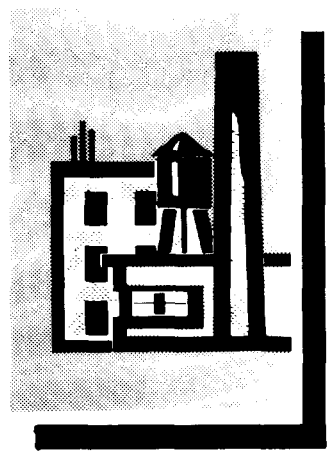
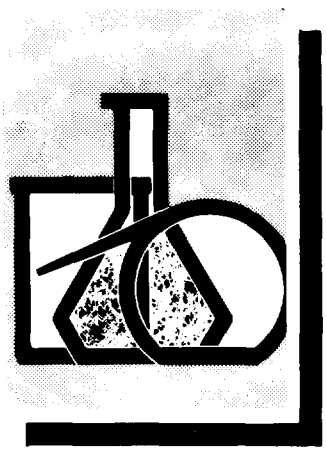
NIOSH

SPECIAL OCCUPATIONAL HAZARD REVIEW for





BENZIDINE-BASED DYES

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Center for Disease Control
National Institute for Occupational Safety and Health

SPECIAL OCCUPATIONAL HAZARD REVIEW

for

BENZIDINE-BASED DYES

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Center for Disease Control
National Institute for Occupational Safety and Health

JANUARY 1980

For sale by the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402

DISCLAIMER

Mention of company name or product does not constitute endorsement by the National Institute for Occupational Safety and Health.

DHEW (NIOSH) Publication No. 80-109

PREFACE

The Occupational Safety and Health Act of 1970 emphasizes the need for standards to protect the health and safety of workers exposed to an ever-increasing number of potential hazards in their workplace. Consequently, the National Institute for Occupational Safety and Health (NIOSH) has implemented a program to evaluate the adverse health effects of widely used chemical and physical agents. This program includes the development of Special Hazard Reviews that serve to support and complement the other major health assessment activities of the Institute.

The intent of a Special Hazard Review is to analyze and document, from a health standpoint, the problems associated with a given industrial chemical, process, or physical agent. Generally, a Special Hazard Review is concerned with those hazards of a chronic nature such as cancer, mutagenicity, teratogenicity, or effects on reproduction. However, they may also deal with other effects that have been identified as being harmful to workers when these effects result from exposure to substances found in the workplace. Special Hazard Reviews also recommend control measures, work practices, or other appropriate action to assist employers in protecting the health and well-being of workers.

Anthony Robbins, M.D.
Director
National Institute for Occupational
Safety and Health

SYNOPSIS

This Special Hazard Review evaluates available information concerning the carcinogenicity and metabolism of benzidine-based dyes and concludes that all these dyes should be recognized as potential human carcinogens.

This conclusion is based on evidence that four of the dyes have rapidly induced tumors in animals; that two studies of dye workers demonstrate an association between benzidine-based dye exposure and bladder cancer in workers; that all of the benzidine-based dyes thus far tested have been metabolized in animals to the carcinogen, benzidine; and that the enzyme (azoreductase) which breaks down these dyes to benzidine is found in both animals and humans. This enzyme acts upon a multitude of azo compounds to chemically reduce and break the azo linkage and, therefore, it is highly probable that the azo-linkage in the as yet untested benzidine-based dyes is also cleaved forming benzidine. In addition, two carcinogenic impurities, 4-aminobiphenyl (an OSHA regulated carcinogen) and 2,4-diaminoazobenzene (an animal carcinogen) have been identified in one commercial sampling of a benzidine-based dye and may contribute to the carcinogenic potential of these dyes.

Dyes constitute a large and diverse group of chemicals that have many applications for imparting color to diverse products. More than 1,200 chemically unique and structurally different dyes are manufactured in the United States, and an additional 800 are imported. Dyes are classified according to their chemical structure as well as by the method of application in the dyeing process.

A major class of dyes are those derived from benzidine, an aromatic amine acknowledged by both industry and government as causing bladder cancer in humans.

These recommendations include those dyes containing an unsubstituted benzidine structure in their makeup. Such dyes are largely classified as direct dyes, since they may be applied directly to fabrics or other substrates without pretreatment or subsequent processing.

Of some 200 benzidine-based dyes, about 30 are now marketed in the United States. The most recent estimate of employee exposure is about 79,000 workers in 63 occupations. Market trends show a decreased usage from over 7 million pounds in 1976 to about 3.3 million pounds in 1978. All but one US company has phased out the manufacture of these dyes, but imports have increased to 1.6 million pounds in 1978 indicating continued worker exposure. Benzidine-based dyes are used primarily to color textiles, leather, and paper. However, diverse industries consume 20% of the total.

ACKNOWLEDGEMENTS

The Division of Criteria Documentation and Standards Development (DCDSD), NIOSH, had primary responsibility for development of this Special Hazard Review on Benzidine-Based Dyes. Arthur R. Gregory, Ph.D., was responsible for compilation and technical evaluation of data in this document, and assembled it for review. Technical review was provided by Imogene Sevin, Ph.D., of DCDSD; Bryan Hardin, Larry Lowery, Ph.D., and Richard W. Niemeier, Ph.D., of the Division of Biomedical and Behavioral Science; Mark Boeniger of the Division of Surveillance, Hazard Evaluations, and Field Studies; and Charles Neumeister of the Division of Physical Sciences and Engineering. Editorial review was provided by William N. LeVee, Division of Criteria Documentation and Standards Development. Irwin P. Baumel, Ph.D. and Frank Mackison had NIOSH program responsibility and assisted in preparation of the document.

CONTENTS

I. INTRODUCTION

Dyes constitute a large and diverse group of chemicals that have diverse applications for imparting color to many types of products. They may be broadly defined as water-soluble chemical substances that contain chromophoric structures and thus may be used to color another substance by becoming attached to it by molecular bonding, adsorption, absorption, or mechanical adherence.

Pigments, in contrast to dyes, are insoluble and impart their color to another substance by being physically attached to or incorporated into it. Detailed information on dyes and pigments may be found in the Colour Index [1], a compendium of technical information on dyes and their use.

The use of dyes dates as far back as prehistoric man, but the synthetic dye industry began in 1856 when William H. Perkin first synthesized the dye mauve [2]. Since that time, the synthetic dye industry has grown extensively in the United States as well as in other countries.

More than 1,200 chemically unique and structurally different dyes are manufactured in the United States, and an additional 800 dyes are imported [3]. Dyes are classified according to their chemical structure as well as by the method of application in the dyeing process. The dyes evaluated in this review are those containing the benzidine moiety without any substitution on the diphenyl portion of the molecule, ie, those having a para diamino diphenyl grouping in their structural configuration. They are hereafter referred to as benzidine-based dyes. Benzidine-based dyes are largely classified as direct dyes, since they may be applied directly to fabrics or other substrates without pretreatment or without subsequent processes that firmly attach the dye to the substrate (mordanting) [2]. More than 200 benzidine-based dyes are listed in the Colour Index or are used commercially (see Appendix II). Over 30 benzidine-based dyes have commercial importance in the United States, according to the US International Trade Commission [3]. Seventeen are manufactured in this country, all by a single company; the remaining dyes are all imported (see Appendix III). Benzidine-based dyes are used chiefly in the leather, textile, and paper industries [1,3,4], but they are also used by beauticians, craft workers, and the general public [5].

The common starting material for the manufacture of these dyes, benzidine, is acknowledged by both industry [6] and government (Federal Register 39(20):3756-97, January 29, 1974) to cause bladder cancer. This is based on considerable evidence from studies with humans as well as with animals. The carcinogenicity of benzidine was reviewed by Clayson [7] and by Haley [8]. The evidence presented in those reviews demonstrates that both brief and prolonged exposures to benzidine have been associated with the development of bladder cancer in workers [9].

Aromatic amines, as a class of chemicals, are generally carcinogenic. Benzidine is an outstanding carcinogen within this class; it causes cancer in humans, rats, hamsters, and mice [9-22]. The structure-activity relationship between aromatic amines and carcinogenic potency has been reviewed in detail [12,23,24]. In addition, benzidine has also been used as a reference mutagen (a known positive control) for several years by the Huntingdon Research Center [25]. In light of all the extensive evidence in both humans and experimental animals, NIOSH recommended in 1973 that benzidine be regulated as a carcinogen; in 1974, the Occupational Safety and Health Administration of the Department of Labor promulgated a regulatory standard for benzidine.

Until recently there had been little concern regarding the dyes derived from and which contain benzidine as an integral portion of their chemical structure. Benzidine chemically united to other substances was not considered dangerous; in 1959, Billiard-Duchesne [26] stated there was "no danger in using the finished dyes." Only recently has there been serious consideration given to the likelihood that processes in the body could free the benzidine that was originally used in the manufacture of the dyes [27]. The process of metabolizing or converting a substance back to a starting component or to another compound is commonly referred to as biotransformation.

In April 1978, NIOSH [28] and the National Cancer Institute (NCI) jointly recommended that three widely used benzidine-based dyes (Direct Black 38, Direct Blue 6, and Direct Brown 95) be handled as if they were carcinogens. The NIOSH/NCI recommendation was based on two major findings. First, NCI had determined in a short-term animal feeding study that rats administered the above dyes developed a high incidence of hepatocarcinomas and neoplastic nodules in 5-13 weeks. No similar lesions developed in control animals [29]. Second, NIOSH field studies demonstrated that some workers who had been exposed to these dyes had benzidine in their urine [27]. The amount found in the urine was greater than could be expected from benzidine contamination in the dyes. This demonstrated that the dye itself had broken down, releasing benzidine.

Since that time, NIOSH has examined and evaluated the available literature concerning these and related dyes. This Special Hazard Review appraises the carcinogenic potential of those dyes known to contain the unsubstituted benzidine moiety.

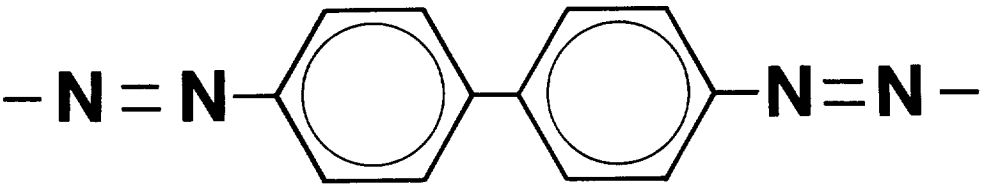
II. CHARACTERISTICS OF BENZIDINE-BASED DYES

Chemical and Physical Characteristics

At room temperature, benzidine-based dyes are all colored solids with negligible vapor pressure. The specific water solubility varies from dye to dye, but all are sufficiently water-soluble to be used for dyeing in an aqueous solution. They are all relatively stable in air or solution at ambient temperatures, and possess considerable fastness to light. Unlike some of the anthraquinone vat dyes, benzidine-based dyes do not accelerate the tendering of cellulosic type fabrics under the influence of light [30]. They exhaust onto (combine with) cellulosic fibers from a salt bath without other treatment. Based on this property, these dyes are called substantive [4]. They usually contain two or three azo groups.

Direct dyes give excellent coverage and generally do not otherwise alter the appearance or character of the material to be colored. They cover a wide hue range [1,4]. Many direct dyes now on the market do not contain the benzidine moiety and, therefore, are not considered in this review. These nonbenzidine-derived direct dyes provide industry with a wide range of colors and hues which may be considered for use as substitutes for benzidine-based dyes [1,30-32].

The chemical structures of benzidine-based dyes currently of commerical importance are shown in Appendix III; all have the characteristic diazotized benzidine nucleus as shown in Figure II-1 and differ only in the substituents attached at either diazo linkage, ie, -N=N-. This linkage is considered the most labile portion of each of these dyes, and, in each case, enzymatic [29,33-35] or thermal breakdown [2,36] results in the production of benzidine.



DIAZOTIZED BENZIDINE MOIETY
FIGURE II-1

Purity and Stability

Benzidine and other carcinogenic substances such as 4-amino biphenyl and 2,4-diaminoazobenzene may be present in benzidine-based dyes both as the result of impurities introduced in manufacturing processes or may be present in benzidine-based dyes as products of thermal or enzymatic decomposition. NIOSH has measured the amount of benzidine present in direct dyes from imported and domestic sources [27]. For 26 samples of 11 types of dyes imported from seven countries, the benzidine concentration ranged from less than 1 ppm to 224 ppm. Only six samples contained greater than 10 ppm of benzidine. Similar results were found for 26 samples from domestic sources. No single type of dye accounted for the high results. Few investigators have attempted to analyze impurities other than benzidine present in direct dyes. However, in one commercially produced lot of Direct Black 38 (a benzidine-based dye), 150 ppm of 4-amino biphenyl and 9,200 ppm of 2,4-diaminoazobenzene (Basic Orange 2) were found even though only 0.1 ppm of benzidine was detected [37].

The concentration of impurities such as benzidine can be increased by decomposition of dyes containing benzidine as a portion of the structure. A measurable increase in the concentration of benzidine and 4-amino biphenyl impurities was found when Direct Black 38 in aqueous solution was stored at 25 or 37.5 C for 48 hours [37]. In hamster urine stored at room temperature, substantial increases in the concentrations of benzidine, 4-amino biphenyl, and 2,4-diaminoazobenzene were found 48 and 96 hours after the addition of Direct Black 38 to the urine; at 5 C the dye in hamster urine was stable at least 96 hours [37].

Although all decomposition products have not been identified, benzidine-based dyes have been shown to break down in aqueous solution at elevated temperatures [36]. Mel'nikov and Kirillova [36] examined a number of technical grade direct dyes, including some that were benzidine-based. They found that the rate of decomposition of the dyes was accelerated by increased temperature and greatly accelerated in the presence of iron. For example, at 140 C after 6 hours, 17.3% of Congo Red (Direct Red 28) decomposed, and, under the same conditions in the presence of iron, 83% decomposed. When a 0.1% aqueous solution of Congo Red (Direct Red 28) was stored at 100 C for 1 hour, no decomposition was observed. The presence of textile additives such as urea, triethanolamine, and cellosolve also increased the rate of decomposition.

III. CHARACTERISTICS OF EXPOSURE

Manufacture and Uses

The synthesis of dyes is briefly described in the Colour Index [1]. Generally, the diazotized benzidine is reacted (coupled) with suitable secondary components such as hydroxylated aromatics or aromatic amines [1,2,4]. Since the manufacture of dyes is directed toward producing a particular color and not to producing a specific chemical entity, impurities such as benzidine, 4-amino biphenyl, 2,4-diaminoazobenzene, diphenyline, and semidine find their way into the final dye product. If these substances do not interfere with the dyeing process, ordinarily they are not removed [4,38]. Additional substances, including other dyes, may be required to produce the particular shade or hue desired in a product. Thus, dyes in general represent mixtures of substances rather than a particular chemical entity [4,38-40]. This is further complicated by the fact that manufacturers may use differing conditions of synthesis, starting materials of different purity, and even different methods to manufacture dyes [30,39,41]. These facts are important since each dye is depicted as a definitive chemical structure in textbooks [2] and in the Colour Index [1].

More than 30 manufacturers in the United States now produce various direct dyes. Only one of the nine US manufacturers that produced benzidine-based dyes in 1974 still does so in 1979 [3,40,42]. Some distributors import the benzidine-based dyes for resale in the United States [27]. The 1979 Buyer's Guide published by the American Association of Textile Chemists and Colorists (AATCC) lists 20 unsubstituted benzidine-based dyes for sale in the United States [43]. Not all dyes sold in the United States, however, are listed in the Buyer's Guide (W Martin, verbal communication, July 1979).

Since 1974, the eight US manufacturers that phased out the synthesis of benzidine-based dyes [3] have replaced them with phthalocyanine, o-tolidine, o-dianisidine, phenylenediamine, and dioxyazine-type dyes [40]. Nonbenzidine-based substitutes appear to be in demand; five manufacturers now sell nonbenzidine-based substitutes for Direct Black 38 [30,40,43]. A suitable substitute for Direct Blue 6 in the leather dyeing industry has been difficult to find [40]. NIOSH considers the need for appropriate toxicologic testing of all dye substitutes to be essential before they are used in the workplace.

Benzidine-based dyes are used extensively to color textiles, leather, and paper. Ease of application has been a major asset to their general availability and widespread usage. Of the total use, 40% is used to color paper, 25% to color textiles, 15% for leather, and 20% for diverse applications in the petroleum, rubber, plastics, wood, soap, fur, and hair dye industries [1,3,28,32].

Production Volume

Seventeen benzidine-based dyes are produced in the United States by one manufacturer, Fabricolor, Inc., of New Jersey. Table III-1 gives the US production figures for benzidine dyestuffs manufactured during 1978. These figures were provided to NIOSH through the courtesy of Fabricolor (E Angstadt, written communication, January 1979).

TABLE III-1

1978 US PRODUCTION FOR BENZIDINE DYESTUFFS

C.I. Generic Name	C.I. Number	kg	Pounds
Acid Red 85	22245	17,980	39,639
Direct Orange 8	22130	12,341	27,208
Direct Brown 2	22311	12,576	27,725
Direct Brown 6	30140	3,884	8,563
Direct Brown 31	35660	16,967	37,406
Direct Brown 74	36300	14,703	32,414
Direct Brown 95	30145	34,452	75,953
Direct Brown 154	30120	28,946	63,816
Direct Green 1	30280	5,745	12,666
Direct Green 6	30295	49,476	109,076
Direct Red 1	22310	11,961	26,370
Direct Red 28	22120	16,931	37,327
Direct Blue 2	22590	99,080	218,435
Direct Blue 6	22610	27,907	61,524
Direct Black 4	30245	11,995	26,444
Direct Black 38	30235	373,336	823,065
Resin F. Black WP	--	38,383	84,620
Total		776,664	1,712,251

Adapted from E. Angstadt, written communication, January 1979

Extent of Occupational Exposure

Based on a national survey conducted from 1972 to 1974 [44], NIOSH estimated that approximately 79,200 workers in 63 occupations were potentially exposed to benzidine-based dyes. These potential exposures occurred in the dye manufacturing, textile dyeing, printing, paper, and leather industries. Appendix III lists the estimated worker exposure for

each commercially available benzidine-based dye. Dye use in arts and crafts was not included. An estimate of worker exposure to benzidine was given by Ferber et al in 1976 [45]. However, no estimate was made of worker exposure to the dyes derived from benzidine.

NIOSH has contacted the one US manufacturer of benzidine-based dyes as well as four previous US manufacturers. While US production has decreased from a high of 2,919 Mg (6,436,000 lbs) in 1976 to 777 Mg (1,712,251 lbs) in 1978, imports have increased from 272 Mg (600,000 lbs) in 1976 to 726 Mg (1,600,000 lbs) in 1978. The present actual worker exposure to benzidine-based dyes cannot be ascertained precisely, but appears substantial in light of the continuing production, sale, and use of these dyes [30,41,43,46].

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.

VIII. REFERENCES

1. Colour Index, ed 3 rev. Research Triangle Park, NC, The American Association of Textile Chemists and Colorists, 1971, 1975, vol 1-6

2. Rys P, Zollinger H: Fundamentals of the Chemistry and Application of Dyes. New York, John Wiley and Sons, 1972, pp 58-71

3. Synthetic Organic Chemicals--United States Production and Sales, 1977, USITC Publication 920. US International Trade Commission, 1978, pp 87-132

4. Gantz GM, Ellis JR (eds.): Dyes-application and evaluation, in Kirk-Othmer Encyclopedia of Chemical Technology, ed 2 rev. New York, John Wiley and Sons, 1967, vol 7, p 535

5. Jenkins CL: Economic impact and available substitutes after regulation of dyes based on benzidine, 3,3'-dimethylbenzidine and 3,3'-dimethoxybenzidine. New York, Center for Occupational Hazards, 1978, pp 1-16 (unpublished)

6. Gehrmann GH: The carcinogenetic agent--Chemistry and industrial aspects. J Urol 31:126-37, 1934

7. Clayson DB: Occupational bladder cancer. Prev Med 5:228-44, 1976

8. Haley TJ: Benzidine revisited--A review of the literature and problems associated with the use of benzidine and its congeners. Clin Toxicol 8:13-42, 1975

9. Case RAM: Tumours of the urinary tract as an occupational disease in several industries. Ann R Coll Surg Engl 39:213-35, 1965

10. Pliss GB: Carcinogenic properties of benzidine. Fed Proc Am Soc Exp Biol (Trans Suppl) 24(3) Part II:T529-32, 1964

11. Report of a paper by TS Scott, Occupational Bladder Cancer, presented at the 132nd Annual Meeting of the British Medical Association, Manchester, 1964. Br Med J 2:302, 1964

12. Weisburger EK, Russfield AB, Homburger F, Weisburger JH, Boger E, Van Dongen CG, Chu KC: Testing of twenty-one environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity. J Environ Pathol Toxicol 2:325-56, 1978

13. Griswold DP, Casey AE, Weisburger EK, Weisburger JH: The carcinogenicity of multiple intragastric doses of aromatic and heterocyclic nitro or amino derivatives in young female Sprague-Dawley rats. Cancer Res 28:924-33, 1968

14. Spitz S, Maguigan WH, Dobriner K: The carcinogenic action of benzidine. Cancer 3:789-804, 1950

15. Zabezhinskii MA: Effectiveness of inhalation as a method of administration of atomized carcinogens. Bull Exp Biol Med USSR (Eng transl) 69:68-70, 1970

16. Zavon MR, Hoegg U, Bingham E: Benzidine exposure as a cause of bladder tumors. Arch Environ Health 27:1-8, 1973

17. Mancuso TF, El-Attar AA: Cohort study of workers exposed to betanaphthylamine and benzidine. J Occup Med 9:277-85, 1967

18. Saffiotti U, Cefis F, Montesano R, Sellakumar AR: Induction of bladder cancer in hamsters fed aramatic (sic) amines. Ind Med Surg 35:564, 1966

19. Prokof'era OG: [Induction of hepatic tumors in mice by benzidine.] Vopr Onkol 17(5):61-64, 1971 (Rus)

20. Frith CH, Dooley K: Hepatic cytologic and neoplastic changes in mice given benzidine dihydrochloride. J Natl Cancer Inst 56:679-82, 1976

21. Bonser GM, Clayson DB, Jull JW: The induction of tumours of the subcutaneous tissues, liver and intestine in the mouse by certain dyestuffs and their intermediates. Br J Cancer 10:653-67, 1956

22. Sellakumar AR, Montesano R, Saffioti U: Aromatic amines carcinogenicity in hamsters. Proc Am Assoc Cancer Res 10:78, 1969 (Abst)

23. Clayson DB, Garner RC: Carcinogenic aromatic amines and related compounds, in Chemical Carcinogens, ACS Monograph Series, No. 173. Washington, DC, American Chemical Society, 1976, pp 366-461

24. Weber WW: Genetic variability and extrapolation from animals to man--Some perspectives on susceptibility to chemical carcinogenesis from aromatic amines. Environ Health Perspect 22:141-44, 1978

25. Urwin C, Richardson JC, Palmer AK: An evaluation of the mutagenicity of the cutting oil preservative Grotan BK. Mutat Res 40:43-46, 1976

26. Billiard-Duchesne J: [Amino tumors of the bladder--42 unpublished cases.] J Urol Med Chir 65:748-91, 1959 (Fre)

27. Boeniger M: An investigation of the metabolic reduction of benzidine azo dyes to benzidine and its metabolites, and their possible relationship to carcinoma of the bladder in man. Cincinnati, US Dept of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, 1978, 160 pp (unpublished)

28. Direct Blue 6, Direct Black 38, and Direct Brown 95--Benzidine Derived Dyes, Current Intelligence Bulletin 24, April 17, 1978, DHEW (NIOSH) Publication No. 78-148. Cincinnati, US Dept of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, 1978, 11 pp

29. 13-Week Subchronic Toxicity Studies of Direct Blue 6, Direct Black 38, and Direct Brown 95 Dyes, NCI-CG-TR-108, DHEW Publication No. (NIH) 78-1358. US Dept of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Cancer Institute, 1978, 127 pp

30. Gerber SM: Substitutes for Benzidine--New Structures, presented before the Southeastern Regional Meeting, American Chemical Society, Savannah, GA, Nov 9, 1978, 17 pp (unpublished)

31. Duff DG, McKinnon H: II--Replacement of one dye class by another. J Soc Dyers Colour 93:45-51, Feb 1977

32. Clayson DB: Case study 2: Benzidine and 2-naphthylamine--Voluntary substitution or technological alternatives. Ann NY Acad Sci 271:170-75, 1976

33. Niitsu K: [Studies on the metabolism and carcinogenicity of azo dyes used for food colors and direct dyestuffs--Part II. Studies on the metabolism and carcinogenicity of direct dyestuffs Blue BB and Black EX.] Tokyo Jikeikai Ika Daigaku Zasshi 88:467-71, 1973 (Jap)

34. Rinde E: A specific assay for the detection of trace amounts of the bladder carcinogen benzidine and other aromatic amines. Dissertation, New York University, Oct 1976, pp 24-109

35. Cueto C, Douglas JF: A basis for short-term in vivo carcinogenesis bioassay. Pharmacologist 20(3):175, 1978 (Abst)

36. Mel'nikov BN, Kirillova MN: Thermal stability of direct dyes in solution. J Appl Chem USSR (Eng Transl) 42(11):2418-22, 1969

37. NCTR Technical Report for Experiment Number 196: Metabolism of Azo Dyes to Potentially Carcinogenic Aromatic Amines. Final Report of Interagency Agreement No. FDA 224-78-0004 Between the National Institute for Occupational Safety and Health and the Food and Drug Administration. Jefferson, AR, National Center for Toxicological Research, Mar 1979, 81 pp

38. Ferber KH, Benzidine and related biphenyldiamines, in Kirk-Othmer Encyclopedia of Chemical Technology, ed 3. New York, John Wiley and Sons, 1978, vol 3, pp 772-77

39. Catino SC, Farris RE: Azo dyes, in Kirk-Othmer Encyclopedia of Chemical Technology, ed 3. New York, John Wiley and Sons, 1978, vol 3, pp 387-433

40. Determination of Chemicals Contained in Wearing Apparel--Draft Final Report. Unpublished report submitted to the US Consumer Product Safety Commission by Kearney Management Consultants, Washington, DC, Nov 1977, pp III-10 to III-14 (Contract No. CPCS-C-77-0009)

41. Sheldrick JE, Helper EW, Steadman TR: Final Report on Product/Industry Profile: Dyes Based on Benzidine and Benzidine Congeners. Unpublished report submitted to the US Consumer Product Safety Commission by Battelle, Columbus Division, Columbus, OH, Apr 1979, 31 pp

42. Imports of Benzenoid Chemicals and Products, 1978, USITC Publication 990. US International Trade Commission, July 1979, pp 38-55

43. Buyer's Guide. Text Chem Color 11(7A):11-280, 1979

44. National Occupational Hazard Survey--Survey Analysis and Supplemental Tables, DHEW (NIOSH) Publication No. 78-114. Cincinnati, US Dept of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, 1977, vol 3, 792 pp

45. Ferber KH, Hill WJ, Cobb DA: An assessment of the effect of improved working conditions on bladder tumor incidence in a benzidine manufacturing facility. Am Ind Hyg Assoc J 37:61-68, 1976

46. Kasprzak SJ: DETO Responses to TITC Questions on Dyes Dated March 13, 1979. Submitted to the Toxic Substances Control Act Interagency Testing Committee by the Dyes Environmental and Toxicology Organization, June 27, 1979, 132 pp

47. Yoshida O, Miyakawa M: Etiology of bladder cancer--"Metabolic" aspects, in Nakohara W, Hirayama T, Nishioka K, and Sugano H (eds.): Analytic and Experimental Epidemiology of Cancer, Proceedings of the 3rd International Symposium of the Princess Takamatsu Cancer Research Fund, 1972. Baltimore, University Park Press, 1973, pp 31-39

48. Genin VA: [Formation of blastomogenic diphenylamino derivatives as a result of the metabolism of direct azo dyes.] Vopr Onkol 23(9):50-52, 1977 (Rus)

49. Yoshida O, Harada T, Miyagawa M, Kato T: [Bladder cancer in workers of the dyeing industry]. Igaku No Ayumi 79:421-22, 1971 (Jap)

50. Criteria for a Recommended Standard--Occupational Exposure to Ethylene Dibromide, DHEW (NIOSH) Publication No. 77-221. Cincinnati, US Dept of Health, Education, and Welfare, Public Health Service, Center for

Disease Control, National Institute for Occupational Safety and Health, 1977, p 83

51. Fujita K, Mine T, Iwase S, Mizuno T, Takayanagi T, Sugiyama Y, Arai T: The carcinogenicity of certain compounds related to trypan blue. Br J Exp Pathol 38:291-98, 1957

52. Korosteleva TA, Skachkov AP, Kondrat'yeva AF: [Carcinogen-protein antigens and the blastomogenic activity of aniline dyes.] Vopr Onkol 23(7):72-73, 1977 (Rus)

53. Okajima E, Hiramatsu K, Ishu I, Matsujima S, Yamada K, Arai M: [Multiple tumors in rats after oral administration of the benzidine type dye Direct Deep Black EX.] Igaku No Ayumi 92:291-92, 1975 (Jap)

54. Manchon PH, Lowy R: [Study of the azoreductase activity of supernatants of rat-liver homogenates--Part II. Comparison with other preparations, variations with age and ingestion of dye.] Food Cosmet Toxicol 3:783-87, 1965 (Fre)

55. Walker R: The metabolism of azo compounds--A review of the literature. Food Cosmet Toxicol 8:659-76, 1970

56. Fouts JR, Kamm JJ, Brodie BB: Enzymatic reduction of prontosil and other azo dyes. J Pharmacol Exp Ther 120:291-300, 1957

57. Roxon JJ, Ryan AJ, Wright SE: Reduction of water-soluble azo dyes by intestinal bacteria. Food Cosmet Toxicol 5:367-69, 1967

58. Arcos JC, Argus MF: Chemical Induction of Cancer. New York, Academic Press, 1974, vol IIB, pp 205-301

59. Nony CR, Bowman MC: Carcinogens and analogs--Trace analysis of thirteen compounds in admixture in wastewater and human urine. Int J Environ Anal Chem 5:203-20, 1978

60. Some Aromatic Azo Compounds. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. Lyon, France, World Health Organization, International Agency for Research on Cancer, 1975, vol 8, pp 91-96

61. Korosteleva TA, Skachkov AP, Shwaydestskiy II: [Detection of carcinogen-protein antigens in serum from workers exposed to aniline dyes.] Gig Tr Prof Zabol 18(5):21-24, 1974 (Rus)

62. Sisley P, Porcher C: [Physiology. On the fate of staining substances in the animal organism.] C R Seances Acad Sci 152:1062-64, 1911 (Fre)

63. Morton KC, King CM, Baetcke KP: Metabolism of benzidine to N-hydroxy-N, N'-diacetylbenzidine and subsequent nucleic acid binding and mutagenicity. Cancer Res 39:3107-13, 1979

64. Rinde E, Troll W: Metabolic reduction of benzidine azo dyes to benzidine in the rhesus monkey. J Natl Cancer Inst 55:181-82, 1975

65. Dieckhues B: [Experiments on the reductive splitting of azo dyes by bacteria.] Zentralbl Bakterol Parasitenkd [Orig] 180:244-49, 1960 (Ger)

66. Goldin B, Dwyer J, Gorbach SL, Gordon W, Swenson L: Influence of diet and age on fecal bacterial enzymes. Am J Clin Nutr (Suppl) 31:S136-40, 1978

67. Yoshida O, Miyakawa M, Okada Y, Ohshiro K, Harada T, Machida S, Kato T: [The disintegration of a benzidine dye, Direct Deep Black EX, by Escherichia coli and soil bacteria.] Igaku to Seibutsugaku 86:361-63, 1973 (Jap)

68. Smith AL: Principles of Microbiology, ed 7. St Louis, The CV Mosby Co, 1973, p 349

69. IBM report to EPA on Dermal Absorbtion of Direct Black 38 in Rabbits. Submitted to NIOSH by the Environmental Protection Agency, Aug 31, 1979 (unpublished)

70. Matthews HB: Metabolism studies in the dog--Benzidine congener dyes (A preliminary report from the NTP), presented at the Interagency Collaborative Group on Environmental Carcinogens, Bethesda, MD, Aug 1979, 3 pp (unpublished)

71. Diazonium Salts and Azo Dyes in Air--Physical and Chemical Analysis Branch Method No. 234, in NIOSH Manual of Analytical Methods, ed 2, DHEW (NIOSH) Publication No. 77-157-B. Cincinnati, US Dept of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, 1977, vol 1, pp 234-1 to 234-6

72. Lazear EJ, Louie SC: Mutagenicity of some congeners of benzidine in the Salmonella typhimurium assay system. Cancer Letters 4:21-25, 1977

73. Criteria for a Recommended Standard--Occupational Exposure to o-Tolidine, DHEW (NIOSH) Publication No. 78-179. Cincinnati, US Dept of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, 1978, p 58

74. Hadidian Z, Fredrickson TN, Weisberger EK, Weisberger JH, Glass RM, Mantel N: Tests for chemical carcinogens. Report on the activity of derivitives of aromatic amines, nitrosamines, quinolines, nitroalkanes, amides, epoxides, aziridines, and purine antimetabolites. J Natl Cancer Inst 41:985-1025, 1968

75. Marshall AHE: The production of tumours of the reticular tissue by diazo vital dyes. Acta Pathol Microbiol Scand 33:1-9, 1953

76. Lagneau G: [Bladder cancer and dye manufacture.] Dissertation, University of Paris, Faculty of Medicine Necker-Enfants Malades, 1978, 93 pp (Fre) (Abst)

77. Bowman MC, King JR, Holder CL: Benzidine and congeners--Analytical chemical properties and trace analysis in five substrates. Int J Environ Anal Chem 4:205-23, 1976

78. Riggin RM, Howard CC: Determination of benzidine, dichlorobenzidine, and diphenylhydrazine in aqueous media by high performance liquid chromatography. Anal Chem 51:210-14, 1979

79. Benzidine in urine (screening test), in NIOSH Manual of Analytical Methods, ed 2, DHEW (NIOSH) Publication No. 79-141. Cincinnati, US Dept of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, 1979, vol 5, pp 315-1 to 315-6

APPENDIX I - ANALYSIS

(A) METHOD FOR MONITORING DIAZONIUM SALTS AND AZO DYES IN AIR

A screening method for analysis of benzidine-based dyes found in the workplace has been developed by NIOSH [71] and is presented below. A negative survey using this methodology is considered adequate to establish that workers are not significantly exposed to the benzidine-based dyes.

Analyte: Diazonium Salts and Azo Dyes	Method No.: P&CAM 234
Matrix: Air	Range: 0.01 to 0.4 mg/cu m in a 500-liter sample of air
Procedure: Filter collection; UV-VIS spectrophotometry	Precision (CV): 0.12 (analytical)
Date Issued: 6/30/76	Classification: D(Operational)

Principle of the Method

Duplicate samples of airborne particulate material are collected on cellulose acetate membrane filters. One filter is extracted with dilute hydrochloric acid and analyzed for diazonium salts spectrophotometrically at 375 nm. The second filter is extracted with an appropriate solvent and a spectrophotometric scan of the solution is made in the 400- to 700-nm range. The absorbance maxima are compared with the absorbance maxima of standard solutions prepared from bulk samples of the azo dyes.

Range and Sensitivity

(a) Diazonium salts and azo dyes follow a Beer's law relationship in the range of 5-200 μg in 40 ml of solvent, the volume used to extract the filters. This corresponds to 0.01-0.4 mg/cu m in a 500-liter air sample.

(b) Dilution of the sample solution with the appropriate solvent may be used to extend the upper limit of the range.

(c) The lower limit of the range can be extended by decreasing the volume of the extracting solvent or by using an absorption cell of longer length.

(d) The sensitivity is determined by the absorptivities of the individual salts and dyes.

Interferences

Any compound that absorbs radiation at the wavelengths corresponding to the absorbance maxima of the diazonium salts and azo dyes is a positive interference.

Precision and Accuracy

(a) An averge coefficient of variation of 0.12 has been established for the analytical procedure over the concentration range of the method.

(b) The recovery of salts and dyes from filters with the appropriate solvent yielded an efficiency of 95±5% in the range of 5-200 μg/40 ml of solvent.

(c) The accuracy of the overall sampling and analytical method has not been determined.

Advantages and Disadvantages

(a) The sampling procedure is rapid, simple, and involves no liquids.

(b) The procedure can be used to distinguish between diazonium salts and azo dyes but not between two salts or two dyes absorbing at the same wavelength.

(c) Since diazonium salts exhibit maximum absorption at approximately 375 nm, the method cannot be used for qualitative identification of individual salts. Thus the total diazonium salt concentration must be reported if more than one salt is being processed in the testing area on the day of sampling. Since each salt has a characteristic absorptivity, the concentration of total diazonium salts must be given as a range. The salt having the highest absorptivity determines the upper limit of the range and the salt having the lowest absorptivity determines the lower limit.

(d) Diazonium salts deteriorate in the presence of light and moisture. Care must be taken to store them in a dry environment and to minimize exposure to light.

(e) Bulk samples of all of the diazonium salts processed in the testing area on the day of sampling must be collected and a calibration curve must be established for each.

(f) The solubilities of the various classes of azo dyes differ widely. Therefore, an appropriate solvent for each azo dye must be determined. Azo dyes that contain one or more sulfonic or carboxylic acid groups are usually soluble in dilute acid. Azo dyes with sulfonated naphthol groups are usually soluble in water or dilute base. Azo dyes with no solubilizing groups are usually insoluble in water but are soluble in polar or nonpolar organic solvents.

(g) Absorption curves must be established for all azo dyes processed in the plant on the day of sampling. Azo dyes exhibit maxima in the 400- to 700-nm range, the exact wavelength being dependent on the structure of the dye molecule.

(h) If more than one azo dye is being processed on the day of sampling and those dyes absorb at a common wavelength, the total azo dye concentration must be reported. The concentration of total azo dye must be given as a range with the dye exhibiting the highest absorptivity determining the upper limit and the dye exhibiting the lowest absorptivity determining the lower limit at a specific wavelength.

Apparatus

(a) Air Sampling Equipment

(1) Cellulose ester membrane filters, 0.8-μm pore size, 37-mm diameter, Millipore Type AA, or equivalent.

(2) Filter holder for 37-mm filters, Millipore MAWP 037 A0, or equivalent.

(3) Personal sampling pump, calibrated with a representative filter unit in the line. A wet or dry test meter or a glass rotameter capable of measuring a flowrate of 2 liters/min to within 5% may be used in the calibration.

(b) Beckman Model 25 Scanning Spectrophotometer or equivalent.

(c) Matched fused silica cells, 1, 5, and 10 cm.

(d) Balance capable of weighing accurately to 1 mg.

(e) Pipets, various sizes.

(f) Beakers, 100 ml.

Reagents

(a) Hydrochloric acid, 0.1 N(pH = 0.9).

(b) Bulk samples of all diazonium salts and azo dyes processed in the testing area on the day of sampling.

(c) Appropriate solvents for dissolving the azo dyes.

(1) Toluene, ACS reagent grade.

(2) Distilled water.

(3) pH 5 buffer solution. Prepare by adding potassium hydrogen phthalate to distilled water and adjusting the pH to 5.0 ± 0.2 with a calibrated pH meter.

(4) pH 9 buffer solution. Prepare as above with ammonium acetate, adjusting the pH to 9.0 ± 0.2.

Procedure

(a) Cleaning of equipment. Wash all glassware in hot detergent solution and rinse well with hot tap water, then rinse several times with double distilled water.

(b) Collection and Shipping of Samples

(1) Place a clean filter and backup pads into the cassette filter holder.

(2) Connect the cassette to the vacuum pump. No tubing should be placed in front of the filter.

(3) Turn on the pump to begin sample collection. Measure the flowrate and time, or volume, as accurately as possible. Sample 500 liters or more at 1.5-2 liters/min.

(4) Take duplicate samples at each sampling site. One is for the diazonium salt analysis; the other for the azo dye analysis. Protect the filters from light to prevent photodecomposition of the diazonium salts.

(5) Ship the filters in a suitable container designed by NIOSH to minimize contamination and to prevent damage in transit. Include two or more blank filters, which are handled in the same manner as the sample filters except that no air is sampled through them.

(c) Analysis of Samples

(1) Diazonium Salts

Extract one of the duplicate sample filters from each site with 40 ml of 0.1 N HCl in a 100-ml beaker. Agitate the mixture in an ultrasonic bath for 5 minutes. Allow the filter and any suspended solids to settle. Extract a blank filter in the same manner. Perform the extractions with minimum exposure to light. Transfer a portion of each solution to a 1-, 5-, or 10-cm fused silica cell, depending on the anticipated diazonium salt concentration. Determine the absorbance of the blank and the sample of 375 nm. Use 0.1 N HCl as the reference solution.

(2) Azo Dyes

Select an appropriate solvent by experimentation with the bulk samples. (See Advantages and Disadvantages, section (f).) Extract one of the duplicate sample filters and a blank filter with the solvent using the extraction procedure outlined in section (1) on Diazonium Salts. Tranfer a portion of each solution to a 1-, 5-, or 10-cm fused silica cell, depending on the anticipated concentration. Using the solvent as a reference, scan the 400- to 700-nm range to locate the absorbance maxima. Measure the absorbance at each wavelength where the bulk azo dyes absorb. (See Calibration and Standards (b) Azo Dyes.)

Calibration and Standards

(a) Diazonium Salts

(1) Prepare a set of five standards for each diazonium salt that may be present in the sample. Each set should consist of standards containing 5, 25, 50, 100 and 200 μg of one diazonium salt in 40 ml of 0.1 N HCl.

(2) Transfer a portion of each solution to a 1-, 5-, or 10-cm fused silica cell.

(3) Using the 0.1 N HCl solution as the reference solution, determine the absorbance of each standard at 375 nm.

(4) Construct a standard curve for each diazonium salt by plotting the absorbance against the amount (in micrograms) of the individual diazonium salt in the corresponding standard.

(b) Azo Dyes

(1) Prepare a set of standards for each azo dye as in Section (a) (1) using the appropriate solvent.

(2) Tranfer each solution to a 1-, 5-, or 10-cm fused silica cell.

(3) Scan the spectrum of one standard solution for each different dye to determine the wavelength of maximum absorption. Use the solvent as the reference solution.

(4) Determine the absorbance of each standard at the wavelength of its maximum.

(5) Construct a standard curve for each azo dye by plotting the absorbance against the amount (in micrograms) of the individual azo dye in the corresponding standard.

Calculations

(a) Diazonium Salts

(1) If only one diazonium salt is present in the sample, read from the appropriate calibration curve the amount (in micrograms) corresponding to the absorbance of the sample.

(2) If more than one diazonium salt is present, the results must be given as a range. (See Advantages and Disadvantages (f).) From the calibration curve for each diazonium salt, read the amount (in micrograms) corresponding to the absorbance of the sample. The upper limit of the range is taken from the curve yielding the largest value. The lower limit of the range is taken from the curve yielding the lowest value.

(b) Azo Dyes

(1) If only one azo dye is present in the sample, read from the appropriate calibration curve the amount (in micrograms) corresponding to the absorbance of the sample at the wavelength of maximum absorption.

(2) If more than one azo dye is present, the results must be given as a range. (See Advantages and Disadvantages (h).) From the calibration curve for each azo dye absorbing at the same wavelength as the sample, read the amount (in micrograms) of azo dye corresponding to the absorbance of the sample. The upper limit of the range is taken from the curve

yielding the largest value. The lower limit of the range is taken from the curve yielding the lowest value.

(c) Correct the calculated amount or range of amounts of the appropriate diazonium salt(s) or azo dye(s) for any corresponding value found by the analysis of blank filters.

(d) The concentration or range of concentrations of diazonium salts and azo dyes may be expressed in mg/cu m:

$$\text{mg/cu m} = \frac{\text{Amount ((ug)g)}}{\text{Vs (in liters)}}$$

where: Vs = volume (liters) of air sampled.

(B) METHOD FOR MONITORING FOR BENZIDINE IN URINE

NIOSH has reviewed the various methods of biological monitoring for benzidine and related chemicals. There are two acceptable methods: the recommended method that follows [79] and the fluorescamine method given in the o-tolidine criteria document [73]. The recommended method presented here is less expensive and more sensitive than the fluorescamine method; however, it requires a longer working time. Neither method is specific for benzidine.

Any other method of at least equal sensitivity and precision may be substituted for the recommended method [37,59,78].

Aromatic Amines in Urine
BENZIDINE IN URINE (SCREENING TEST)

Analyte:	Aromatic Amines (as Benzidine)	Range:	100-20,000 ng/ 100 ml urine
Matrix:	Urine	Precision:	Not determined
Procedure:	CHC13 extraction HCl reextraction Spectrophotometry (Benzidine confirmation by thin-layer chromatography)	Classification:	D (operational)
		Date Issued:	8/3/79

Principle of the Method

Aromatic amines, including benzidine, are extracted from urine with chloroform after pH adjustment. The chloroform extract is back extracted into 0.1 N HCl and derivatized with 2,4,6-trinitrobenzene sulfonic acid (TNBS). The TNBS derivatives are extracted with chloroform to remove interfering chromophores and the final chloroform extract quantitated spectrophotometrically at 400 nm while using benzidine as a standard. The resulting chloroform extract is concentrated to droplet volume for benzidine identification by thin-layer chromatography (TLC). Attention is called to the poor stability of the chloroform solution of the TNBS derivative of benzidine. Quantitation of this derivative by colorimetry should be performed within 30-45 minutes after preparation of the final chloroform solution.

Range and Sensitivity

(a) The detection limit of this spectrophotometric procedure is 100 ng of benzidine (aromatic amine) per 100 ml of urine. The detection limit for benzidine by TLC is 300 ng/100 ml urine.

(b) The range is from the detection limit up to 20,000 ng/100 ml of urine.

(c) The UV and/or visible detector (maximum absorbance, 400 nm) has a linear response up to 0.02 mg of benzidine per 100 ml of urine.

Interferences

(a) Any other aromatic amine having an absorbance maximum at 400 nm and an Rf value identical to that of benzidine when chromatographed by TLC would interfere.

(b) Aromatic amines in substances that are normally present, ingested, or produced by metabolic processes in the worker will produce a chromophore (false positive).

(c) Extraction at pH 5 with chloroform and subsequent back extraction into HCl reduce interference from other compounds.

Precision and Accuracy

(a) Recovery studies conducted on benzidine indicate 70% at the 500 ng/100 ml level and a marked decrease (about 20%) at the 100 ng/100 ml level.

(b) The precision has not been evaluated at this time.

Advantages and Disadvantages

(a) The principal advantage is that the method is specific for aromatic amines in the nanogram range.

(b) Another advantage is that benzidine at 300 ng/100 ml can be confirmed by TLC in conjunction with the method. It should be noted that the TLC confirmation data does not prove that the chromophore is benzidine. More rigorous methods must be used for absolute confirmation.

(c) The disadvantages of the method are the complexities of the procedure (emulsions and losses in extractions), nonspecificity for benzidine, and the increased time for confirmation by TLC.

(d) A rigorous specific method for benzidine in urine has recently been published [37,59]. It uses fluoroanhydride derivatization and electron capture gas chromatography, but requires considerable more time and equipment to perform.

Apparatus

(a) Spectrophotometer capable of measuring absorbance at 400 nm and accommodating semimicrocuvettes (1-ml capacity).

(b) Centrifuge with speed range to 4000 rpm.

(c) Rotator for mixing test tubes (25x200 mm).

(d) pH meter.

(e) TLC plates precoated with silica gel and without fluorescence indicator (0.5-mm thickness, E. Merck, Darmstadt, Germany).

(f) Chromatographic tank for thin-layer chromatography.

(g) UV source for reading TLC plates.

(h) Graduated cylinders (100 ml).

(i) Glass bottles with Teflon-lined caps (180-ml capacity, 6-ounce size).

(j) Volumetric glass pipets (2 and 5 ml).

(k) Separatory funnels (125 ml).

(l) Volumetric flasks (25 and 100 ml) and a 10-ml amber flask.

(m) Glass culture tubes with Teflon-lined caps (16x125 mm and 25x200 mm).

(n) Microliter pipets (0.01, 0.1, and 0.7 ml).

(o) Nitrogen source for concentrating organic samples.

(p) Disposable Pasteur glass pipets and bulbs.

(q) Disposable plastic gloves.

(r) Desiccator.

(s) Polyethylene bottles, 250 ml for urine collection.

Reagents (All reagents must be ACS reagent grade except where otherwise noted.)

(a) Benzidine, 99% (available from RFR Corporation, 1 Main Street, Hope, Rhode Island 02831) (CAUTION: CARCINOGEN).

(b) Chloroform.

(c) 2,4,6-Trinitrobenzene sulfonic acid (TNBS) (Eastman Organic Chemicals, reagent grade). 100 mg TNBS per ml of water. Stable for up to 7 days in the dark.

(d) Methyl alcohol.

(e) Sodium hydroxide, 1 N.

(f) Sodium chloride.

(g) Sodium acetate buffer pH 5.5, 2 M (refrigerate).

(h) Hydrochloric acid, 1 N and 0.1 N.

(i) Acetone.

(j) Formic acid.

Procedure

(a) Cleaning of Glassware

(1) All glassware used for the laboratory analysis should be treated with chromic acid, rinsed with tap water and washed in detergent. The glassware is rinsed thoroughly in distilled water and air dried.

(2) Samples of "spot" urine (150 ml) are collected following 6 hours of suspected exposure to benzidine-based azo dyes.

(3) Samples should be collected in polyethylene bottles and, if not analyzed on the same day, should be frozen until analysis can be done.

(b) Analysis of Samples

(1) One hundred milliliters of well mixed urine is adjusted to pH 5.0 to 6.0 (1 N HCl or 1 N NaOH) in a glass bottle (180-ml capacity). A control urine sample (100 ml) and a control urine sample (100 ml) spiked with benzidine (300-1000 ng) should be analyzed concurrently with the unknown samples.

(2) Add 0.2 g NaCl crystals to the pH adjusted urine.

(3) The urine is extracted with 10 ml of chloroform for 2 minutes. If an emulsion is formed, then centrifuge to separate the two phases. The chloroform fraction (organic phase) is collected and saved.

(4) The urine is extracted twice more with chloroform (10 ml), and all three of the chloroform fractions are combined.

(5) Reextract the combined chloroform mixture with 2 ml of 0.1 N HCl for 30 minutes on a rotator.

(6) Transfer the aqueous phase (about 2 ml) into a culture tube (16x125 mm) using a Pasteur pipet.

(7) Add 2 ml of pH 5.5 buffer and 0.7 ml of TNBS reagent, mix well, and let stand for 15 minutes at room temperature. A reagent blank is prepared by adding 2 ml of 0.1 N HCl to 2 ml of pH 5.5 sodium acetate buffer and 0.7 ml TNBS reagent and is treated as a sample.

(8) Add 2 ml of CHC13 and shake for 1 minute.

(9) Measure the absorbance of the organic phase at 400 nm on a spectrophotometer.

(10) Retain the organic phase for the benzidine-TLC confirmation.

(c) TLC Confirmation of Benzidine

(1) The TNB-derivative (chloroform extract) is concentrated by evaporating with nitrogen to about 0.2-ml aliquot.

(2) Ten microliters of the aliquot is spotted on a silica-gel TLC plate that was activated at 110 C for 30 minutes.

(3) The plate is then developed in chloroform-formic acid 90:10, volume to volume (prepared daily).

(4) The Rf of the unknown amine derivative is compared with that of a benzidine spiked derivative, which should always be run as a standard. Benzidine produces a spot on the TLC plate having an Rf = 0.41, visualized by both visible and UV light. The spot is yellow in visible light and appears as a dark spot under UV light.

Calibration and Standardization

(a) CAUTION: Benzidine is a known human carcinogen and appropriate precautions should be utilized to minimize exposure. All wastes including acetone rinsed dirty glassware should be collected and disposed by approved methods.

(b) Prepare a working standard solution containing 10 μg of benzidine per ml of methyl alcohol. A series of benzidine spiked urine samples are prepared from a urine pool sample that was previously shown to have less than 100 ng benzidine per 100 ml of urine. The spiked urine samples serve as standards and are analyzed by the colorimetric method. The calibration curve is established by plotting benzidine concentration (ng per 100 ml of urine) vs the absorbance at 400 nm.

Calculations

(a) The concentration of the analyte in the urine sample is compared with a standard curve prepared with benzidine spiked urine samples as described in Calibrations and Standardization. All samples are read against a reagent blank as described in Procedure.

(b) No corrections for extraction efficiency are needed since standards are prepared in urine and both standards and samples are treated the same way.

(c) If the calculated concentration exceeds 300 ng/100 ml, the chloroform extract should be analyzed by TLC to tentatively confirm the presence of benzidine.

(d) In this laboratory, normal rangds of urine specimens from NIOSH employees not exposed to benzidine or aromatic amines are reported below:

Number of Urine Specimens	Aromatic Amine Conc. (ng/100 ml)
10	less than 100
2	100 - 120
1	200
1	300

Total Number: 14.
Benzidine was not detected by TLC.

APPENDIX II

NAME AND COLOUR INDEX NUMBER OF SOME DIRECT DYES

CONTAINING THE BENZIDINE MOIETY*

Benzidine-Based Dye	C.I. No.
1. Pyramidal Brown (LDC)	21060
2. Congo GR(A)	22000
3. Direct Yellow 24	22010
4. Diazo Violet R	22020
5. Direct Brown 86	22030
6. Diazo Brown R Extra	22035
7. Direct Brown 56	22040
8. Direct Brown 165	22045
9. Direct Violet 88	22046
10. Diamine Brown S	22050
11. Pyramine Orange 3G	22060
12. Pyramine Orange RR	22070
13. Paranil Bordeaux B	22080
14. Oxamine Scarlet B	22090
15. Oxamine Red B	22095
16. Oxamine Orange G	22100
17. Diazo Black R Extra	22110
18. Direct Red 28	22120
19. Glycine Red	22125
20. Direct Orange 8	22130
21. Direct Orange 25	22135
22. Direct Dye	22140
23. Direct Red 10	22145
24. Direct Red 17	22150
25. Direct Red 13	22155
26. Brilliant Congo G	22160
27. Direct Dye	22165
28. Direct Red 74	22170
29. Chlorazol Orange 2R	22175
30. Direct Red 42	22180
31. Direct Orange 101	22190
32. Acid Orange 45	22195
33. Direct Red 60	22200
34. Direct Red 43	22205
35. Zambesi Brown GG	22210
36. Glycine corinth	22220
37. Para Green BBL	22230
38. Acid Red 323	22238
39. Direct Red 37	22240
40. Acid Red 85	22245
41. Direct Yellow 1	22250
42. Cloth Orange	22255
43. Brilliant Direct Orange G	22260
44. Mordant Dye-Cloth Brown R	22270
45. Palatine Chrome Red RX	22275
46. Direct Red 18	22280
47. Cloth Brown G	22285
48. Direct Red 52	22290
49. Ozamine Maroon	22300
50. Direct Red 29	22305
51. Direct Red 33	22306
52. Direct Red 1	22310
53. Direct Brown 2	22311
54. Direct Green 60	22315
55. Diazol Brown MA	22320
56. Direct Green 21:1	22322
57. Direct Brown 60	22325
58. Triazol Red 6B	22330
59. Diphenyl Brown RN	22335
60. Direct Brown 58	22340
61. Direct Brown 59	22345
62. Direct Red 88	22360
63. Direct Orange 1	22370
64. Direct Orange 1	22375
65. Direct Orange 2	22380
66. Direct Orange 33	22385
67. Alkali Yellow R	22390
68. Wool Red G	22400
69. Direct Red 53	22405
70. Direct Yellow 20	22410
71. Oxamine Red BN	22415
72. Direct Red 59	22420
73. Direct Orange 1	22430
74. Direct Violet 43	22440
75. Direct Violet 3	22445
76. Direct Violet 42	22450
77. Direct Blue 230	22455
78. Direct Violet 27	22460
79. Direct Violet 17	22465
80. Direct Violet 36	22470
81. Direct Blue 16	22475
82. Direct Violet 22	22480
83. Direct Blue 19	22485
84. Direct Blue 58	22490
85. Naphthamine Blue 3R	22495
86. Direct Red 44	22500
87. Direct Blue 42	22505
88. Direct Violet 45	22510
89. Direct Violet 85	22520
90. Alkali Dark Brown G, V Alkali Red Brown RR, 3R, T	22530
91. Direct Blue 49	22540
92. Direct Grey R	22545
93. Direct Violet 12	22550
94. Direct Violet 4	22555
95. Direct Blue 48	22565
96. Direct Violet 1	22570
97. Direct Black 29	22580
98. Naphthamine Black RE/ Naphthylamine Diazo Black	22585
99. Direct Blue 2	22590
100. Direct Blue 64	22595
101. Diamine Nitrazol Green BB	22600
102. Naphthamine Blue 2B	22605
103. Direct Blue 6	22610
104. Direct Black 15	22620
105. Direct Blue 177	22625
106. Direct Violet 38	22630

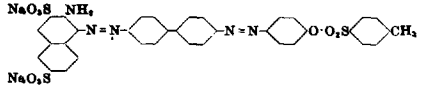
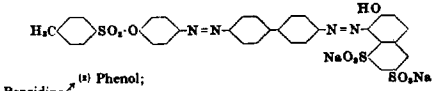
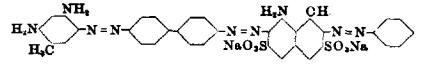
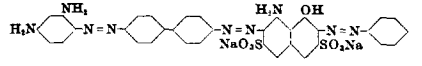
107.	Direct Dye	22640
108.	Direct Brown 7	30035
109.	Direct Brown 171	30040
110.	Direct Brown 1	30045
111.	Direct Brown 79	30050
112.	Direct Brown 61	30055
113.	Direct Brown 20	30060
114.	Direct Dye	30065
115.	Direct Brown 158	30070
116.	Direct Dye	30075
117.	Direct Dye	30080
118.	Direct Dye	30085
119.	Direct Blue 38	30090
120.	Direct Dye	30095
121.	Direct Brown 17	30100
122.	Direct Dye	30105
123.	Direct Brown 1:2	30110
124.	Direct Dye	30115
125.	Direct Brown 154	30120
126.	Direct Brown 68	30125
127.	Direct Dye	30130
128.	Direct Brown 5	30135
129.	Direct Brown 6	30140
130.	Direct Brown 95	30145
131.	Direct Brown 175	30150
132.	Direct Brown 21	30155
133.	Direct Dye	30160
134.	Direct Brown 173	30165
135.	Direct Dye	30170
136.	Direct Dye	30175
137.	Direct Dye	30180
138.	Direct Dye	30190
139.	Direct Dye	30195
140.	Direct Dye	30200
141.	Direct Blue 43	30205
142.	Direct Dye	30210
143.	Direct Dye	30215
144.	Direct Green 39	30220
145.	Direct Green 58	30225
146.	Direct Dye	30230
147.	Direct Black 38	30235
148.	Direct Black 11	30240
149.	Direct Black 4	30245
150.	Direct Dye	30250
151.	Leather Dye	30255
152.	Acid Black 69	30260
153.	Direct Black 41	None
154.	Direct Dye	30265
155.	Direct Black 131	30270
156.	Acid Black 66	30275
157.	Direct Green 1	30280
158.	Direct Green 10	30285
159.	Direct Green 12	30290
160.	Direct Green 6	30295
161.	Direct Dye	30300
162.	Direct Green 19	30305
163.	Direct Green 9	30310
164.	Direct Green 8	30315
165.	Direct Dye	30320
166.	Direct Brown 75	30325
167.	Direct Green 7	30330
168.	Direct Dye	30335
169.	Acid Black 94	30336
170.	Direct Blue 51	30340
171.	Direct Black 14	30345
172.	Direct Blue 11	30350
173.	Acid Black 70	30355
174.	Direct Dye	30360
175.	Direct Brown 151	31685
176.	Direct Dye	31690
177.	Direct Dye	31695
178.	Direct Brown 24	31700
179.	Direct Brown 57	31705
180.	Direct Brown 51	31710
181.	Direct Dye	31715
182.	Direct Brown 62	31720
183.	Direct Brown 27	31725
184.	Direct Brown 26	31730
185.	Direct Brown 54	31735
186.	Direct Brown 101	31740
187.	Direct Dye	31745
188.	Direct Brown 190	31750
189.	Direct Brown 159	31755
190.	Direct Black 40	31760
191.	Direct Dye	31765
192.	Direct Dye	31770
193.	Direct Green 22	31775
194.	Direct Dye	31780
195.	Direct Brown 46	31785
196.	Direct Green 21	31790
197.	Direct Dye	31793
198.	Direct Dye	31795
199.	Direct Dye	31800
200.	Direct Dye	31805
201.	Direct Black 27	31810
202.	Direct Dye	31815
203.	Direct Dye	31820
204.	Direct Dye	31825
205.	Direct Dye	31830
206.	Direct Dye	31835
207.	Direct Dye	31840
208.	Direct Dye	31845
209.	Direct Black 83	31850
210.	Direct Dye	31855
211.	Direct Brown	35060
212.	Direct Dye	35065
213.	Direct Dye	35070
214.	Direct Black	35075
215.	Direct Dye	35080
216.	Direct Blue 131	35085
217.	Direct Dye	35240
218.	Direct Dye	35400
219.	Direct Black 100	35415
220.	Direct Brown 33	35520
221.	Direct Brown 70	35530
222.	Direct Brown 73	35535
223.	Direct Dye	35650
224.	Direct Brown 31	35660
225.	Direct Brown 43	35700
226.	Direct Brown 13	35710
227.	Direct Brown 14	35715
228.	Direct Brown 215	35720
229.	Direct Dye	35900
230.	Direct Brown 25	36030
231.	Direct Dye	36040
232.	Direct Dye	36210
233.	Direct Brown 74	36300
234.	Direct Brown 111	None
235.	Direct Black 31	"
236.	Resin F Black WP	"

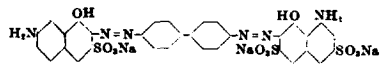
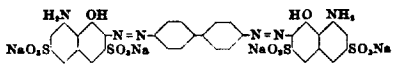
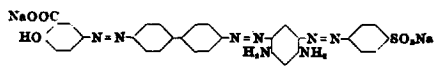
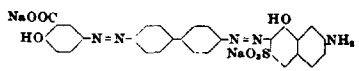
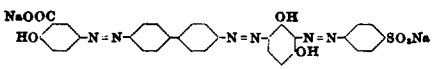
*Synonyms and trade names are listed in the Colour Index [1]

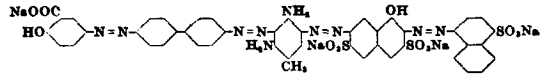
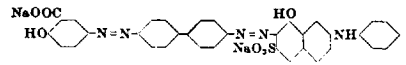
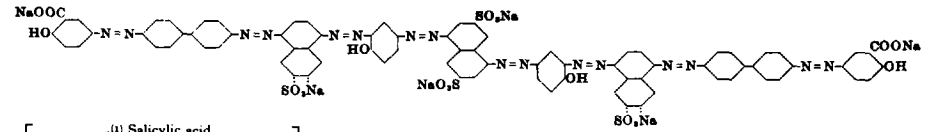
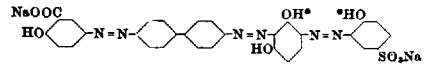
Adapted from references 1,43,(E Angstadt, written communication, January 1979)

APPENDIX III

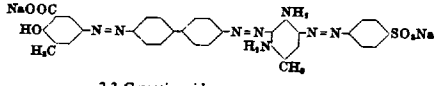
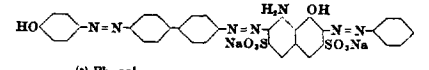
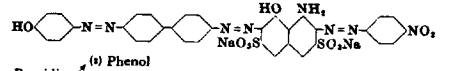
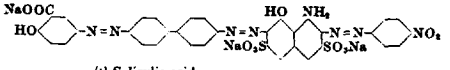
BENZIDINE-BASED DYES REPORTED TO BE COMMERICALLY AVAILABLE IN THE UNITED STATES*

Chemical Structure	Colour Index No. [1]	Chemical Abstracts Service No.	Total Produced lb/y	Total Imported lb/y	Uses	Estimated No. of Workers Exposed**
C.I. Acid Orange 45 (Reddish orange)  Benzidine → 3-Amino-2,7-naphthalenedisulfonic acid Benzidine → Phenol; *then esterify the hydroxy group with p-toluenesulfonyl chloride*	22195	2429-80-3	Not reported; less than 3 manufacturers	Not listed	Dyeing of cotton, silk, nylon, and leather; heavy metal salts used as pigments	Unknown
C.I. Acid Red 85 (Yellowish red)  Benzidine → (2) Phenol; Benzidine → (1) G acid *then esterify the phenol hydroxy group with p-toluenesulfonyl chloride* There are closely related dyes in which benzidine may be replaced by tolidine and other esterifying agents may be used. See C.I.23635 and C.I.24125	22245	3567-65-5	67,000(1975) 22,245(1978)	2,190(1976) 1,000(1978)	Dyeing of cotton, wool, silk, nylon, and viscose; Viqoureux printing	525
C.I. Direct Black 4 (Black)  Benzidine → (2) Toluene-2,4-diamine Benzidine → (1) (*acid*) H acid (*alk.*) (s) ← Aniline Aqueous solution + HCl conc. — corinth ppt; NaOH conc. — greyish blue ppt.	30245	2429-83-6	26,444(1978)	Not listed	Dyeing of cotton, wool, silk, nylon, leather, and paper	Unknown
C.I. Direct Black 38 (Black)  Benzidine → (2) *m*-Phenylenediamine Benzidine → (1) (*acid*) H acid (*alk.*) (s) ← Aniline	30235	1937-37-7 RTECS No. JM7170000	3,760,000(1976) 823,000(1978)	70,753(1976) 49,525(1977) 170,442(1978)	Dyeing of leather, plastics, cotton, wool, and silk; aqueous inks, biological stain; wood flour used as a resin filler, wood stain; typewriter ribbons	13,072

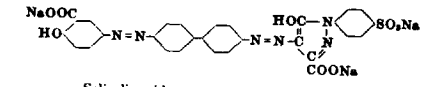
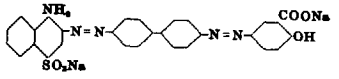
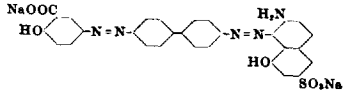
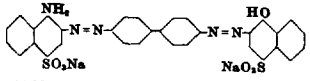
Chemical Structure	Colour Index No. [1]	Chemical Abstracts Service No.	Total Produced lb/y	Total Imported lb/y	Uses	Estimated No. of Workers Exposed**
C.I. Direct Blue 2 (Dull Blue)  Benzidine → (s) (*alk.*) Gamma acid Benzidine → (t) (*alk.*) H acid	22590	2429-73-4	218,435(1978)	38,478(1976) 30,755(1978)	Dyeing of cotton, leather, and paper	1,958
C.I. Direct Blue 6 (Blue)  Benzidine → (*alk.*) H acid (2 mol.) Aqueous solution + HCl conc. — navy blue, ppt; + NaOH conc. — dark violet, ppt.	22610	2602-46-2 RTECS No. QJ6400000	327,000(1976) 61,524(1978)	4,409(1978)	Dyeing of leather, cotton, silk, paper; aqueous writing inks, biological stains	832
C.I. Direct Brown 1 (Brown)  Benzidine → (t) Salicylic acid Benzidine → (u) [*m*-Phenylenediamine (*acid*) ← Sulfanilic acid]	30045	2586-58-5	Not listed	4,409(1978)	Dyeing of leather, paper, silk, nylon, wool and cotton	Unknown
C.I. Direct Brown 2 (Reddish brown)  Benzidine → (t) Salicylic acid Benzidine → (s) (*alk.*) Gamma acid	22311	2429-82-5	125,000(1975) 27,725(1978)	18,739(1976) 2,205(1977)	Dyeing of leather, paper, silk, nylon, wool, and cotton; heavy metal salts used as pigments	106
C.I. Direct Brown 6 (Brown)  Benzidine → (t) Salicylic acid Benzidine → (s) Resorcinol (s) ← Sulfanilic acid This sequence of operations, which is that recorded for Congo Brown G, differs slightly from C.I. 1st Edition 598	30140	NA	8,563(1978)	Not listed	Dyeing of leather, paper, silk, wool, and cotton	Unknown

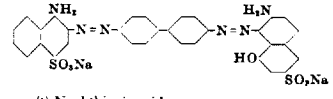
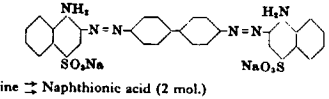
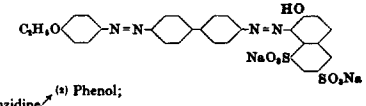
Chemical Structure	Colour Index No. [1]	Chemical Abstracts Service No.	Total Produced lb/y	Total Imported lb/y	Uses	Estimated No. of Workers Exposed**
C.I. Direct Brown 31 (Reddish brown)  Benzidine → (1) Salicylic acid; (2) (Toluene-2,4-diamine (acid) ← *O*-Phenylsulfonyl 2R acid); *then hydrolyse the benzenesulfonic ester group* (3) ← Naphthionic acid (In some brands 2R acid is used instead of its *O*-phenylsulfonyl derivative* as in C.I.35650)	35660	2429-81-4	37,406(1978)	Not listed	Dyeing of leather and paper; heavy metal salts used as pigments; printing on cellulosics (concentrated dye only)	Unknown
C.I. Direct Brown 59 (Blackish brown)  Benzidine → (1) Salicylic acid; (2) (*alk.*) *N*-Phenyl Gamma acid	22345	NA	Not listed	"	Dyeing of cotton, wool, and silk; leather; occasional use on chrome and vegetable tannages	"
C.I. Direct Brown 74 (Brown)  [Benzidine → (1) Salicylic acid; (2) 1,6(and 1,7)-Cleve's acid] (2 mol.) ⇄ (3) [Phenol (2 mol.) ⇄ 4,8-Diamino-2,6-naphthalenedisulfonic acid] HNO_3 conc. — dull red solution, turns yellow brown Aqueous solution + HCl conc. — brownish yellow to olive ppt; + NaOH conc. — orange brown	36300	NA	32,414(1978)	"	Dyeing of cotton, wool, silk, leather, chrome tannage (occasional)	"
C.I. Direct Brown 95 (Reddish brown) Copper complex derived from  Benzidine → (1) Salicylic acid; (2) [Copper complex formed at * from 2-Amino-1-phenol-4-sulfonic acid → Resorcinol] In Sirius Supra Brown BRLN 20% of the salicylic acid is replaced by 2,3-cresotic acid	30145	16071-86-6 RTECS No. JM78780000	346,000(1975) 595,000(1976) 75,953(1978)	8,205(1976) 15,962(1977) 5,512(1978)	Dyeing of cotton, wool, silk paper, plastics, and leather; heavy metal salts used as pigments	714

APPENDIX III (CONTINUED)

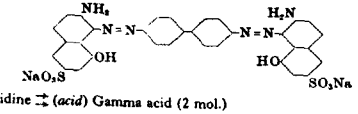
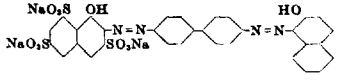
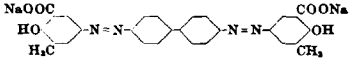
Chemical Structure	Colour Index No. [1]	Chemical Abstracts Service No.	Total Produced lb/y	Total Imported lb/y	Uses	Estimated No. of Workers Exposed**
C.I. Direct Brown 111 (Reddish brown) Structure Unknown	No C.I. No.	NA	Not listed	Not listed	Dyeing of cotton and leather; chrome tannage (occasional)	Unknown
C.I. Direct Brown 154 (Brown)  Benzidine ← 2,3-Cresotic acid; [Toluene-2,4-diamine ← Sulfanilic acid]	30120	6360-54-9	63,816(1978)	"	Dyeing of cotton, wool, silk, leather, and paper; direct printing on cellulosic weave and silk fabrics	322
C.I. Direct Green 1 (Dull green)  Benzidine ← (2) Phenol; (1) (*acid*) H acid (*alk.*) (2) ← Aniline	30280	3626-28-6	57,000(1974) 12,666(1978)	"	Dyeing of cotton, wool, silk, nylon, leather, and paper; aqueous inks; direct printing on cellulosic, silk, and nylon fabrics	1,850
C.I. Direct Green 6 (Dull green)  Benzidine ← (2) Phenol; (1) (*alk.*) [H-acid (*acid*) ← *p*-Nitroaniline] Aqueous solution + HCl conc. — dull bluish green ppt; + H_2SO_4 (10%) — bluer; + NaOH conc. — dark green to olive	30295	4335-09-5	143,000(1974) 109,076(1978)	4,659(1978)	Dyeing of cotton, wool, silk, and nylon; aqueous inks, pigments, leather, paper, and soap; direct printing on nylon	1,095
C.I. Direct Green 8 (Dull green)  Benzidine ← (1) Salicylic acid; (2) (*alk.*) [H acid (*acid*) ← *p*-Nitroaniline]	30315	5422-17-3	Not reported; less than 3 manufacturers	250(1977)	Dyeing of cotton, wool, silk, nylon, leather, and paper	Unknown

APPENDIX III (CONTINUED)

Chemical Structure	Colour Index No. [1]	Chemical Abstracts Service No.	Total Produced lb/y	Total Imported lb/y	Uses	Estimated No. of Workers Exposed**
C.I. Direct Orange 1 (Yellowish orange)***  Benzidine → Salicylic acid; Benzidine → 3-Carboxy-1-(*p*-sulfophenyl)-5-pyrazolone	22370	6459-87-6	Not reported; less than 3 manufacturers	Not listed	Dyeing of cotton, wool, silk, nylon, paper, and leather direct printing on cellulosics and nylon	Unknown
C.I. Direct Orange 8 (Reddish orange)  Benzidine → (s) Naphthionic acid; Benzidine → (1) Salicylic acid *In some brands part of the salicylic acid is replaced by 2,3-cresotic acid* (C.I.22140) *and part of the naphthionic acid by other aminonaphthalene-sulfonic acids* (C.I.22165)	22130	2429-79-0	86,000(1976) 27,208(1978)	4,066(1976)	Dyeing of cotton, wool, silk, nylon, and paper	"
C.I. Direct Red 1 (Bluish red)  Benzidine → (1) Salicylic acid; Benzidine → (s) (*acid*) Gamma acid	22310	2429-84-7	132,000(1975) 26,370(1978)	4,409(1977)	Dyeing of cotton, wool, silk, nylon, paper, and leather	55,508
C.I. Direct Red 10 (Bordeaux)  Benzidine → (1) Naphthionic acid; Benzidine → (s) Nevile and Winther's acid	22145	2429-70-1	Not reported; less than 3 manufacturers	100(1975)	Dyeing of cotton, wool, silk, and leather; biological stain	Unknown

Chemical Structure	Colour Index No. [1]	Chemical Abstracts Service No.	Total Produced lb/y	Total Imported lb/y	Uses	Estimated No. of Workers Exposed**
C.I. Direct Red 13 (Bordeaux)  Benzidine → (1) Naphthionic acid; (2) *(acid)* Gamma acid (In *Diamine Bordeaux N* (By) part of the Gamma acid was replaced by J acid)	22155	1937-35-5	Not reported; less than 3 manufacturers	Not listed	Dyeing of cotton, wool, nylon, paper, and leather (chrome tannage); printing of cellulosics	1,640
C.I. Direct Red 28 (Yellowish red) Classical name **Congo Red**  Benzidine → Naphthionic acid (2 mol.) Soluble in water (yellowish red) and ethanol (orange); very slightly soluble in acetone H_2SO_4 conc. — deep blue; on dilution — paler blue, blue ppt. Aqueous solution + HCl conc. — reddish blue ppt; + Acetic acid — bluish violet, then reddish blue ppt.; + NaOH conc. — yellower	22120	573058-0 RTECS No. QK1400000	37,327(1978)	11,000(1974) 33,069(1978)	Dyeing of cotton, wool, silk, and paper; biological stain and indicator; (first synthetic direct cellulose dye)	523
C.I. Direct Red 37 (Red)  Benzidine → (1) Phenol; (2) G acid *then ethylate the phenol hydroxy group by heating under pressure with ethyl chloride in aqueous ethanol solution in the presence of sodium carbonate*	22240	3530-19-6	63,000(1975)	Not listed	Dyeing of cotton, wool, silk, leather and paper; direct and discharge printing of cellulosics and nylon	1,052

APPENDIX III (CONTINUED)

Chemical Structure	Colour Index No. [1]	Chemical Abstracts Service No.	Total Produced lb/y	Total Imported lb/y	Uses	Estimated No. of Workers Exposed**
C.I. Direct Violet 1 (Violet)  Benzidine → (*acid*) Gamma acid (2 mol.)	22570	2586-60-9	Not reported; less than 3 manufacturers	Not listed	Dyeing of cotton, wool, silk, leather, and paper; biological stain	Unknown
C.I. Direct Violet 22 (Bluish violet)  Benzidine → 1-Naphthol-3,6,8-trisulfonic acid; → 2-Naphthol	22480	6426-67-1	" manufacturers	"	Dyeing of cotton, wool, silk, nylon, leather	"
C.I. Direct Yellow 20 (Yellow)  Benzidine → 2,3-Cresotic acid (2 mol.) Aqueous solution + HCl conc. — brownish yellow, ppt; + NaOH conc. — reddish yellow, ppt.	22410	6426-62-6	Imported only	3,900(1977)	Dyeing of cotton, silk, wool, nylon, leather	"
Resin Fast Black WP	No C.I.#	NA	84,620(1978)	Not listed	Dyeing of textiles, especially those subsequently finished with resins	"

*This table lists the benzidine-based dyes that were reported as being commerically available by DETO [46] and reported as produced or imported by the US International Trade Commission (ITC) [3,42] or those to which potential exposure was found [44]. If less than three manufacturers make a dye, ITC does not publish the production figures.
**A discussion of limitations of the estimation of worker exposure is contained in reference 44.
***This dye may also be synthesized with cresotic acid in place of salicylic acid. The Colour Index designates both dyes as Direct Orange 1.

☆ U.S. GOVERNMENT PRINTING OFFICE: 1980 -657-147/5844

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

OFFICIAL BUSINESS
PENALTY FOR PRIVATE USE, $300

THIRD CLASS

POSTAGE AND FEES PAID
U.S. DEPARTMENT OF H.E.W.
HEW 396

DHEW (NIOSH) Publication No. 80-109