkey, dog, and rabbit; but except for one bladder tumor in a dog after 19 months, no significant findings were reported.

Lamonier and Laquerriere applied a daily application of benzidine (3% in benzene) to the lumbar skin of the Wistar rat. After only 15 days jaundice due to toxic hepatitis appeared in the rats. They then entered a latent period of 2 to 4 months, followed by a neoplastic phase, during which malignant tumors appeared, 60% of which were hepatomata (23).

In 1964, Pliss produced hepatomata, zymbal gland tumors, and sarcomata in the underlying fat of rats that were subcutaneously administered a total individual calculated dose of 300 mg of benzidine. These tumors were observed in 14/20, or 70%, of the rats that were subcutaneously injected intermittently with benzidine and that survived for at least 5.5 months (24).

In 1970, Zabezhinskii administered benzidine base to rats by inhalation. Non-inbred albino rats of both sexes were exposed to a concentration of 10 to 20 mg of benzidine per cubic meter of air 4 hours a day, 5 times a week for 20 months. The total calculated individual dose was about 27 mg. Tumors appeared in 8/27 experimental rats and 2/21 control rats. In 29% of the experimental rats inhalation of benzidine led to the development of leukemias, tumors of the liver, and/or carcinomas of the female and male mammary glands. The two control rats developed adenomas of the mammary glands at a later date. (The carcinomas in this experiment were similar to those observed by Pliss.) Zabezhinskii had earlier intermittently administered total individual doses of 70 mg of benzidine by intratracheal injection, and tumors appeared in 11/14, or 79%, of the surviving rats after 8 months of the experiment. He noted that the sites of these tumors were very similar to those observed by other researchers who had administered benzidine by feeding or by subcutaneous injection. He concluded, therefore, that the character of action of aromatic amines is dependent mainly on the species of the experimental animal rather than on the mode of administering the substance (25).

Griswold et al. reported a high incidence of mammary carcinomas in Sprague-Dawley rats that were intragastrically administered benzidine in 10 individual doses at three day intervals. Administration started at 40 days of age and the surviving animals were sacrificed after 9 months. Out of the 4 groups administered a total of 50, 35, 25, and 12 mg per animal, the number in each group of autopsied rats with mammary lesions was 4/5, 0/0, 7/9, and 5/10, respectively. Only 5/132 rats administered sesame oil developed such lesions (26).

Experiments using hamsters, mice, dogs, and rabbits produced some tumors, but no bladder tumors, except in the dog and possibly in the rabbit. Bonser administered 20 mg/week of benzidine orally to rabbits for 3.5 years and found one urinary bladder tumor in 13 rabbits (27). Spitz et al. produced bladder carcinoma in 3/7 mongrel

dogs (1 male and 6 female). They had been administered benzidine orally by capsule for a period of five years; the total dose was 325 g. Three of the dogs developed bladder carcinoma 7.8 years after the start of the treatment and two dogs after 9 years. Spitz also weekly administered 50 to 200 mg of benzidine in butyl succinate subcutaneously to four female monkeys for periods of 2 to nearly 5 years, with individual cumulative dosages amounting to 6.2 to 9.1 gm. All of the monkeys died, mainly of respiratory infections, and no evidence of cancer of any type was noted at the necropsies (22).

Haley et al. noted that it was strange that all congeners of benzidine known to produce urinary bladder cancer in man also produce it in the mouse. Moreover, bladder cancer has been induced in most animal species with the congeners, but not benzidine. It may be that the acute toxicity of benzidine and the long latency period needed for the appearance of tumors require that smaller quantities of benzidine be administered to reveal its tumorogenic effect (19).

Hydrazobenzene, the current intermediate for benzidine synthesis in this country, is also a suspected human carcinogen. It is chemically possible that hydrazobenzene, if swallowed, may be converted to benzidine upon reaching the acidic environment of the stomach. Experimental animal data also have demonstrated the carcinogenic potential of hydrazobenzene.

Troll reported the urinary excretion of benzidine in dogs dosed orally with hydrazobenzene (28). The Russian experimenter Pliss investigated the carcinogenic properties of hydrazobenzene by chronic experiments with 163 rats and 110 2-month-old C57 mice. For about 588 days, a suspension of hydrazobenzene in sunflower seed oil was injected subcutaneously into the animals (40 mg/wk per rat and 5 mg/wk per mouse), or added to their fodder (30 mg 5 times/week for both rats and mice), or applied to their skin (30 mg 5 times/week per rat and 2 mg 3 times/week per mouse). Individual rats received a total of 3.8 g by injection, or 360 mg on the skin. Neoplasms occurred after subcutaneous injection in 36.6% of the mice and 22.6% of the rats. Neoplasms appeared after skin application in 22.2% of the mice (no data are given for skin application to rats) and after oral feeding in 50% of the mice and 50% of the rats. Lymphoid leukemia and tumors of the uterus, mammary and zymbal glands, and liver and spleen were observed in the rats. Subcutaneous rhambdomyosarcoma, pulmonary adenoma, leukemia, liver tumors, and skin cancer were observed in the mice (29). Troll concluded that since intoxication and cirrhotic liver changes were absent in these experiments, the entire carcinogenic effect of hydrazobenzene cannot be attributed to its chemical rearrangement and conversion to benzidine.

Recently, the National Cancer Institute (NCI) reported on a 78-week continuous feeding study of hydrazobenzene to Fisher 344 rats and B6C3F1 mice. Dosage levels were 0.001 and 0.004 percent in the feed of female rats and 0.008 and 0.03 percent in the feed of male rats. Both low and high doses produced mammary and zymbal gland tumors and hepatocarcinomas. Dosage levels for male mice were 0.008 and 0.04 percent; neither dose produced tumors. However, at the 0.04 and 0.004 percent dosage levels female mice had an increase in hepatocellular carcinomas (30).

Chapter 4

CARCINOGENICITY OF BENZIDINE IN MAN

Today, the fact that benzidine is a human carcinogen is accepted by both industry and regulatory agencies because of the extensive epidemiological data from past high levels of exposure (31-33). The main target organ is the bladder; and, if a bladder tumor is malignant, death frequently results. Of all patients with carcinoma of the bladder, 70% to 75% die of the disease (34). Between 1955-64, the median survival time for all patients with bladder tumors was 3.2 years; between 1965-69, 87% of all patients with such tumors underwent surgery, indicating poor treatability (35). It appears that the prognosis for survival is still poor.

The American Cancer Society estimates that 6300 men and 2900 women died of bladder cancer in the United States in 1973, representing about 3.5% of all cancer deaths. In recent years the incidence rate each year has been approximately 25 cases per 100,000 people for males and 7 cases per 100,000 people for females (36). Approximately 30,000 new cases of bladder cancer were diagnosed in 1976, and the overall bladder cancer mortality rate in the United States has increased about 7% in the past 25 years (37). It is uncertain how many of these cases can be attributed to environmental agents. Occupational involvement may account for 10% to 50% (38-41).

The causes behind the majority of "spontaneous" urinary bladder tumors is still open to speculation, although several possible causal factors have been presented. Possible agents include cigarette smoking, the formation of nitrosamines from the combination of nitrite amines or nitrate and secondary amines in the diet, natural and artificial carcinogens in food, and abnormal tryptophan metabolism (42). However, much additional work must be performed in these areas before any conclusions can be drawn.

The first suspicions of the carcinogenicity of benzidine did not evolve until the 1920's, and in the United States it was not accepted as a carcinogen until about 1950. As early as 1898 2-naphthylamine was suspected (43), but not until 1938 was it demonstrated that it could produce experimental bladder tumors in dogs (44). Because both of these aromatic amines were often produced in the same facilities, it was not possible for many years

to conclusively demonstrate that excessive bladder tumors occurred in workers exposed solely to benzidine. Nevertheless, in 1927, Oppenheimer reported 5 cases of bladder cancer and 1 case of papilloma after examining 52 cases of urinary tract disease related to exposure to benzidine (45). In Britain, Scott reported on 66 cases of workers who had worked with either alpha-naphthylamine, beta-naphthylamine, benzidine, or a combination of these. He was able to isolate 23 cases of benzidine-induced bladder tumors from this cohort. The average latent period for tumor development was 16 years (46).

Case traced 4,622 dyestuff workers who had been in contact with the suspect amines and discovered 341 cases of bladder cancer. This indicated a 30 times greater risk among these workers. Moreover, he showed that the relative potencies for 2-naphthylamine, benzidine, and 1-naphthylamine were in the ratios of 5, 1.7, and 1, respectively (47).

In Italy, benzidine-induced tumors were also being reported. In 1952, 13 benzidine bladder carcinomas were diagnosed among 83 workers in the benzidine department of a dyestuffs plant. The latent period varied between 4 and 28 years, again in agreement with reports from other countries (48). In addition, in 1964, Maltoni et al. attributed four cases of cancer of the kidneys and ureters in dyestuffs workers to benzidine (49).

Mancuso and El-Attar obtained the death certificates of 171 of 640 workers employed during 1938 and 1939 in a dyestuffs factory producing B-naph-thylamine and benzidine. They reported 18 cases of bladder and kidney cancer, 30 times above the expected number. A significant number of malignancies of the digestive tract were also noted, especially of the pancreas (50).

In France, 17 cases of benzidine tumor and 15 cases of benzidine azo dye bladder tumor were reported in 1958 (51). It is interesting that reports from Switzerland linked cases of benzidine exposure to tumors of the stomach, lung, rectum, and prostate (52). In Czechoslovakia, eight cases of bladder papilloma and one of ureteral carcinoma were reported in workers exposed to benzidine; one person also worked with hydrazobenzene (53).

In the United States, Goldwater et al. reported the incidence of malignancies among 366 workers at a dyestuffs factory manufacturing beta naphthylamine and benzidine. The cohort consisted of all the male workers employed between 1912 and 1962. Of the 76 workers exposed to only benzidine, 17 (or 21.3%) developed bladder malignancies. Workers exposed to both B-naphthylamine and benzidine had an incidence rate of 45.5%; but since levels of exposure could not be determined, it is not possible to state whether a synergistic effect existed. Goldwater also recorded the incidence of other

malignancies: three lung, three prostate, two stomach, two brain, and one lymphoma. The incidence of these latter malignancies was not great enough to suggest that malignancies in organs other than the bladder occur from exposure to B-naphthylamine or benzidine (54).

Smart studied the occurrence of tumors in the urological tract to determine if a correlation existed between the time bladder tumors appeared and the time tumors appeared at other sites in the tract. However, he did not suggest that these incidences of tumors were related to occupation (55).

Although the aforementioned studies attempted to correlate the incidence of tumors to exposure to benzidine only, frequently B-naphthylamine was also produced, and tumors caused by occasional exposure solely to B-naphthylamine cannot be ruled out. Probably the most convincing data, therefore, are from the study performed by Zavon et al. They observed a group of 25 workers exposed to benzidine; 13 of these workers, or 52%, developed bladder tumors, of which 11 were malignant. Workers who developed tumors had an average exposure to benzidine of over 13 years, while the non-tumor group averaged less than 9 years of exposure (56). Environmental air monitoring in 1958 revealed levels as high as 17.6 mg/m^3 of benzidine when the final product was hand shoveled into drums. Of the 25 men exposed to these high levels, 3 had 1 year of exposure to B-naphthylamine, 3 had some exposure to o-toluidine, and 7 had exposure to dichlorobenzidine. However, these exposures were considered insignificant when compared to the long term heavy exposure to benzidine.

Chapter 5

METABOLISM OF AZO COMPOUNDS

Azo compounds comprise approximately 60% of all organic coloring dyes in use today (57). They are most important in dyeing cellulostic materials (e.g., cotton, paper, animal hides) and are widely used in the coloring of foods, pharmaceuticals, and cosmetics (16). Though attempts have been made to relate azo dye structures with carcinogenic activity, little knowledge has been gained in this area. Of prime importance here is whether a dye itself is carcinogenic, or whether the amines produced by the reductive fission from the release of the azo bonds is responsible.

Before addressing this question, it is first appropriate to review the experimental data indicating some of the possible metabolic processes that azo compounds may undergo. Probably the earliest realization of the metabolism of azo compounds in mammals came as a result of feeding Orange I to dogs in 1911. Sulphanilic acid was identified in the urine, demonstrating for the first time that azo compounds may be metabolized by reductive cleavage of the azo group (58). Two active metabolic sites that are important in azo dye metabolism are the liver and the intestinal tract. The major determinant of which of the two sites is more important will vary with the molecular size and polarity of the dye ingested into the gastrointestinal system. A basic principle, then, is that complex polar molecules (e.g., direct class azo dyes) will be poorly absorbed from the gut. Sulfonated dyes, therefore, are too highly ionized, and will most likely remain in the gut until expelled or metabolized to a smaller or less ionized compound by the gut flora, at which time they can be absorbed. On the other hand, non-polar dyes tend to be readily absorbed intact from the gut and may be metabolized mainly by the liver.

The major metabolic processes of specific organ sites is discussed below.

A. Intestinal Reduction

Much information exists confirming the importance of the intestinal flora in the reductive fission of the azo bond. The classical work performed by Radomski and Mellinger on water-soluble

azo dyes used as food colorings demonstrated the importance of the gut flora in altering the dye structure. When these sulfonated dyes were given orally to rats, little or no intact dye was found in the feces. However, when antibiotics were administered and the gut flora suppressed, a definite increase in the excretion of the dye was observed, indicating the importance of the gut flora in this process. Furthermore, when the dye was administered intravenously or by intrasplenic infusion, 86% to 93% was excreted in the bile over an 8-hour period, showing that reduction of water-soluble dyes by the liver is not of much quantitative significance. When the dyes were administered orally to series of rats with cannulated bile ducts and cannulated urinary bladders and collected for a 48-hour period, only 1.7% to 3.6% of the administered dye was absorbed intact. Moreover, the rats absorbed from the gut 18% of a primary cleaved product of FD & C Orange No. 2, 1-amino-r-naphthalene sulfonic acid, demonstrating the extent of the absorption of the metabolites from the gut (59).

In vitro studies using various species of gut bacteria further demonstrated their biological ability to reduce the azo bond. Dieckhues screened 21 species, including many common to the mammalian gut flora, against 14 azo compounds, and showed that azo bond reducing activity for all bacteria tested was fairly general and relatively nonspecific. When a Bacillus and a Proteus species (both particularly active) were used in combination to study the reduction of 102 azo compounds, only 12 of those compounds were resistant to reduction (60).

Yoshida and Miyakawa found that when sulfonated benzidine azo dyes were injected into mouse intestines that had been removed and then incubated, free benzidine was later found. They also showed that Escherichia coli and soil bacteria, when incubated at 37 degrees C, are capable of reducing benzidine dyes (61).

Daniel investigated the effect of sulfonation and nuclear methyl substitution on the metabolism of the azo food colors. He reported that the number of sulfonic acid groups or methyl groups appeared to have little quantitative effect on azo reduction (62).

When comparing the abilities of fortified rat-liver homogenates with cell-free extracts of a Proteus species to meatabolize azo dye compounds, Roxam et al. found that the Proteus extract was far more efficient in reducing the azo bond of water-soluble dyes (63).

A significant portion of metabolized and intact dye is excreted via the bile, which returns it to the large intestine. This biliary excretion or recycling of the intact dye or its metabolites has been termed "enterohepatic circulation." Therefore, intact lipid-soluble dyes may be absorbed from the small intestine, conjugated in the liver, and excreted back into the large intestine where they are

acted upon by the gut flora and very possibly reduced to the component amines if they were not previously reduced before absorption (59, 64).

Azo bond reduction is the major metabolic process that occurs within the gastrointestinal tract, and it is the only activity that occurs in the gut. Conjugates excreted into the gut via the bile may undergo hydrolysis (from B-glucuronidase), and these products may be again reabsorbed and acted upon by the liver (64). Another possible modification occurring in the gut is acetylation (65).

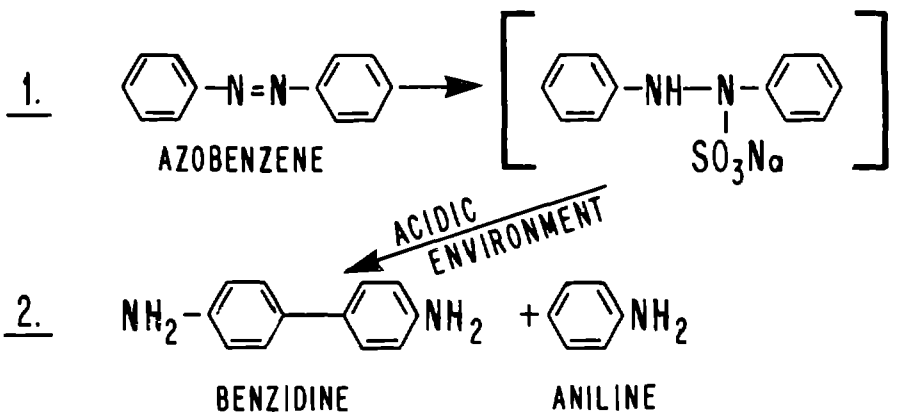
B. Hepatic Transformations

Again, only lipid-soluble dyes are absorbed from the gut to any appreciable extent. For orally administered doses, 2% to 4% of sulfonated dyes may be absorbed, the absorbed dye being rapidly excreted, principally in the bile (66). Lipid-soluble dyes probably undergo azo reduction in both the gut and the liver. Gingel et al. supported this assumption with their work on Prontosil, a fat-soluble dye, and Neoprontosil, a water-soluble dye. Both dyes have long been known for their antibacterial properties, which are due to the metabolic release of sulfanilamide, the active component of the drug (67). To demonstrate that the liver plays an important part in the reduction of Prontosil, it was administered in various ways to rats. Given orally, the sulfanilamide excreted in the rats' urine over a three day period was 53% of the administered dose; given intraperitoneally (i.p.), the sulfanilamide output was 32% of the dose; and given orally after suppressing the gut bacteria with oral antibiotics, it was 31%. The biliary excretion of Prontosil was low, 5% of the oral dose and 10% of the i.p. injection in a 24-hour period. On the other hand, Neoprontosil (given by i.p.) was excreted 60% unchanged in the bile after 6 hours. These observations suggest that in the rat the fat-soluble dye is split by both the liver and gut flora, whereas the water-soluble dye is reduced mainly by the gut flora.

The generalization that highly sulfonated dyes are not readily absorbed from the gastrointestinal tract does not appear to apply to the sulfonated amine metabolites of these dyes. Thus, polar dyes given orally appear in greater quantities in the urine than in the feces. Rabbits given oral doses of tartrazine excreted 96% of the dose in the urine as free and acetylated sulfanilic acid (62). This also follows for Neoprontosil, which could not act if the sulfonated amines were not absorbed. Though the absorption of these amino-sulfonic acids appears to conflict with the earlier statement that strong polar acids are not readily absorbed, this may partially be explained by the basic free amino group and smaller molecular size of the cleavage products. At neutral and acid pH sulfanilic acid, furthermore, exists as the sparingly soluble isoelectric or zitter ion (68).

In vitro studies have identified azo-reductase activity in the liver of the guinea pig, mouse, rabbit, rat, and chicken (69). It is now generally held that the enzyme is located mainly in the endoplasmic reticulum (70). Several investigators have linked NADPH-cytochrome reductase and $FADH_2$ with a very nonspecific reduction of azo compounds (68).

The actual reduction of the azo bond probably occurs in two steps. When azobenzene is injected intraperitoneally into rats and their urine acidified, a compound thought to be benzidine is found. Hydrazobenzene, or its N-sulfonate or N-glucuronide, is the expected intermediate; and the reaction would proceed in the following generalized steps (71).



Step 2, known as the Benzidine Rarrangement, occurs in strong acid. The author suggests that this reaction occurs in vivo to a limited extent (71).

Other evidence that supports this two-step mechanism was obtained from two experiments on rabbits that were fed azobenzene. Bray et al. found both hydrazobenzene and benzidine in the rabbits' urine (72); and Walker discovered that azo reductase activity occurs in rabbits mainly in the liver, but also occurs in the kidney, lung, and other tissues (68). Both of the above studies are rather dated, however, and it would probably be of much interest to perform similar studies for confirmation, using current analytical instrumentation.

C. Conjugation

A number of conjugation products are formed with aromatic amines and azo dyes. Among the most common conjugates are those formed with naturally occurring glucuronic acid, acetic acid, sulfuric acid, or phosphoric acid.

Conjugation is an important means of biologically solubilizing a compound with a polar group, allowing renal excretion. Conjugation occurs primarily in the amino or hydroxyl groups (hydroxylation will be discussed more fully in the next section). Hepatic enzymes are predominantly responsible for in vivo conjugation.

Conjugation with glucuronic acid appears very important and can occur by attachment to a hydroxy or carboxylic acid group and less frequently to an amino group (73). Glucuronidation of the N-hydroxy group of fluorenylacetamide is one of the excretion metabolites of this carcinogen (74). Axelrod et al. demonstrated that an enzyme in guinea pig liver microsomes was capable of conjugating amines with glucuronic acid (74). In some cases, however, the formation of N-glucuronides appears to be formed spontaneously from amines and glucuronic acid (75). Glucuronide conjugates can be hydrolyzed to both the parent amine and glucuronic acid by a B-glucuronidase enzyme present in the urine (76).

D. Hydroxylation

Hydroxylation occurs primarily at an amino group, or ortho to an amino group. Ortho-hydroxylation was long thought to be an important requirement for carcinogenicity, based on the bladder implanation work of Bonser (77-78). While this hypothesis has lost appreciable ground, the importance of o-hydroxylation as a metabolic step still must be recognized as a major site for further conjugation. The dog, for instance, excretes from 55% to 70% or more of a dose of 2-naphthylamine as 2-amino-1-naphthol conjugates (77). Glucuronic acid and, to a lesser extent, sulfuric acid preferably bind a hydroxyl ortho to the amino group. For example, 29% of orally administered doses of benzidine given to mice were excreted as 3-OH glucuronide, while only 18% were excreted as N-ethereal sulfate and/or glucuronide (79). In humans exposed to benzidine, approximately 80% to 90% of the excreted benzidine was found as the 3-hydroxy-benzidine metabolite (from conjugates) (79). Ortho-, meta-, and para-aminophenols given orally to rabbits were excreted as less site-specific conjugates. Conjugation of these three isomers with glucuronic acid occurred in amounts of 65%, 64%, and 61%; with acetic acid in amounts of 17%, 30%, and 45%; with sulfuric acid in amounts of 15%, 15%, and 12%; and unchanged as 11%, 0%, and 2% of the administered dose, respectively (80). Thus, in the above instances conjugation with glucuronic acid was the major excretion product.

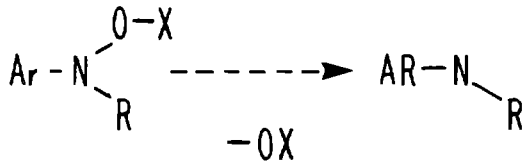
Hydroxylation ortho to an amino group is NADPH-dependent and requires molecular oxygen; it is carried out by enzymes of the liver (73).

N-hydroxylation is now recognized as an important metabolic step in carcinogenesis and as an intermediate step in the formation of several ethereal conjugates. N-hydroxylation occurred in both monkeys and dogs fed 2-naphthylamine; the residual amounted to about 5% of the total dose, and the remainder was excreted as ortho hydroxylamine conjugates (81). Evidence also shows that N-hydroxylation occurs to a minor extent in rats. Fisher strain rats excreted only 1% to 2% as biliary and 0.7 to 1% as urinary N-hydroxy compounds (82). A quantitative estimation can also be made from Radonski's data on N-oxidation products excreted by dogs given 2-naphthyl-amine (83). In dogs administered a 5 mg/kg dose, only 0.2% was excreted as an N-hydroxylated product. Following N-hydroxylation, conjugation with several of the naturally present acids mentioned previously can occur. This occurred in the mouse, rat, guinea pig, rabbit, dog, monkey, and man (19).

Chapter 6

METABOLIC CONVERSION OF AROMATIC AMINES TO THE ULTIMATE CARCINOGEN

It is now generally believed that N-hydroxylation is the primary step in the conversion of many aromatic amines to their ultimate carcinogenic form. Furthermore, it appears that a secondary activation step also occurs. Evidence points to the formation of a highly unstable ester of sulfate, glucuronide, or acetate, with the resultant disassociation to a positively charged ion. The general reaction scheme is:



where Ar = aromatic structure;
R = H, alkyl, or aryl group;
X = H or esterifying group.

Evidence for this mechanism has been demonstrated for 2-fluorenyacetamide (FAA) by Miller (84-86). The inhibitory effect of non-carcinogenic acetanilide (AA) on FAA, when given together to rats, suggests that the sulfate ester is the key ultimate carcinogen. Seventy percent of the rats fed AA and N-OH-FAA with Na_2SO_4 added developed hepatoma and hyperplasia, whereas only 10% of the rats fed AA and N-OH-FAA without Na_2SO_4 did so. The fact that AA inhibits the hepatocarcinogenicity of N-2-FAA and that the addition of sulfate fails to restore the carcinogenic effect of N-2-FAA shows that the initiation step for tumor induction lies in the N-hydroxylation. Sulfate ester formation constitutes a required second activation step. The required sulfotransferase enzyme system is found mainly in the liver cytosol (87). Similar work with hepatic sulfotransferase activity was performed using N-OH-FAA derivatives, in vitro, to determine the susceptability of rats and other species to liver tumor induction by this substance (88).

Evidence for o-glucuronide ester activation of arylamine-like metabolites of N-hydroxy-FAA exists, but its rate of reaction with nucleophiles is much slower (89-91). Thus, it appears that hepatocarcinogenic arylamines, through metabolic activation, are

N-hydroxyarylacetamide N-O-sulfates, at least for most species. The sulfate ester group then disassociates into an arylamidonium ion. (See Figure I).

It is interesting that the ultimate carcinogenic form of arylamines, with respect to bladder carcinogenesis, does not appear to be the same as that of the hepatocarcinogenic form. For example, the dog is susceptible to bladder carcinogenesis, is refractory to liver tumors, and does not acetylate arylamines (92-93). This suggests that N-acetylation is not required for bladder carcinogenesis, though its occurrence certainly does not prevent carcinogenesis of the bladder from occurring in other species (73, 79, 94). Lower and Bryan found a correlation between species variation in arylacetamide deacetylase enzyme systems and susceptibility to bladder carcinogenesis, indicating that removal of the N-acetyl group is first required (95). Thus, in the dog, which does not acetylate, but does N-hydroxylate (96), the ultimate carcinogen may be a N-hydroxyarylamine, N-glucuronide or N-hydroxyarylamine-N-O-glucuronide ester, which subsequently disassociates to the arylnitrenium ion (97). Radomski et al. demonstrated that up to 25% of radio-labeled 4-aminobiphenyl was excreted in the urine of dogs as the glucuronic acid conjugate of N-hydroxy-4-aminobiphenyl (98). The conjugate was very acid labile and readily liberated N-hydroxy-4-aminobiphenyl in the presence of beta-glucuronidase.

Using radio-labeled N-OH-AAF and N-OH-DABz, evidence has been presented that in hamsters the N-hydroxyaryl acetamide is the ultimate hepatocarcinogen upon acyltransferase-catalyzed esterification and disassociation to the arylnitrenium ion. N-O-acyltransferase activation was demonstrated by the liver cytosol with resultant binding to tRNA (99). Man has been shown to N-hydroxyacetylate amino compounds, but to a very small extent. Thus, when humans were orally given 2-naphthylamine and the non-carcinogen N-phenylacetamide, they produced an average urinary excretion of 0.42% and 0.45% of the dose, respectively, as N-hydroxyacetyl compounds. The formation of this metabolite was not the sole requirement for carcinogenic activity, and its formation was not restricted to the carcinogenic aromatic amines (100). Radomski et al. collected 24-hour urine samples from dogs given 2-acetylaminonaphthalene; only 0.035% of the dose was recovered as N-hydroxy-2-acetylaminonaphthalene (101).

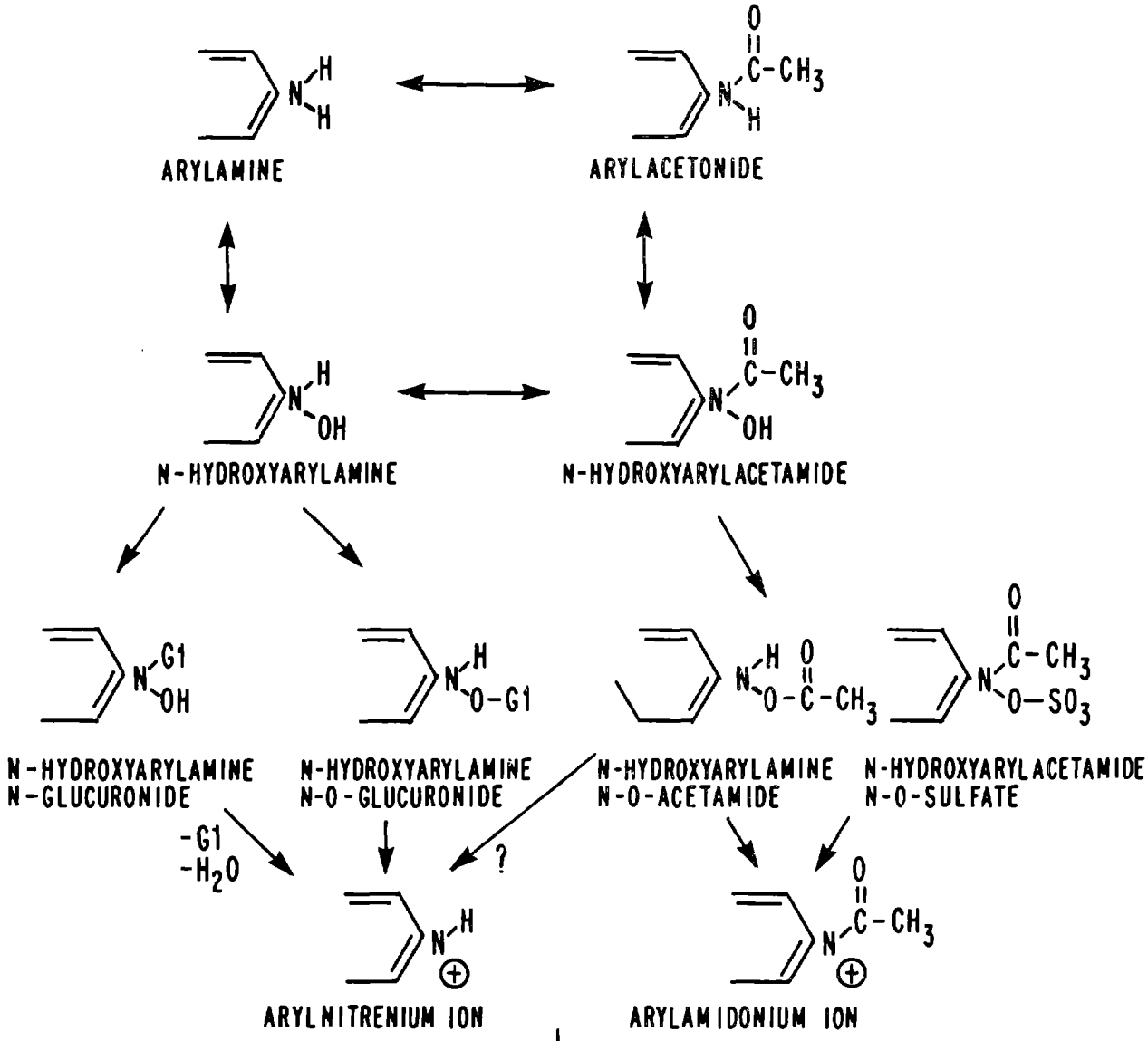
Therefore, from the experimentation performed to date, it appears that:

1) Potent carcinogenicity is associated with two or more conjugated or fused aromatic rings.

2) The position para to the amine group must be occupied.

FIGURE I

POSSIBLE METABOLIC TRANSFORMATIONS LEADING TO THE ULTIMATE CARCINOGEN



S_N1 REACTION

MODIFICATION OF CELLULAR MACROMOLECULES (THROUGH ELECTROPHILIC BINDING ?) INVOLVED WITH THE STORAGE AND EXPRESSION OF GENETICALLY CONTROLLED STRUCTURAL AND FUNCTIONAL CELLULAR PHENOTYPE.

TUMOR FORMATION

3) The groups attached on the amino nitrogen can have a substantial effect in interfering with N-hydroxylation.

4) Substitution in the aryl ring can profoundly influence the carcinogenicity.

5) N-hydroxy esters may be concerned in aromatic amine carcinogenesis.

The ultimate carcinogenic form may vary from tissue to tissue and from species to species. The ultimate form responsible for human bladder cancer is still not known. However, it has been suggested by one author that the N-hydroxy-glucuronide form as found in the dog may be of importance (102).

Chapter 7

CARCINOGENIC AZO COMPOUNDS

Several excellent reviews of the structural activity relationships of various carcinogenic azo dyes have been compiled (4, 73). However, only two carcinogenic dyes, 4-dimethylaminoazobenzene (DAB) and O-aminoazotoluene (O-AAT), have been studied in any great detail in this respect. Appendix I summarizes published data on many of the dyes found carcinogenic in animals.

DAB, also known as C.I. Solvent Yellow 2, is used today as an industrial coloring dye. DAB carcinogenic activity is specific for the livers of rodents (rats are much more susceptible than mice), and there is some indication that it may also be active in hamsters (4). Bladder tumors were also found in 2 of 19 dogs fed DAB for 3 to 4 years at a daily dose of 20 mg/kg of body weight (105).

The carcinogenic form of DAB is not now considered related to the reduced amines. Upon feeding DAB to rats, at least 50% of the substance was excreted in the urine as acid-hydrolyzable conjugate forms of p-phenylenediamine and p-aminophenol (106). However, the reduced amines have either not been carcinogenic or are only slightly so (103, 107). Furthermore, a diet rich in riboflavin, which increases azo reductase activity, has a definite masking effect on tumor formation in rats fed DAB (4).

Experiments with various ring and amino nitrogen substitutions of DAB have revealed several characteristics:

1) Monodemethylation or deethylation of the amino nitrogen appears to have equal activity. Substitution to the N-diethyl compound abolishes carcinogenic activity. Monodemethylation precedes the formation of the proximate carcinogenic N-hydroxy and/or N-acyloxy dye (108).

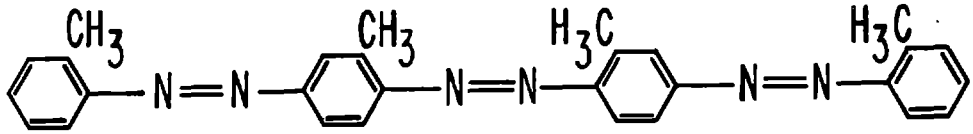
2) Substitution of the 4' position with an ethyl or fluoro group greatly enhances carcinogenic activity, while substitution of this position with higher alkyl groups or with other halogens lessens such activity (109).

From this evidence, it is felt that protection of the 4' position from hydroxylation, which abolishes DAB carcinogenic activity, with a group with low or positive inductive character and with low steric hindrance is required to prevent carcinogenic activity. Substitution of powerful inductive groups completely abolishes such activity when the groups are in the 4' position. The substitution of the 4' methyl causes deactivation, probably because of its enhanced exposure to metabolic oxidation and, hence, resultant 4' hydroxylation. In conclusion, steric hindrance and inductive effects can be compromised for optimum effects. Information from two studies suggests that the ultimate carcinogenic form is the positively charged arylamidonium ion (see Figure I); this assumption is based on the fact that the ion binds to liver proteins (110, 111). Continuing research is needed, however, to unravel the many aberrations to the above observations.

Much work has also been performed in an effort to establish why o-amino azotoluene (o-AAT) is carcinogenic. It induces multi-organ cancer in mice and rats, bladder tumors and gallbladder cancer in dogs, and urinary bladder cancer in hamsters (112). When o-AAT is fed to mice, which are more susceptible in this case than rats, liver tumors form almost exclusively. However, when administered subcutaneously, AAT reveals a multitarget action, causing high incidences of hepatomas, hemangio-endotheliomas, reticulum cell sarcomas, pulmonary tumors, fibrosarcomas, and dermal squamous cell carcinomas (113-115).

Experimental substitution of the methyl and amino group in AAT give some interesting results on the structural activity relationships. Methylation of the amino group inactivates O-AAT (116). An amino group is not essential in the 4' position. Furthermore, total removal of an amino group (2,3'-dimethylazo-benzene) in AAT induces low incidences of sarcomas and hepatomas when AAT is subcutaneously administered to mice (117) and low incidences of papillomas and carcinomas of the bladder when it is fed to rats (4, 107). Hydroxylation in the 4' position only slightly decreases potency (117). The most potent variant of AAT is 2', 5-dimethyl-2-amino-azobenzene (118).

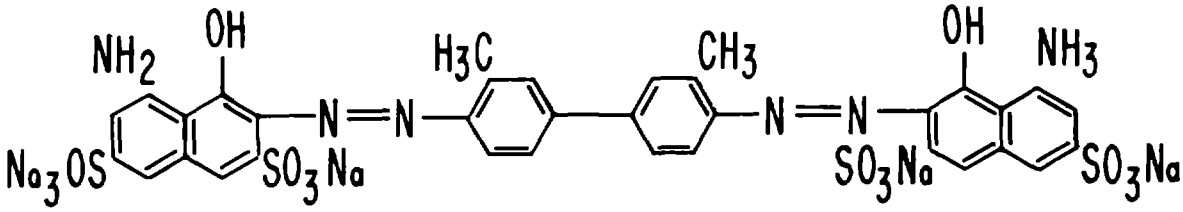
Of all the variations tried, carcinogenic activity occurred only in those studies with the methyl group ortho to the azo linkage and where conjugating ability was greatest and the resonance could be reinforced by the inductive effect of the second methyl group. Thus, conjugation and resonance through the AAT and DAB azo compounds, aided by ring-substituted electron donating and pulling groups, form highly reactive electrophiles. It has recently been found that the ultimate carcinogenic forms of O-AAT and DAB may be similar to those for the carcinogenic aromatic amines. The finding of 4,4' bis(orthotolylazo)-2,2'-dimethylazobenzene in the livers of rats and mice fed AAT was highly suggestive of a N-OH or nitroso-metabolite (119).



Subsequently, the N-glucuronide of AAT and the N-glucuronide-O-sulfate of 4' hydroxyazotoluene were found. Similarly, 50% to 60% of the urine products of DAB in rats were sulfates or glucuronides of N-acetylated metabolites (104). Esterification of N-OH-MAB may cause reactive electrophilic metabolites (110). This was further supported by in vitro experimentation where N-OH-MAB was converted by soluble sulphotransferase of rat liver to a metabolite that reacted with liver proteins (111). This mechanism is reminiscent of that for FAA, which leads to the ultimate hepatocarcinogen.

While structural activity relationships for DAB and AAT can be postulated, very little can be said about the phenylazo napthol and azonaphthalene type dyes, many of which appear carcinogenic (see Appendix I).

The following discussion of Trypan Blue (C.I. Direct Blue 14) gives further evidence that some intact dyes containing azo linkages are carcinogenic and that the azo-reduced components are not.



Investigators have confirmed that the subcutaneous administration of Trypan Blue induces reticulum cell sarcomas (120-123), as well as pleomorphic and spindle-cell neoplasms (124-125). Reticuloendothelial tumors occurred in as little as 100 days in the subcutaneous tissue near the injection site. Subcutaneous administration of six structurally related o-tolidine dyes did not produce tumors, indicating a structure-related specificity of the intact dye. Thus, by this route of administration the intact dye and not its metabolite (o-tolidine) was responsible for the local and distant site tumorgenesis at the dosages administered. Since liver sarcomas were specifically produced, one would suppose that the administered dyes would all be subject to hepatic enzymic azo-reductase activity with the resultant release of o-tolidine. However, the lowest reported subcutaneous dose of o-tolidine that produced liver tumors was 2880 mg/kg of body weight, administered intermittently over a 35-week period (126); the lowest dose for subcutaneous administration of Trypan Blue that produced tumors was 1088 mg/kg of body weight, administered

intermittently over an 87-week period (127). The lower carcinogenic dose concentration for Trypan Blue (o-tolidine only comprises about 25% of the intact dye) further indicates that the intact dye is much more carcinogenic than its intermediates. (For a good review of the teratogenic effects of Trypan Blue and other azo dyes, see reference 128.)

Recently the National Cancer Institute released the results of a 90-day oral feeding study on Fisher 344 rats and mice (129). The three benzidine-derived dyes, Direct Black 38, Direct Blue 6, and Direct Brown 95, induced hepatocellular carcinomas and neoplastic nodules in the liver of both male and female rats, while no such changes were observed in the control rats. In mice, all three dyes were found harmful to the liver, but no cancerous changes were found. All three dyes contained less than 4 ppm residual free benzidine. However, urine collected at the 4th and 12th weeks of dosing showed substantial benzidine, thus indicating metabolic reduction of the dyes to benzidine. NCI stated in a joint NIOSH/NCI Current Intelligence Bulletin (see reference 130 and Appendix IV) that the findings from the tests with rats and mice were "compatible with the interpretation that the toxic effects may be related to benzidine, because benzidine is more likely to produce cancer in rats than in mice." However, one investigator disagrees with the interpretation of the observations, which were related solely to benzidine and based on the small amount of benzidine obtainable from the given dose of the metabolized dye (131). It was reasoned that a 100% metabolic conversion of the lowest carcinogenic dose of the dye given in the NCI experiment (1.13 grams of dye per kilogram of body weight, after 29 days of continuous oral feeding) would release only 0.16 grams of benzidine per kilogram of body weight. Presently, the lowest dosages of benzidine known to have caused cancer in rats are much greater than the dosages of benzidine given in the NCI bioassay. For example, a dosage of 4.5 grams of benzidine per kilogram of body weight after intermittent feeding for 30 days produced mammary carcinoma in rats (26), and 1.2 grams of benzidine per kilogram of body weight after 6 months of oral feeding produced sarcomas, hepatomas, zymbal gland tumors, and cirrhosis of the liver in rats (24). To date these are the lowest carcinogenic dosages reported. Therefore, in the NCI study the greatest possible quantity of benzidine given to rats at the cancer-causing low dose was 4% to 13% of the previously reported lowest cancer-causing dose. A possible suggestion for this potentiated carcinogenic effect may be that, like Trypan Blue, Butter Yellow, and o-amino-azo toluene, the intact benzidine-derived dyes may be more carcinogenic than the component metabolic amines.

Chapter 8

EPIDEMIOLOGIC STUDIES CONCERNING DYE USERS

The textile industry uses two-thirds of all organic dyestuffs; the paper and pulp industry uses about one-sixth; and the leather and plastics industries uses the rest, chiefly for dyeing and the production of organic pigments (132). A comprehensive search of the literature reveals only a few case comparison type mortality studies (or proportionate mortality studies) related to these industries. Several individual studies, primarily in Great Britain and the United States, have indicated significant increased mortality from bladder cancer among textile and leather workers; however, data concerning such cancer in the paper manufacturing industry is primarily limited to one study. This latter study found no increased risk from bladder cancer, but did find an increase in the expected rate of cancers of the small intestine and cancers of the lymphatic and hematoporotic tissues. Incidences of Hodgkins disease, lymphosarcoma, and multiple myeloma were also higher (133).

One case comparison study involving leather workers from New York City hospitals (300 male and 70 female) who were bladder cancer patients indicated a higher risk to those workers. Personal information, including smoking habits and past occupations, was collected; and from the data collected on workers employed for 5 or more years in a given occupation, a comparison of the study population with matched controls was made. Following is the distribution of cases: Shoe repairers--6 study cases and 1 control case; leather workers--3 and 0; textile workers--7 and 1; tailors and dress pressers--4 and 1; and hair dressers--3 and 0. Factory shoemakers only involved 2 study cases compared to 2 control cases (134).

Another case comparison study in eastern Massachusetts of 356 males with transitional or squamous cell carcinoma of the lower urinary tract further identified increased risk to leather industry workers (135). Overall relative risk, after adjustment for age and smoking, was 2.0. When further broken down by occupation, relative risk for preliminary processes and tanning was 1.45; finishing and associated processes, 2.65; and contact with the finished product, 1.73. These results were based on 65 cases.

Undoubtedly, the greatest relative risk was found in a study of 17,000 patients admitted to a northern New York State hospital between 1956 and 1965 (136). The survey included 13 occupations and 17 cancer sites. Among leather operatives, high relative risks for carcinomas of the larynx (6.9), of the buccal cavity and pharynx (3.4), and of the bladder (12.9) were observed among those employed in this industry for more than 5 years. The number of cases involved were 7, 18, and 11, respectively. Only one worker diagnosed as having bladder cancer was classified as a shoemaker/ repairer.

A study was also made of 210 male members of a New York City local chapter of the United Shoe Workers of America Union. Of the 60 deaths among members due to cancer, only 3 were bladder cancer. The union's membership included cutters, polishers, dyers, clerks, and retailers. This limited data does not suggest an unusual frequency of bladder cancer (134).

In Europe, Anthony conducted a case comparison study of 1030 persons (812 men and 218 women) admitted to 2 British hospitals between 1959-67. All were admitted for treatment of carcinoma or papilloma of the urinary bladder. These patients were matched with two control groups by age, residence, and smoking habits. However, none of the male bladder tumor patients reported leather working as his predominant occupation (10 or more years of employment), whereas several control patients did report such occupation (137). Thus, this study was not consistent with American studies. One possible reason might be the nature of the leather industry in the area studied; that is, the specific occupations encountered in this study that were classified in the general category "leather workers" may not have been associated with processes involving carcinogenic exposure.

General population mortality data for England and Wales have been classified periodically by occupation, and the comparative mortality by cause has been analyzed. An elevated risk to those employed as curriers or leather dressers was noted from the Registrar General's Occupational Mortality Tables for the years 1949-55. For those workers dying after the age of 65, the excess of cancer incidence was significant at the 5% level (138). Similar results were seen for the years 1959-63, with increased mortality rates from both kidney and bladder cancer for those under age 65 and from bladder cancer for those over age 65 (139).

Decoufle attempted to relate an increased incidence of bladder cancer with those U.S. counties having a high involvement with the leather industry (140). Counties were selected in which the leather industry employed 1,000 or more workers, comprising at least 5% of the total employment. Decoufle found 24 counties in 6 states that met these criteria. While a strong relationship between bladder

cancer and work in the leather industry was not seen in all of the counties studied, significant elevations in many New England and New York State counties were seen (141). Again, the discrepancies could be due to several possibilities; for example, workers might not reside in the county where they are employed, or they might not work in processes where exposure to a carcinogen occurred.

Higher incidences of cancer in sites other than the urinary bladder (usually cancers of the lung and nasal cancers) have been noted in shoe workers and repairers. The principal etiological agents identified were leather and wood dusts (the latter derived from waste paper used in shoe soles). Other possible agents were vegetable infusions (from wood, bark, leaves, etc.), previously used in tanning leather for soles and heels, and the dyes used in the preparation of fiberboard. Shoe shiners too use various dyes in their work. The extensive use of chromate salts also warrants concern since this contaminant is also found in leather (142-147).

Hoover and Fraumeni noted a correlation in the same counties between nasopharyngeal and bladder cancer. They stated that "this relationship is consistent with evidence that workers in the finishing rooms of leather and shoe factories are prone to nasal and bladder cancers, suggesting that chemicals may be carcinogenic at the sites of absorption and excretion" (148).

A weaker relationship exists between working in the textile industry and having an increased risk of developing bladder cancer.

In a study by Wynder et al., 300 male bladder cancer patients were matched by sex and age with a control group. Seven patients who had worked in the textile industry developed bladder cancer, while only one patient in the control group did (134).

In another proportional mortality study on bladder cancer patients in Leeds, England, textile finishers, dyers, weavers, and tailors were seen at a relative risk of 2.0, 3.6, 6.5 and 12.2, respectively. The high relative risk among tailors is interesting because no increase was noted for machine sewers. A hypothesis for this difference is that hand sewers frequently draw the thread along their lips to steady it before intricate basting and sewing, while machine sewers do not, and such use of poorly dyed thread allowed some ingestion. The high incidence of bladder cancer among tailors might also be explained if the rubber padding in the presses contained biphenylamine antioxidants that were volatilized during pressing (137). However, no apparent evidence exists for these hypotheses. Among those patients diagnosed as having cancer, no reduction in mean age at onset was seen, only an increased incidence. This might suggest moderate to high exposures to a relatively weak carcinogen.

Newhouse performed a proportional mortality study of 1,429 dyers and bleachers who had died between 1957-68 and whose relatives received union death benefits. Of these deaths, 301 had died of cancer. The ratio of the observed to the expected number of deaths from cancers of the digestive system, lung, bladder, and other sites were 122/106, 103/109, 14/13, and 62/66, respectively. The digestive system was the only site for which an excess of cancers was noted; a slight increase of deaths from cardio-vascular and respiratory disease was also recorded (149).

The above study had several serious shortcomings. In order for death benefits to be paid, a worker had to be in the union only 12 months. Some of the men had joined the union at an early age and later changed jobs, and their occupations since that time were not always known. Also, by including bleachers, who are not exposed to any suspected carcinogens, any effect to dyers, who are exposed to such compounds, would be diluted. In addition, more bleachers than dyers were probably included in this cohort since more bleachers are employed in the textile mills. Nonetheless, the slight increase of some fatal diseases found by this study is reason for concern and warrants further studies of textile dyers.

A strong association relating previous human exposure to benzidine-based dyes with a subsequent increase in bladder tumors was presented after a case-control mortality study of 200 bladder cancer patients in Japan. The patients had worked as kimono painters and dyers and used Direct Red 28 and Direct Black 38. Ingestion of these dyes may have occurred since 47% of those workers reportedly licked their brushes or spatulas to moisten them. The relative risk to this cohort was 6.8 times higher than the general population (150).

Chapter 9

PRESENT STUDY

Benzidine, a known human carcinogen, and the congeners of benzidine, suspected carcinogens in man, have been widely used in making dyestuffs for many decades; and many industries have used these for the dyeing of their products.

Historically, studies of worker exposure to carcinogenic amines and other carcinogenic compounds have been aimed primarily at the manufacture of those compounds. Little or no effort has been made in these studies to look critically at the metabolisms of the vast number of workers exposed to chemicals in industrial environments that contain carcinogenic intermediates. This is true also for occupational environments in which dyestuffs are made from carcinogenic amines. What is known about the metabolism of azo compounds in man has resulted from animal feeding studies designed to evaluate the safety of the use of food colors in the human diet.

The present study, therefore, was designed to identify those industries where the use of and potential exposure to benzidine azo dyes occurs and to determine if metabolic reduction to the component amines occurs in man. The information obtained on the metabolism of these dyes, in conjunction with historical and epidemiological data, will be used to attempt to establish a correlation between the use of benzidine-based dyes in the work environment and a higher incidence of cancer among workers involved with such use.

Chapter 10

STUDY DESIGN

The selection of the benzidine-based dye manufacturing and consuming facilities to be visited was restricted to those plants in which both environmental and biological monitoring of the production employees could be performed. Consuming facilities were selected according to the degree of the "pure" exposure of workers to benzidine-based dyes and according to the number of employees potentially exposed. (This preliminary information was obtained through telephone and mail correspondence.) A study protocol was mailed to those facilities queried (see Figure II).

Plant visits to textile and leather facilities were arranged through the American Textile Manufacturers Association and the Tanners Council of America. Labor union participation by the International Chemical Workers Union, Amalgamated Meat Cutters Union, United Paper Workers Union, and the Amalgamated Clothing and Textile Workers Union was also encouraged.

A. Field Monitoring

1. Environmental Sampling

Workers included in the environmental and urine monitoring were selected with the aid of the management at each facility. In all cases, those workers who were asked to submit urine samples were also monitored environmentally through personal air filter sampling.

Personal air filter samples were collected on tared closed-face Gelman 37-mm glass fiber filters in three-piece cassettes. The sampled air was drawn through the filters by Mine Safety Appliance Model G pumps at approximately 1.8 liters per minute. Throughout each shift monitored, the filters were periodically checked and changed, if necessary. Generally, only one shift of workers wore sampling pumps, but in some facilities more than one shift was monitored. Information on engineering controls,

personal protective equipment, eating and locker facilities, and historical information on the facility was also obtained.

Bulk samples of the benzidine-based dyes present during the monitoring period were obtained for determination of residual-free benzidine base and for the salts of benzidine. The bulk samples were also used for a color differentiation of the air filter samples, allowing a semi-quantitative determination of the proportion of benzidine-based dyes collected in comparison to the total particulates collected on the filters. Most filters were weighed for total weight, and the atmospheric concentrations were calculated in milligrams per cubic meter. In one manufacturing facility of benzidine-based dyes, airborne free benzidine was also determined. This was done using a sampling train consisting of a glass fiber filter with a silica gel backup. In some consuming facilities, airborne benzidine was also sampled near processes where the use of the free amine might occur. All environmental samples were submitted to NIOSH for analysis.

Urine Monitoring

Urine samples were collected in 180-mL polyethylene bottles. The period of collection usually began during the beginning of the work shift that was sampled environmentally and continued with a first catch voiding the next day and throughout the first half of the next day's shift. Thus, this monitoring usually was continued for about a 28-hour period. The time was recorded on each sample submitted, which was frozen immediately on dry ice in a thermal insulated carrying box.

Normally, the biological monitoring of workers requires prior Human Subjects Review Board (HSRB) clearance to safeguard workers from harm that might result from such testing. However, in this case clearance was waived by the HSRB chairperson because of the limited involvement of the workers and because no information was collected that could possibly threaten their privacy. NIOSH regulations do require that the workers voluntarily give their consent to participate in a study of this kind and that they be fully informed of the need for such testing and the benefits that are afforded. In addition, written permission must be obtained from each worker before the results of biological testing may be released to anyone. (For a sample of the required forms see Appendix III.)

Urine samples were analyzed by both the Clinical and Biochemical Support Section, Division of Behavioral and Biomedical Science, NIOSH, and the Chemistry Division, National Center for Toxicological Research (NCTR).

B. Analytical Procedures

1. Environmental Analysis

As was described previously, the environmental air filter samples were analyzed for total weight, color differentiation, and, in some cases, for benzidine, both as the amine and its salt. Bulk samples of benzidine-based dyes were collected for total benzidine determination. The individual analyses are described below:

a) Gravimetric analysis of tared (pre-weighed) glass fiber filters is a routine determination used to measure total gross particulates in the air. The filters are first allowed to reach equilibrium in a constant temperature and humidity environment and then weighed. After the sampled air is drawn through the filters and the cassettes sealed, they are returned to the lab for reweighing. Before reweighing the sample filters, they are again allowed to reach equilibrium in the constant temperature and humidity environment. Sample "blanks" were treated like the samples, except no air was drawn through them. The total sample weight per filter is determined by subtracting the initial weight of the filter from the weight of the filter after sampling and adjusting for the blank. The samples are expressed as air particulates per cubic meter of air, dividing the total sample weight in micrograms or milligrams by the total air sample in liters.

b) The method used for the color differentiation of the various dyes collected on a filter sample is P&CAM 234, NIOSH Manual of Analytical Methods, 1977. The principle of the method is that a sample filter is extracted with an appropriate solvent, and a spectrophotometric scan of the solution is made in the 400-700 nm range. The absorbance maxima are compared to the absorbance maxima of standard solutions prepared from the bulk samples of the azo dyes. It is therefore vital that bulk samples be acquired during air monitoring. If two or more bulk samples exhibit a maximum at the same wavelength, concentration of azo dye must be reported as a range within the limits

determined by the absorptivity of the dyes sharing the lambda maximum. After comparison of sample absorbance to standard curve, the value either as a number or a range is calculated as milligrams of azo dye per filter, which, when divided by the total air sampled, is expressed as milligrams of azo dye per cubic meter of air.

A spectrophotometric absorbance curve indicates that a Beer's law relationship in the 5-200 Ng of azo dyes per 40 mL solvent range is followed. The range of detection can be adjusted by diminishing or increasing the amount of solvent or by changing the cell length.

Precision and accuracy are reported with a relative standard deviation of 12% and a recovery efficiency of 95% ($\pm$ 5%) in the 5-200 Ng/40 mL range.

c) Bulk samples of azo dyes are analyzed by liquidchromatography for residual benzidine and its salts. The pH of the dye is measured with an electrode. If the azo dye is basic, the bulk is analyzed only for residual benzidine. Approximately 1 gram of dye is dissolved in water.

The free amine is extracted with chloroform, and the salts are converted with base to the free amine and extracted with chloroform. The chloroform extracts are filtered, reduced to dryness in a vacuum oven, and redissolved in methanol. An aliquot of the methanol is injected onto a reverse phase column and eluted with a 60:40 Clehanol water mixture at 1 mL/min. Detection is made with a 280-nm UV detector. Peak areas of the samples are compared to standard curves, and Ng per sample of benzidine is calculated. The detection limit of benzidine is 1 ppm (w/w) when the initial azo dye extracted is 1 gram.

Studies showed that this method yielded a recovery of approximately 100% for benzidine and its salts. The time per analysis, however, is slow--about three samples per day per HPLC are the maximum number of analyses obtainable. The large number of other contaminants and the time needed for purging the HPLC column are the reasons for this slow rate. (This method was developed as a result of this project, and the method is unpublished.)

2. Biological Analysis

The urine samples were analyzed for total aromatic amines, free benzidine, and monoacetylbenzidine. The procedures used are as follows:

a) Total aromatic amines are determined colorimetrically, using a modified method developed by Rinde (151) and Satake (152).

After thawing the frozen samples, the pH is adjusted to between 5 and 6; and 100 mg of NaCl is added to the urine to facilitate extraction in chloroform. Three chloroform extracts are made in series with 10 mL per extract and the total organic phase is pooled. The chloroform extract is back-extracted with 2 mL of 0.02N HCl and is reduced by drying to 1 mL volume. One mL of a sodium acetate (CH_3COONa) buffer solution and 0.6 mL trinitrobenzene sulfonic acid (TNBS) is added to the 1 mL HCl extract and mixed for 15 minutes. A final extraction with 1 mL $CHCl_3$ is performed and this is read on a spectrophotometer at 400 nm. A chloroform reagent blank is used for zeroing and a standard curve prepared with benzidine is used to determine the sample concentrations.

This method is excellent for determing the excretion of non-specific primary aromatic amines resulting from an exogenous source. The lower limit of detection is 100 ng/100 mL (1.0 ppb) of urine expressed as free benzidine.

For confirmation of the aromatic amines as benzidine, thin layer chromatography (TLC) was used. The TNBS positive samples were chromatographed on silica-gel pre-coated plates. The retention factor was 0.43 in a chloroform/ethyl alcohol (95/5) solvent. The lower limit of detection is 100 ng/100 mL of urine. Recovery was about 50% from standards.

b) For confirmation of the TNBS/TLC method for identifying free benzidine and monoacetylbenzidine, many urine samples were split for shipment to NCTR. Developed by Nony and Bowman, the method employed at NCTR involved electron-capture gas chromatography (EC-GC) (153). Here the urine samples were extracted first with benzene and then back-extracted in HCl. Salient elements of the analytical procedure are: extraction of the urine (adjusted to pH 12 with NaOH) with benzene, conversion of the amine residues to

their pentafluoropropionyl derivatives, and subsequent analysis by EC-GC. The results were corrected on the basis of recoveries from the samples.

The following compounds and their lower levels of detection by EC-GC are:

1. Benzidine	(Bzd)	1.4 ppb
2. Monoacetylbenzidine	(AcBzd)	5.8 ppb
3. 3,3'-dimethylbenzidine	(DiMeBzd)	3.0 ppb
4. 3,3'-dimethoxybenzidine	(DiMxBzd)	3.6 ppb

For the methods used to determine aromatic amines, benzidine, and monoacetylbenzidine in the urine, analytical confirmation was sought to establish that these amines were metabolites and did not result from the chemical reduction of intact dyes by the analytical method. This was confirmed by spiking several NIOSH control urines with substantial quantities of benzidine-based dyes. These samples were thereafter treated like the sample urines. Only a very small quantity of benzidine base could be detected; this presumably resulted from the residual benzidine in the dye samples that were used to spike the urine samples.

Figure II. Dye study protocol.

INTRODUCTION

NIOSH is conducting various studies to evaluate the potential carcinogenic risk from exposure to synthetic organic dyestuffs. Included in the NIOSH studies will be various attempts to determine through animal experiments the metabolism and carcinogenicity of several suspected dyes.

The current effort is to gather basic information concerning the extent of exposure, the routes of absorption and excretion, and the production of certain metabolites found in the urine of workers. The possible metabolic products of azo compounds made from benzidine or other carcinogenic intermediates has long been a matter of curiosity. Azo compounds can be metabolically reduced in vivo by either the gut flora or by hepatic (liver) microsomal enzymes. Troll et al. found free benzidine and monoacetylbenzidine in the urine of Rhesus monkeys fed benzidine azo dyes containing no free benzidine (159). The effect of the inhalation of these dyes by man is a current occupational health concern; and benzidine-containing dye has been selected for the initial field study because of the superior analytical possibilities of this class of dye.

PLANT SELECTION

Essentially, NIOSH is interested in locating any facility that uses benzidine azo dyes in manufacturing its product. These facilities might typically include textile mills, leather tanneries, and paper and pulp dyeing mills. The manufacturers of these dyes have already been studied.

The facilities selected will include those that use a high proportion of benzidine azo dyes in their total daily consumption. Facilities that use these dyes continuously, or as infrequently as once a month, suit the needs of this study. The facility should include at least four to six workers who have potential exposure to these dyes by inhalation, skin absorbtion, or ingestion.

FIELD SAMPLING PROTOCOL

After a minimum notification of 5 working days before the intended visit to a facility using benzidine azo dyes, a NIOSH team (usually two members) will arrive at that facility. An opening meeting will be held with appropriate representatives from both management and labor. The functions of NIOSH and the scope and design of the present study will be discussed. During the meeting a list of the materials that are handled by the workers who will be included in this study will be requested. This will allow NIOSH to

Figure 11 (continued).

determine if any materials other than benzidine dyes could interfere or conflict with the findings obtained. The workers to be studied will generally be selected prior to the survey. A brief review of existing health, safety, industrial hygiene, and medical programs at the facility will be discussed. After this meeting a brief walk-through survey of the facility will be conducted. This will allow the survey team to familiarize themselves with the facility and its processes and enable them to monitor the areas where exposure is likely to occur. Space will be requested for use of the survey equipment during the stay; this should preferably be a small area that has electrical outlets and can be secured at night. The survey team will most likely remain for one full shift the first day and return for half of a shift the following day.

After selecting the workers and determining the job areas to be monitored, the NIOSH team will begin sampling. Environmental monitoring will consist of collecting personal air filter samples from portable sampling pumps attached to the workers' belts. Area samples of a similar nature will also be taken. The filters will be changed as needed throughout the shift. At that time, the monitored workers will be asked to shower and submit a urine sample. Those workers will also be given a sample bottle to take home and fill the next morning upon arising. These samples will be returned to the NIOSH investigators as soon as possible for preservation. About 15 pounds of dry ice will be needed for freezing the urine samples. The companies' cooperation in locating a supplier of dry ice will be appreciated.

Observations of local and general ventilation controls, the use of personal protective equipment, work practices, job responsibilities, and personal habits will be recorded.

ANALYTICAL PROTOCOL

The air filter samples will be taken on pre-weighted glass fiber filters and analyzed gravimetrically for total dust and quantitatively for each specific dye. One ounce bulk samples of each of these dyes will be needed to provide standards for analysis. Free benzidine may also be analyzed from these filters. Methods of analysis for each of these procedures can be obtained upon request.

Urine analysis will include a quantitative determination of the total aromatic amines and free benzidine, o-tolidine, and o-dianisidine that that are of an exogenous nature. The results of all samples taken will be forwarded in a summarized report to the employer and, if applicable, the union. Individual resuts will be sent to the employees.

Figure 11 (continued).

Before finalizing, draft reports of the survey will be written and sent to management for their review. The final reports shall be forwarded to management, the local union, if applicable, NIOSH regional consultants, and the OSHA regional office responsible for the facility that was surveyed. Data and information collected at each facility but not included in the final report is obtainable through the Freedom of Information Act. No data collected by NIOSH can be used by OSHA for compliance purposes. In addition, proprietary information shall be retained in a confidential manner by NIOSH.

Every effort will be made to minimize interference with the normal work practices at the facility during the NIOSH survey.

Further information can be obtained from Mark Boeniger, NIOSH Project Officer, at (513) 684-2876.

Chapter 11

RESULTS

Environmental and urine monitoring data were collected from six facilities: two benzidine-derived dye manufacturers, two textile dyeing facilities, a leather tanning and finishing facility, and a speciality paper mill. Reference to these facilities in this report will be given by a code letter. The individual results from each facility will first be presented; a discussion of the overall results will follow.

A. Dyestuff Manufacturers

1. Facility A

At the time of this survey, Facility A was the major domestic producer of benzidine-derived dyes. Recently, the new ownership of this facility discontinued its manufacture of such dyes.

Description of Process

The production processes for benzidine azo dyes at Facility A were well controlled and left little potential for exposure. The production facilities at the wet end were not in operation at the time of our survey, but the procedure for its operation was explained and the equipment inspected.

Hydrazobenzene had been substituted for benzidine base because it is a safer material to transport and handle. The hydrazobenzene is dumped by hand into a charging chute leading to a reaction vessel. This operation is isolated by an enclosure, and the worker wears personal protective equipment, including a full-face respirator, during the charging. Decontamination of the work area and worker with a sodium hypochlorite solution follows operations. The hydrazobenzene is then rearranged to benzidine dihydrochloride by the addition of strong hydrochloric acid and water. Upon complete rearrangement, a portion of the slurry is pumped under pressure to a second closed unit for

a tetrazotization process that the company considers unique The residual benzidine slurry is stored in the rearrangement kettle until it is needed.

Tetrazotization is accomplished in a reaction vessel on the fourth floor of the building. The reaction product is then pumped to other vessels where the dyes are made by coupling the reaction product with appropriate compounds. It is at this stage that benzidine-containing dyes are considered innocuous. The dyes are then pumped to the filter presses where they are unloaded manually into tubs by workers wearing rubber gloves. The filter press removes unwanted reaction products. The filter press cake is re-slurried and then is finally dried by drum dryer. These dryers are well controlled by local ventilation. The dyes are automatically loaded into drums through cloth chutes. Very little dust is released in this latter process.

In another building the drum is unloaded manually into hammer mills where the dye is ground to a fine powder. Next, the dye is transferred through chutes into ribbon blenders. Appropriate additional dyes and dedusting oil is added at this point to bring the dyestuff to a specific shade and to reduce dustiness. When this is accomplished, the dyestuff is loaded from cloth chutes into drums, which are below the blenders. The drums must be periodically adjusted for weight by an employee. (An adjustable local exhaust slot hood was used during this re-weighing with excellent results.) Finally, the drums are covered and sealed and are ready for transfer to the shipping department.

It should be noted that workers handling these dry dyes conscientiously wore cartridge respirators when working. Because of the engineering controls and personal protective equipment, probably very little exposure to the finished dyestuff occurred. Eight spot urine samples were collected to determine if any exposure to benzidine was occurring among workers in job titles that are given in Figure III. However, a repeat survey was not possible because of the discontinuance of benzidine-derived dye production. Results of the spot urine sampling are given in Table V.

Figure III. Number of production workers in the dry end at Facility A.

Shift	Title	Number	Description of duties
Bldg. A			
1	material handler	6	Unload filter presses
2	" "	2	
3	" "	1	
		9 total	
Bldg. B			
1	material processor	5	Drum dry loading, drum loading
2	" "	1	
3	" "	1	
		3 group ops.	
		10 total	
Bldg. C			
1	group operator	2	Grinding, blending, drumming
2	" "	1	
1	material processor	3	
2	" "	2	
1	material handler	5	Fork truck operator, receiving/shipping
2	" "	2	
		15 total	
		34 Grand Total	

Table V. Results of spot urine monitoring of workers at dye manufacturing Facility A.

Urine Specimen	Benzidine or Monoacetylbenzidine (MAB)	Aromatic Amines (ng/100 mL)	Thin-Layer Chromatography
1	3 ppb MAB	120	N.D.
2	N.D.	80	N.D.
3	N.D.	100	N.D.
4	N.D.	80	N.D.
5	N.D.	90	N.D.
6	N.D.	80	N.D.
7	7 ppb MAB	90	N.D.
8	N.D.	60	N.D.

N.D.--not detected

2. Facility B

Description of Plant and Process

Facility B is a dyestuff manufacturer that presently markets approximately 60 dyes; 70% of this production volume is benzidine derived. Prior to 1960, both benzidine dyestuffs and benzidine were manufactured at this facility. (The production of benzidine dyes is expected to increase at this site.)

The company employs 73 workers, 54 of whom work in the production area. There are 3 production shifts, and the day shift employs 35 workers. All production workers are male. Worker distribution is presented in Figure IV.

Of the four major buildings at this facility, one houses the reaction vessels, below which are the filter presses and some tray dry ovens. There is also a drum dryer in this building. The second building contains additional oven dryers and press cake pulverizers. There is one spray dryer on the premises, and its operating temperatures range from about 450-500 degrees F. In this building the dried dyestuff is automatically dropped into 55-gallon drums, which are then sealed by the operator. A dedusting oil is added to the press cake to minimize dusting of the dry dyestuff. A third building is used for storage and contains six ribbon blenders with which dyestuffs are color-blended and standardized by adding salts. The final product is then loaded from the blenders into drums.

Facility B obtains its benzidine primarily from a U.S. supplier in the form of hydrazobenzene. This is received as a white flaky material in drums and is treated as a suspected carcinogen. Dianisidine (3,3'-dimethoxybenzidine) o-tolidine base and various anilines and aminonapthols are also used.

Description of Survey Methods and Results

The work at Facility B is divided into three shifts, but since the predominating production period is the day shift, only that shift was involved in the field sampling. Sampling was conducted for two days, and the following personal monitoring was performed:

I. Environmental Sampling--Air sampling with 37-mm glass fiber filters in three-piece cassettes was done for processes involving tray-drying, pulverizing of press cake,

spray drying, and blending of dyestuffs. The results are reported in Table VI. The drum dryer was not operating during the survey. However, accumulated dyestuff in the room housing this dryer suggests that additional controls should be instituted to reduce the potential exposure of the operators to benzidine-based dyes.

In three instances, samples taken with 10-mm prefilter cyclones preceding the glass fiber filters were compared with non-prefiltered samples to estimate the proportion of respirable particulates among the total airborne particulate concentration. These results are shown in Table VII.

During the first day, most filters were backed up with a silica gel tube for the collection of airborne benzidine that might pass through the glass fiber filters. This was done to determine if residual benzidine present in the dyestuffs was volatized during hot processing. In addition, the preceding filters were also analyzed for benzidine content. No benzidine was detected in either the silica gel samples or the glass fiber filter samples. A detection limit of 0.5 μg per 500 μL of desorbing solution was obtained using NIOSH P&CAM method 243.

Swab swipe samples using cotton swabs in pH buffered solution were also taken in the azo department for analysis of benzidine contamination of the work area. These results are reported in Table VIII. These swipe samples were kept frozen until analyzed by high pressure liquid chromatography. The detection limit was 0.5 μg/sample.

Low volume pumps with silica gel air collection tubes were placed in the general areas of the liquefaction tubs for the spray dryer and at the spray dryer itself. Sampling was performed during the entire shift. Considerable heat and steam was evident in these areas; in addition, the wide variety of potentially volatilizable chemicals processed subjected the operators in these areas to possible exposure. The samples were frozen in dry ice immediately after collection and were kept frozen until analyzed. The analytical methods used were qualitative determinations using gas chromatography and mass spectrometry and a quantitative analyses for benzidine (as hydrochloride and base) using gas chromatography. These results are presented in Table IX.

Bulk samples of benzidine-based dyes were collected for analysis of their residual free benzidine content. The names and quantities of free benzidine in 11 dyes that were

collected during this survey are given in Table XI. The content of residual benzidine varied; however, none were found to contain greater than 25 ppm residual benzidine.

II. Urine Sampling--Urine samples were taken from workers who wished to participate in this portion of the study. In most cases, those workers from whom urine samples were taken also participated in the personal breathing zone sampling. Unfortunately, many workers who were monitored environmentally and who appeared to have high levels of exposure did not provide urine samples. To minimize contamination of the samples, end-of-work samples were collected after the worker showered. The samples were immediately numbered and placed on dry ice for preservation.

Using a spectrofluorometric procedure and confirmed with thin-layer chromatography, the urine samples were analyzed for aromatic amines expressed as benzidine; and using an electron-capture gas chromatographic procedure after several extractions of the urine samples, they were analyzed for benzidine, o-tolidine, and o-dianisidine. Both methods were used for comparison in an attempt to develop the first as a cheap, reliable method for the screening of aromatic amines and benzidine in the urine. Investigators found that although the first method was not as selective as the second, it can be used as an easy screening tool. Table X summarizes the results of samples obtained from workers who were monitored environmentally and from workers who submitted urine samples. These data were collected to allow a quick visual inspection by job category of exposure and excretion. A job dictionary for Table X is given as Figure VI.

Evaluations of Ventilation Systems

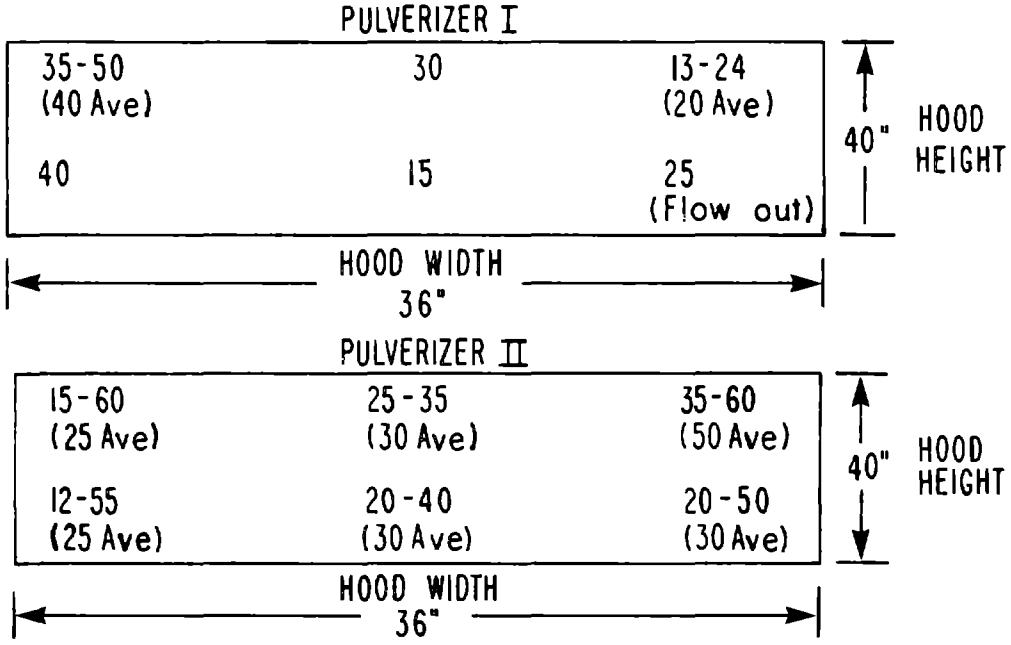
The local ventilation systems for the control of dry dyestuff particulate emissions into the pulverizer and ribbon blender work areas were evaluated to determine their efficiency. Face velocities were determined with a thermoanemometer and smoke tubes.

Three-sided ventilation hoods are installed over two pulverizer mills (I and II). These hoods are constructed of plywood (approximately 36" x 64" x 36") and connected by flexible tubing to two separate portable dust collection units. These units, designed in-house, contain two-horsepower (3450 RPM) centrifugal fans attached directly to the tops of 55-gallon steel drums. The fans are rated at

1100 cubic feet of air per minute. The collected air is first exhausted into the 55-gallon drums where the heavy particulates settle out. The air is then exhausted out of the drums through cloth bags (also attached to the top of the drum) that filter the fine particulates out of the air.

The local ventilation hood installed over pulverizer II was located approximately north of pulverizer I and was similar to pulverizer I in all respects, except that it was nearer (within 4') to wall windows that were sometimes open. These windows could definitely have interfered with the hood's collection efficiency.

Air velocities were measured with a thermoanemometer at six points at the face of each hood. The air velocities in linear feet per minute are shown below. (Air velocities of the local ventilation hoods for pulverizer I and II are reported in linear feet per minute.)



From the above data, it is evident that the capture velocities needed for the control of dyestuff particulates were not approached and, in fact, were not appreciably different from general room air currents. Average face velocities should be at least 100 linear fpm for the adequate control of nuisance or low toxicity materials (154). Such velocities are easily obtainable with conventional exhaust systems.

The above ventilation systems were installed one month prior to this survey. During this survey several leaks

developed in the flexible tubing, at joining points, and in the exhaust filter bag. (The systems, which require almost constant attention, have obvious disadvantages and more permanent and conventional systems are recommended.)

At the time of the survey, a third unit similar to those just described was being installed to control dust emissions during tray dumping of the oven dried press cake.

The local dust control ventilation system in the blending department was also evaluated. This system was installed in 1976. There are six ribbon blenders in this department, each fitted with a three-sided hood located over the charge port on the blenders. Each hood is connected to a central duct system that loads to a baghouse-type dust collector located at one end of the room so that the cleaned air is exhausted back into the room. Each hood is fitted with a shutter (or blastgate) located in the duct just above each hood so that unused hoods do not draw air. All six hoods were inspected with the blastgates shut on the unused hoods, and six points were measured at the face of each hood with the thermoanemometer. The results are recorded in Figure IV, and the values obtained, all of which fall between the recommended 100-200 linear fpm face velocities, indicate that this system adequately controls dust emissions during operations in which materials are dumped into the blenders. However, no local control ventilation exists on the drumming operation below the blenders, and a significant dust exposure to workers exists during this operation, as evidenced by the results of samples 11 through 15 presented in Table VI. Additional potential dust exposure exists during weight adjustment of the dyestuff in the drums; however, little general air movement was observed in this department when smoke tubes were used.

Since this survey, Facility B has reportedly taken measures to improve the control of dyestuff exposures and to monitor the urine of the production workers for benzidine.

Figure IV. Face velocities obtained on six hoods (in linear feet per minute).

Hood Number	Position Measured	Hood Number	Position Measured
1) 190		1) 180	
2) 200		2) 190	
3) 185		3) 150	X
4) 190	X	4) 160	
5) 200		5) 170	
6) 190		6) 135	
1) 145		1) 135	
2) 185		2) 110	
3) 160	X	3) 170	X
4) 160		4) 130	
5) 170		5) 170	
6) 175		6) 110	
1) 100		1) 85	
2) 180		2) 140	
3) 135	X	3) 125	X
4) 120		4) 130	
5) 130		5) 140	
6) 200		6) 130	

Hood arrangement for ribbon blenders.

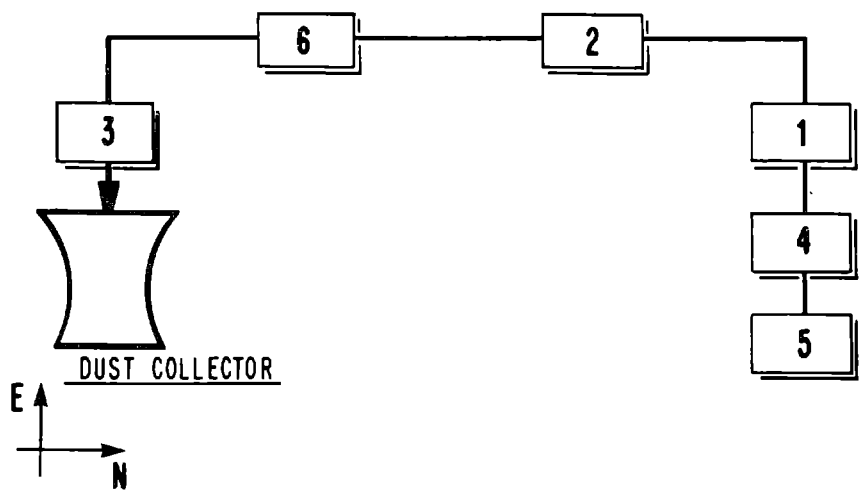


Figure V. Worker distribution at Facility B.

JOB OR DEPARTMENT	NUMBER OF WORKERS
Receiving	2 on 1st shift
Azo	9 on 1st shift 4 on 2nd shift 3 on 3rd shift
Tray Drying	11 on 1st shift 4 on 2nd shift 2 on 3rd shift
Drum Drying	1 each shift (3)
Spray Drying	1 each shift (3)
Blending	3 on 1st shift
Shipping	2 on 1st shift
Cleaning	2 on 1st shift
Maintenance	6 on 1st shift
	54 Total

Table VI. Environmental air sampling results--Facility B.

Filter Number	Airborne Concentration*	Job Description
4	12.0	Pulverizer
5	6.0	" "
6	12.8	" "
7	5.8	" "
8	6.2	" "
9	4.9	Area sample 8" (N) distance from pulvizer at 4' height
10	7.2	6" (S) from pulverizer charge chute
11	4.7	Blender
12	8.7	" "
13	1.9	" "
14	92.7	Blender--16 minute sample during drum filling
15	13.1	Area in blending department near drumming chute
16	17.4	Spray dryer area--floor level near drumming chute
17	1.8	Tray dryer operator
18	7.4	" "
19	6.5	" "

*Total particulate concentrations are given in in milligrams per cubic meter of air. All samples were taken at worker's breathing area unless otherwise indicated.

Table VII. Comparison of respirable to total sample particulates-- Facility B.

	Respirable concentrations*	Total concentrations*	Time sampled (hours)	Ratio respirable to non-respirable dust	Job Description
Sample 1	0.5	1.9	3.3	1.4	Blending department adjusting weight of loaded drums and sealing
Sample 2	2.5	5.8	2.8	1:2.3	Pulverizing department manned pulverizer and material transfer
Sample 3	0.5	4.7	2.7	1:9.4	Blending department loading dyes into pulverizer

*mg/m^3 airborne particulate concentration

Based on three samples, the above results show that the proportion of non-respirable to respirable airborne particulates is two to nine times greater.

Table VIII. Swipe sampling results for benzidine. (Minimum level of detection is 1 μg/sample.)

Swipe sample	Location taken	Result
1	Inside bzd. azo reactor	ND
2	Azo charging chute opening	ND
3	Bottom area diazo tank wall	ND
4	Inside bottom outlet spigot of diazo tank	ND
5	Door knob of change room used for decontamination of worker	ND
6	Charging chute for hydrazo benzene dumping into reactor vessel	ND

Table IX. Chemical identification of volatile emissions during spray drying.

Sample	Location	Compounds Found*
1	Area sample taken at breathing level over spray dryer reliquification tanks	azobenzene
2	Area sample taken above drum loading chute below spray dryer unit	azobenzene

*Only benzidine was quantitated.

Results: Benzidine was not found in either of these samples; however, the shelf life of these samples over long periods is not known. GC/MS identification of azobenzene, which is very closely related to hydrazobenzene, is presumably a rearrangement reduction by-product. Azo benzene has an appreciable vapor pressure, especially at elevated temperatures.

Table X. Environmental and biological sampling data from a dye manufacturer--Facility B.

Job Description	Env. Conc.	Urinary Excretion*	Notes
Pulverizer 1	1) 12 mg/m^3 total 2) 5.8 mg/m^3 total 3) 6.0 mg/m^3 total 4) 2.5 mg/m^3 resp. 5) 4.9 mg/m^3 upper level 6) 7.2 mg/m^3 lower level	52 ppb Bzd; 248 MAB† 18 ppb Bzd	Wore cartridge respirator occasional exposure to very high levels during adjustments
Spray dry	7) 17.4 mg/m^3 - area near chute	112 ppb Bzd; 590 ppb MAB: 50 ppb DiMeBzd	Wore no respirator - most of day spent outside building. The presence of DiMeBzd would indicate previous exposure as no DiMeBzd dyes were being used on day of sampling.
Pulverizer 2	8) 6.2 mg/m^3 total 9) 12.8 mg/m^3 total 10) 16.2 mg/m^3 total	10 ppb aromatic amines expressed as Bzd; Bzd confirmed on TLC 5 ppb aromatic amine as benzidine	Wore cotton gauze respirator; became very dirty from dyestuffs
Tray Oven	11) 1.8 mg/m^3 total 12) 7.4 mg/m^3 total 13) 6.5 mg/m^3 total	11 ppb Bzd; 22 ppb MAB; 15 ppb, DiMeBzd	Wore cartridge dust respirator; emptied dried oven trays into drums

Abbreviations:
Bzd - benzidine
MAB - monoacetylbenzidine
DiMeBzd - 3,3' -dimethylbenzidine

*Limit of Detection (ppb)
Bzd - 1.4
MAB - 5.8
DiMeBzd - 3.0

Figure VI. Facility B job duties.

Pulverizer--Dumps drums of dried dye presscake into the pulverizing bin. Occasionally shovels and pushes the presscake into the grinding element. Replaces filled drums at the bottom of the pulverizer with new drums.

Tray Dryer--Shovels wet presscake onto metal trays for drying in the ovens. After drying, the tray dryer removes the trays and dumps the presscake into the drums. Insulated rubber gloves and a half-face cartridge respirator are worn.

Spray Dryer--Monitors mechanical controls on the spray dryer unit. Removes and replaces drums as needed (about 4 times per shift).

Blending Department Operator--Operates forklift truck. Dumps dry presscake from the tray ovens into the ribbon mill blenders through the top chute. Removes and replaces drums at the bottom chutes below the blenders. Adjusts weight on the drums before sealing and labeling. Cleans and washes the ribbon blenders after run.

Azo Department Operator--Transfers wet and dry intermediate chemicals to the loading chutes above the reactor vessels. Unloads wet presscake from the frame filter presses.

Table XI. Residual benzidine in 11 benzidine-based dyes.*

Trade name	Residual benzidine (ppm)
1) Direct Brown M (100%) (C.I. Direct Brown 2)	1
2) Direct Brown 3 GN (200%) (C.I. Direct Brown 154)	15
3) Fast Green WS (100%) (C.I. Direct Green 1)	1
4) Catechine 3G (C.I. Direct Brown 74)	4
5) Catechine Fast Brown B (125%) (C.I. Direct Brown)	3
6) Diazo Black BH (100%) (C.I. Direct Blue 2)	1
7) Direct Fast Blue 2B (250%) (C.I. Direct Blue 6)	12
8) Diazo Fast Green BX (100%)	3
9) Direct Brown BRL (200%) (C.I. Direct Brown 95)	19
10) Direct Black Gx (200%) (C.I. Direct Blk 38)	10
11) Congo Red 4B (C.I. Direct Red 28)	2

*Chemical structures and synonyms can be obtained from: The Society of Dyers and Colorists--American Association of Textile Chemists and Colorists. The color index. 3d ed. Vol. 5. London: Lund, Humphries, Bradford, London; 1971.

B. Textile Industry

1. Facility C

Description of Plant and Process

Facility C is a moderate-sized textile dyeing and finishing facility in which 450 workers are employed in the production areas in 3 work shifts. Almost all of the production workers are male. The total work force numbers about 550.

The principal production operations are the dyeing of broad woven cotton goods and the dyeing of synthetic materials, blends, and non-woven gray goods. Only solid shades are dyed. Other operations include bleaching, applying fillers (e.g. talc), starching, calendering, and final pressing.

Pad dyeing has almost entirely replaced jig dyeing. The pad dyeing process involves the use of a series of liquor vats for bleaching and dyeing and the use of rinsing vats and drying ovens. The gray goods are fed in continuously through a system of guiding rollers.

Jig dyeing is a much simpler process. The fabric job consists of a system of rolls and a liquor trough through which the fabric is passed. The dye bath is commonly associated with steam evolution (and in this facility a canopy hood was provided for comfort control purposes). The dye liquor is prepared by drug room operators. It is their responsibility to weigh and solvate the required quantities of dry dyestuffs needed in formulating any given shade. The total number of employees with potential exposure to either dry or liquid dyestuff is quite small in relation to the total employment. The following are estimates of the workers potentially exposed to dyestuffs:

Drug Room---2 per shift
Jig Dyeing--1 per shift
Pad Dyeing--7, 5, 5 on 1st, 2nd, and 3rd shift, respectively

Total: 26 workers.

Currently only about 20% of the consumed dyestuffs are of the benzidine-based type.

Description of Medical, Industrial Hygiene, and Safety Programs

This facility has active medical, industrial hygiene, and safety programs. The medical program includes pre-placement and annual physical examinations for all employees. A visiting physician is available every Tuesday and the first Thursday of every month. There is not a licensed nurse at the facility; however, at least one employee trained in first aid is available on each work shift. The industrial hygiene program includes sampling provided by the company's insurance carrier and is available upon request. The company reports that the samplings for chlorine bleach, vinyl chloride polymer, and other substances show that exposure levels are below the current permissible standards in all cases. A formalized safety program, led by one individual and supported by a safety committee, is responsible for safety surveillance.

Exposure Control Measures

The identification of the use of hazardous material control measures in this facility was limited to measures for workers weighing and mixing dyestuffs and to measures for workers operating dyeing machines. No personal respiratory protective measures were used in any of these operations. Occasionally, insulated rubber gloves were used when boiling-up dyes by hand; however, no other personal protective equipment was worn.

The drug room was kept under positive pressure for humidity control through forced air vents in the ceiling. A long, rectangular local exhaust hood was located above the boil-up trough to remove steam. Using smoke tube indicators, the investigators discovered that this design provided little protection. There was no local ventilation near the dye weighing scales, nor was there ventilation when the dyes were scooped out of the drums to be measured.

A large canopy-type hood was installed over the jig tub dyers, and ventilation was primarily used for control of the high temperatures and steam evolved during dyeing. An employee periodically worked under the hood when changing rolls or making adjustments. During this operation, his exposure to volatilized materials may have been high; and, by testing the canopy hood with a smoke tube indicator, an investigator found the control efficiency of the hood low.

The operator of the pad dyer was responsible for diluting a concentrated dye liquor in a formulation tank before the liquid was drained to the dye bath trough. There was no

local exhaust at the tank for the pad dyer. Only minor steaming was evident, and no local ventilation was used during this operation.

Survey Description

The plant management and the American Textile Manufacturers Institute (ATMI) were notified in advance of the survey and its intent and design, and the study protocol was sent to each for review before the survey was conducted. After an opening meeting with representatives of the plant and the ATMI (no union exists at the plant), a walk-through survey of the drug room and dyeing departments was conducted. This familiarized the NIOSH investigators with the operations in which potential exposure to benzidine dyes might occur and allowed them the opportunity to select the employees that should be asked to participate in the study. In all, seven employees (four dye weighers, two pad dyeing operators, and one jig dyeing operator) were included. Each worker was informed of the reasons for the study and the benefits of participation; in addition, each was given a consent form, which confirmed an understanding of the study's purpose and a willingness to participate.

The survey, which intended to identify benzidine in the urine of workers, was limited to monitoring these employees for only one day. All air filter and urine samples were collected in the manner described previously. The air filter and urine sampling continued throughout most of the work shift, and some urine samples were returned after first void the following morning. All samples were frozen in dry ice. Two benzidine-derived dyes, Direct Black 38 and Direct Blue 2B (both from GAF Corp.), were heavily used on the day of the survey. Approximately 2 ounces of each were taken for residual free benzidine analysis.

Analysis and Results

Urine sample results from 23 NIOSH office workers, which were used as controls and are reported in Table XII, were compared with the survey results that were expressed as "aromatic amines as benzidine." Table XIII shows that production worker results were higher than office worker results in all cases.

The environmental and urinary sampling results have been summarized in Table XIII according to job category. A detailed description of the environmental sampling parameters is given for each sample in Table XIV. Airborne dust concentrations were in all cases below the OSHA

nuisance dust standard of 15 mg/m3 of air. Bulk sample analysis results for residual benzidine in the three benzidine-derived dyes being used at the facility are given below:

Dye Used	Residual benzidine (w/w)
GAF Black JXA (Direct Black 38)	13 ppm
GAF Blue 2B* (Direct Blue 2)	1 ppm
GAF Black ER-200* (Direct Black 38)	4 ppm

*Used during the time of the survey.

Table XIII shows that urinary benzidine and/or monoacetylbenzidine were found in three of seven workers who were monitored. Two of these workers were dye weighers; one was a pad dyer operator.

Table XII. NIOSH control urine results.

Individual	Aromatic Amines* (ng/100ml)	Approximate ppb	TLC** Confirmation
1++	205	2	ND
2	ND	< 1	ND
3	ND	< 1	ND
4	ND	< 1	ND
5	100	1	ND
6++	ND	< 1	ND
7	ND	< 1	ND
8	120	1.2	ND
9+	300	3.0	ND
10	ND	< 1	ND
11	ND	< 1	ND
12	ND	< 1	ND
13	ND	< 1	ND
14	ND	< 1	ND
15++	ND	< 1	ND
16	ND	< 1	ND
17	ND	< 1	ND
18	145	1.4	ND
19	100	1.0	ND
20	100	1.0	ND
21	200	2.0	ND
22	ND	< 1	ND
23	ND	< 1	ND
Standard 1 (Bzd)	100	1.0	Positive
Standard 2 (Bzd)	250	2.5	Positive
Standard 3 (Bzd)	350	3.5	Positive

*Expressed as benzidine.
**Thin-layer chromotography
+Allergy medication taken
++Pipe smokers

Note: Lower limit of detection is 100 ng/100 mL of urine.

Table XIII. Environmental and urinary excretion in textile dryers at Facility C.

Job Description	Envir. Conc.*	Urinary Excretion			Notes
		Aromatic Amines	Benzidine	Monoacetyl-benzidine	
Dye Weigher I	1) 1.39 mg/m^3	3.2 ppb 4.4 ppb	ND	ND	Wore no respirator. General ventilation only.
Dye Weigher II	2) 1.06 mg/m^3	8 ppb	benzidine confirmed by TLC		Wore no respirator. General ventilation only. Boiled up dye by hand.
		5.8 ppb	39 ppb	5 ppb	
Dye Weigher III	3) 2.99 mg/m^3 4) 1.15 mg/m^3 1.68 TWA	5 ppb	1 ppb	7 ppb	No respirator worn. Beginning of shift-dustiest.
Dye Weigher IV	5) 3.93 mg/m^3 6) 1.20 mg/m^3	3 ppb	ND	ND	No respirator worn. Beginning of shift dustiest.
Pad Dye Operator I	7) 1.07 mg/m^3	4.5 ppb	ND	ND	Carried dye solution to dye baths and diluted to desired conc. Spent 80% of time in non-exposure area loading gray goods into Pad Dyeing Machine. Wore no respirator. No contact with dry dye.
Pad Dye Operator II	8) 1.12 mg/m^3	9 ppb	benzidine confirmed by TLC		
		13 ppb	16 ppb	38 ppb	

Table XIII. (continued).

Job Description	Envir. Conc.[1]	Urinary Excretion: Aromatic Amines	Urinary Excretion: Benzidine	Urinary Excretion: Monoacetyl-benzidine	Notes
Jigg Dyer	9) 3.88 (?)	3.4 ppb			Wore no respirator.
	10) 1.40	3.0 ppb			Spent much time near
	1.98 TWA	4.0 ppb	ND	ND	steamy jigg baths making
		3.2 ppb	ND	ND	adjustments on cloth rolls.
					No contact with dry dyes.
	Area Sample				
	11) 2.14 mg/m^3				
	Area Sample during next work shift				
	12) 2.14 mg/m^3				
	13) 1.70 mg/m^3				
	1.81 TWA				

*Concentrations expressed as airborne particulates per cubic meter of air samples.

Table XIV. Environmental data for Table XIII.

Field No.	mg/filter Total wt.	Air Volume (liters)	mg/m^3	λmax* (nm)	mg/filter Azo Dyes	Percent Azo Dyes
1	0.61	437.5	1.39	N	<0.010	0
2	0.46	432	1.06	N	<0.010	0
3	0.69	231	2.98	577	0.148	21.4
4	0.57	495	1.15	N	<0.010	0
5	0.93	236	3.94	577	0.270	29.0
6	0.60	499	1.20	577	0.133	22.2
7	0.46	430.5	1.07	N	<0.010	0
8	0.82	735.0	1.12	N	<0.010	0
9	0.53	136.5	3.88	N	<0.010	0
10	0.65	463	1.40	N	<0.010	0
11	0.27	126	2.14	N	<0.010	0
12	0.53	136	3.89	N	<0.010	0
13	0.57	355	1.70	N	<0.010	0

*λmax.: 577 for Black ER-200;
577 for Blue 2B

2. Facility D

Description of the Plant

Facility D is a moderate-sized manufacturer of textiles for use in making cotton disposable work gloves. The plant consists of the main production building where raw cotton is received, cleaned, spun into thread, weaved into cloth, and finished before shipping. Most of the 370 workers employed over 3 shifts are employed in areas other than the finishing departments, where potential chemical exposure can occur. There are also two warehouses adjacent to the main building. The workforce is approximately evenly distributed by sex.

Description of Process

Since worker exposures to dyes was the only area of concern in this study, the process under observation was limited to the finishing department. The work process in this department is as follows: The knitted cloth is sent to the finishing department in rolls. There the cloth is dyed, then napped, dried, and steam pressed. After this it is rolled up or folded for shipment. The dye bath solution is prepared in a separate room where it is weighed on scales, put into boil-up tubs, and then gravity-drained to loop dyeing machines, as needed. Four benzidine-based dyes in moderate use were Direct Blue 6, Direct Brown 95, Direct Black 38, and Direct Red 8. One dye weigher is on duty per shift, and four workers attend the dyeing tubs. Dye tub operators load the fabric into loop dyers where it is agitated for approximately 2 hours at 200-212 degrees F. The dyed fabric is removed manually and the excess liquid is squeezed out through ringers. The fabric is then transferred to napping machines and afterwards dried in ovens at approximately 300 degrees F. Finally, the cloth is steam pressed and rolled up for shipment.

Medical, Safety and Industrial Hygiene Programs

A full-time nurse and a physician on call are available as needed by the employees. An assigned safety supervisor is responsible for periodic meetings on safety and plant inspections. No formal industrial hygiene program exists; however, this facility has been monitored for cotton dust by its insurance carrier.

Survey Methods

The plant management and the ATMI were notified in advance of this survey, and the study protocol was sent to them. After an opening meeting with the company's management, a walk-through survey was conducted of the drug room and finishing department. Certain workers were then selected and asked to participate in this study, at which time the need for the study and the benefits to them were explained. In all, ten workers participated in the survey, which combined environmental airborne dust monitoring with urine sampling. This continued for two continuous work shifts. During this time, these workers wore Mine Safety Appliance (MSA) Model G pumps calibrated to filter air through 37-mm glass fiber filters in three-piece cassettes at 1.75 liters per minute. Air sampling continued throughout most of the two work shifts. Urine samples were taken during the work period and were frozen in dry ice immediately after collection. Eight bulk dye samples were collected for use as standards for azo dye determination on the filter samples and for free residual benzidine analysis.

Results and Discussion

Table XV summarizes the environmental and urine sampling results according to job description. All environmental results are breathing zone air filter samples expressed as time-weighted averages (TWA's), unless otherwise indicated. The sampling time was 6 hours or more. When more than one urine specimen was collected, the results are reported in the order of collection. Individual samples from the same worker are separated by a dotted line.

Supplemental information on the environmental data in Table XV is reported in Table XVI. This table shows the sample air volumes, gravimetric filter sample results, calculations to airborne concentration, spectrophotometric absorbance maxima (λ max.), azo dye concentrations (calibrated from calibration curves for that corresponding dye), and the estimated azo dye percentage, by weight, on each sample. It is difficult, however, to accurately determine the azo dye content on air filter samples because the absorbance spectra for each dye submitted as a standard may overlap. Therefore, only the dye with the greatest corresponding peak is calculated and reported. Thus, the total quantity of azo compounds is not reported but only the concentration that is estimated from one standard dye. Those samples with no reported azo dye concentration probably did not contain any dye since most dyes being used during this survey were submitted as standards.

Eight bulk benzidine-based dye samples were collected for the above procedure. In addition, the concentration (in parts per million) of residual-free benzidine was determined. These results are reported in Table XVII.

Aromatic amines found in the urine of 23 NIOSH office workers (Table XII) indicate that fewer than 35% excreted more than one ppb, while 40% of the sample subjects at Facility D excreted greater than 1 ppb. Analysis did not reveal benzidine in any individual's urine. However, a trace quantity of monoacetylbenzidine was found in Dye Weigher I. It is interesting that the environmental exposure of this individual (1.45 mg/m^3) was the highest of all the workers who were monitored in this facility. Also, one of two dye tub operators with elevated aromatic amine levels had an estimated environmental exposure to 1.58 mg/m^3 of total particulate, while the average for five other operators was only 0.60 mg/m^3. One explanation for these differences is individual work practices since each worker, by job description, performed the same tasks. Of course, ingestion of dyestuffs through poor personal hygiene habits may also play an important role as a route of exposure to aromatic amines and benzidine. The role of skin penetration of direct class dye is not clearly understood, but is presumed to be negligible since these dyes are sulfonated and thus not lipid or fat soluble.

C. Leather Finishing

1. Facility E

Description of Plant

Facility E is a relatively large tannery and finisher of cattlehide leather. About 365 workers are employed in production jobs and about 35 in administrative jobs. The majority of the workers are employed during the first and second shifts. Nearly all production workers are male. Structures on the site are between 5 and 100 years old; most of the present facility was built within the past 15 years.

Table XV. Results of urine and environmental monitoring by job classification at Facility D.

Job	Environmental Conc.*	Urinary Concentrations			Notes
		Aromatic Amines	Benzidine	Monoacetyl-benzidine	
Dye Weigher I	1) 1.54 mg/m^3	4 ppb			Weighed dyes in Drug Room before dissolving in boil-up tubs. Worker sometimes wore a half-face pad type respirator and gloves when weighing dyes. General ventilation from roof exhaust fans only.
	2) 1.31 mg/m^3	4.8 ppb	ND	4 ppb	
	1.45 mg/m^3 TWA	3.6 ppb	ND	ND	
Dye Weigher II	3) 1.15 mg/m^3	ND			Same as above.
	4) 1.11 mg/m^3	ND	ND	ND	
	1.13 TWA	1.3 ppb			
	Area sample over scales				
	5) 0.55 mg/m^3				
Dye Tub Operator I	6) 5.31 mg/m^3 (void)	ND			Worker loads and unloads cloth from rolls to and from dye tubs. Tubs were ventilated by top hood exhaust and had front hood moveable doors. No respirators worn. Rubber gloves worn sometimes. Worker is splashed by dye liquor during work.
		ND			
Dye Tub Operator II	7) 0.90 mg/m^3	ND			Same as above.

*Environmental concentrations expressed as total airborne particulates per cubic meter of samples air.

Table XV (continued).

Job	Environmental Conc.*	Urinary Concentrations			Notes
		Aromatic Amines	Benzidine	Monoacetyl-benzidine	
Dye Tub Operator III	8) 1.58 mg/m^3	3.2 ppb			Same as above.
Dye Tub Operator IV	9) 0.67 mg/m^3	ND			Same as above.
Dye Tub Operator V	10) 0.63 mg/m^3	3.2 ppb			Same as above.
Dye Tub Operator VI	11) 0.20 mg/m^3	ND ND ND			Same as above.
Dye Tub Operator VII	12) 0.60 mg/m^3	ND			Same as above.
Roll-up Machine Operator	13) 0.48 mg/m^3	ND			Worker operates steam-press roll-up machine. Considerable heat and resultant steam evolved. No respirator worn.

Table XVI. Supplemental environmental data to Table XV.

Sample No.	Air Volume Samples (liters)	Total Dust per filter	Total Dust in mg/m^3	λ Max.*	Azo Dye per filter	Percent Azo Dye per filter
1	455	0.85	1.54	450	0.076	9
2	320.2	0.57	1.31	450	0.060	10.5
3	407.8	0.62	1.15	495	0.065	10.5
4	488.3	0.69	1.11	495	0.160	23.2
5	728.0	0.55	0.55	N	-	-
6	740.2	4.08	5.31	N	-	-
7	735.0	0.81	0.90	N	-	-
8	728.0	1.30	1.58	N	-	-
9	731.5	0.64	0.67	N	-	-
10	666.8	0.57	0.63	N	-	-
11	750.8	0.30	0.20	N	-	-
12	749.0	0.60	0.60	N	-	-
13	745.5	0.51	0.48	N	-	-

*λmax.: 450 for Brown 3GN;
495 for Black GX and Seal Brown C.F.

Table XVII. Residual benzidine analysis results.*

Dye Name	Benzidine Concentration in ppm (w/w)
1. Direct Brown 3GN (C.I. Direct Brown 95)	<1
2. Direct Scarlet 8B (C.I. Direct Orange 8)	<1
3. Direct Orange WS (C.I. Direct Orange 8)	7
4. Direct Black GX (C.I. Direct Black 38)	20
5. Seal Brown CF (C.I. Direct Brown 95 and Direct Black 38)	4
6. Direct Blue 2GF (C.I. Direct Blue 2)	<1
7. Direct Black OB (C.I. ?)	<1
8. Direct Black BH (C.I. ?)	<1

*Residual level of detection is less than 1 ppm (w/w).

Description of Process

Both raw and salted cattlehides are received by truck at the facility. The hides are quickly dehaired in rotating bins containing calcium hydroxide and sodium sulfhydrate. Next, the skins are washed and bated (this removes the outer epidermis and residual proteins), pickled in an acid solution, and tanned with basic chromium sulfate. The above three operations can be performed in the same vessel. The tanned skins are trimmed and split and shaved to the desired thickness. Coloring is performed in rotating drums where either direct, acid, or basic dyestuffs are used. The dye drum operators are responsible for dissolving the dyestuffs they receive from the dye weigher. There are between five and seven dye drum operators and one dye weigher working with dyes each day. The dyed skins are dried either by the vacuum drying or pasting method. Finishing may include applying pigments, lacquers, and other chemicals to obtain the desired color and texture in the product.

Description of Survey

Facility E used only a minor amount of benzidine-based dyes when compared to the total amount of dyestuffs it consumed. However, by scheduling production orders to coincide with this survey, only benzidine-based dyes were handled during the monitored period. Personal air sampling (using 37-mm glass fiber filters in three-piece closed face cassettes with MSA Model G sampling pumps) was performed on three workers, and the sample flow rate was 1.75 liters per second. All workers were monitored during the entire length of their work shift.

Urine samples were taken the next day during the first shift at the first void and during the morning of the next day's shift. All samples were immediately frozen in dry ice and analyzed by the electron-capture gas chromatographic method (177).

Results and Discussion

A summary of the environmental air concentration results and the urinary benzidine excretion results are reported by job classification in Table XVIII. Only the dyestuff weigher had substantial relative potential exposure to airborne dyestuff particulates; his 8-hour time weighted average exposure (TWA) was 10.65 mg/m^3. However, this worker's actual respiratory exposure was almost certainly

much lower because he wore a half-face (NIOSH-approved) cartridge respirator during the weighing operation.

None of the urine samples collected from the dye weigher or the two dye drum operators contained any detectable benzidine or monoacetylbenzidine. Since urine samples were collected during the exposure day, after a first morning void, and on the following morning, we are confident that any benzidine in the workers' bodies would have been excreted during the collection period.

Because the potential exposure group at this facility was very small, the conclusions based upon the recorded data should be interpreted with caution. Nonetheless, it is highly probable that appropriate work practices prevent the detection of discernable amounts of benzidine in the urine of these workers. The practices of wearing respirators, eating in a clean lunch facility, using shower and wash facilities after work, and changing out of work clothing after work probably contribute to these negative results.

Supplemental data on the environmental samples is presented in Table IXX. The results are given as milligrams per cubic meter of total particulates and as percent azo dyes. The latter results are not absolute. The primary absorbance peak in all samples is for C.I. Direct Brown 95; therefore, only the approximate dust concentration for this dye is reported. Additional exposure to C.I. Direct Black 38 may also have occurred but cannot be defined by this method.

In summary, the percent azo dye (C.I. Direct Brown 95) on each sample in which it was detected ranged from 22% to 48% by weight. This confirms that the airborne dust exposure to the dye weigher contained a significant percentage of benzidine-derived dye in addition to other non-dye or non-benzidine dye particulates.

D. Paper Company

1. Facility F

Description of Plant

Facility F dates back to 1867 when it originally produced newspaper stock. The present building structures are approximately 30 years old. Today, the facility produces only speciality papers of various colors and textures. The plant employs approximately 80 production workers (mostly male) and 20 administrative personnel. There are three work shifts, but most employees (58) work the day shift.

Table XVIII. Summary of results by job.

Job Description	Environmental Airborne Particulates	Urinary Excretion	Notes
Dyestuff Weigher	1) 12.05 mg/m^3 2) 12.95 mg/m^3 3) 14.72 mg/m^3 4) 1.27 mg/m^3 10.65 mg/m^3 TWA*	Day 1 ND† ND Day 2 ND ND	Only benzidine-derived C.I. Direct Black 38 and C.I. Direct Brown 95 were used. Dye weigher spent 80% of time weighing these dyes into paper bags. Half-face cartridge respirator was worn during weighing.
Dye Drum Operator I	5) 1.42 mg/m^3 6) 0.44 mg/m^3 7) 0.00 0.69 mg/m^3 TWA	Day 1 ND ND Day 2 ND ND	Operator picks up bagged dyes from weigher and empties bag into solvating tub. Potential exposure would only occur during emptying. Both operators wore half-face cartridge respirators during dye handling. Other responsibilities include loading and unloading dyed hides from dye bin.
Dye Drum Operator II	8) 1.12 mg/m^3 9) 1.65 mg/m^3 10) 16.79 mg/m^3 5.79 mg/m^3 TWA	Day 1 ND ND Day 2 ND ND	

*Time Weighted Average
†Benzidine not detectable

Table XIX.Supplemental environmental data for Table XVIII.

Sample No.	mg/filter total wt.	Air Volume (liters)	Airborne Concentration mg/m^3	λ max* (nm)	mg/filter Azo Dye	Percent Azo Dye
1	1.41	117.0	12.05	450	0.68	48
2	2.59	200.0	12.95	450	1.82	70
3	2.57	174.6	14.72	450	1.16	45
4	0.18	142.2	1.27	N†	ND‡	0
5	0.33	232.2	1.42	450	0.07	21
6	0.04	203.4	0.44	N	ND	0
7	-0.07	168.8	0	N	ND	0
8	0.27	241.2	1.12	450	0.06	22
9	0.27	163.8	1.65	N	ND	0
10	2.75	163.8	16.79	N	ND	0

*Dye Name — Absorbance λmax

GAF Direct Black 38 — 525

GAF Direct Brown 95 — 450

†No absorption max observed in the visible spectrum

‡Not Detectable

Description of Process

The overall production process can be briefly described as follows. Bleached kraft paper and/or recycled paper is added to large agitating tubs called "beaters." The paper is quickly pulped in a water solution to a uniform consistency. To this pulp, dyes, fillers (such as talc), clay, and titanium dioxide are added. Three workers are responsible for the general material handling; however, only one worker per shift weighs the dyes. When ready, this homogeneous mixture is pumped to holding tanks and is then continuously fed to the fourdrinier machine, which makes the paper. The combined action of pressure and the drying heat forms the paper. Using modified starch, dry sizing is applied to the paper surface. A finishing operation, which some paper undergoes, is performed by texture presses. Cutting and trimming of the paper into sheets is the final step before shipment.

The only job categories with which this study was concerned were those of the beater operators and dye weighers. The beater room contained three beater tubs where the paper was pulped. On each shift two beater operators and a beater operator engineer were present. The engineer was responsible for weighing the dyes and adding them to the beater tubs. Small quantities of dye (less than ten pounds) were weighed in a room containing three small weighing scales. A permanent wall exhaust fan was installed over these scales to remove airborne dust away from the workers' breathing zone. Another larger scale was located nearby for weighing large quantities of dyes. (Dye-filled drums were stored in this area for future use.) A high volume propeller-type fan was mounted in the ceiling near the large weigh-scale and near a beater tub. This fan was primarily used for general ventilation purposes; however, part of the fan was blocked off and duct work leading to the large weighing scale installed. It is questionable whether the system provided enough exhaust to adequately control airborne dusts that are generated when weighing dyes. At the time of this survey no hood was attached to the duct over the scales, but the company indicated that it was obtaining one. The exhaust fan was used only when particularly dusty operations were being performed. When the fan was not turned on a downdraft from the duct over the scale was noted. Using ventilation smoke tubes, investigators noted little other general air movement in the room.

Description of Medical, Industrial Hygiene, and Safety Programs

Facility F requires all new employees to take a general medical exam. No future examinations are provided. This exam, however, does not include a chest X-ray, hearing test, lung function test, blood test, or urine test. A nearby physician is on call.

Industrial hygiene measurements had not been taken in the facility before the survey. The facility has a formal safety program; however, the presence of at least one individual on each work shift with formal first-aid training is doubtful. Four management personnel are involved in this program. (One aspect of this program is respiratory protective equipment maintenance.) The facility requires the use of protective equipment; this includes wearing protective goggles when near beaters, wearing safety shoes (only for certain individuals), and wearing a face respirator when weighing dyes. However, the use of such equipment is not enforced. There is not a separate lunch room, and washing facilities are not readily available in designated "clean areas." Shower facilities are available in locker rooms, but these were not used during this survey. Workers usually wear their work clothes home.

Description of Survey Methods

The company reveived notice of the intended study 1 month in advance. The survey team arrived at the facility on Sunday, March 5, 1978, and departed on Friday, March 10, 1978. It had two objectives: (1) Long-term urine monitoring to obtain excretion data before, during, and after environmental exposure to benzidine-derived dyes, and (2) personal and area air filter sampling to obtain data on airborne particulates.

The survey team conducted the monitoring during all three work shifts. Sampling began on day two, shift three, and continued until day five, shift one. Sampling was performed only during that period in which benzidine-derived dyes were used. Six operators wore personal air filter pumps during the exposure period. Each sample represents at least a 7-hour sample period. In addition, area samples were collected near the weigh scales and near the beater tubs. Four respirable samplers (with 10-mm pre-cyclones) were placed next to a total particulate air filter sampler near the large weighing scale. (This type of testing has been performed in other facilities where benzidine-derived dyes were used, and the ratio of

respirable to total dust averaged one to four). Glass fiber filters were used in three-piece plastic cassettes. Total dust samples were collected at a flow rate of 1.75 liters per minute. In all, 28 filter samples were collected.

Nine bulk dye samples were collected for use as color standards and for residual benzidine analysis.

All urine samples were collected when the workers could provide them, and the samples were immediately labeled and frozen on dry ice in an insulated storage container.

Results

Table XX summarizes in chronological order the environmental exposure data and the urinary excretion findings. Prior to day two, shift three, of the study period only non-benzidine dyes were used. The use of benzidine-derived dyes began on day two and continued through day five, shift one. Of the 21 study samples analyzed for aromatic amines, 57% showed benzidine levels greater than 1 ppb; 7% showed levels greater than 1.5 ppb. While the study group samples generally contained greater than 1 ppb more often than the NIOSH non-exposed comparison group, excretion of 1.5 ppb or greater was seen less often in the study group than in the control group. Based on this screening technique, the study group did not appear to have been largely exposed to azo dyes at the time of this survey.

Using electron capture gas chromatography, 47 urine samples were analyzed for benzidine and monoacetylbenzidine. Of the seven workers monitored, four excreted benzidine and/or monoacetylbenzidine. Concentrations excreted were near the minimum level of detection. Another excretion product associated to the use of Direct Black 38 was also identified in three workers. This was 2,4-diaminoazobenzene (DAAB) - a confirmed animal carcinogen. The detail s of metabolism of this compound are not known but it is possible that it is a contaminant or intentional additive to Direct Black 38 in some formulations, rather than a metabolite of Direct Black 38. This has been shown to be the case with Direct Black 38 fed to the hamster at NCTR, but the metabolism appears different.

The personal and area air filter samples are shown in Table XX as milligrams of total particulates per cubic meter of air. Both the dye weigher and operator had similar potential total dust exposures. However, further analysis of the data in Table XXI shows that, as expected, the dye weigher's

exposure to azo compounds was greater than the operator's. It should be noted that each dye weigher was wearing a half-face respirator with a toxic insecticide cartridge, so his actual exposure would have been lower than that reported in Table XX.

The airborne dust concentrations near the weigh scales were low in all cases. The degree of dustiness of the various dyes can be seen by the three-fold increase in airborne dust when Direct Black 38 from an alternate manufacturer was used (see sample 22, Table XXI).

An attempt to compare respirable airborne dust with total airborne dust was not wholly successful since a wide range of ratios was obtained. However, as Table XXII shows, three of the four sample pairs contained greater total dust than respirable dust.

It is surprising that benzidine was not found more often in the urine of some of these workers. An estimated 2500 pounds of Direct Black 38 was used during this survey. Since 18 pounds to 52 pounds of Direct Black 38 was used in each beater batch, a large number of individual weighings took place--each with the potential for generating airborne dust. Two possible explanations for the marginal findings include: (1) the combination of engineering controls and personal protective equipment was sufficient to control exposure to near the lower limits of that which could be monitored during this survey, and (2) body burden and/or enzyme induction may play some role in the human excretion of benzidine. All previous workers who excreted benzidine had potentially long-term exposure to benzidine-derived dyes while the present workers did not. While the first explanation appears more acceptable, animal metabolism studies that are underway may test the second hypothesis more closely.

Table XX. Summary of monitoring results from a paper plant.

Day	Shift	Sample Location	Env. Conc. (mg/m^3)	Urine Conc. (Parts/billion) Aromatic Amines	Benzidine	Notes
1	1	Dye Weigher I		ND		Dye Weigher weighed all dyes and delivers to dye tub. Worker wore respirator during weighing.
		Operator I			ND	Operator delivered pulp by forklift and maintained dye tub operation.
1	2	Dye Weigher II		1	ND	
2	1	Dye Weigher I			ND	Worker wore respirator when weighing dyes.
					ND	
				ND	ND	
		Operator I			ND	
2	2	Dye Weigher II			ND	Worker wore respirator when weighing dyes.
				2	ND	
		Operator II			ND	
				ND	ND	
2	3	Dye Weigher III		1	ND	Began using Direct Black 38 towards end of this shift.
				ND	ND	
		Operator III		1.3	ND	
		Lg. Weigh Scale	1) 0.65 mg/m^3		ND	
		Sm. Weigh Scale	2) 0.36 mg/m^3			
3	1	Dye Weigher I	3) 3.30 mg/m^3	1.4	ND	First shift using Direct Black 38
		Operator I	4) 2.31 mg/m^3		ND	First shift using Direct Black 38
		Lg. Weigh Scale	5) 0.36 mg/m^3			
		Sm. Weigh Scale	6) 0.70 mg/m^3			

Table XX (continued)

Day	Shift	Sample Location	Env. Conc. 1 (mg/m^3)	Urine Conc. (Parts/billion) 2		Notes
				Aromatic Amines	Benzidine	
3	2	Dye Weigher II	7) 1.61 mg/m^3	2.2	ND	Using Direct Black 38
		Operator II	8) 3.70 mg/m^3	1.3	ND	
		Lg. Weigh Scale	9) 0.17 mg/m^3			
			10) 0.17 mg/m^3			
3	3	Dye Weigher III	11) 2.85 mg/m^3	ND	ND	Using Direct Black 38. Worker wore respirator when weighing dyes.
				ND	ND	
		Operator III	12) 2.64 mg/m^3	1.3	ND	
		Lg. Weigh Scale	13) 0.53 mg/m^3			
		Sm. Weigh Scale	14) 0.50 mg/m^3			
4	1	Dye Weigher	15) 3.43 mg/m^3	ND	ND	Using Direct Black 38.
				ND	3MAB	
				1		
		Operator I	16) 5.10 mg/m^3		ND	
		Area at Pulper 2	17) 1.41 mg/m^3		ND	
		Lg. Weigh Scale	18) 0.58 mg/m^3			
		Sm. Weigh Scale	19) 0.91 mg/m^3			
4	2	Dye Weigher II	20) 2.51 mg/m^3		ND	
					ND	
					ND	
		Operator II			ND	
		Area at Beater 2	21) 0.56 mg/m^3			
		Lg. Weigh Scale	22) 1.43 mg/m^3			

Table XX (continued)

Day	Shift	Sample Location	Env. Conc.* (mg/m^3)	Urine Conc. (Parts/billion)2 Aromatic Amines**	Benzidine+	Notes
4	3	Dye Weigher III	23) 2.26 mg/m^3		ND ND ND	Using Direct Black 38
		Operator III	Void		2MAB	
4	3	Operator IIIA			3MAB 3DAAB	
5	1	Dye Weigher I		4.9	3MAB 1Bzd 32DAAB	Stopped using Direct Black 38 towards end of this shift
		Operator I		2.9	1Bzd 5DAAB	
5	2	Dye Weigher II		2.6	ND ND	
		Operator II Area at Pulper I	24) 2.93 mg/m^3	ND	ND	Using Carbon Black
5	3	Dye Weigher III			2DAAB	Washed down equipment for next color run
		Operator III			ND 2MAB	
		Operator IV A			2MAB	
6	1	Dye Weigher I			8MAB 2DAAB	
		Operator I			ND	

*Concentrations expressed as milligrams of total airborne particulates per cubic meter air
**Concentration of non-specific colorimetric procedure with the limit of detection at 1 ppb
+Concentration of specific aromatic amines in ppb with the following detection limits: benzidine 1.0 ppb, MAB, 1.8 ppb; DAAB, 0.8ppb; diacetylbenzidine, 0.2ppb.

Table XX (continued)

	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6	
	Env. Conc.* (mg/m^3)	Urine (ppb)	Env. Conc. (mg/m^3)	Urine (ppb)	Env. Conc. (mg/m^3)	Urine (ppb)	Env. Conc. (mg/m^3)	Urine (ppb)	Env. Conc. (mg/m^3)	Urine (ppb)	Env. Conc. (mg/m^3)	Urine (ppb)
Worker I	-	N.D+ (N.D)++	-	N.D (N.D)	3.3	N.D (1.4)	3.4	3 MAB (1)	Void	3 MAB 1 Bzd 32 DAAB (4.9)	-	8 MAB 2 DAAB
Worker II	-	N.D	-	N.D (N.D)	2.3	N.D -	5.1	N.D -	-	1 Bzd 5 DAAB (2.9)		N.D
Worker III	-	N.D (1.0)	-	N.D (2.0)	1.6	N.D 2.2	2.5	N.D -	-	N.D (2.6)		
Worker IV	-		-	N.D (N.D)	3.7	N.D (1.3)	Void	N.D	-	(N.D) (N.D)		
Worker V				N.D (1.0)	2.9	N.D (N.D)	2.3	N.D -	-	2 DAAB (N.D)		
Worker VI			-	N.D (1.3)	2.6	N.D (1.3)	Void	2 MAB -	-	2 MAB		
Worker								3 MAB 3 DAAB		2 MAB		

* environmental concentrations expressed as milligrams total airborne particulates per cubic meter of air.
\+ concentration of specific aromatic amines in ppb with the following detection limits:
benzidine, 1.0 ppb; monoacetylbenzidine, 1.8 ppb; 2,4-diaminoazobenzene, 0.8 ppb; diacetylbenzidine, 0.2 ppb.
++ concentration of non-specific colorimetric precedure with the limit of detection at 1 ppb.

NOTE: Area within heavy black line signifies period of Direct Black 38 useage.

Table XXI. Supplemental data to Table XX.

Field No.	mg/filter Total Wt.	Air Volume (liters)	Calculated mg/m^3	λ max* (nm)	mg/filter Azo Dyes	Percent Azo Dyes
1	0.49	749	0.65	N	<0.01	--
2	0.24	744	0.35	400	0.19	79
3	2.42	734	3.30	410	0.19	7
4	1.84	796	2.31	N	<0.01	--
5	0.29	807	0.36	550	U	U
6	0.56	796	0.70	400	0.10	18
7	1.28	793	1.61	410	0.42	33
8	2.93	791	3.70	485	0.04	1
9	0.13	773	0.17	N	<0.01	--
10	0.13	768	0.17	405	0.17	100
11	2.20	773	2.85	490	0.08	4
12	2.06	779	2.64	N	<0.01	--
13	0.42	793	0.53	N	<0.01	--
14	0.40	791	0.50	N	<0.01	--
15	2.52	735	3.43	490	0.12	5
16	2.90	569	5.10	N	<0.01	--
17	1.12	796	1.41	485	0.12	11
18	0.46	796	0.58	485	0.09	20
19	0.32	350	0.91	N	<0.01	--
20	1.93	786	2.51	485	0.25	13
21	0.22	394	0.56	N	<0.01	--
22	1.09	760	1.43	485	0.40	37
23	1.69	749	2.26	485	0.32	19
24	1.94	663	2.93	N	<0.01	--

Key to Abbreviations:

N	None
U	Unknown dye
*	Absorbance wavelength maximum

Absorbance Maxima*

λ	Dye Name	λ	Dye Name
400	Paper Direct Brown NPC	490	Orange RO
405	Paper Direct Brown NPC	510-535	Direct Black 38E
410	Paper Direct Brown NPC	595	Phenamine Black E 200
485	Orange 5R	580	Phenamine Blue BB

Table XXI. Comparison of respirable to total air particulate samples at a large dye weighing scale.

Sample Type	Environmental Concentration*	Ratio
Respirable	0.35	
Total	0.53	1:1.2
Respirable	0.11	1:5.2
Total	0.58	
Respirable	0.00	--
Total	0.36	
Respirable	0.26	1:0.7
Total	0.17	

*Given in (mg/m^3).

Chapter 12

DISCUSSION

In all, urine samples were collected over varying lengths of time from 38 industrial employees who were potentially exposed to benzidine-derived dyes. In 12 of the 38 workers monitored, benzidine was found in quantities ranging from 1 ppm to 112 ppb and/or monacetylbenzidine (MAB) found in quantities ranging from 1 ppm to potentially exposed workers.

Evidently, benzidine-derived dyes can be used in the work place without detecting benzidine or its metalolites in the urine if more prudent controls can be defined for their use. Inadequate environmental control of exposures to these dyes may be defined by analyzing those instances that led to the excretion of benzidine and/or MAB in the urine of the twelve workers. A comparison of the exposure data from the positive benzidine-excretion workers with exposure data from the nonbenzidine-or MAB-excretion workers could presumably reveal an acceptable level of work exposure to benzidine-derived dyes. Some individual case discusstions follow.

At Facility B, a dye manufacturer, all workers who were monitored excreted detectable levels of benzidine and MAB. The level of exposure was obvisously unacceptable. Environmental air filter sampling at each worker's breathing zone and general area samples revealed elevated airborne concentrations of particulates above 5 mg total particulates per cubic meter of sampled air (see Table X). Frequent leaks and uncontrolled sources of emissions allowed potentially very high, short term exposures. Table VI shows that a blending department operator experienced a 92.7 mg/m^3 exposure over a 16-minute period while filling dye drums for shipment.

The greatest excretion concentration of benzidine in Facility B was observed in a spray dryer operator who excreted 112 ppb benzidine, 590 ppb MAB, and 50 ppb 3,3'-dimethoxybenzidine. Since only benzidine-derived dyes were being processed during that day's workshift, the presence of the dimethoxy derivatives may suggest a previous day's exposure to that compound. Furthermore, the 17.4

mg/m^3 area sample results collected near the drumming chute are likely to be greater than this worker's actual exposure, based upon observations of his work routine.

An investigation of the thermal stability of 15 direct dyes in aqueous solutions at various temperatures ranging from 100 degrees C to 140 degrees C indicates that benzidine-derived dyes may substantially decompose in the higher temperature range. Little or no decomposition occurred at the boiling point over a 1- to 2-hour period. However, at 140 degrees C (284 F), 6% to 24% of the initial dye concentrations decomposed after two hours (155). The exact decomposition products are unknown; however, the weak nature of the azo bonds would suggest a possible reduction to the component amines. Furthermore, many textile drying ovens operate between 140-205 degrees C (284 F) but average 163 degrees C (325 F). The decomposition of benzidine-derived dyes in textiles at the temperatures reached in drying ovens should be evaluated to determine if a potential exposure to benzidine might occur in textile workers handling the finished goods.

The worker who excreted 112 ppb benzidine and 590 ppb MAB operated a spray dryer that operated at temperatures ranging from 232 degrees C (450 F) to 260 degrees C (500 F). At these temperatures it is feasible, based upon the reported thermal instability of dyes at elevated temperatures, that this worker's exposure were due to inhalation and skin absorption of the volatilized amine. Gas chromatography/mass spectrometer analysis of two silica gel air sample tubes collected at the spray dryer and primary solution tanks identified only azo benzene as a major compound. The presence of azo benzene may be accounted for in the in situ rearrangement of hydrazobenzene, which was a primary starting material (156). However, benzidine was not identified in these samples; this weakens support for the above dye decomposition hypothesis.

While the spray dryer operator's exposure to benzidine may not have been entirely due to only inhalation of the finished benzidine-derived dyes, all other workers who were monitored and who were exposed only to the finished dyestuffs also excreted benzidine in their urine. Airborne dust levels of the dyestuffs were quite high; thus, a potential inhalation exposure to benzidine-derived dyes existed.

In Facility C, a textile finishing plant, two out of four workers who weighed dyestuffs had benzidine or MAB in their urine. Dye Weigher II, in whom 39 ppb urinary benzidine and 5 ppb MAB were found, apparently had exposure to only 1.06 mg/m^3 total airborne particulates. Two other dye weighers with slightly greater airborne particulate exposures did not excrete benzidine or MAB. This inconsistency in the exposure and urinary excretion data may be due

to a variety of factors, but individual work practices and personal hygiene are likely to play the greatest role in determining the extent of worker exposure.

Industrial exposures to benzidine-derived dyes most likely occur primarily through inhalation of the dust or mist and through accidental ingestion, which could occur after the dust is trapped in the upper respiratory passageways and inadvertently swallowed (157, 158). The optical sizing of benzidine dye samples and the prefiltering of environmental air filter samples with a 10-mm cyclone show that much of the airborne dye is not respirable. A dry smear of several benzidine dye samples shows that much of the dye particles are 20 μm or greater in diameter. Preparing a wet slide with ethyl alcohol as the diluent indicates that 60% to 70% of the particles are greater than 2.6 μm in diameter. Tables VII and XXI indicate that about 75% of total airborne particulates are typically greater than 10 aerodynamic equivalent micrometers in diameter. Thus, many of the dye particulates that are inhaled would be trapped in the upper respiratory tract. The addition of dedusting oils by the manufacturers is an important contributor to the low dusting observed.

Little is known about the rate of direct absorption from the lung of such compounds as the direct dyes, which are generally polar and cationic in nature. However, a benzidine-derived dye that is captured in the upper respiratory tract and is inadvertently swallowed would be subject to intestinal flora reduction of the azo bonds, thus releasing benzidine, which would be readily absorbed. Urinary excretion of benzidine following oral feeding of benzidine-derived dyes to both rats and monkeys has been shown (129, 159).

Because of their polar nature, the skin absorption of direct benzidine-derived dyes would not supposedly be significant. To prove this, rhesus monkeys were painted on their arms and backs with Direct Black 38 dissolved in dimethyl sulfoxide. No benzidine was present in their urine after three days of monitoring. The limit of detection was reportedly in pico mole quantities (160). Several industrial workers who participated in the NIOSH study were repeatedly splashed with hot dye liquid; however, no benzidine was found in their urine. Because of the limited data on the role of skin penetration as a means of absorption of benzidine-derived dyes, additional experimentation under a variety of conditions should be initiated.

Recently, concern over the residual benzidine content in imported benzidine-derived dyes prompted NIOSH to investigate this problem. Arrangements were made with the Customs Department of the U.S. International Trade Commission to submit to NIOSH dye samples each month. At this time, 26 samples have been analyzed for

benzidine amine and benzidine salt; the results are reported in Table XXIII. Of these randomly chosen dyes, 76% contained less than 10 ppm (w/w) of both the salt and base amine; one sample, however, contained 224 ppm total free benzidine. Nonetheless, no apparent relationship exists between the amount of salt and base amine and the country of export or the dye color. A similar compilation of randomly collected domestic dyes are presented in Table XXIV for comparison; of these samples, 73% contained 10 ppm or less of benzidine and its salts. One sample contained 265 ppm benzidine dihydrochloride and 5 ppm benzidine base.

Dyestuff manufacture is a batch operation, and variation in the quality of the finished product is expected. Because benzidine and its salts are readily absorbed through the skin and add to any exposure by inhalation or ingestion of the finished dyes, a good quality control program or stricter control during manufacturing would help assure that residual benzidine in dyes that are sold is kept to a minimum.

Fifteen consumer retail dyes bought at arts and crafts shops in New York City by the Center for Occupational Hazards, Inc., were chemically reduced and analyzed qualitatively for benzidine, o-tolidine, o-dianisidine, and aniline. NIOSH analyzed these dyes to ascertain whether professional artists, as well as the general public, might be at risk when using them. While most dye formulations are a complex mixture of individual dyes, the four reduction components mentioned above are reported in Table XXV in percent concentration relative to each other. The results indicate that most of the dye formulations tested contained dyes derived from benzidine.

Under ideal conditions, hypothetical calculations can be used to estimate the quantity of benzidine that will be excreted by man after exposure to a given dose of a benzidine-derived dye. Assuming exposure primarily occurs through inhalation, the dose via this route can be estimated by multiplying the average eight hour inhalation volume ($8m^3$) by the airborne particulate concentration. The estimated benzidine dye exposure may be further defined by knowing the percent of these dyes (by weight) in the total dust exposure. Such data is given in Tables XIV, XVII, IXX, and XXI. Animal experimentation indicates that about 1.25% of the administered benzidine-derived dye is excreted in the urine as benzidine (159). It is estimated that the unaltered quantity of benzidine excreted in the urine of humans exposed to benzidine-derived dyes averages 7% of the total benzidine (161, 162, 163, 164). These human estimates may be elevated because of the unknown role of percutaneous absorption. Next, the concentration of excreted benzidine in the urine can be calculated by estimating the urine volume during a given time period. The average urine volume voided during one-half day is 1000 mL. And finally, the molar

weight of benzidine in a dye compound is approximately 14% to 23%. Almost 100% metabolic conversion of the dye to benzidine may be assumed in the following calculations (159).

Using the above calculation parameters, it can be shown that inhaling 2 mg/m^3 of a benzidine-derived dye over an 8-hour workday can result in the excretion of between 28ppb to 48 ppb of benzidine in the urine (assuming complete excretion over one half of a day) or 14 to 24 ppb benzidine over a full day. However, the above calculation is only for a pure airborne dyestuff exposure to a benzidine-derived dye. The proportion of such dyes found in the work air environment will vary considerably; and that proportion multiplied by the estimated pure dye excretion quantity may give a rough estimate of the amount of benzidine in the urine, if the airborne concentration of benzidine-derived dyes is known. From the limited historical data, it would appear that human urinary excretion of greater than 100 ppb of benzidine constitutes a noticeable increased risk of cancer in man (164). Thus, such hypothetical calculations of excretion may be applied to defining a safe exposure level.

Hypothetical calculations can also help to demonstrate the improbability that the benzidine found in those positive urines resulted from the residual benzidine in the dyes. Balanowska reported than an average of 9 ppb benzidine was excreted by workers in a benzidine manufacturing plant where 9 μg of benzidine per cubic meter of air was determined to be the average exposure (163). It can be calculated that from a 6 mg/m^3 8-hour exposure to benzidine-derived dyes that contain 10 ppm of residual benzidine, an exposure to 60 ng of benzidine per cubic meter of air would occur. This would theoretically correspond to an expected urine benzidine concentration equaling 0.06 ppb (w/w) in 100 mL of urine.

Additional calculations similar to those used before to estimate the relationship between respiratory exposure to benzidine-derived dyes and an expected urine concentration of benzidine can be used again to estimate an expected urine concentration based soley on exposure to 10 ppm of residual benzidine in dyes. Using this approach only 0.007 ppb of benzidine would be expected to be found in 1000 mL of urine. Both means of calculation clearly indicate that if exposure to benzidine was solely due to the residual benzidine quantities and not also to the benzidine released through metabolic conversion of the dyes then the current analytical methods would not be able to detect this low concentration of benzidine.

Recently, a Russian study reported finding benzidine in 8 of 22 workers in a dye manufacturing plant. All the workers were employed in the drying, grinding, and packing of the finished dyes. Urine concentrations ranging from trace quantities (unspecified) to 300 ppb were reported. In addition, five cases of urinary bladder

cancer in that factory were reported among workers engaged in the drying and grinding of direct azo dyes over the period 1965 to 1968. The latent periods ranged from 18 years to 43 years (165).

Also in Russia, a medical study concerning the early detection of bladder tumors among textile dyers using benzidine-derived dyes found an unusual incidence of bladder lesions, some of which were suggested as being of a pre-cancerous nature. A correlation between the apparent extent of exposure and the incidence of lesions was observed (166).

Apparently only two benzidine-derived dyes, Congo Red and Chlorazol Violet N, have been tested for mutagenicity. Preincubation with S-9 mix plus riboflavin and TA 98 Salmonella typhimurium histidine-requiring strains showed moderate mutagenicity. No mutagenicity was observed, however, without the addition of riboflavin (167).

Table XXIII. Residual benzidine in import dye samples.

Dye Name	Exporting Country	Concentration of residual benzidine in ppm (w/w)
C.I. Direct Black 38	Egypt	1,254
C.I. Direct Red 1	Belgium	224
C.I. Direct Orange 8	India	143
C.I. Direct Blue 2	Belgium	87.4
C.I. Direct Green 6	Holland	70
C.I. Direct Black 38	Egypt	53
C.I. Direct Black 38	Poland	40
C.I. Direct Black 38	Poland	38
C.I. Direct Blue 2	Holland	24
C.I. Direct Blue 6	India	10
C.I. Direct Black 38	India	9
C.I. Direct Blue 2	Belgium	8
C.I. Direct Blue 2	Romania	8
C.I. Direct Blue 2	India	7
C.I. Direct Red 28	Romania	7
C.I. Direct Blue 6	Belgium	6.6
C.I. Direct Red 28	India	6
C.I. Direct Green 1	Poland	3
C.I. Direct Black 38	Holland	3
C.I. Direct Red 1	Poland	3
C.I. Direct Red 28	Polant	2
C.I. Direct Red 28	Belgium	2
C.I. Direct Blue 2	Poland	2
C.I. Direct Red 28	Korea	2
C.I. Direct Black 38	Holland	2
C.I. Direct Blue 2	Poland	1
C.I. Direct Red 37	Holland	1
C.I. Direct Brown 54	Poland	1
C.I. Direct Brown 1:A	Poland	1
C.I. Direct Red 28	Poland	1
C.I. Direct Brown 1/154	Poland	1
C.I. Direct Blue 2	Poland	0.4
C.I. Direct Red 28	Korea	0.5

Table XXIV. Residual benzidine in direct dyes from domestic sources.

Company and Dyes	Residual Benzidine in ppm (w/w)
GAF Corporation	
Black JXA (Blk. 38)	13
Black ER-200 (Blk. 38)	4
Black EA (Blk. 38)	2
Black BRL (Brn. 95)	2
Blue 2B (Blue 6)	4
Scarlet 4BGP (Red 39)	1
Fabricolor	
Brown 3GN (Brn. 95)	270
Black GX (Blk. 38)	20
Brown BRL 200% (Brn. 95)	19
Brown 3GN (Brn. 154)	15
Green WS 133% (Grn. 1)	12
Fast Blue 2B 250% (Blue 6	12
Grown Brown B 125 (Brn. 31)	10
Black GX 200% (Blk. 38)	10
Catechine 3G (Grn. 74)	4
Brown 3GN (Brn. 154)	4
Fast Brown B 125% (Brn. 31)	3
Fast Green BX 100% (Grn. 6)	3
Phenamine Black E-200 (Blk. 38)	2
Congo Red 4B (Red 28)	2
Brown M 100% (Brn. 2)	1
Green WS 100% (Grn. 1)	1
Diazo Black BH (Blue 2)	1
Brown 3GN (Brn. 154)	1
Allied Chemical	
Niagara Blue	1
Erie Green GPD	1

Table XXV. Relative percent quantities of four derivitives in retail dyes.+

Field	Company	Dye Name	Aniline	Benzidine	o-Tolidine	o-Dianisidine
1*	FBS, Inc.	Fazan Brown #4029	1	99	0	0
2	FBS, Inc.	Black #1628	2	98	0	0
3*	FBS, Inc.	Dark Blue #4025	0	0	0	0
4	Tintex	Black-Powder	2	98	0	0
5	Tintex	Brown-Powder	1	99	0	0
6*	Tintex	Navy Blue #25	1	74	0	25
7*	Tintex	Royal Blue-Powder	0	1	98	1
8	Tintex	Chocolate Brown-Powder	0	99	1	0
9*	Aljo	Black-Cotton	12	88	0	0
10	Aljo	Dark Brown	0	100	0	0
11	Aljo	Imperial Blue	0	96	4	0
12*	Aljo	Royal Blue	0	2	96	2
13	RIT	Chestnut Brown #43	2	6	87	5
14	RIT	Navy Blue 30	2	0	93	5
15*	RIT	Black 15	5	2	92	1

+ Dyes purchased Fall, 1978.
* Indicates GC/MS verification.

Chapter 13

CONCLUSIONS

This study, which is probably the most comprehensive investigation of its kind performed to date in this country, supports other data that indicate the potential carcinogenicity of benzidine and the metabolism of benzidine-derived dyes to the carcinogenic amine. Presently, the literature reports two instances in which bladder cancer in humans has been directly attributed to exposure to benzidine-based dyes (150, 165) and one instance in which benzidine was found in the urine of a worker exposed only to the dye (65). There is also a study on the metabolism of benzidine-derived dyes to benzidine in rats (129) and in monkeys (159). In the present study, benzidine or monoacetylbenzidine was detected in 12 of 38 workers potentially exposed to benzidine dyes. Although data on the carcinogenic risk to workers exposed to these dyes are incomplete, temporary measures should be taken to ensure minimal potential exposure to such dyes. In addition, all substitutes for benzidine-derived dyes should be evaluated toxicologically for carcinogenic potential. Appendix I shows that many dyes (in addition to the benzidine-derived dyes) may be potentially carcinogenic. Data from the National Occupational Hazards Survey, conducted by NIOSH from 1972-74, show that possibly 47,282 workers are exposed to various dyes and that 79% have no protective equipment. It is probable that about 1200 different dyes are made in the United States and that 800 other dyes are imported. It would be prudent to minimize worker exposure to all dyes whose toxicity has not been determined. Any necessary exposure should be reduced to the lowest feasible level.

Chapter 14

RECOMMENDATIONS

During the course of this study, several concerns were identified for possible future study:

1. The extent of dermal absorption of dyestuffs should be investigated under a variety of physical and chemical conditions that may alter the rate of penetration. Urine metabolites should be monitored to assess absorption.

2. The current study failed in its attempt to define the rate and pattern of excretion in workers who were exposed to benzidine-derived dyes. It is thus unknown when best to monitor workers by urine sampling to attain a peak excretion level. NIOSH has allocated funds to the National Center for Toxicological Research (NCTR) to study the excretion metabolites, excretion kinetics, and mutagenicity of select excretion products in the urine of both hamsters and rats fed azo dyes derived from benzidine, o-tolidine, o-dianisidine, and 3,3'-dichlorobenzidine. (Further information can be obtained from Dr. Larry Lowry, (513) 684-8338 at NIOSH or from Mr. Malcomb Bowman, (501) 740-4556 at NCTR.)

3. A systematic effort to test organic dyes and non-metallic pigments for long-term chronic effects should be undertaken through the joint involvement of both industry and government. Substitutes for benzidine and benzidine-derived dyes should not be used prior to toxicological verification of their safety.

4. More in-depth industrial hygiene studies to identify potentially exposed individuals should be undertaken. Priority should be given to studies of dyes and pigments suspected or known to be carcinogenic to animals.

5. It would be of scientific interest to determine the extent of pulmonary deposition and clearance of water-soluble organic dyes. Such knowledge would help in understanding the active sites of metabolism and fate after inhalation exposure to such dyes.

6. It would be useful to publish a work practice guide on dye manufacture and use that defines all reported potential health and safety hazards and lists methods of controlling exposure.

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APPENDIX 1

DYES EXHIBITING EXPERIMENTAL CARCINOGENIC ACTIVITY*

*The chemical structures for the 44 dyes listed in Appendix 1 appear on pages 120 to 123.

Dyes Reported as Exhibiting Experimental Carcinogenic Activity (continued)

Dye Name	Class	Producers 1974	Producers 1976	Production Fig. 1974	Production Fig. 1976	Carcinogenic Data
1. Acid Yellow 73 C.I. 45350	Xanthene	2	2			Oral-rat 3300 mg/kg/24 WC:car
2. Acid Orange 20 C.I. 14600	Monoazo	N.L.	N.L.			Scu-rat 9400/kg/zyz:neo
3. Acid Red 18 C.I. 16255 Scarlet 3R, Food Red 7	Monoazo	4	2	129,000		Oral-rat 2600 gm/kg/z10 DC:car Oral-rat 524 gm/kg/74 WC:car
4. Acid Red 26 C.I. 16150 Acid Red 14, Food Red 5	Monoazo	1	2			Oral-rat 35 gm/kg/52 WC:car Oral-mus 10 gm/kg/76 WC:car
5. Acid Red 27 C.I. 16185 FDC Red Dye #2 (Amatanth)	Monoazo	1	N.L.			Oral-rat 1080 gm/kg/540 DC:car Oral-rat 1500 µg/kg/(preg): TER
6. Acid Red 148 C.I. 26665	Diazo	N.L.	N.L.			Scu-mus 80 mg/kg/4 WC:car
7. Acid Green 3 C.I. 42085 (Food Green 3)	Triphenylmethane	4	3	164,000	Acid Green Total 485,000	Oral-rat 600 gm/kg/43 WC:car
8. Acid Green 5 C.I. 42095	Triphenylmethane	1	N.L.			Oral-rat 1290 gm/kg/86 WC:car Scu-rat 6825 mg/kg/46 WC:car

Dyes Reported as Exhibiting Experimental Carcinogenic Activity (continued)

Dye Name	Class	Producers 1974	Producers 1976	Production Fig. 1974	Production Fig. 1976	Carcinogenic Data
9. Acid Green 50 C.I. 44090 (Acid Leather Green)	Triphenylmethane	2	N.L.			Inh-rat 20,000 ppm/154 DA:car Scu-rat 250 gm/kg/30 WI:car
10. Acid Blue I C.I. 42045	Triphenylmethane	2	N.L.			Scu-rat 6500 mg/kg/33 WI:neo ims-rat 960 mg/kg/25 W:neo
11. Acid Blue 3 C.I. 42051	Triphenylmethane	N.L.	N.L.			
12. Acid Blue 9 C.I. 42090 Neptune Blue and Brilliant Blue	Triphenylmethane	3	3	1,937,000	1,420,000	Oral-rat 3000 mg/kg -:car Scu-rat 17 gm/kg/2y:car par-rat 4580 mg/kg/434 DI:car
13. Acid Blue 74 C.I. 73015 (Food Blue 1, Indigo carmine)	Indigiod	2	1		N.L. Total Acid Blue 4,401,000	Scu-rat 10 gm/kg/2 YI:car
14. Acid Violet 49 C.I. 42640 (Food Violet 2, D&C Violet 1, wool violet 4BN)	Triphenylmethane	4	N.L.	122,000		Oral-rat 800 gm/kg/46 WC:car Scu-rat 9400 mg/kg/2 YI:neo
15. Basic Orange 2 (Chrysoidine) C.I. 11270	Monoazo	7	5	403,000	488,000	Oral-mus 31 gm/kg/52 W:car

Dyes Reported as Exhibiting Experimental Carcinogenic Activity (continued)

Dye Name	Class	Producers 1974	Producers 1976	Production Fig. 1974	Production Fig. 1976	Carcinogenic Data
16. Basic Orange 3 Basic Green 4	Monoazo	N.L.	N.L.			See 13
17. Basic Yellow 2 Auramine C.I. 41000	Ketone Imine	2	1			Oral-rat 40 m/kg/87 WC:car Scu-rat 2388 mg/kg/21 W:car
18. Basic Violet 10 C.I. 749 (Rhodamine B)	Xanthene	3	4		Basic Violet total 3,232,000	Scu-rat 3825 mg/kg/68 WI:neo
19. Direct Blue 6 C.I. 22610 Direct Blue 2B	Diazo	2	3		N.L.	Scu-rat 50 mg/kg (8.5 preg):ter par-rat 150 mg/kg (9D preg):ter Scu-rat 750 mg/kg/127 WI:car
20. Direct Blue 14 (Trypan Blue) C.I. 23850	Diazo	3	1			Oral-mus 72 mg/kg/52 WC:car Scu-rat 1088 mg/kg/87 WI:car
21. Direct Blue 53 C.I. 23860 (Sky Blue FF, Evans Blue)	Diazo	N.L.	1			Scu-rat 150 mg/kg (7-9D preg):ter par-rat 100 mg/kg (7.5D preg):ter IARC 8, 151, 75
22. Direct Brown 78 C.I. 40290	Stilbene	N.L.	N.L.			See 12
23. Disperse Yellow 3	Monoazo	8	6	2,870,000 3,125,000		bladder implant/25 WC:car

Dyes Reported as Exhibiting Experimental Carcinogenic Activity (continued)

Dye Name	Class	Producers 1974	Producers 1976	Production Fig. 1974	Production Fig. 1976	Carcinogenic Data
24. Disperse Black 6	Diazo	1	N.L.			Oral-rat 13 gm/kg/52 WI:neo Oral-harm 560 gm/kg/70 WI:neo
25. Reactive Blue 19 (See Acid Red 148)	Anthranquinone vinylsulphonyl	1	1			Orl-rat 87/mg/Kg/2 YI:neo Scu-mus 19/mg/Kg/48 WI intermittent:neo
26. Natural Blue 2 Acid Blue 74	Indigo	2	1			Scu-rat 10 gm/kg/2 YI:car
27. Solvent Yellow 1 C.I. 11000	Monoazo	N.L.	N.L.			Skn-rat 1600 mg/kg/2.3 YI:car Ivn-frg 11 mg/kg:car
28. Solvent Yellow 2 Butter Yellow	Monoazo	3	N.L.	11,000		orl-rat 800 mg/kg/40D:car skn-rat 155 mg/kg/4 WI:car
29. Solvent Yellow 3 C.I. 11160 (o-aminoazotoluene)	Monoazo	2	1			orl-mus 11 gm/kg/112 WC:neo orl-ham 9600 mg/kg/42 WI:neo
30. Solvent Yellow 5 C.I. 11380	Monoazo	N.L.	N.L.			Orl-rat 12 gm/kg/57 WC:car Orl-mus 7000 mg/kg/70D:car Scu-mus 330 mg/kg:car Imp-mus 80 mg/kg:neo Orl-ham 840 mg/kg/6 WC:neo
31. Solvent Yellow 6 C.I. 11390	Monoazo	N.L.	N.L.			Orl-rat 17.5 mg/kg/DC:neo Scu-rat 17.5 mg/kg/DC:neo
32. Solvent Yellow 14 C.I. 12055	Monoazo	6	5	511,000		Orl-mus 56 gm/kg/52 WC:neo Imp-mus 80 mg/kg/neo

Dyes Reported as Exhibiting Experimental Carcinogenic Activity (continued)

Dye Name	Class	Producers 1974	Producers 1976	Production Fig. 1974	Production Fig. 1976	Carcinogenic Data
33. Solvent Yellow 34 (Auramine, C.I. 4100B)	Ketone Imine	2	N.L.			Oral-mus 29 mg/kg/52 WC:car See 15
34. Solvent Orange 2 C.I. 12100	Monoazo	1	N.L.			Scu-mus 6 gm/kg/52 W:car
35. Solvent Orange 3 C.I. 11270 (Basic Orange 2, Chysoidine)	Monoazo	5	3			Orl-mus 158 gm/kg/58 WC:neo Orl-mus 2 g/kg/52 WC:car
36. Solvent Orange 7 C.I. 12140	Monoazo	5	3			Imp-mus 80 mg/kg/40 WC:car Orl-mus 1 gr/kg/8952 WC:neo
37. Solvent Orange 15 C.I. 46005B (Acridine Orange waxoline orange A)	Acridine	N.L.	N.L.			Scu-rat 2500 mg/kg:neo Skn-mus 6630 mg/kg/65 WI:neo
38. Solvent Red 19 C.I. 26050	Diazo	N.L.	N.L.			Orl-rat 17 gm/kg/50 WC:neo
39. Solvent Red 24 C.I. 26105	Diazo	5	4			Scu-rat 512 mg/kg/58 WC:car
40. Solvent Red 80 C.I. 12156 (Citrus Red 2)	Monoazo	N.L.	N.L.			Orl-mus 365 gm/kg/2 YC:car Ipl-mus 80 mg/kg:neo Scu-mus 200 gm/kg/80 WI:car Orl-mus 2.5 gm/kg/96 WC:car Orl-rat 2.5 gm/kg/96 WC:car Imp-mus ? /kg/40 WC:car

Dyes Reported as Exhibiting Experimental Carcinogenic Activity (continued)

Dye Name	Class	Producers 1974	Producers 1976	Production Fig. 1974	Production Fig. 1976	Carcinogenic Data
41. Solvent Blue 7 (Acid Blue 74)	Azine	1	1			See Solvent Yellow 1
42. Solvent Brown 1 C.I. 11285	Monoazo	N.L.	N.L.			Orl-rat 34 gm/kg/89 WC:car
43. Food Red 6 C.I. 16155 Poncean 3RN VSCERT Red No. 1	Monoazo	N.L.	N.L.			Orl-rat 730 gm/kg/2 YC:car Orl-mus 1900 gm/kg/68 WC:car Imp-mus 80 mg/kg:neo Orl-rat 30 gm/kg/65 WC:car Orl-rat 5-50 gm/kg/2 YC:car Orl-rat 30 gm/kg/70 WC
44. Food Green 3 C.I. 42053 (Fast Green FCF)	Triphenylmethane	1	N.L.			Scu-rat 5925 mg/kg/48 WI:car

Data for Appendix I were taken from:

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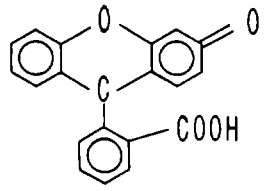
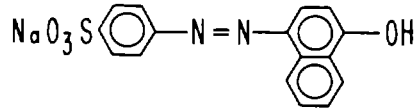
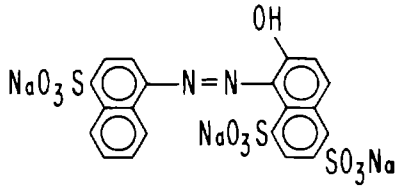
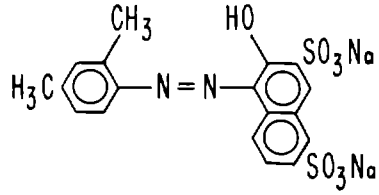
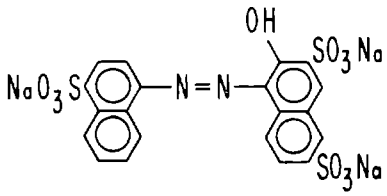
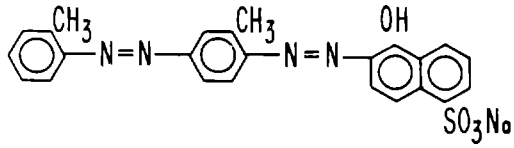
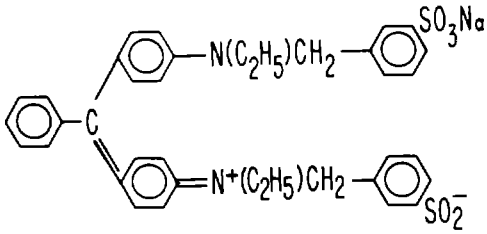
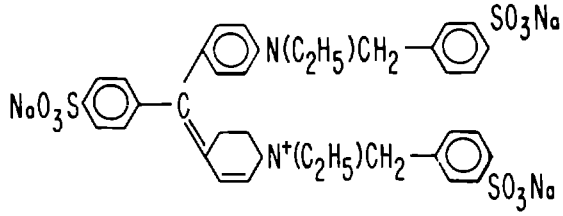
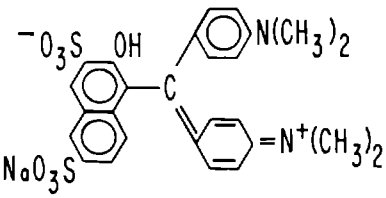
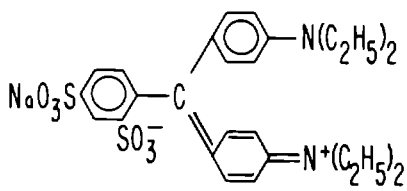
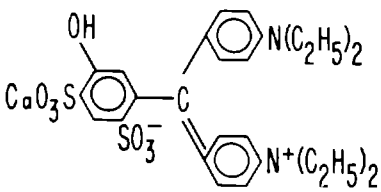
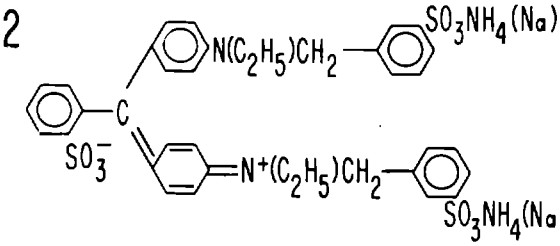
1 	2 
3 	4 
5 	6 
7 	8 
9 	10 
11 	12 

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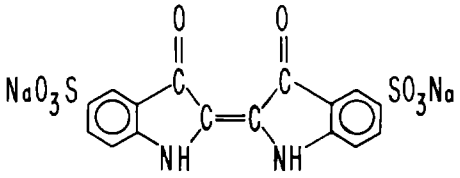

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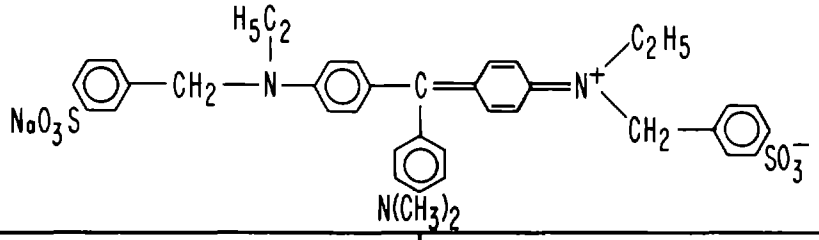

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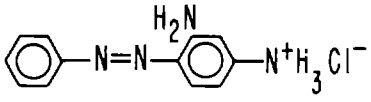

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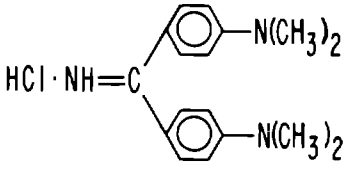

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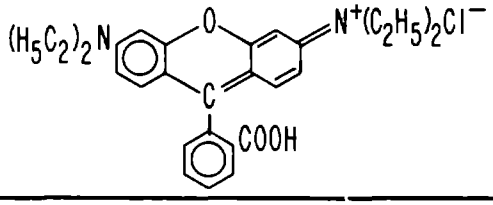

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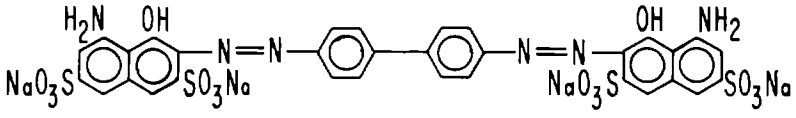

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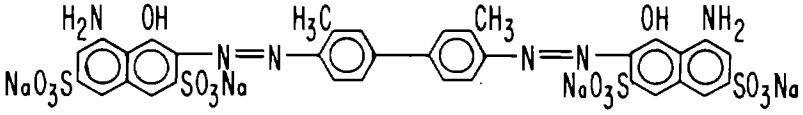

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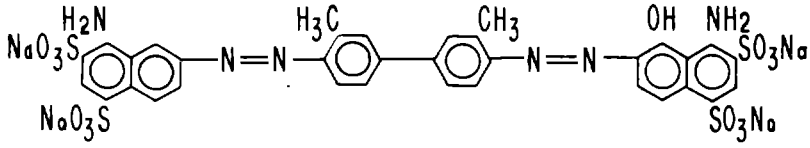

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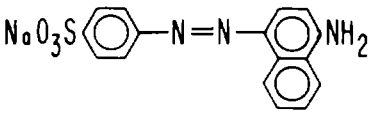

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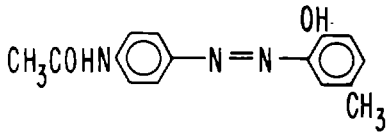

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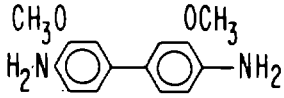

AZOIC COMPOUND
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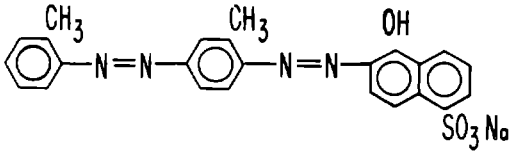

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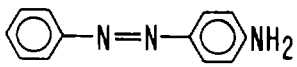

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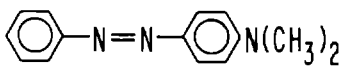

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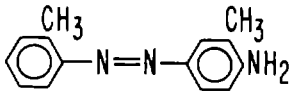

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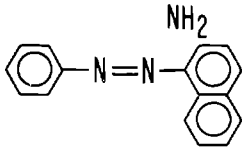

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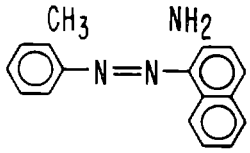
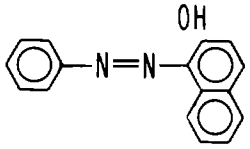
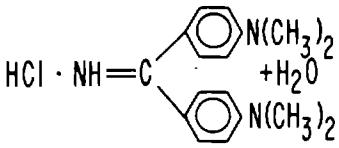
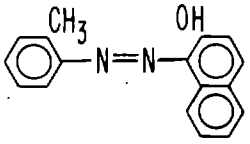
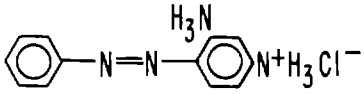
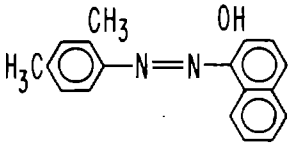
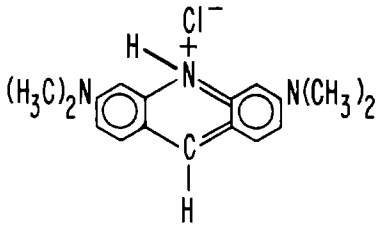
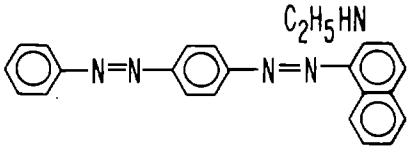
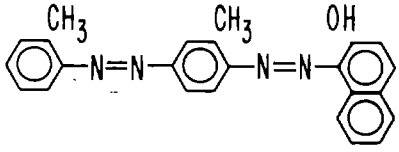
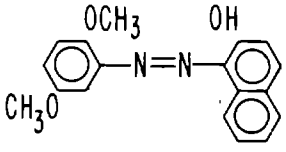
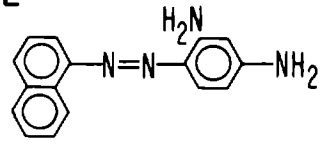
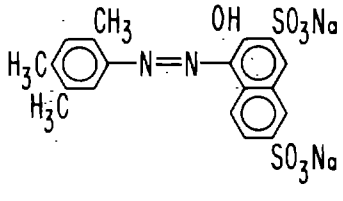
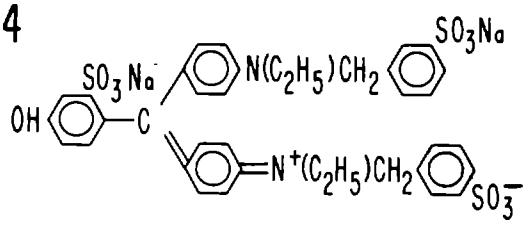


32 	33 	
34 	35 	
36 	37 	
38 	39 	
40 	41 NO KNOWN STUCTURE	42 
43 	44 	

APPENDIX 2

PHYSICAL AND CHEMICAL DATA

Benzidine

(4,4'-diaminobiphenyl, 4,4'-biphenyldiamine)

PHYSICAL STATE:	White or slightly reddish crystals or powder	
PHYSICAL PROPERTIES:	Molecular Weight	184.23
	Density	1.250 (20g/4 degrees C)
	Melting point	116.5 degrees C
	Boiling point	401.7 degrees C
SOLUBILITY:	0.04% in H_2O at 12 degrees C 0.94% in H_2O at 100 degrees C 2.2% in ethyl ether at 20 degrees C	
EXPOSURE STANDARD:	Benzidine is a regulated human carcinogen. No exposure should occur.	
NOTE:	Benzidine, whether free base, sulfate or chloride, is reported to have a low vapor pressure. It can be sublimed.	

o-Dianisidine

(3,3'-dimethoxybenzidine)

PHYSICAL STATE:	Colorless crystals that turn violet on standing.	
PHYSICAL PROPERTIES:	Molecular weight	244.3
	Density	N.A.
	Melting point	137-138 degrees C
	Boiling point	N.A.
SOLUBILITY:	Very slightly soluble in water, soluble in most organic solvents.	
EXPOSURE STANDARD:	No regulations. Positive animal carcinogen.	

3,3'-dichlorobenzidine

PHYSICAL STATE:	Colorless crystals	
PHYSICAL PROPERTIES:	Molecular weight	253.13
	Density	N.A.
	Melting point	133 degrees C
	Boiling point	N.A.
SOLUBILITY:	Practically insoluble in water, but easily soluble in organic nonpolar solvents. Slightly soluble in dilute hydrochloric acid.	
EXPOSURE STANDARD:	FDA carcinogen. Positive animal carcinogen. OSHA regulated carcinogen.	

o-Tolidine

(3,3'-dimethyl-4,4'-diaminobiphenyl, 3,3'-dimethylbenzidine)

PHYSICAL STATE:	White to reddish crystals or crystaline powder The monohydrochloride and dihydrochloride are in the form of flakes.	
PHYSICAL PROPERTIES:	Molecular weight	212.28
	Density	N.A.
	Melting point	129-131 degrees C
	Boiling point	N.A.
SOLUBILITY:	Slightly soluble in water: very soluble in organic solvents. Monohydrochloride 0.89% in H_2O at 12 degrees C; dihydrochloride, 5.77% in H_2O at 12 degrees C. The disulfate is sparingly soluble in water.	
EXPOSURE STANDARD:	Not regulated. Positive animal carcinogen.	

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IARC monographs on the evaluation of carcinogenic risk of chemicals to man. Vol. 4. Lyon: International Agency for Research on Cancer; 1974.

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Personal communication with Dr. Winfred Craig, GAF, Dye and Color Div., Rensealear, N.Y.

APPENDIX III

CONSENT FORM

I _____, age _____voluntarily agree to participate in this study to evaluate my exposure to benzidine-based dyes so as to determine whether or not I may be potentially at risk through metabolizing these dyes to benzidine, which is a known human bladder carcinogen. The authority to conduct this study is provided to the National Institute for Occupational Safety and Health (NIOSH) by Section 20(a)(7) of the Occupational Safety and Health Act (1970) and in accordance with Federal Regulations (42 Code of Federal Regulations, Part 85a).

I understand that I will be asked to provide urine samples. The benefit to me is that any urine specimens I provide will be tested and evaluated by NIOSH and the results will be made known to me, and if I request, to my private or company physician. I understand that at any time during the study I have the right to ask questions of NIOSH and that I am free to withdraw my consent and to discontinue participation in the study at any time without prejudice to myself.

All information gathered in this evaluation will not be disclosed in a manner which will identify me except with my written permission (see below) or except as required by law. This information will be used by NIOSH primarily for purposes of occupational health research.

SIGNATURE_____DATE_____

INVESTIGATOR_____DATE _____

REQUEST FOR RELEASE OF INFORMATION

I hereby request and authorize NIOSH to inform the following person(s) listed below of any of my findings from the current study. I will also personally receive a copy of my individual findings from NIOSH.

COMPANY PHYSICIAN _____ YES_____NO_____
(Name)

ADDRESS _____

_____

PRIVATE PHYSICIAN _____ YES_____NO_____
(Name)

NAME_____

ADDRESS_____

CITY, STATE _____ZIP CODE_____

SIGNATURE _____DATE _____

NIOSH/NCI JOINT CURRENT INTELLIGENCE BULLETIN

DIRECT BLACK 38, DIRECT BLUE 6, AND DIRECT BROWN 95 BENZIDINE-DERIVED DYES

April 18, 1978

The National Institute for Occupational Safety and Health (NIOSH) recommends that three widely used benzidine-derived dyes, Direct Black 38, Direct Blue 6, and Direct Brown 95, be handled in the workplace as if they were human carcinogens. This recommendation is based primarily upon a preliminary analysis of National Cancer Institute (NCI) data from short-term feeding studies, and on early results from NIOSH field studies. Cancerous and precancerous liver conditions were found in rats, similar to the damage produced by known liver carcinogens. Degeneration of liver cells was found in mice. Although the dyes tested by NCI contained less than 4 ppm residual benzidine when fed to the test animals, greater quantities of benzidine were found in the urine of dosed rats and mice. Caution is also indicated by preliminary results from NIOSH field studies showing that humans working with these same dyes also excrete higher than expected levels of benzidine in their urine. Both laboratory and field studies indicate that these benzidine-derived dyes can be metabolized to benzidine which is present in the urine of animals and humans.

Based on the data from the short-term study, NCI scientists believe a cancer-causing potential exists upon exposure to the benzidine-derived dyes, most likely through the mechanism of metabolic conversion of the dyes to benzidine in the animal system. This NIOSH/NCI Joint Bulletin summarizes the results of the NCI animal study, the NIOSH field studies, other pertinent data, their implication for occupational health, and suggested guidelines for minimizing employee exposure to the three dyes.

Potential Occupational Exposures

The National Occupational Hazard Survey (NOHS), conducted between 1972 and 1974 by the National Institute for Occupational Safety and Health, indicates that workers are occupationally exposed to Direct Black 38, Direct Blue 6, and Direct Brown 95 in a variety of industries including: paper and allied products, petroleum and related industries, rubber and plastic products, leather and leather products, instrumentation and measuring devices, and banking. In addition, the

textile industry accounts for a substantial occupational exposure. It is estimated that 25 percent of the benzidine-derived azo dyes are applied to textiles, 40 percent to paper, 15 percent to leather, and the remainder to other diverse applications. (1)

The Colour Index, (2) a reference by Great Britain's Society of Dyers and Colourists, reports the following uses for the three dyes:

- Direct Black 38: Dyeing or staining of wool, silk, fibers for rope and matting, hogs hair, cellulose, acetate, nylon, and biological stains.

- Direct Brown 95: Dyeing or staining silk, cotton, acetate, cellulose, wool, nylon, leather, paper, and certain plastics.

- Direct Blue 6: Dyeing or staining silk, wool, cotton, nylon, leather, paper, biological stains and writing inks.

Historically, benzidine has been an important intermediate in dye production since its introduction to the dyestuff industry around 1890. (3,4) In 1948, production of benzidine-derived dyes was 35 million pounds which accounted for 25% of all domestic dyes manufactured and almost all of the direct class dyes. (5) Domestic production of direct benzidine-derived dyes has dropped to 11.4 million pounds in 1971. (6) Domestic production in 1978 should be limited to 12 benzidine dyes. The latest domestic production figures for Direct Black 38 show 7.3 million pounds in 1971, (6) down to 2.2 million pounds in 1975,(7) which rose to 3.76 million pounds in 1976. (8) Direct Brown 95 has shown an increased production to 600,000 pounds in 1976 which is up from 406,000 pounds in 1975 and 343,000 pounds in 1974. (8) Domestic production for Direct Blue 6 indicates that 327,000 pounds were produced in 1973, which is the last available figure for that dye. (9)

In the general population, unspecified exposure levels to the three dyes are thought to occur through the use of retail packaged dyes for home dyeing and for home and school use in art and craft projects such as tie-dyeing or batik. The Art Hazards Project of the Center for Occupational Hazards, New York City, has reported that package dyes sold in supermarkets, variety stores and hardware stores are combinations of direct, acid and basic dyes, and thus may contain benzidine-derived dye components. (10) Two of these dyes, Direct Black 38 and Direct Blue 6, have been used in hair dyes. (11)

NCI Dose-Ranging Feeding Study (11)

Ninety-day animal feeding tests have been completed by the National Cancer Institute for three widely-used dyes, Direct Black 38, Direct Blue 6, and Direct Brown 95. The first-phase tests are conducted routinely to establish dosage levels

in mice and rats for chemicals being screened for cancer-causing activity. The dose-ranging studies commonly precede standard bioassays--animal lifetime tests at dosages that do not shorten lifespans or impair growth.

All three of the dyes are benzidine-derived, having a unit of benzidine in their chemical structure. Benzidine is a known animal and human cancer-causing agent. (12) Residual free benzidine in the feed was below 4 parts per million (ppm).

A total of 120 rats and 120 mice were divided into groups of 10. One group of each sex and each species was reserved as undosed controls, while five groups of each sex and species received differing dosages of the dyes in feed. At the 4th and 12th week of the study, urine was collected from dosed rats and mice for benzidine analysis. After 91 days of feeding and one day of observation, the surviving animals were killed and their tissues examined.

After the 90-day trial, significant incidences of cancerous and precancerous changes in the liver were found in both male and female rats dosed with any of the three dyes, whereas, untreated control rats had no liver damage. The liver changes in dosed animals were similar to changes caused by benzidine.

In mice, all three dyes were found harmful to the liver, but no cancerous changes were found. This finding is compatible with the interpretation that the toxic effects may be related to benzidine, because benzidine is more likely to produce cancer in rats than in mice. No liver abnormalities or damage to any internal organs were found in the control animals of either species.

In addition, although the dyes were essentially benzidine-free when fed to the animals, substantial benzidine was found in the urine of dosed rats and mice, an indication that animal systems metabolize the dyes to benzidine.

The technical report "13-Week Subchronic Toxicity Studies -- Direct Blue 6, Direct Black 38, and Direct Brown 95 Dyes" is available from the Office of Cancer Communications, National Cancer Institute, Bethesda, Maryland 20014.

Other Laboratory Animal Studies

As an historical point, an early realization of the metabolism of azo compounds in mammals came as a result of feeding Orange I, an azo dye, to dogs in 1911. (13) An intermediate of the dye, sulfanilic acid, was identified in the urine demonstrating for the first time that azo compounds may be metabolized by reductive cleavage of the azo group. Since then it has been repeatedly demonstrated that the intestinal flora within animals and many animal hepatic enzyme systems are capable of splitting the azo bond. (14,15) The majority of this work was performed with azo dyes intended for food coloring.

Yoshida and Miyakawa found that when sulfonated benzidine azo dyes were injected into surgically removed mice intestines and then incubated, free benzidine was

later found. They also showed that Escherichia coli as well as soil bacteria were quite capable of reducing benzidine dyes when incubated at 37 C. (16)

Another study showed that rhesus monkeys, fed benzidine-derived dyes with no residual benzidine, excreted benzidine in their urine. The levels excreted were estimated as being almost as high as if an equal amount of pure benzidine, as found in the dye moiety, were fed instead to the monkeys. (17) These studies lead NIOSH to believe that when dyes of these types are ingested by man, they result in benzidine in the urine thus posing a potential carcinogenic hazard.

NIOSH Field Studies

Preliminary results from NIOSH studies indicate the presence of benzidine, or monacetylbenzidine (MAB), a metabolite of benzidine, in the urine of workers in four out of five industrial facilities in which urine samples were collected. The facilities surveyed to date include two benzidine dye manufacturers, two textile finishing companies and a leather tannery.

In one dye manufacturing plant, benzidine and MAB were found in all dye workers who worked solely with the finished dyes. Bulk benzidine-based dyes were quantitatively analyzed for residual free benzidine content which ranged from less than 1 to 19 ppm. It was conservatively estimated that about 20 times more benzidine and up to 200 times more MAB were present in the urine of these dye workers, than if they had been exposed only to the residual benzidine content in the dyes. The concentrations of benzidine found in the urine of these workers were significant fractions of those concentrations associated with a high incidence of bladder cancer as reported in the scientific literature. Among textile dyers included in the NIOSH study, four of ten had benzidine or MAB present in their urine and all had elevated aromatic amine levels. No benzidine or MAB was detected in urine samples collected at a leather tannery where good work practices were observed. While there was biological variability in body burden tolerance and differences in metabolism among those studied, it appears likely that work practices and personal hygiene played a major role in minimizing exposure. Analyses of data from these NIOSH studies are ongoing.

The relatively large particle size of the dry dye powders causes inhaled dyes to be deposited largely in the upper respiratory tract and then ingested. Prevention of worker inhalation or ingestion of these dyes would greatly reduce absorption into the body and subsequent benzidine exposure.

Epidemiological and Medical Studies -- Benzidine-Based Dyes

A strong association relating human exposure to benzidine-based dyes with the subsequent development of bladder tumors was presented after a case-control mortality study of 200 bladder cancer patients in Japan. (16) The patients were found to have been predominantly kimono painters and dyers. The kimono painters

had the habit of forming a point on their brushes by drawing the brush between their lips, which allowed for ingestion of the dyes.

Several other case-control mortality studies indicate an increased risk of developing bladder cancer in the textile and leather industries, both large users of direct dyes. However, only a few references have been made concerning benzidine-derived dyestuffs.

In Russia, a medical study concerning the early detection of bladder tumors among textile dyers using benzidine-derived dyes found an unusual incidence of bladder lesions, some of which were suggested as being of a precancerous nature. The greatest number of such lesions were found in those workers with the highest potential exposure to these dyes.(18)

The National Institute for Occupational Safety and Health and the National Cancer Institute have jointly prepared this Bulletin to facilitate the rapid dissemination of our preliminary findings on three benzidine-derived dyes: Direct Black 38, Direct Blue 6, and Direct Brown 95. NIOSH recommends that these dyes be handled in the workplace as if they were human carcinogens and requests that producers, distributors, professional associations, and unions transmit the information in this Bulletin to their customers, employees, associates, and members.

Arthur C. Upton, M.D.
Director
National Cancer Institute

J. Donald Millar, M.D.
Assistant Surgeon General
Acting Director,
National Institute for Occupational Safety and Health

NIOSH Action on Benzidine-Derived Dyes

1. The NIOSH Industrial Hygiene Section will shortly conduct a study in which workers who have had a one-day exposure to benzidine-based dyes are monitored over several days. This will hopefully confirm some assumptions on the excretion kinetics of such exposure and better establish the best time to monitor a worker's urine for benzidine.

2. The NIOSH Clinical and Biochemical Support Section has an interagency agreement with the National Center for Toxicological Research to conduct animal metabolism studies. Test animals will be fed Direct Black 38, Direct Blue 6 and Direct Brown 95. Metabolites will be identified in the urine and evaluated for mutagenic activity. Additional dyes made from 3,3-dimethylbenzidine, 3,3-dimethoxybenzidine, and 3,3-dichlorobenzidine will also be tested.

SUGGESTED GUIDELINES FOR MINIMIZING EMPLOYEE EXPOSURE TO DIRECT BLACK 38, DIRECT BLUE 6, DIRECT BROWN 95 -- BENZIDINE-DERIVED DYES

NIOSH recommends that it would be prudent to handle Direct Black 38, Direct Blue 6, and Direct Brown 95 in the workplace as if they were human carcinogens. Exposure to Direct Black 38, Direct Blue 6, and Direct Brown 95 should be limited to as few employees as possible, while minimizing workplace exposure levels. The area in which they are used should be restricted to only those employees essential to the process or operation.

EXPOSURE MONITORING

Initial and routine employee exposure surveys should be made by competent industrial hygiene and engineering personnel. These surveys are necessary to determine the extent of employee exposure and to ensure that controls are effective.

The NIOSH Occupational Exposure Sampling Strategy Manual, NIOSH Publication #77-173, may be helpful in developing efficient programs to monitor employee exposures to Direct Black 38, Direct Blue 6, and Direct Brown 95. The manual discusses determination of the need for exposure measurements, selection of appropriate employees for exposure evaluation, and selection of sampling times.

Employee exposure measurements should consist of 8-hour TWA (time-weighted average) exposure estimates calculated from personal or breathing zone samples (air that would most nearly represent that inhaled by the employees). Area and source measurements may be useful to determine problem areas, processes, and operations.

MINIMIZING EMPLOYEE EXPOSURE

There are four basic methods of limiting employee exposure to Direct Black 38, Direct Blue 6, and Direct Brown 95. None of these is a simple industrial hygiene or management decision and careful planning and thought should be used prior to implementation of any of these.

- Product Substitution

The substitution of an alternative material with a lower potential health and safety

risk is one method. However, extreme care must be used when selecting possible substitutes. Alternatives to Direct Black 38, Direct Blue 6, and Direct Brown 95 should be fully evaluated with regard to possible human effects. Unless the toxic effects of the alternative have been thoroughly evaluated, a seemingly safe replacement, possibly only after years of use, may be found to induce serious health effects.

o Contaminant Controls

The most effective control of Direct Black 38, Direct Blue 6, and Direct Brown 95, where feasible, is at the source of contamination by enclosure of the operation and/or local exhaust ventilation.

If feasible, the process or operation should be enclosed with a slight vacuum so that any leakage will result in the flow of air into the enclosure.

The next most effective means of control would be a well designed local exhaust ventilation system that physically encloses the process as much as possible, with sufficient capture velocity to keep the contaminant from entering the work atmosphere.

To ensure that ventilation equipment is working properly, effectiveness (e.g., air velocity, static pressure, or air volume) should be checked at least every three months. System effectiveness should be checked soon after any change in production, process, or control which might result in significant increases in airborne exposures to Direct Black 38, Direct Blue 6, and Direct Brown 95.

o Employee Isolation

A third alternative is the isolation of employees. It frequently involves the use of automated equipment operated by personnel observing from a closed control booth or room. The control room is maintained at a greater air pressure than that surrounding the process equipment so that air flow is out of, rather than into, the room. This type of control will not protect those employees that must do process checks, adjustments, maintenance, and related operations.

o Personal Protective Equipment

The least preferred method is the use of personal protective equipment. This equipment, which may include respirators, goggles, gloves, and other devices should not be used as the only means to prevent or minimize exposure during routine operations.

Exposure to Direct Black 38, Direct Blue 6, and Direct Brown 95 should not be controlled with the use of respirators except:

-- During the time period necessary to install or implement engineering or work practice controls; or

-- In work situations in which engineering and work practice controls are technically not feasible; or

-- For maintenance; or

-- For operations which require entry into tanks or closed vessels; or

-- In emergencies.

Only respirators approved by the National Institute for Occupational Safety and Health (NIOSH) should be used. Refer to NIOSH Certified Equipment, December 15, 1975, NIOSH publication #76-145 and Cumulative Supplement June 1977, NIOSH Certified Equipment, NIOSH publication #77-195. The use of faceseal coverlets or socks with any respirator voids NIOSH approvals.

Quantitative faceseal fit test equipment (such as sodium chloride, dioctyl phthalate, or equivalent) should be used. Refer to A guide to Industrial Respiratory Protection, NIOSH publication #76-189 for guidelines on appropriate respiratory protection.programs.

In addition, proper maintenance procedures, good housekeeping in the work area and education of employees concerning the nature of the hazard, its control and personal hygiene are all aspects of a good control program.

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			- August 22, 1975
			- October 7, 1975
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*	20.	Tetrachloroethylene (Perchloroethylene)	- January 20, 1978
	21	Trimellitic Anhydride (TMA)	- February 3, 1978
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*Cancer related alerts

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
CENTER FOR DISEASE CONTROL
NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH
ROBERT A. TAFT LABORATORIES
4676 COLUMBIA PARKWAY, CINCINNATI, OHIO 45226

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