IV. BIOLOGIC EFFECTS OF BENZIDINE-BASED DYES

Human Cancer Studies

Two epidemiological studies have reported that exposure to benzidine-based dyes produces cancer in humans [47-49]. Yoshida et al [47,49] examined the possibility of a relationship between employment in the dyeing industry and an increased risk of developing bladder cancer. Occupational histories were available for 200 male patients, all of whom had bladder cancer and who resided in Kyoto, Japan. One control group consisted of 148 men, at least 45 years old, who had been admitted to hospitals in the area with urinary disorders other than malignant tumors. Of the 200, 17 had worked in the dyeing industry. Bladder cancer patients were 6.8 times more likely to have been employed as dyers than patients with other urinary disorders. Ten of the 17 had been Kimono painters. In a subsequent survey of Kimono painters in the Kyoto area, Yoshida and Miyakawa [47] found that the practice of wetting the brush or spatula on the tongue was common. Of 141 persons interviewed, 47% admitted to this practice. This indicated that ingestion of benzidine-based dyes was likely. No information on specific dyes to which the bladder cancer patients were exposed was available. However, Yoshida and Miyakawa referred to four benzidine-based dyes, Direct Black 38, Direct Green 1, Direct Red 17, and Direct Red 28, that had widespread use in Japan in the early 1970's [47].

Genin [48] also studied worker exposure to benzidine-based dyes. After preliminary work in which benzidine or dianisidine had been found in the urine of rats given dyes based on these substances, Genin examined the urine of 22 workers engaged in the drying and grinding of direct azo dyes [48]. Benzidine was present in the urine of eight persons and dianisidine was present in three. The quantities varied from what were described as trace amounts to 0.3 μg/ml. Although the dyes being worked with at the time of urine sample collection were not described, Genin referred to two benzidine-based dyes, Direct Black 38 and Direct Blue 2, as being of greatest commercial importance in the USSR. Genin then examined the company records and found five cases of bladder tumors. Three had occurred between 1965 and 1968 among workers engaged in drying and grinding of direct azo dyes. The persons were 68, 70, and 72 years old; had latent periods of 18, 33, and 43 years; and had been exposed 24, 3, and 18 years, respectively. Genin concluded that exposure to direct azo dyes, synthesized from diphenyl amino derivatives, is potentially a cancer hazard. A dose-response relationship could not be established, since the intensity of exposure to the dyes was not measured.

Animal Cancer Studies

The National Cancer Institute (NCI) conducted a 93-day study with mice and rats that were fed three commercially available benzidine-based dyes: Direct Black 38, Direct Blue 6, and Direct Brown 95 [29]. The three dyes

were technical grade, factory-strength direct dyes. The molecular structures are shown in Appendix III, and the benzidine moiety can be identified in each structure. Direct Blue 6 was 66% pure, Direct Black 38 was 86% pure, and Direct Brown 95 was 79% pure, according to the manufacturer. Midwest Research Institute confirmed similar values upon reanalysis of the materials. The balance of the constituents was mostly salt and water, but analyses by thin-layer chromatography (TLC), which used two different solvent systems, demonstrated the presence of 8-15 minor impurities in each dye. No attempt was made to identify or quantitate these individual impurities. A specific analysis by high-pressure liquid chromatography was done to determine the presence or absence of benzidine. No benzidine was detected at the lowest detectable limit of 0.004% (40 ppm) in any of the dyes. Corn oil was added to the dyes at 1.3% to suppress dust formation. While no specific analyses were done for benzidine in the food, spectrophotometric analyses of extracts of the diet showed that each of the dyes was stable in feed for up to 2 weeks at temperatures up to 45 C.

For the bioassay in Fischer 344 rats, each dye was fed to 10 male and 10 female rats at 190, 375, 750, 1,500 or 3,000 ppm [29]. Each dye was fed to 10 B6C3F1 mice of each sex at 750, 1,500, 3,000, 6,000, or 12,500 ppm, except that females were given Direct Brown 95 only up to 6,000 ppm. Ten matched mice and rats of each sex served as controls at each dose level. All animals except controls received one of the three dyes in their feed for approximately 13 weeks.

The first observed tumor in the rats given dye occurred before 5 weeks in each case, regardless of the type of dye fed [29]. This time-to-tumor interval is the shortest encountered in the NCI bioassay program thus far. Dibromoethane has the next shortest time-to-tumor interval, which was 10 weeks [50]. The shortest time-to-tumor for benzidine itself that has been observed in adult rats is 6 months [7,8,13]. Tumor incidences in the rats given 1,500 ppm of a benzidine-based dye in the food are shown in Table IV-1.

The single most important aspect of this study [29] was that significant numbers of cancerous and precancerous lesions developed in the rats within 93 days; hepatocarcinomas and/or neoplastic nodules developed in a significant number of rats, except in the males exposed to Direct Brown 95. With this dye the lesions found in male rats were limited to those described in the NCI report as precancerous. Neither cancerous nor precancerous lesions occurred in any of the control animals, nor had such lesions ever been observed in any of the controls in previous studies within a 93-day time period.

The investigators [29] concluded that the cancer observed was caused by the dyes themselves or a metabolite and was not due to benzidine as an impurity. Since the benzidine concentration in each dye was less than 40 ppm, and since the time-to-tumor interval for benzidine in rats at even higher levels has always been much longer than that observed for the dyes [7,8,10,13-15,18-21], this conclusion appears to be justified. The

possible role of other impurities in the demonstrated carcinogenicity of the dyes cannot be established from the information contained in the report.

TABLE IV-1

NEOPLASTIC RESPONSES IN FISCHER 344 RATS
TO THREE BENZIDINE-BASED DYES AT 1,500 ppm

	Hepatocellular Carcinoma		Neoplastic Nodules		Basophilic Foci	
	Male	Female	Male	Female	Male	Female
Direct Blue 6	2/10	0/10*	6/10	0/10	1/10	0/10
Direct Black 38	4/9	0/10	5/9	8/10	3/9	0/10
Direct Brown 95	0/9	1/8	0/8	4/8	7/8	3/8
Matched Controls	0/10	0/10	0/10	0/10	0/10	0/10

*At 3,000 ppm in female rats, the incidence of hepatocellular carcinoma was 4/10.

Adapted from reference 29

The Clearinghouse on Environmental Carcinogens, a group representing industry, government, and the public, makes final evaluation of the experimental results on substances tested by the NCI bioassay program. The group concluded that Direct Blue 6 and Direct Black 38 dyes caused cancer in both sexes of Fischer 344 rats under the conditions of this bioassay procedure, and that Direct Brown 95 dye caused cancer only in female rats [29]. The Clearinghouse on Environmental Carcinogens concluded that testing at lower levels for longer durations was unnecessary because of the rapid appearance of the tumors. The premature termination of the study, originally contracted to be carried out over the normal lifetimes of the rats and mice and at nontoxic levels, precluded demonstration of any carcinogenic effect in the mouse or a dose-response relationship in the rat.

In contrast to the NCI study, two other investigations of the carcinogenic properties of benzidine-based dyes have given equivocal results. In a 270-day study by Fujita et al [51], Direct Blue 6 was

injected subcutaneously into 20 male and 20 female rats of an unspecified strain. Total doses were 170 and 180 mg, respectively, given at weekly or biweekly intervals. The daily dose was 1 ml of an aqueous 1% solution of the dye. Total survival at 270 days was 50%. In 2 of the 20 females, injection site sarcomas developed at 211 and 216 days, respectively. While atrophy of the parenchymal cells of the liver and dilatation of the liver sinusoids were found during pathological examination of the rats, no pre-neoplastic or neoplastic lesions were reported. This study did not support the NCI findings of a rapid neoplastic response. The appearance of local sarcomas at injection sites may or may not indicate carcinogenicity in this study since no tumors were observed at remote sites [23].

Niitsu [33] studied the carcinogenicity of two benzidine-based dyes, Direct Blue 6 and Direct Black 38 (sources unspecified). Wistar rats were given the dyes via their drinking water (0.04%). Twenty male and twenty-five female rats were used to test Direct Black 38. Because Direct Blue 6 was found to be particularly toxic to male rats, the assay of this dye was limited to 20 female rats. The observation period was limited to only 14 months, since by that time a large number of animals had developed infections and died. The author [33] concluded that the immunological competence of the rats had been compromised. With Direct Black 38 administration, 14-month survival was 4/20 for males and 2/25 for females. One of the two surviving females had cancer of the breast (pathological designation not specified). With Direct Blue 6 administration, 12/20 female rats were alive at 12 months; 1 of the 12 surviving Wistar female rats had a glandular tumor of the outer ear. No tumors were found in the controls. Niitsu concluded that the carcinogenicity or lack of carcinogenicity of these two direct dyestuffs could not be determined from the results of the experiment [33].

Different strains of rats and different sources of dye were used in the Niitsu and the NCI studies. Also, the NCI results were found using concentrations of 1,500 and 3,000 ppm in the food [29], while the Niitsu study used 400 ppm in the drinking water [33]. The marked susceptibility of the rats to infection noted in the Niitsu 14-month study was not found in the shorter 93-day NCI study, except for one rat that died of bacterial infection. The 93-day period may have been too short a time to expect the type of infection noted by Niitsu [33].

Marshall [79], in 1953, reported that Vital Red (a benzidine-based dye) injected intraperitoneally (ip) into 27 Wister albino rats, aged 3-4 months, at a dosage of 1 ml of a 2% aqueous solution of the dye every 2 weeks for 7 months, produced no pathological changes. A detailed pathology description was not given in the report. Twenty animals maintained on the same diet were the negative controls for this study. In contrast, rats injected with Trypan Blue or Evans Blue (o-tolidine-based dyes) developed lymphomatous tumors. While this study cannot be considered a negative lifetime study, it is apparent that ip-injected Vital Red did not, under the experimental conditions stated, rapidly produce liver tumors as did oral adiministration of Direct Black 38, Brown 95, and Blue 6 in the NCI study.

Korosteleva et al [52] reported a study in 1977 designed to test the carcinogenicity of Direct Red 10. They utilized a total of 75 white rats of unspecified strain without stating the number used as controls. The purity of Direct Red 10 was not described. The dye was administered to the rats at 500 mg per rat daily in the food. Nephrotoxic and hepatotoxic effects were evident by the 100th day of observation. More severe kidney effects were noted at a still later stage. Direct Red 10, a benzidine-based dye, produced tumors in 10 of the 18 male rats that had survived 500 days. The type of neoplasms found in the study were four microcholangiomas (malignancy of mixed masses of liver cord cells and bile ducts), three leukemias (malignant transformation at white bood cells), two plasmacytomas (multiple myeloma, a neoplasm of plasma cells), and one hypernephroma (kidney neoplasm with structure that resembles cortical tissue at the adrenal gland).

These types of tumors, which are rare in rats, were markedly different from the hepatocarcinomas found in the NCI study [29]. Korosteleva et al also designed the study to determine whether Direct Red 10 could be metabolized to benzidine (see section on Animal Metabolic Studies).

Another chronic test of the carcinogenicity of Deep Direct Black EX (Direct Black 38) [28] was reported by Okajima et al in 1975 [53]. The dye was administered to male Wistar rats in the drinking water for 60 weeks. At that time all were killed since a significant number had developed neoplasms. Results are given in Table IV-2. This report confirmed that Direct Black 38 is carcinogenic in rats when administered at lower doses over a longer period of time than used in the NCI study [29]. Because the 60-week experimental period is much shorter than the rat's normal life span, a more extended experiment would be expected to result in still more tumors. As in the NCI bioassay [29], it would appear as though it was not necessary to continue this experiment [53] over the lifetime of the rats since a significant number of tumors has resulted by 60 weeks.

TABLE IV-2

HISTOLOGICAL FINDINGS IN THE URINARY BLADDER, LIVER, AND COLON IN RATS FED DIRECT BLACK 38 (DIRECT DEEP BLACK EXTRA)

Experimental Group	Effective No. of Rats	Urinary Bladder			Liver		Colon	Rats with Tumor
		Hyper-plasia	Papilloma	Carcinoma	Hyper-plasia	Carcinoma	Adeno-carcinoma	
		NUMBER OF OBSERVATIONS						
100 ppm Direct Black 38	8	1	0	0	1	0	0	0
500 ppm Direct Black 38	13	9	2	3	8	3*	2	6
Control	9	0	0	0	0	0	0	0

*One hemangioendothelioma

Adapted from reference 53

Okajima et al [53] and Niitsu [33] both administered Direct Black 38 to rats in the drinking water at similar concentrations (500 and 400 ppm, respectively). It is not apparent why carcinogenicity was established only in the study by Okajima et al [53]. Undoubtedly, the low survival rate in the Niitsu study was a major factor, since early deaths may have precluded the development of cancer in this study. The mortality rate in the Niitsu study was 97% for Direct Black 38, while the mortality rate in the Okajima study was 13% for the same dye.

Human Metabolic Studies

Humans as well as other mammals can change (metabolize) the benzidine-based dyes back to benzidine [27,47,48,52,54]. The major organ in which benzidine-based dyes are metabolized to benzidine is the liver, but other organs also can do this to a greater or lesser degree [54,55].

Various bacteria and yeast normally found in the small and large intestine can also reduce the azo bond in benzidine-based dyes and release benzidine [55-57]. This occurs both in vivo and in vitro [27,29,33,55].

The major enzyme capable of lysing (breaking) the azo linkage of benzidine-based dyes is a cytochrome oxidase. This enzyme, termed azoreductase, is associated with the cytochrome P-450 microsomal fraction of the cellular homogenate. The enzyme was extensively studied with relation to the azo dye prontosil [56], and has been described as an "enzyme par excellance" for lysing the azo linkage of dyes [58]. Azoreductase is an extremely nonspecific enzyme found in all mammals tested so far [55], as well as in various microorganisms [57]. It is not necessary that the substance be a dye for this enzyme to exert its action. The only requirement is the diazo linkage, ie, -N=N-. No exceptions have been reported [33,55]. Knowledge of this enzymatic capability in humans led to the following study [27].

In 1977, a NIOSH investigation was undertaken to survey industrial hygiene practices in the dye industry and to examine urine samples from workers exposed to benzidine-based dyes [27]. The study protocol included prior notification of the benzidine-based dye manfacturer and team sampling of environmental levels of total particulate. In most cases, it also included determination of the amount of dye present in the particulate and analysis of the dye samples for residual benzidine. In addition, determination of the urinary levels of benzidine through, in most cases, controlled dual analytical procedures was also performed. These procedures were developed through the efforts of the Clinical and Biomedical Support Section, Division of Biomedical and Behavioral Science, and the Measurement Services Section, Division of Physical Sciences and Engineering, NIOSH. Both procedures are described in the report [27]. One of the procedures is also given in Appendix I of this Special Hazard Review. The lowest detectable limit for nonspecific primary aromatic amines by this method was 1 ppb with the provision that at least 100 ml of urine be available for analysis. This method required confirmation of the nonspecific primary

aromatic amines as benzidine or monoacetyl benzidine by TLC. The other procedure used to confirm the split samples was electron-capture gas chromatography as developed by Nony and Bowman [59]. The lowest detectable concentration of benzidine in urine stated by these authors [59] was 1.4 ppb; for monoacetyl benzidine, it was 5.8 ppb.

Environmental and urinary samples were collected at six facilities where workers were potentially exposed to benzidine-based dyes. These facilities were: two benzidine-based dye manufacturers, two textile dyeing plants, a leather tanning plant, and a specialty paper mill [27].

In the first dye manufacturing facility, two of eight workers potentially exposed to benzidine-based dyes had monoacetyl benzidine in their urine. The concentration of primary amines was 3 ppb in one case and 7 ppb in the other. These spot samples of urine were positive despite the fact that the workers observed were using cartridge-type face respirators at the time of the sampling and that the environment appeared to be dust free. This facility has since discontinued the manufacture of benzidine-based dyes [27].

In the other dye manufacturing facility, four workers were monitored for exposure to benzidine-based dyes. The average environmental exposure levels of four of the workers were 4.3, 5.2, 11.7, and 17.4 mg total particulate/cu m. The corresponding urinary concentrations of benzidine averaged 52, 11, 10, and 112 ppb, respectively. The worker having 112 ppb benzidine in his urine (spray dry operator) also had 590 ppb monoacetyl benzidine in the same sample. This facility has also taken measures since then to control dyestuff exposures and to monitor the urine of workers for benzidine [27].

Three of the above four workers had benzidine congeners other than monoacetyl and diacetyl benzidine in their urine. Two of these workers had o-tolidine in their urine at 15 and 50 ppb. The third had o-dianisidine in his urine at 1 ppb. At the time of the investigation, these three workers were not exposed to o-tolidine, o-dianisidine, or to dyes derived from these two substances. However, prior exposure to any of these substances could not be ruled out. Therefore, the source of o-tolidine and o-dianisidine in the urine of these workers could not be established. Many workers, who were monitored and found to have short-term environmental exposures as high as 92.7 mg total particulate/cu m, did not provide urine samples to the investigators and thus their urine could not be evaluated for the presence of benzidine [27].

In one textile dye manufacturing facility, 7 potentially exposed workers were compared with 23 nonindustrially exposed office workers who were used as controls. Direct Black 38 and Direct Blue 2, both benzidine-based dyes, were being used at this facility. No benzidine was detected in the urine of any control. Urinary concentrations of benzidine in the seven potentially exposed workers ranged from below the limit of detection to 39 ppb; three had both benzidine and monoacetyl benzidine in the urine (one dye tub operator and two dye-weighers). The four other potentially exposed

workers had no benzidine or monoacetyl benzidine in the urine. The total airborne particulate material (measured gravimetrically) ranged from 1 to 4 mg/cu m. Neither the dye concentration nor the types of dye in the particulates were determined.

In the other textile plant, 10 workers were also monitored in the same way. The dyes used were Direct Blue 6, Direct Black 38, Direct Brown 95, and Direct Red 8. All airborne concentrations were less than 2 mg total particulate/cu m. Some exposures were equivalent to those in the first textile dye facility. At this facility, the amount of benzidine-based dye in the particulate samples was measured colormetrically. The amount ranged from 0 to 29% by weight in the different samples. Presence of benzidine in the urine was expected but not found [27].

Dye exposure in the leather finishing facility was limited to Direct Black 38 and Direct Brown 95. Time-weighted average (TWA) environmental concentrations were 0.69, 5.79, and 10.65 mg/cu m (three samples each). Each of the three workers potentially exposed wore NIOSH-approved half-face cartridge respirators. No benzidine was found in the urine of the three workers [27].

In the speciality paper processing facility, 23 environmental samples and 47 urine samples were analyzed. The environmental samples were all less than 6 mg/cu m (range, 0.17-5.10 mg total particulate matter/cu m). In this facility, management had recently initiated a program in which respirator use was strictly enforced. Approximately 1,667 kg (3,000 lb) of Direct Black 38 were consumed during the 3-day survey. Despite this heavy consumption, no urine samples contained benzidine [27].

Time limitations prevented extended and repeated monitoring of the environment and the workers under a variety of conditions [27]. The time of day for urine collection, for example, may be critical, because exposure is most likely during work hours and a major portion of the benzidine metabolites may have been eliminated in the urine before spot sampling the following day. This possibility of a cyclic type of excretion pattern is made more apparent by work in experimental animals showing that the benzidine metabolites of Direct Black 38 were largely excreted within the first 16 hours after dye intake [37]. Since spot samples were taken during the workshift, the peak excretion phase may have been missed.

This study [27] demonstrated that benzidine can be found in the urine of workers who have contact only with the finished dyes under the present working conditions of the industry. No dye samples contained more than 25 ppm benzidine. Calculations provided in the NIOSH report demonstrated that the amount of benzidine found in urine of the workers was too great to have come only from benzidine impurity in the dye, and thus was a metabolic breakdown product of the dye. Even when it appeared that standard protective equipment such as cartridge respirators were used, the occurrence of benzidine in the urine was not necessarily prevented. One pulverizer operator who was observed to be using a half-face respirator had 52 ppb benzidine in his urine. However, this worker was only observed a

short time and it is not known whether he used the respirator throughout the day.

Following identification of 2,4-diaminoazobenzene (at 9,200 ppm) and 4-amino biphenyl as contaminants in Direct Black 38, the National Center for Technical Research (NCTR) [37] reanalyzed some of the the urine samples from workers studied in the above NIOSH investigation [27]. Although quantitative data were not given, 2,4-diaminoazobenzene was reported to be present in some urine samples but not 4-amino biphenyl [37]. The International Agency for Research on Cancer (IARC) has reviewed the carcinogenic effect of 2,4-diaminoazobenzene, and designated it an animal carcinogen [60]. OSHA regulates 4-amino biphenyl as a carcinogen in the same manner as benzidine.

Genin [48] analyzed the urine of 22 workers who had potential long-term contact with benzidine-based dyes during the manufacture of the direct azo dyes Direct Black 38, Direct Blue 2, Direct Blue 15, and Direct Blue 218. He found benzidine in the urine of 8 of the 22 workers potentially exposed and dianisidine in 3. The concentrations of benzidine or dianisidine in the urine ranged from what were described as "trace amounts" to 300 ppb, but the individual levels were not reported. Although this study demonstrated that workers exposed to benzidine-based or dianisidine-based dyes may have benzidine or dianisidine in their urine, quantitative exposure could not be measured under the conditions present, and dose-response data were not reported.

Korosteleva et al [52,61], in studies from 1966 to 1977, identified a benzidine complex in the serum of workers in a textile factory. The amount of the benzidine complex in the serum depended on the extent and duration of exposure to direct dyestuffs in the workplace [61]. In this study, the author compared the blood serum of female textile mill workers (18-60 years old) with that of nonindustrially-exposed blood donors. He found 22 of 77 workers potentially exposed to any type of dye had benzidine complexed to albumin in the serum, compared with no instances of this complex in 24 nonindustrially-exposed blood donors. Further, those workers exposed only to direct dyes showed an incidence of 19 of 40, while 21 workers exposed to dyes that were not direct dyes, or to other industrial substances, had no benzidine-albumin complex in their serum. Since the major direct dyes reported were benzidine-based [1,2,38], and the benzidine-albumin complex was only found in the workers exposed to direct dyes, the only reasonable source of the benzidine in the blood was from the benzidine-based dyes.

Thus, two Russian studies [48,61] and one US study [27] have demonstrated that benzidine or benzidine complexes are present in the body fluids of humans exposed to benzidine-based dyes.

Animal Metabolic Studies

As early as 1911, it was known that azo dyes could be metabolized to simpler components. Sisley and Porcher [62] reported that when dogs

received oral doses of Orange 1, a monoazo dye, the dye was reductively cleaved at the azo linkage, resulting in sulfanilic acid production in the urine. Sisley and Porcher further demonstrated that it was necessary for Orange 1 to pass through the intestinal tract to be lysed. They suggested at that time that the microbial flora of the digestive tract was essential for the reduction of this dye [62]. In 1970, Walker reviewed the metabolism of azo compounds and concluded that many species of animals, yeast, and bacteria could reduce the azo linkage [55]. The nonspecificity of azoreductase has been repeatedly demonstrated [54-57]. This enzyme rapidly and efficiently breaks the double bond in the N=N linkage regardless of the other entities in the substrate.

The National Cancer Institute found that both rats and mice can metabolize benzidine-based dyes to benzidine [29]. Prior to feeding Direct Black 38, Direct Brown 95, and Direct Blue 6 in the diet, the investigators analyzed each batch of dye, and detected no free benzidine (detection limit was 0.004%). The amounts of benzidine found in urine of the animals are given in Tables IV-3 and IV-4.

Although there was no direct correlation between the amount of benzidine excreted in the urine and the incidence of tumors in rats, each animal fed dye excreted benzidine, and the amount excreted was dose-related in most cases to the amount of dye administered [29]. Since food consumption was not reported, individual dose-excretion ratios could not be calculated.

Benzidine was measured in the urine of mice and rats 3 and 11 and 4 and 12 weeks, respectively, after the experiment began [29]. The mice excreted approximately the same amount of benzidine at 3 weeks as the rats did at 4 weeks, and, in general, the mice excreted considerably more at 11 weeks than rats excreted at 12 weeks. There were no tumors found in mice by 93 days, while high incidences were found in rats exposed for the same period. Since benzidine alone did not produce tumors in rats until approximately 6 months of exposure at high doses [14], the production of tumors in rats by 93 days suggested that the parent dye (or a metabolite other than benzidine) was the active carcinogen and that carcinogenicity did not depend exclusively on the presence of benzidine, per se. The NCI report concluded that the benzidine found in urine was a product of dye biotransformation and not from a benzidine contaminant in the dye [29]. The role of other known carcinogens in these dyes, such as 4-amino biphenyl or 2,4-diaminoazobenzene [37], was not examined; since they were not suspected contaminants at that time, no analysis was carried out to establish their presence or absence. It should be noted that the analytical techniques used in the NCI study were colorimetric assays similar to the one described in Appendix I. These methods are not specific for benzidine, and it is possible that metabolites and/or other aromatic amines were responsible for the colorimetric response [29].

TABLE IV-3

BENZIDINE EXCRETION PER RAT (μg/24 h)*

Dye Dietary Concentration, ppm	Weeks on Diet: 4 — Male	4 — Female	12 — Male	12 — Female
Direct Blue 6				
3,000 or 1,500**	5.8 (0.9)***	8.0 (6.7)	0.77 (0.65)	0.55 (0.29)
750	1.4 (0.8)	0.94 (0.27)	0.32 (0.10)	0.29 (0.18)
190	0.85 (0.18)	0.62 (0.17)	0.44 (0.41)	0.16 (0.10)
Direct Black 38				
1,500	3.6 (4.8)	16.8 (n=2)	0.16 (0.03)	0.31 (0.16)
750	1.7 (n=2)	2.1 (0.06)	0.46 (0.09)	1.4 (0.35)
190	0.55 (0.31)	0.44 (0.13)	0.49 (0.39)	0.43 (0.32)
Direct Brown 95				
750	4.2 (1.3)	3.7 (2.9)	0.44 (0.12)	1.1 (n=1)
375	1.0 (0.77)	4.2 (1.3)		5.8 (9.7)
190	0.80 (n=2)	0.66 (0.24)	0.29 (0.11)	0.27 (0.05)

*Samples from untreated controls taken at weeks 4 and 12 showed no benzidine when spotted on TLC plates.

**Female rats at week 4 were from the 3,000-ppm group; male rats at week 4 and both males and females at week 12 were from the 1,500-ppm group.

***Numbers in parentheses are standard deviations. If fewer than three samples were averaged, the number of samples is given in parentheses instead.

Adapted from reference 29

TABLE IV-4

BENZIDINE EXCRETION PER MOUSE (μg/24 h)*

Dye Dietary Concentration, ppm	Weeks on Diet: 3 — Male		3 — Female		11 — Male		11 — Female	
Direct Blue 6								
12,500	5.2	(0.85)**	5.1	(n=2)	2.4	(1.3)	5.5	(1.0)
3,000	0.97	(0.32)	1.1	(0.35)	1.7	(0.62)	3.1	(0.94)
750	0.55	(0.65)	0.31	(0.023)	1.1	(0.72)	0.52	(0.17)
Direct Black 38								
12,500	12.8	(2.8)	6.08	(1.8)	14.4	(2.7)	8.6	(1.0)
3,000	3.5	(2.1)	7.3	(n=2)	7.3	(2.2)	7.4	(1.7)
750	3.6	(3.4)	3.0	(2.7)	2.8	(3.2)	2.0	(1.8)
Direct Brown 95								
12,500	9.4	(n=2)			7.5	(0.90)		
3,000	4.7	(0.93)			1.2	(0.20)		
750	0.39	(0.09)			0.49	(0.23)		
6,000			3.5	(1.8)			3.2	(0.59)
1,500			2.8	(0.85)			0.35	(0.12)
375	•		0.56	(0.19)			0.19	(0.12)

*Samples from untreated controls taken at weeks 3 and 11 showed no benzidine when spotted on TLC plates.

**Numbers in parentheses are standard deviations. If fewer than three samples were averaged, the number of samples is given in parentheses instead.

Adapted from reference 29

Aromatic amines, such as benzidine, produce tumors only indirectly [24]. They are first converted by the body to a more reactive substance, which has been termed the ultimate (or proximate) carcinogen [24]. The NCI investigators [29] did not attempt to identify specific metabolites of benzidine, and it is not known if N-hydroxy diacetyl benzidine (one metabolite suggested as the ultimate carcinogen [24,63]) was among the metabolites in either the rat or mouse urine.

Rinde [34] and Rinde and Troll [64] reported that when any of four benzidine-based dyes, Direct Blue 6, Direct Black 38, Direct Brown 95, and Direct Red 28 (Congo Red), was administered to rhesus monkeys by gavage, benzidine and its monoacetyl derivative could be detected in the urine on an average of 1.25% of the benzidine moiety in the dyes studied. When benzidine itself was fed, free benzidine and its monoacetyl derivative were detected in the urine on the average of 1.45% of the original benzidine fed. The authors [34,64] concluded on the basis of the above evidence that nearly total conversion of the dye to benzidine took place. However, this may not be the case because water-soluble metabolites of benzidine or the dye may constitute a differing proportion of the metabolites than the sparingly-soluble portion [37].

Because each dye was administered in dimethyl sulfoxide (DMSO), absorption of the dye from the intestine would be expected to be greater than when administered in aqueous solution, since DMSO enhances solubility and absorption. Since no other metabolites were investigated, NIOSH does not consider the report's conclusion of complete conversion of benzidine-based dyes to benzidine as appropriate. Nevertheless, the fact was established that there is at least partial conversion of each of the four dyes to benzidine under the conditions of the experiment.

Direct Black 38 and Direct Blue 6 dyes at 0.04% (400 ppm) were injected by Niitsu [33] into the ligated, incubated intestines of mice. Benzidine was isolated from the intestinal contents after introducing Direct Black 38 but not after introducing Direct Blue 6. Direct Black 38, Direct Green 1, Direct Red 17, and Direct Red 28 dyes were injected by Yoshida and Miyakawa into ligated, incubated intestines of mice and rats in similar experiments [47]. Benzidine was detected as a metabolic product of the dye in each case. In control experiments in which the dye solution was instilled into the intestines that were turned inside out, no free benzidine was detected [47].

Dieckhues [65] investigated the ability of 21 common bacterial species to reduce azo dyes. All species tested were capable of this action. Direct Red 10, Direct Red 17, Direct Red 28, Direct Orange 8, and Direct Black 38 were found to be susceptible to azo reduction in this study. Thus, bacterial lysis of the azo bond in the intestine is probably a basic means of producing benzidine from the benzidine-based dyes. This benzidine is then available to be absorbed into the body and excreted by the kidney to produce an effect on the bladder. An increase in the bacterial azoreductase enzyme level in the intestine was brought about in Fischer 344 rats by feeding a meat-based diet in place of the normal grain-based diet

[66]. It is not known whether rats given benzidine-based dyes together with a meat-based diet would excrete more benzidine in the urine than when fed the dyes together with a grain-based diet (such as used in the NCI study), but such an effect would not be unexpected. This is significant, since man generally consumes a diet high in meat protein.

A review article on benzidine metabolism [55] called attention to results from various investigations [57,67] that showed that a variety of azo dyes including benzidine-based dyes were reducible at the azo linkage. In the case of azonaphthols, reduction occurred far more readily in the bacterial system than the liver preparation [57]. Direct Blue 6, however, while reducible in vivo [2,34], was not reported to be reducible by the intestinal bacteria of female mice of a strain designated as dd [33].

Yoshida et al reported in 1973 that Direct Black 38 (Direct Deep Black EX) was reducible both by E coli and common soil bacteria [67]. The E coli used were isolated from humans. The common soil bacteria were those taken from soil as well as from raw river water. Thin-layer chromatography was used for benzidine detection, and adequate negative and positive controls were used. Benzidine was found to be a reduction product from all bacterial samples used. In addition, Yoshida et al demonstrated that 3 g of cotton cloth dyed with Direct Black 38 yielded benzidine when incubated with the bacterial flora of raw river water for 72 hours. The color of the fabric faded under the action of the bacteria but not when incubated for 2 weeks with distilled water. This is the only investigation found in the literature that dealt with bacterial breakdown of benzidine-based dyes once the dye is attached to a fabric. The importance of this investigation can also be appreciated by considering that while intact benzidine-based dyes may not penetrate the skin [34], the benzidine portion of the molecule is readily absorbed through the skin [7-9,11,14,16,17,45]. Since E coli is a bacteria commonly found on the skin, it is likely that the dye attached to a benzidine-based dyed fabric that contacts the skin will break down to benzidine. Since benzidine can be absorbed directly through the skin, fabric with benzidine-based dyes can be a source of this compound. E coli is unusually resistant to the bacteriostatic effect of dyes in general and grows at temperatures as low as 20 C [68].

In 1977, Korosteleva et al [52] demonstrated that rats of an unspecified strain, given 500 mg of Direct Red 10 orally each day, could metabolize this benzidine-based dye to benzidine, which then acted as a hapten forming a complex with protein in the liver and kidney within 4 days after the initial dose. By the 30th day, the benzidine complex was present in the blood. The investigators reported a correlation between the carcinogenicity of Direct Red 10 and its ability to form antigens containing the benzidine moiety in vivo.

Metabolism studies on Direct Black 38 were recently completed for NIOSH at the National Center for Toxicological Research, in Jefferson, Arkansas [37]. Sensitive as well as specific analytical chemical methods were developed for assay of the known and the proposed impurities in Direct Black 38 as well as its known and proposed metabolites. Similar studies

were carried out for possible metabolites of the substance Pigment Yellow 12, a pigment containing and derived from 3,3'-dichlorobenzidine. This pigment was not metabolized to benzidine, dichlorobenzidine, 2,4-diaminoazobenzene, or 4-amino biphenyl, confirming prior studies in other species [35].

Fifteen male Syrian golden hamsters weighing approximately 110 g were administered Direct Black 38 at 100 mg/kg by gastric lavage [37]. Urine was collected for analysis at intervals up to 7 days. Three hamsters were used as controls. The dry, purified dye was analyzed and found to contain 3 ppm benzidine, 6 ppm 4-amino biphenyl, and 670 ppm 2,4-diaminoazobenzene. Further attempts at purification to eliminate the 2,4-diaminoazobenzene were unsuccessful.

The major portion of all metabolites of Direct Black 38 was excreted within 16 hours after administration. The average totals of metabolites excreted by 16 hours are shown in Table IV-5.

TABLE IV-5

METABOLITES OF DIRECT BLACK 38 EXCRETED WITHIN 16 HOURS

Metabolite	(mg)
Benzidine	7.4
Monoacetyl benzidine	424
Diacetyl benzidine	21.0
4-Amino biphenyl	9.9
Hydrolyzable benzidine*	257
Hydrolyzable 4-amino biphenyl*	5.1

*Hydrolyzable means that these substances were originally present as conjugates and were divided by adding sodium hydroxide.

Adapted from reference 37

This analysis would account for approximately 10% of the benzidine moiety available in the Direct Black 38 originally fed to these animals.

The presence of 4-amino biphenyl as a metabolite in the hamster urine is significant in that 4-amino biphenyl is a substance regulated by OSHA as

a carcinogen in the same manner as benzidine [7]. In addition, the presence of relatively high concentrations of monoacetyl benzidine and hydrolyzable benzidine in the urine means that at some point the total exposure of each animal to benzidine in this experiment must have been several orders of magnitude greater than that indicated by the concentration of benzidine itself in the urine [34,37]. In monkeys, Rinde [34] was able to recover approximately 1.5% of the benzidine contained in each of four benzidine-based dyes as benzidine or monoacetyl benzidine. A major portion of benzidine would be expected to be excreted in a conjugated form and would not be detected unless the urine is first treated with an alkali to hydrolyze the conjugates of benzidine. Methods developed up to this point have not included a hydrolysis step. Future methods for monitoring human urine should utilize a hydrolysis step to provide the most sensitive indicator of exposure to benzidine-based dyes [37].

The International Business Machines Corporation recently reported preliminary data to the Environmental Protection Agency on a test to determine possible skin absorption of Direct Black 38 in rabbits [69]. The diphenyl portion of Direct Black 38 was first labeled with carbon-14. A proprietary mixture of Direct Black 38 was then applied to the skin of two rabbits. At the end of 144 hours, 91% of the radioactivity was recovered in the urine and feces of the rabbits. This indicates that Direct Black 38 or a portion of the molecule had penetrated the skin. Previous work by Rinde in monkeys had not shown skin absorption in that species [34].

In another preliminary study, Matthews [70] examined the following benzidine-based dyes: Direct Blue 2, Direct Black 4, Direct Brown 2, Direct Red 28, Direct Orange 8, and Direct Green 1. Each was fed to one of six female mongrel dogs at 100 mg/kg. Benzidine itself was fed to a seventh dog as a positive control. The treated dogs were held in individual metabolism cages, where they received food and water ad libitum. The urine was collected daily for 3 days, and analyzed for benzidine. The amount of benzidine measured in each urine varied from 320 to 1,675 μg, but, in every case, dye administration resulted in the excretion of benzidine. In the case of Direct Brown 2, benzidine excretion after administration of the dye exceeded total urinary benzidine excretion observed in the positive control dog given pure benzidine.

These results increase to 11 the number of dyes that have been demonstrated to be metabolized to benzidine in humans, monkeys, rats, hamsters, mice, or dogs [27,29,33,34,47,48,61,64,70].

V. EVALUATION AND CONCLUSIONS

Benzidine, an intermediate in the synthesis of most benzidine-based dyes, is controlled as a human carcinogen in the workplace. When a Federal standard for benzidine (29 CFR 1910.1010) was promulgated in 1974, there was little evidence to suggest that dyes prepared from benzidine were carcinogenic. Since then, a number of cases of bladder cancer have been reported in two groups of workers with exposure to benzidine-based dyes [47,48]. These reports are meaningful in that they provide evidence that man is susceptible to the carcinogenic action of these dyes. However, the major evidence for the carcinogenic action of benzidine-based dyes is found in controlled animal studies. Rats fed Direct Blue 6, Direct Black 38, and Direct Brown 95 developed tumors in as little time as 5 weeks [29]. By 13 weeks, many exposed rats developed hepatocarcinomas or neoplastic nodules. In a separate study in which rats were fed Direct Red 10, carcinogenic activity was also demonstrated [52]. In yet another study, Direct Black 38 was found to be carcinogenic in rats when given at lower doses over a longer period of time [53]. Since the results in animals support the findings in humans, it must be concluded that benzidine-based dyes may cause cancer in humans.

Studies on ligated intestine [33] and bacteria commonly present in the intestine [65] have shown that the azo linkage can be broken to yield benzidine from Direct Black 38, Direct Red 10, Direct Red 17, Direct Red 28, and Direct Orange 8. While inhalation is a major route of employee exposure to benzidine-based dyes [27,28,48], many of the inhaled dye particles may be too large to reach and be retained in the lung. They then would be returned to the epiglottis by the cilial action of the bronchial mucosa or trapped by nasal impaction, and then swallowed so that they become available for absorption by the body. In addition, hand to mouth transfer, contamination of foods, or poor work practices would lead to oral ingestion of the dyes. Bacterial reduction in the intestine would represent one source of benzidine in such cases.

Other available evidence suggests that cleavage of the azo linkage of the dye also occurs after absorption of the dye, resulting in the release of benzidine in vivo. Benzidine has been found in the urine of workers who handled benzidine-based dyes in the dye manufacturing and textile industries [27]. In a Russian study, about half of the textile mill workers examined who handled direct dyes had benzidine-albumin complexes in the blood [61]. In another Russian study, 8 of 22 workers who handled benzidine-based and o-dianisidine-based dyes had benzidine in the urine [48]. In this latter study, examination of company records revealed five cases of bladder cancer. Benzidine has also been identified in the urine of mice [29], rats [29], hamsters [22,37], dogs [70], and monkeys [34,64] exposed to a number of benzidine-based dyes.

Further research is needed to clarify the issue of skin absorption of the dyes. However, it is known that Direct Black 38 can be reduced to

benzidine by bacteria commonly found on the skin [67]. This suggests that the dermal route could be a source of employee exposure since benzidine is readily absorbed through the skin [9]. Preliminary work in rabbits supports this as a possible route of exposure [69].

The evidence presented above demonstrates that benzidine is a metabolic product of at least 11 benzidine-based dyes. The azoreductase enzyme that breaks down these dyes to benzidine is ubiquitous and generic. It acts on a multitude of azo compounds, containing a large variety of individual components and has been observed to cleave the N=N linkage common to these compounds. The ability to be metabolized in vivo to a known carcinogen is sufficient evidence to necessitate regulation of all benzidine-based dyes. In addition, animal experiments have suggested that these dyes could have a greater potential for carcinogenicity than benzidine alone, since the dyes have been reported to form tumors much more quickly than benzidine [29]. Therefore, benzidine-based dyes may be a more robust source of the ultimate carcinogen. Impurities introduced in the manufacture of the dye may also be a factor. For example, 4-amino biphenyl and 2,4-diaminoazobenzene were identified in commercially prepared Direct Black 38 [37]. Both contaminants are important because 2,4-diaminoazobenzene is considered a carcinogen by IARC [50] and 4-amino biphenyl is regulated as a human carcinogen (29 CFR 1910.1011). Mutagenesis tests using the Salmonella typhimurium (TA-98 and TA-100) assay with activation were positive for the following substances: the urine of hamsters fed Direct Black 38, the major metabolites of this dye (benzidine, monoacetyl benzidine, diacetyl benzidine, and 4-amino biphenyl), and the dye itself [37,72].

Studies have reported that four benzidine-based dyes rapidly induce cancer in experimental animals, suggesting that these substances may have a greater carcinogenic potential than can be attributed to their metabolite benzidine alone. Other studies have reported a number of cases of bladder cancer in two groups of workers exposed to benzidine-based dyes, but not to benzidine. In addition, all of the 11 benzidine-based dyes thus far tested have consistently been metabolized in animals to the carcinogen benzidine. The azoreductase enzyme responsible for formation of benzidine in the body is known to be nonspecific in its action and is found in bacteria, animals, and humans. Occupational exposure to benzidine-based dyes has also resulted in benzidine formation in the bodies of workers. This then indicates that there is an extremely high probability that those yet untested benzidine-based dyes can be metabolized to benzidine also. Based on a combination of the above factors, NIOSH concludes that all benzidine-based dyes, regardless of their physical state or proportion in a mixture, should be recognized as potential human carcinogens. In addition, NIOSH recommends that the production, use, storage, packaging, and distribution of all benzidine-based dyes be discontinued in light of present evidence of potential carcinogenic risks. The replacement of benzidine-based dyes with less toxic substitutes should be initiated immediately. As an interim measure, stringent controls and work practices are recommended to minimize exposure to any of the benzidine-based dyes.

During this interim period, since the carcinogens benzidine, 4-amino biphenyl, and 2,4-diaminoazobenzene have been identified as contaminants or breakdown products of a commercially prepared benzidine-based dye [37], particular attention should be given to the possible increase in concentration of these impurities in the cleanup of spills or leaks, waste disposal, and hot dyeing processes.

A number of reports have considered or referred to the use of substitutes to replace benzidine-based dyes [2,5,28,32,40]. Several companies now market substitutes for each commercially important benzidine-based dye (including Direct Black 38) [43]. A number of the direct dye substitutes, however, are based on the benzidine congeners o-tolidine and o-dianisidine. NIOSH has previously concluded that there is reason to believe that o-tolidine will induce bladder cancer in humans [73]. A study conducted for NCI has demonstrated the carcinogenic activity of o-dianisidine in animals [74]. Information available, however, on the metabolism and carcinogenic effects of the dyes containing these congeners is extremely limited. Although there is as yet no information on the carcinogenic potential of o-dianisidine dyes, limited animal studies have demonstrated a carcinogenic effect of two o-tolidine dyes [75]. In the absence of additional information, NIOSH recommends that the benzidine congener dyes be handled with extreme care in the workplace and that exposure to these dyes be minimized.

Substitution of noncarcinogenic dyes for those that are benzidine-based is essential. However, it must be recognized that the structural requirements for a compound to impart color leads to the use of dyes containing moieties that tend to be chemically reactive and toxic. Thus, the possibility of metabolic conversion to even more toxic compounds, as well as the effects of the dye itself, must be considered. In addition, toxic impurities can be introduced in manufacture. Information on the toxic effects of possible substitutes should be taken into account during replacement of benzidine-based dyes. If such information is incomplete or suggests that the dye might also have carcinogenic potential, other substitutes must be used.

VI. WORK PRACTICES AND CONTROL RECOMMENDATIONS

This section evaluates the conditions under which employee exposure to benzidine-based dyes is likely. It also delineates those operations in which the most intense exposures would be predicted in the absence of controls. Emphasis is placed on work practices and control recommendations to limit excessive employee exposure to benzidine-based dyes in operations that can be particularly hazardous. The employer should, in addition, evaluate existing programs for labeling and posting, employee education, cleanup of spills, disposal of waste, emergencies, and general plant sanitation to ensure their adequacy in light of evidence of carcinogenicity of the benzidine-based dyes. If present programs are inadequate, new ones should be implemented to ensure a clean and healthful workplace and to ensure that employees are aware of the hazards involved and of their role in maintaining a safe working environment.

During manufacture, the dyes are generally prepared in a closed system in which benzidine is formed by the reaction of the starting material, hydrazobenzene, with hydrochloric acid [27]. One dye manufacturer has developed a process that uses nitrobenzene as the starting material, thus eliminating the need for employees to handle hydrazobenzene, which forms benzidine in the stomach if ingested [45]. After the dye is precipitated, however, generally it is handled in open systems [27]. The dye is filtered in presses and the press cake is unloaded manually. The press cake is then dried, and the dried dye is ground into a fine powder. This fine powder is then transferred to ribbon blenders where other dyes are often admixed to obtain the desired colors. Sulfonated dedusting oil is usually added at this point to reduce the tendency for the dye to produce an aerosol when poured or mixed. Salt or sugar is nearly always added to dilute the concentrated dye [41]. The final product is then weighed and packaged for marketing.

Processes in which dried dye is handled have the greatest potential for employee exposure during the manufacture or repackaging of benzidine-based dyes; such processes should be performed in closed systems. Access to such areas should be restricted to authorized employees. When such enclosure is not possible, each operation should be provided with continuous local exhaust ventilation so that air movement is always from surrounding work areas to the operation and then through suitable filters as described in OSHA safety and health standards (29 CFR 1910), so as to prevent the release of any benzidine-based dye to the work environment.

Handling of moist press cakes and solutions constitutes a lesser source of employee exposure to benzidine-based dyes than handling dry powders, since solutions and moist materials are less likely to be dispersed into the air or distributed over large areas. Nevertheless, closed systems should be used to further limit employee exposure to benzidine-based dyes. Filter presses that can be emptied and decontaminated without opening the filters have been used for preparation of benzidine sulfate [45], and the

use of this type of filter should also be applicable in the manufacture of the dyes.

Various methods of eliminating the dust hazard associated with dyes have been used. In addition to the treatment with sulfonated oils referred to above, two other methods are presently employed [41]. One method is to form the dye into pellets so that dusting is minimized. The other procedure is to make up the dyes in unitized double packages. The outer package, which is used for protection during shipment, contains an inner package that dissolves in water. Thus, the worker making up a dye bath would add the appropriate number of units of dye to the bath without opening the inner package. These procedures may be combined for added safety (a dye package of pellets in double packets).

Pastes rather than dried dyes have been used by the paper industry for some time [39,41]. In this case, the paste is added to a large container of an aqueous solution and that solution is metered to each batch of paper as it is dyed.

Benzidine-based dyes are used in the paper, textile, and leather finishing industries. Since the industries are diverse, it would be expected that the conditions of potential exposure to benzidine-based dyes are equally diverse. At least 63 occupational categories have been found to be associated with potential exposure to benzidine-based dyes [44]. In the facilities surveyed by NIOSH [27], a three-step process for handling the benzidine-based dyes was characteristic of all three industries. First, a dye weigher dispensed the material into a vessel. In some cases the weigher also dissolved the dry dyestuffs. Next, the material to be dyed and the dissolved dye were placed in a dyeing vat. Finally, the dyed material was dried and finished. The degree of worker exposure in areas where paper, textiles, or leather are being dyed would be expected to vary widely depending on conditions such as the temperature of the dye solution, the amount of manual handling of the dyed material, and the design of the dye vats. Employees who handle paper, textiles, or leather after it is dyed could also be exposed to benzidine-based dyes since excess dye retained on the finished material would be available for release as dried powder.

During use of benzidine-based dyes, the greatest potential for exposure would be expected to be among dye-weighers who handle dry powders [27]. Their operations should be carried out in a hood designed and maintained so as to draw air inward at an average linear face velocity of 150 feet per minute (0.76 m/s) with a minimum of 125 feet per minute (0.64 m/s). Particular attention should be paid to the design of such hoods to ensure that the employee can transfer the material from its original container to the weighing scale without taking any dye outside the enclosure or inserting any part of the body other than hands and arms inside the hood. Containers of benzidine-based dyes should be opened only during the weighing operation. Once opened, they should remain within the hood until disposal or until all the contents have been used. The use of pastes or liquids rather than dried dyes should be considered as a control measure.

The dye weigher should prepare the dye solutions to be placed in the vats. This procedure would eliminate the need to transfer the more hazardous dry material from one station to another. The dye weighing area should be regulated, and access should be limited to authorized employees who are wearing adequate personal protective equipment adequate to prevent skin contact with or inhalation of the dyes. If workers must handle the material in the vats manually, or if adjustments to machinery are necessary while benzidine-based dye is present, the worker must wear impervious clothing and respiratory protection to prevent exposure to the dyes.

Textiles are dyed at various stages in their manufacture, including unspun fibers, unwoven yarn, and finished fabric. Workers who prepare fabrics from unspun fibers are of particular concern, since they could be potentially exposed to benzidine-based dyes contained on dusts generated during manufacture. In addition, some benzidine-based dyes possess much poorer fastness to wet treatment than do others; persons who launder such clothing are potentially exposed to the dyes. Employees and employers should be aware that those who launder, weave, or sew fabrics dyed with benzidine-based dyes are potentially exposed to the dyes. If exposure is considered likely, the employer should institute stringent control measures and work practices to prevent such exposure.

Several generally acceptable practices for the control of hazardous materials are recommended wherever there is potential for exposure [45,76]. For example, pressure failure alarms for closed systems and exhaust ventilation can rapidly indicate a system failure that might result in the release of substantial quantities of benzidine-based dyes. Continuous flow indicators, such as water or oil manometers properly mounted at the juncture of a fume hood and duct throat and marked to indicate acceptable airflow, will give a readily observable indication of decreased efficiency in the ventilation system for the hood. Wet methods, vacuum cleaning, or other methods that do not lead to redispersion of settled dust should be used for plant maintenance and sanitation. Dry sweeping or blowing with compressed air should be prohibited. In the cleanup of leaks or spills and in maintenance or repair operations on contaminated systems or equipment, employees should wear clean impervious garments, including at least gloves, boots, and an air-supplied respirator with positive pressure in the facepiece.

Benzidine is readily absorbed through the skin, and reuse of protective equipment or work clothing contaminated with benzidine can lead to its dermal absorption [7,8]. However, evidence for dermal absorption of the benzidine-based dyes is conflicting [34,69]. As a prudent measure, absorption of benzidine-based dyes through the skin must be considered a real possibility [69]. Thus, employee exposure through contaminated clothing may be as serious a problem for benzidine-based dyes as it is for benzidine [7,9,11,16]. Particulate material containing the dyes can be released into the external environment, including the employee's home, unless clothing potentially contaminated with the dyes is removed before the employee leaves the exposure area. If an employee's skin is potentially contaminated with benzidine-based dyes, the employee should wash or shower as appropriate before leaving the exposure area.

VII. MONITORING METHODS

Workplace Air

At this time, NIOSH is unaware of a practical method for identifying each specific benzidine-based dye workers may be exposed to. It should be possible to identify classes of azo dyes such as the benzidine-based dyes by the use of high performance liquid chromatography or gas chromatography after reduction of the azo linkage. Additional research is generally needed in this area.

Although methods to measure the concentration of an individual benzidine-based dye in air are not available, a screening method for diazonium salts and azo dyes in air has been developed by NIOSH [71] and is described in Appendix I(A). The range of the method is listed as 0.01-0.4 mg/cu m in a 500-liter sample of air. If only one benzidine-based dye is present and other positive interferences are absent, a quantitative estimate of the concentration of the dye present in the air can be made. If more than one azo dye of any type is present, the method is not quantitative and the concentration of total azo dye present must be given as a range. However, the method can indicate the adequacy of work practices and engineering controls employed to minimize the concentration of airborne benzidine-based dyes during the period required to phase in substitute dyes.

Urinary Levels

Aromatic diamines, such as benzidine, are not normally found in the body. However, benzidine, a metabolic product of benzidine-based dyes, has appeared in the urine of employees exposed to the dyes but not to benzidine. This demonstrates systemic absorption of the dye or a portion thereof. Currently, the measurement of urinary benzidine is used more as a diagnostic practice than for use in compliance. The presence of benzidine in the urine of a worker potentially exposed to a benzidine-based dye would verify that such exposure had, in fact, occurred, but its absence cannot be considered verification that no exposure has occurred.

The method recommended in Appendix I(B) can detect the presence of aromatic amines at 100 ng/100 ml of urine, although recovery efficiency at the limit of detection is poor. If aromatic amines are found in the urine, thin-layer chromatography (TLC) can be used to confirm (though not rigorously prove) the presence of benzidine. The total volume of the sample must be no less than 100 ml; the minimum detection limit in such a volume is 300 ng. An Rf value identical to that of benzidine constitutes this confirmation. If benzidine is detected by urinalysis, it would demonstrate that employee exposure to benzidine or benzidine-based dyes has occurred and would suggest inadequacies in either engineering controls or work practices. Thus, urinalysis in addition to environmental monitoring

is necessary to assure the employer that exposure of employees to benzidine-based dyes has been minimized.

Specific methods for the detection of benzidine in human [59,77] and hamster [37] urine and in industrial effluents [78] have been developed. Two [37,59] are based on electron-capture gas chromatography, one is based on high performance liquid chromatography [78], and one is a spectrophotofluorimetric technique [77]. Unlike the fluorescamine method [73] or the method given in Appendix I(B), they can measure benzidine in the presence of other aromatic amines. While all these methods should be readily adaptable to detection of benzidine in an employee's urine, they involve considerably more elaborate instrumentation and analytical techniques than the routine screening method described in Appendix I(B). Several points need further clarification before the amount of benzidine in the urine can be correlated precisely with the concentration of benzidine-based dyes in the air. For example, the optimum conditions for sample collection are not known. The NCTR study [37] recommended alkaline hydrolysis of the urine to measure both free and conjugated benzidine. Since urinalysis is presently useful as a qualitative index of exposure only, the less elaborate screening method constitutes the best approach at this time.