NTP REPORT ON CARCINOGENS BACKGROUND DOCUMENT for DYES METABOLIZED TO BENZIDINE (BENZIDINE DYE CLASS)

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NTP Report on Carcinogens Listing for Dyes Metabolized to Benzidine (Benzidine Dye Class)

Carcinogenicity

Benzidine-based dyes that are metabolized to benzidine are *known to be human carcinogens* based on the fact that 1) benzidine is both an animal and human carcinogen (IARC, 1972, 1979, 1982a, 1987; NTP, 1998), 2) metabolism of benzidine-based dyes to release free benzidine is a generalized phenomenon in all species studied, including humans (Rinde and Troll, 1975; Lynn et al., 1980; Nony et al., 1980; Lowry et al., 1980; Martin and Kennelly, 1985), 3) benzidine exposure following exposure to benzidine-based dyes is equivalent to exposure to equimolar doses of benzidine (Lynn et al., 1980), and 4) all available evidence indicates benzidine-based dyes are animal carcinogens and represent a carcinogenic risk to humans (NCI 108, 1978; IARC, 1982a,b).

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

Benzidine was one of the first chemicals for which an association of occupational exposure and increased cancer was recognized for humans. Increased incidences of urinary bladder cancer in humans were concluded to result from industrial exposure by the International Labour Office in 1921 (International Labour Office, 1921; cited by IARC, 1982a). Since that time, several IARC and NTP committees (IARC, 1972, 1979, 1982a, 1987; NTP, 1998) have concluded that benzidine and its salts are carcinogens in numerous species including rats, mice, hamsters, dogs, and humans. The primary target organs for carcinogenicity induced by benzidine vary with species. Rats, mice, and hamsters develop increased incidences of hepatocellular carcinomas, mammary carcinomas in female rats, and Zymbal gland tumors in both sexes of rats. Dogs and humans develop increased incidences of urinary bladder cancer.

The first dyes based on the benzidine molecule were synthesized more than 100 years ago. Since a wide spectrum of colors could be achieved by varying the molecules' chromophores, linked to benzidine by an azo linkage, this facile and productive synthesis resulted in many excellent dyes. The variety of dyes based on benzidine is exemplified by the fact that 258 benzidine-based dyes were listed in the third edition of the Colour Index (Martin and Kennelly, 1985). Each of these dyes was formed by diazotization of benzidine with nitrous acid and then coupling the resulting diazonium salt with various chromophores to form compounds with azo linkages (-N=N-). Similar or different chromophores may be linked at each amino group of the benzidine to form various bisazobiphenyl dyes. However, regardless of the chromophore(s) involved, the azo linkages of all benzidine-based dyes are essentially chemically equivalent.

Just as the azo linkages between benzidine and chromophores are easily formed chemically, they are also easily broken by chemical or enzymatic reduction. Products of reductive cleavage of the dyes are free benzidine and the respective chromophores. One of the first reports of reductive cleavage of a benzidine-based dye in a biological system was that of Rinde and Troll (1975). That report indicated that each of four benzidine-based dyes was reduced to benzidine by primates, most probably by gastrointestinal bacteria. Later reports provided evidence that benzidine-based dyes are metabolized to free benzidine by humans (Lowry et al., 1980) and also rats and dogs (Lynn et al., 1980), and hamsters (Nony et al., 1980). Lowry et al. (1980) concluded that the amount of benzidine and its metabolites detected in urine

of exposed workers could not have been accounted for by the minute amounts of free benzidine in the dyes to which they were exposed. Thus, evidence was provided to indicate that humans also metabolize benzidine-based dyes to free benzidine. The conclusion to be drawn from this series of studies is that reduction of benzidine dyes to release benzidine was a generalized phenomenon that occurred in most, if not all, species. By determining the quantities of benzidine and its metabolites excreted following administration of free benzidine versus three benzidine-based dyes Lynn et al. (1980) also provided quantitative data for the reduction of benzidine-based dyes to free benzidine. Results of that study indicated evidence that each of the dyes studied was reduced to an amount of free benzidine equal to that observed from an equimolar dose of benzidine. Thus, the first evidence was provided to indicate that ingestion of benzidine-based dyes was equivalent to exposure to an equimolar dose of free benzidine.

Since occupational exposure to benzidine-based dyes has been most frequently associated with co-exposure to benzidine, it has been difficult to clearly establish their carcinogenicity in humans. Two recent studies have endeavored to address this problem by studying Chinese workers who remained in the same jobs for many years. Results of these studies were mixed. Whereas You et al. (1990) observed no increased incidence of tumors in workers exposed almost exclusively to benzidine-based dyes, Bi et al. (1992) reported that cancer incidences were elevated for workers exposed to both benzidine and benzidine-based dyes. Unfortunately, neither report was able to adequately document levels of exposure to either benzidine or the dyes. Evidence for the carcinogenicity of benzidine-based dyes in laboratory animals has been provided by studies in which three dyes, Direct Blue 6, Direct Black 38, and Direct Brown 95, were positive liver carcinogens in rats following an exposure of only 13 weeks (NCI 108, 1978; IARC, 1982a,b). The IARC evaluation of these results and benzidine-based dyes in general reached the following conclusion. "Although the epidemiological data were inadequate to evaluate the carcinogenicity to man of individual benzidine dyes, they, together with the presence of benzidine in the urine of exposed workers, provide *sufficient evidence* that occupational exposure to benzidine-based dyes represents a carcinogenic risk to man."

Listing Criteria from the Report on Carcinogens, Eighth Edition

Known To Be A Human Carcinogen:

There is sufficient evidence of carcinogenicity from studies in humans which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated To Be A Human Carcinogen:

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias or confounding factors, could not adequately be excluded, or

There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor, or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either a known to be human carcinogen or reasonably anticipated to be human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

Supporting Information for Listing

The following table includes information on benzidine-based dyes that have records in the Registry of Toxic Effects of Chemical Substances (RTECS) and other biomedical databases. Those that have undergone carcinogenesis bioassays and/or are known to be metabolized to benzidine are indicated.

A substructure search on benzidine identified approximately 18,000 compounds that included benzidine and its congeners and dyes and pigments based on them. Approximately 700 of the compounds were found in the database CHEMLIST, indicating each compound was on one or more U.S. and foreign regulatory lists. About 120 of the regulated benzidine structural analogues had records in one or more of the biomedical databases BIOSIS, CANCERLIT, EMBASE, MEDLINE, RTECS, and TOXLINE. Those that were benzidine-based dyes and had records in RTECS appear in the table.

It was initially assumed that toxicity testing would be more likely for regulated than for unregulated compounds. However, about 120 of the 17,384 unregulated analogs were indexed in about 750 records in the biomedical databases by terminology included in the Medical Subject Heading (MESH) for all neoplasms compared to about 1,400 records so indexed for the regulated analogues. These records were not examined.

Appendix F lists 236 direct dyes that contain the unsubstituted benzidine moiety. Appendix G lists benzidine-based dyes reported to be commercially available in the United States in the late 1970s. These appendices are reprinted from the NIOSH Special Hazard Review of Benzidine-Based Dyes (NIOSH, 1980).

TABLE 1. SOME REGULATED AZO DYES DERIVED FROM BENZIDINE THAT HAVE CITATIONS IN BIOSIS, CANCERLIT, EMBASE, MEDLINE, RTECS, AND/OR TOXLINE

Name	CASRN	Tumor Data: Y/N	Comment	NTP TR	RTECS	Other Source	Metabolized to Benzidine: Y/N (Source)
C. I. Acid Orange 45, disodium salt; C. I. 22195	2429-80-3	N			QJ6270000		
C. I. Acid Red 85; C. I. 22245	3567-65-5	N			QJ6479000		
C.I. Direct Black 4, disodium salt; C.I. 30245	2429-83-6	N					Y - dogs and rats (Lynn et al., 1980).
C. I. Direct Black 38, disodium salt; C. I. 30235	1937-37-7	Y Positive	NTP results: MR FR MM FM P P N N Study duration 13 weeks.	TR-108 (NCI, 1978)	QJ6160000	IARC (1982a). p. 295. Animal Sufficient Evidence. Human Inadequate Evidence.	Y - humans (Lowry et al., 1980); monkeys (Rinde and Troll, 1975); rats (Kennelly et al., 1982; Zhdan and Pylev, 1982); hamsters (Nony et al., 1980).
C. I. Direct Blue 2, trisodium salt; C. I. 22590	2429-73-4	N			QJ6158000		Y - humans (Genin, 1977); dogs and rats (Lynn et al., 1980)
C. I. Direct Blue 6, tetrasodium salt; C. I. 22610	2602-46-2	Y Positive	NTP results: MR FR MM FM P P N N Study duration 13 weeks.	TR-108 (NCI, 1978)	QJ6400000	IARC (1982a), p. 311. Animal Sufficient Evidence. Human Inadequate Evidence	Y - monkeys (Rinde and Troll, 1975); rats (Kennelly et al., 1982, 1984; Martin and Kennelly, 1985).
C.I. Direct Brown 1:2, disodium salt; C. I. 30110	2586-58-5	N			DG6241500		
C.I. Direct Brown 2, disodium salt; C.I. 22311	2429-82-5	N					Y - dogs and rats (Lynn et al., 1980).
C.I. Direct Brown 2, disodium salt; C.I. 22311	2429-82-5	N					Y - dogs and rats (Lynn et al., 1980).
C. I. Direct Brown 31, tetrasodium salt; C. I. 35660	2429-81-4	N			DG6241000		

TABLE 1. SOME REGULATED AZO DYES DERIVED FROM BENZIDINE THAT HAVE CITATIONS IN BIOSIS, CANCERLIT, EMBASE, MEDLINE, RTECS, AND/OR TOXLINE (Continued)

Name	CASRN	Tumor Data: Y/N	Comment	NTP TR	RTECS	Other Source	Metabolized to Benzidine: Y/N (Source)
C. I. Direct Brown 95, copper, disodium salt; C. I. 30145	16071-86-6	Y Positive	NTP results: MR FR MM FM N P N N Study duration 13 weeks. The benzidine moiety is not complexed in this copper complex.	TR-108 (NCI, 1978)	GL7375000	IARC (1987), p. 125. Animal Sufficient Evidence. IARC (1982a), p. 321. Animal Limited Evidence. Human Inadequate Evidence.	Y - monkeys (Rinde and Troll, 1975); rats (Kennelly et al., 1982).
C. I. Direct Brown 154, disodium salt; C. I. 30120	6360-54-9	N			DG6241600		
C. I. Direct Green 1, disodium salt; C. I. 30280	3626-28-6	N			QJ6195050		Y - dogs and rats (Lynn et al., 1980).
C. I. Direct Green 6, disodium salt; C. I. 30295	4335-09-5	N			QJ6194500		
C.I. Direct Orange 1; C.I. 22370; C.I. 22375; C.I. 22430	54579-28-1	N					Y - dogs and rats (Lynn et al., 1980).
C.I. Direct Orange 8, disodium salt; C. I. 22130	2429-79-0						Y - dogs and rats (Lynn et al., 1980).
C. I. Direct Red 1, disodium salt; C. I. 22310	2429-84-7	N			DG2800350		
C.I. Direct Red 17, disodium salt; C. I. 22150	2769-07-5	N			QK1296000		
C. I. Direct Red 28; Congo Red; C. I. 22120	573-58-0	N	Presumed weak carcinogen (Martin and Kennelly, 1985; pp. 106-107)		QK1400000		Y - monkeys (Rinde and Troll, 1975); dogs and rats (Lynn et al., 1980); rats (Birner et al., 1990; Martin and Kennelly, 1985). DNA binding in rat liver occurred upon metabolism of the compound (Kennelly et al., 1984). Poor rat liver azo reductase substrate (Martin and Kennelly, 1985).

TABLE 1. SOME REGULATED AZO DYES DERIVED FROM BENZIDINE THAT HAVE CITATIONS IN BIOSIS, CANCERLIT, EMBASE, MEDLINE, RTECS, AND/OR TOXLINE (Continued)

Name	CASRN	Tumor Data: Y/N	Comment	NTP TR	RTECS	Other Source	Metabolized to Benzidine: Y/N (Source)
C. I. Direct Violet 1, disodium salt; C. I. 22570	2586-60-9	Y Positive	From RTECS (1997): Tumors in the liver, kidney, ureter, bladder, and blood were observed in rats [Voprosy Onkologii 23(7):72, 1977(Korosteleva et al., 1977); Gigiena Truda i Professional'nye Zabolevaniya 22(10):22, 1978 (Korosteleva et al., 1978)]. Controls and specific information were not provided.		QK1420000	See IARC (1981), p. 377. Listed as a dye used for leather and paper dyeing, but gives no evaluation of carcinogenicity. Abstracts for these papers in other online biomedical databases were not found.	Y - rats (Korosteleva et al., 1978)

Abbreviations: C.I. = Colour Index; Y = Yes; N = No; MR = Male Rats; FR = Female Rats; MM = Male Mice; FM = Female Mice; P = Positive; N = Negative.

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IARC (International Agency for Research on Cancer). 1981. Appendix 6. Some dyes used in wood, leather, and some associated industries, with cross references to IARC Monographs [Evaluations of carcinogenicity are reported only for those chemicals evaluated in IARC Monographs.] IARC Monogr. Eval. Carcinog. Risk Chem. Hum. 25(Wood, Leather and Some Associated Industries):371-379.

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APPENDIX A

Excerpts from the IARC Monograph on the
Evaluation of the Carcinogenic Risk of Chemicals to Humans
Volume 29 (Some Industrial Chemicals and Dyestuffs) 1982
Benzidine and Its Sulphate, Hydrochloride, and Dihydrochloride
pp. 149-183
Supplement 1, Benzidine, 1979, p. 25
Supplement 4, 1982, Benzidine and Benzidine-Based Dyes, pp. 57-59
Supplement 7, 1987, Benzidine and Benzidine-Based Dyes, pp. 123-126

Volume 29, 1982
Benzidine and Its Sulphate, Hydrochloride, and [redacted]
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BENZIDINE AND ITS SULPHATE, HYDROCHLORIDE AND DIHYDROCHLORIDE

These substances were considered by a previous working group, in December 1971 (IARC, 1972). Since that time new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Chemical and Physical Data

Benzidine

1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 92-87-5

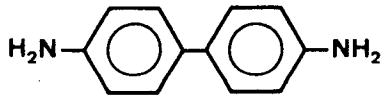
Chem. Abstr. Name: (1,1′-Biphenyl)-4,4′-diamine

IUPAC Systematic Name: Benzidine

Synonyms: Benzidine; benzidine base; 4,4′-bianiline; *p,p*′-bianiline; 4,4′-biphenyldiamine; 4,4′-biphenylenediamine; C.I. 37225; C.I. Azoic Diazo Component 112; 4,4′-diaminobiphenyl; 4,4′-diamino-1,1′-biphenyl; *p,p*′-diaminobiphenyl; 4,4′-diaminodiphenyl; *p*-diaminodiphenyl; 4,4′-diphenylenediamine

Trade Name: Fast Corinth Base B

1.2 Structural and molecular formulae and molecular weight



$C_{12}H_{12}N_2$ Mol. wt: 184.2

1.3 Chemical and physical properties of the pure substance

From Verschueren (1977), unless otherwise specified

(*a*) *Description*: Greyish-yellow, white or reddish-grey crystalline powder (Hawley, 1981)

(*b*) *Boiling-point*: 402°C

(*c*) *Melting-point*: 116/129°C (isotropic forms)

(*d*) *Density*: d_4^{20} 1.250

(*e*) *Spectroscopy data*: λ_{max} 287 mm (in ethanol) (Weast, 1979); nuclear magnetic resonance and mass spectral data have been reported (NIH/EPA Chemical Information System, 1980)

(*f*) *Solubility*: Practically insoluble in cold water (400 mg/l at 12°C); slightly soluble in hot water (9400 mg/l at 100°C); soluble in diethyl ether; slightly soluble in ethanol (Weast, 1979)

(*g*) *Stability*: Darkens on exposure to air and light (Windholz, 1976)

(*h*) *Reactivity*: Undergoes chemical reactions characteristic of primary arylamines (e.g., formation of diazonium salts and acyl and alkyl derivatives) (Ferber, 1978)

(*i*) *Conversion factor*: ppm = 0.133 x mg/m^3

1.4 Technical products and impurities

Benzidine is no longer manufactured for sale in the US or Japan.

Benzidine sulphate

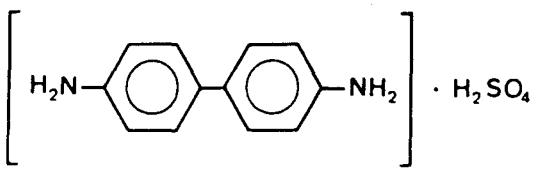
1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 531-86-2

Chem. Abstr. Name: (1,1'-Biphenyl)-4,4'-diamine, sulfate (1:1)

IUPAC Systematic Name: Benzidine, sulfate (1:1)

1.2 Structural and molecular formulae and molecular weight



$C_{12}H_{12}N_2.H_2SO_4$ Mol. wt: 282.3

1.3 Chemical and physical properties of the pure substance

(*a*) *Description*: White crystalline powder (Hawley, 1981)

(*b*) *Solubility*: Soluble in diethyl ether; slightly soluble in water (98 mg/l at 25°C) (Ferber, 1978), ethanol and dilute acids (Hawley, 1981)

1.4 Technical products and impurities

Benzidine sulphate is no longer manufactured for sale in the US or Japan.

Benzidine hydrochloride

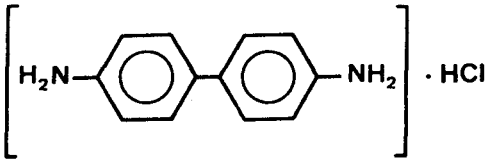
1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 14414-63-7

Chem. Abstr. Name: (1,1'-Biphenyl)-4,4'-diamine, hydrochloride

IUPAC Systematic Name: Benzidine, hydrochloride

1.2 Structural and molecular formulae and molecular weight



$C_{12}H_{12}H_2.HCl$ Mol. wt: 220.7

1.3 Chemical and physical properties of the pure substance

Solubility: 5.345 g/l in water at 25°C (Ferber, 1978)

1.4 Technical products and impurities

Benzidine hydrochloride is no longer manufactured for sale in the US or Japan.

Benzidine dihydrochloride

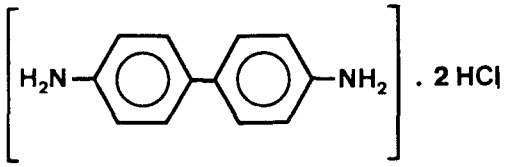
1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 531-85-1

Chem. Abstr. Name: (1,1′-Biphenyl)-4,4′-diamine, dihydrochloride

IUPAC Systematic Name: Benzidine, dihydrochloride

1.2 Structural and molecular formulae and molecular weight



$C_{12}H_{12}N_2.2HCl$ Mol. wt: 257.2

1.3 Chemical and physical properties of the pure substance

(*a*) *Description*: Crystals (Windholz, 1976)

(*b*) *Solubility*: Soluble in water and ethanol (Windholz, 1976)

1.4 Technical products and impurities

Benzidine dihydrochloride is no longer manufactured for sale in the US or Japan.

2. Production, Use, Occurrence and Analysis

Several reviews have been written about benzidine (Shriner *et al.*, 1978; JRB Associates, Inc., 1979; Jones, 1980).

2.1 Production and use

(*a*) *Production*

Benzidine was first synthesized in 1845 by reduction of azobenzene with ammonium sulphide, followed by treatment of the hydrazobenzene with sulphuric acid and treatment with a base to release the free benzidine. Although several methods for the reduction of nitrobenzene can be used, the key reaction in all routes of benzidine synthesis is the rearrangement of the intermediate hydrazobenzene to benzidine (Lurie, 1964). In commercial production, this rearrangement is generally carried out by treating hydrazobenzene with hydrochloric acid (sulphuric acid can also be used); and the resulting benzidine dihydrochloride is used for subsequent reaction rather than converted to the free base (Ferber, 1978).

Benzidine was first produced commercially in Europe in about 1880 (Schwenecke, 1980). This compound and its sulphate were produced commercially in the US for at least 60 years (US Tariff Commission, 1922); commercial production of benzidine hydrochloride was first reported in the US in 1928 (US Tariff Commission, 1930). At present, benzidine dihydrochloride is the only form produced commercially in the US, and this only as an unisolated intermediate which is further converted by the single manufacturer to a variety of dyes. In 1977, one US company reported that its production of benzidine dihydrochloride for captive consumption was in the range of 45.4-454 thousand kg (US Environmental Protection Agency, 1981). Commercial production of benzidine in the US was last reported (by a single company) in 1976 (US International Trade Commission, 1977), and that appears to be the last year of large-scale production of benzidine in the US. An estimated 500 thousand kg were produced in 1974 and 700 thousand kg in 1972 (Ferber, 1978). Even these amounts are small compared with the 1.8 million kg of benzidine produced in the US in 1948 (Boeniger, 1980). A total of 4100 kg were imported through the principal US customs districts in 1980 (US International Trade Commission, 1981).

One company in France probably produces benzidine dihydrochloride as an unisolated intermediate for dyes.

Commercial production of benzidine in Japan was stopped in about 1966. One or more companies in South Korea is believed to produce benzidine dihydrochloride as an unisolated dye intermediate.

The following countries, which are believed to manufacture benzidine-based dyes, may have plants in which benzidine and/or its salts are produced as unisolated dye intermediates: Argentina, Brazil, India, Mexico, the People's Republic of China, Poland, Romania and the USSR.

(*b*) *Use*

Although benzidine has had a variety of applications since the first benzidine-based dye, Congo Red, was prepared in 1884, its principal commercial use has remained the production of direct azo dyes. The amount consumed in all its other uses has been insignificant by comparison.

The Society of Dyers and Colourists (1971, 1975) indicates that 254 dyes or pigments can be prepared from benzidine. JRB Associates, Inc. (1979) stated that only one benzidine-based pigment, Pigment Red 39, had been, but was no longer, produced commercially in the US. They also indicated that only 16 out of a list of 232 benzidine-based dyes were being produced in the US or being imported; three of these 16 dyes, Direct Black 38, Direct Blue 6 and Direct Brown 95, are the subjects of monographs in this volume. Data on US production (or sales), imports and principal uses of the remaining 13 dyes during the period 1971-1979 are given in an appendix to the 'General Remarks on the Substances Considered', as are US sales in 1978 of another benzidine-based dye, Resin F Black WP, the composition of which has not been disclosed. The quantities of benzidine-based dyes produced and their relative importance in the dye industry have been decreasing steadily: their position is insignificant compared with the situation in 1948 when 14 million kg of benzidine-based dyes were produced in the US, representing 21% of all dyes manufactured and almost all of the direct dyes on the market that year (Boeniger, 1980).

Benzidine or its salts has also been reported to be used in the following minor applications: for the detection of blood, both in clinics and in criminal investigations - the latter use has existed for over 50 years (Steinberg, 1977); as a hardener (e.g., for polyurethanes) in the rubber industry (see IARC, 1982) and in the adhesives and plastics industries; for the detection of hydrogen peroxide in milk; in security printing (because it reacts with ink erasers to give coloured products); for the detection of a large number of inorganic ions and compounds; for the quantitative determination of nicotine; as a spray reagent for sugars; in the analysis of metals; as a chromogenic spray reagent in thin-layer chromatography of chlorinated organic pesticides; for the detection of bacterial cytochromes; for the determination of naphthalenesulphonic acids and detergents; in the synthesis of nitrosulphone and sulphonic acid derivatives, which can be used as dye intermediates; in the detection of chlorine or pyridine in drinking-water; and in the detection of *meta*- and *para*-cresols (Lurie, 1964; IARC, 1972; Shriner *et al.*, 1978; Auerbach Associates, Inc., 1978; Jones, 1980). It is not known to what extent, if any, benzidine is still used in any of these applications.

Regulations in the US concerning benzidine designate strict procedures to avoid worker contact: mixtures containing 0.1% or more benzidine must be maintained in isolated or closed systems, employees must observe special personal hygiene rules, and certain procedures must be followed for movement of the material and in the case of accidental spills and emergencies (US Occupational Safety and Health Administration 1980).

Benzidine and its salts have been recognized as carcinogenic by the following 13 countries by regulation or guidelines: Australia, Belgium, Finland, the Federal Republic of Germany, Italy, The Netherlands, Poland, Romania, Sweden, Switzerland, the UK, the USA and Yugoslavia. Those countries which also designate it as a skin irritant are: Australia, Belgium, Italy, The Netherlands, Poland, Romania, Switzerland and the USA. Manufacture of benzidine and its salts is prohibited in Japan; and production of benzidine has been discontinued in the USSR by a decree of the Ministry of Health (International Labour Office, 1980).

The US Environmental Protection Agency (EPA) (1980a) has established the following standards to prevent pollution of navigable waters by industrial discharges: the maximum allowed is 0.1 μg/l; discharges from all benzidine manufacturers and benzidine-based dye applicators shall not contain benzidine concentrations exceeding an average per working day of 10 μg/l, calculated over any calendar month; and various limits apply to the concentrations permissible in any working day. Since the EPA has identified benzidine as a toxic waste, it requires that persons who generate, transport, treat, store or dispose of it comply with the regulations of a Federal hazardous waste management programme (US Environmental Protection Agency, 1980b). The EPA has also proposed a regulation requiring notification whenever discharges of hazardous substances are made into waterways. If this regulation is adopted, notification will have to be given to the EPA of any such discharges containing 4.54 kg or more of benzidine (US Environmental Protection Agency, 1980c).

As part of the US Department of Transportation (1980) Hazardous Materials Regulations, shipments of benzidine are subject to a variety of labelling, packaging, quantity and shipping restrictions consistent with its declaration as a hazardous material.

2.2 Occurrence

(*a*) *Natural occurrence*

Benzidine has not been reported to occur as such in Nature.

(*b*) *Occupational exposure*

Bell (1973) reported that 17 US companies were using benzidine and that 62 employees were potentially exposed. Zavon *et al.* (1973) studied a benzidine manufacturing plant and found airborne concentrations of $\leqslant$0.007 mg/m^3 at four locations, 0.152 mg/m^3 at a salting-out tub, 0.072-0.415 mg/m^3 at a filter press, and 17.600 mg/m^3 where benzidine was shovelled into drums. A sample from a rafter above the reactors in which nitrobenzene was reduced was found to contain 0.27% benzidine.

Steinberg (1977) reported the results of a 1974 survey of US forensic laboratories, which showed that 54 of 276 laboratories were familiar with the benzidine test for blood. The same report cited several steps in blood testing and the enhancement of fingerprints on a bloody substrate during which laboratory or field workers could be exposed to benzidine-containing solutions.

The National Occupational Hazard Survey (Boeniger, 1980) estimated that about 700 people were exposed occupationally to benzidine. However, many more people may be

Table 1. Methods for the analysis of benzidine

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Bulk direct dyes	Extract (chloroform); elute (choloform-ethanol)	TLC	not given	Genin *et al.* (1977)
	Dissolve (water); extract (benzene); dry extract (sodium sulphate); evaporate; synthesize derivative with heptafluoro-butyric anhydride	GC/ECD	not given	Nony and Bowman (1980a)
Air	Collect sample on glass filter and silica gel; desorb (triethylamine in methanol); elute (60:40 methanol:water)	HPLC/UV	3 μg/m^3	Morales *et al.* (1979)
	Collect on filter paper; extract (sodium carbonate); dilute (chloroform/hexane)	LC/UV	not given	Krajewski *et al.* (1979)
Water				
Wastewaters	Acidify (hydrochloric acid); extract (benzene); add sodium hydroxide to aqueous layer; extract (benzene); percolate extract through sodium sulphate; evaporate; synthesize derivatives with pentafluoropropionic anhydride or heptafluorobutyric anhydride	GC/ECD	0.1 μg/l	Nony and Bowman (1978)
	Add sodium hydroxide; filter; extract (diethyl ether); extract (hydrochloric acid); neutralize (sodium hydroxide); extract (diethyl ether); add methanol; concentrate	GC/FID	2-3 μg/l	Jenkins and Baird (1975)
Aqueous media	Three alternative procedures: (1) direct injection; (2) extract (chloroform), wash and concentrate, dilute (acetate buffer); or (3) adjust pH (phosphate buffer), clean by column chromatography, wash and concentrate, dilute (acetate buffer), concentrate	HPLC/E	(1) 1 μg/l (2) 0.05 μg/l (3) 0.1 μg/l	Riggin and Howard (1979)

Municipal sludge	Dilute (phosphate buffer); extract (chloroform); extract (sulphuric acid); neutralize (sodium phosphate); extract (chloroform); add methanol and concentrate; dilute (acetate buffer)	HPLC/E	10 µg/kg	Warner *et al.* (1980)
Biological samples				
Urine	Add ammonia/ammonium chloride buffer and diethyl ether; centrifuge; repeat ether extraction; add perchloric acid; centrifuge; analyse aqueous layer	HPLC/E	0.01 µg/l	Rice and Kissinger (1979)
Hamster urine	Dissolve (methanol); evaporate; elute (1:1 methanol:potassium phosphate)	HPLC/UV	1 µg/l	Nony and Bowman (1980b)
Monkey urine	Adjust pH; extract (chloroform); extract (hydrochloric acid); add 2,4,6-trinitrobenzenesulphonic acid; extract (chloroform); elute (9:1 chloroform: ethanol)	TLC	not given	Rinde and Troll (1975)
Human urine	Add sodium hydroxide; extract (benzene); dry (sodium sulphate); evaporate; synthesize derivatives with pentafluoropropionic anhydride and heptafluorobutyric anhydride	GC/ECD	1 µg/l	Nony and Bowman (1978)
	Adjust pH (sodium carbonate); extract (diethyl ether or 3:2 diethy ether: benzene); wash (sodium carbonate); extract (hydrochloric acid); add chloramine T; extract (chloroform)	S	10 µg/l	Dangwal *et al.* (1978)

Abbreviations : TLC, thin-layer chromatography; GC/ECD, gas chromatography/electron capture detection; HPLC/UV, high-performance liquid chromatrography/ultra-violet detection; LC/UV, liquid chromatography/ultra-violet spectrometry; GC/FID, gas chromatography/flame-ionization detection; HPLC/E, high-performance liquid chromatography/electrochemical detection; S, spectrometry (colorimetric analysis)

exposed as a result of manufacturing or of using dyes based on benzidine. Twenty-six US-produced dyes based on benzidine were found to contain <1-20 mg/kg benzidine and one had 270 mg/kg. Eight of 33 benzidine-based dye samples obtained from Belgium, Egypt, India, The Netherlands, Poland, Romania, and South Korea were found to contain 38-1254 mg/kg of benzidine, and the others had 24 mg/kg or less. Benzidine was detected in swipe samples taken during a benzidine-dye manufacturing operation; and some of the workers in the factory who were exposed to the dyes were found to have benzidine in their urine (proposed to have arisen from metabolism of the dyes). Similarly, some workers exposed to benzidine-based dyes in a textile dyeing operation were found to excrete benzidine; however, it was detected in the urine of only a very few workers in a paper dyeing operation and in no workers at a leather dyeing plant where benzidine-based dyes were used (Boeniger, 1980).

The National Occupational Hazard Survey estimated that approximately 79 000 workers in 63 occupations were potentially exposed to benzidine-based dyes (National Institute for Occupational Safety and Health, 1980).

(*c*) *Water and sediments*

Effluents from factories where textiles are dyed with benzidine-based dyes were found to contain an average concentration of 3.5 µg/l; those from a leather factory and another manufacturing plant with 'heavy benzidine-dye use' contained 0.25 and 3.5 µg/l, respectively (Jones, 1980).

Benzidine concentrations of up to 233 µg/l were found downstream from a dye plant on the Sumida River in Japan. These were attributed to reduction of the dye molecules to benzidine when either hydrogen sulphide or sulphur dioxide was present in the water (Takemura *et al.*, 1965).

Benzidine has been detected in oil refinery, municipal and industrial effluents and in surface water (Hushon *et al.*, 1980). It has also been found in river water and in raw sewage effluents (Shackelford and Keith, 1976).

2.3 Analysis

Procedures for the detection of benzidine in air, clothing and miscellaneous deposits by colorimetry and paper chromatography have been described (Butt and Strafford, 1956). More recently, analytical methods for the determination of benzidine in air, wastewaters and biological media based on spectrometry, spectrofluorometry, thin-layer chromatography and gas chromatography have been reviewed (Shriner *et al.*, 1978; US Department of Commerce, 1978). An IARC Manual (Egan *et al.*, 1981) gives selected methods for the analysis of aromatic amines and azo dyes, including benzidine.

Typical methods for the analysis of benzidine in various matrices are summarized in Table 1.

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals[1]

(*a*) *Oral administration*

Mouse: Benzidine dihydrochloride (certified ACS grade) was fed at a level of 150 mg/kg [ppm] of diet to B6C3F_1 mice, from the 6th to 45 weeks of age. Groups of 50 animals were killed at 45, 60, 75 and 90 weeks of age to evaluate the occurrence of liver-cell tumours (LCT). When the treatment was terminated at 45 weeks, 8/50 (16%) mice had LCT, 4% of which were hepatocellular carcinomas; at the successive serial killings at 60, 75 and 90 weeks, the proportions of LCT were 20/50 (40%), 31/50 (62%) and 35/50 (70%), 10%, 28% and 48% of which were hepatocellular carcinomas, respectively. In historical controls, the incidence of LCT was 1/98 (1%) in males and 0/100 (0%) in females (Vesselinovitch *et al.*, 1975).

Benzidine dihydrochloride (certified ACS grade) was fed at a level of 150 mg/kg [ppm] of diet for periods of 39, 54 or 84 weeks to 6-week-old B6C3F_1 male mice. All animals were killed at 90 weeks of age. The incidences of mice bearing LCT, mostly hepatocellular carcinomas, are given in Table 2. Thus, a negative relationship was observed between the incidence of LCT and duration of treatment; this may have been related to toxicity (Vesselinovitch *et al.*, 1975).

Table 2. Incidences of liver-cell tumours (LCT) in mice fed benzidine

Duration of treatment (weeks)	Estimated consumption of benzidine (mg/mouse)	Effective no. animals	LCT No.	%
39	117	50	35	70
54	162	50	25	50
84	188	50	22	44

A study was carried out to evaluate the role of age and sex in benzidine hepatocarcinogenesis. Doses of 50 or 100 mg benzidine dihydrochloride (certified ACS grade) per kg of diet [ppm] were given by stomach tube or in food to groups of 50 male and 50 female infant or adult B6C3F_1 mice; a further group was treated pre- and postnatally. No effects on survival were noted; all animals were killed at 90 weeks of age. The incidences of LCT are shown in Table 3. Continuous feeding of benzidine in the diet was found to produce LCT in a positive-dose response relationship in animals of both sexes: in 3/50 and 11/50 males receiving 50 and 100 mg/kg, respectively; and 13/50 and 32/50 in females,

[1] The Working Group was aware of a study, completed but not yet published, of s.c. administration in rats (IARC, 1981).

revealing a greater susceptibility of animals of this sex. The incidence of LCT in untreated controls was negligible (1/98 in males and 0/100 in females). Twice-weekly administration of benzidine by stomach tube showed a lesser hepatocarcinogenic effect that continuous feeding: 3/75 and 12/75 of males and 4/75 and 17/75 of females given the low and high dose of benzidine, respectively, developed LCT. The incidences of tumours at other sites (Harderian tumours and lung adenomas) were also affected by benzidine treatment; the compound also had a marginal effect upon the development of lymphoreticular tumours (Vesselinovitch *et al.*, 1975).

Table 3. Incidences of liver-cell tumours (LCT) in mice of different ages fed benzidine

Period of treatment (days of age)	Estimated total intake of benzidine dihydrochloride (mg/mouse)		Incidence of LCT			
			Males		Females	
	Males	Females	No.	%	No.	%
7-27 (by stomach tube)	0.63	0.63	59/89	66	0/82	0
1-27 (150 mg/kg diet to) mother and offspring from delivery to weaning)	4.20	3.36	62/65	95	2/43	5
42-630 (150 mg/kg diet)	187.87	150.29	22/50	44	47/50	94

Groups of $B6C3F_1$ mice were fed a diet containing 150 mg/kg [ppm] benzidine dihydrochloride (purity unspecified) (1) from the 12th day of gestation (prenatal) to delivery; (2) to mothers with litters from delivery to weaning; (3) to offspring from weaning to 90 weeks of age; (4) during prenatal and preweaning period; or (5) prenatally, during preweaning and in adulthood. Groups of untreated controls were also available. Administration prenatally or during preweaning induced a marked increase in the incidence of LCT in male mice (31 and 95%, respectively) but not in females (3 and 5%, respectively). In mice treated from weaning to 90 weeks of age, the incidences of LCT were 59% in males and 96% in females. In the group treated both prenatally and during preweaning, the incidences of LCT were 100% in males and 25% in females. When mice were treated prenatally, during preweaning and up to 90 weeks of age, the incidences of LCT were very high in animals of both sexes (100% in males and 94% in females) (Vesselinovitch *et al.*, 1979).

In studies designed to assess the biological significance of liver tumours in mice, groups of F_1 (C57BL/6Jf C3Hf/Nctr females x BALB/cStCr1fC3Hf/Nctr males) and F_2 (F_1 females and F_1 males) weanling mice were fed diets containing 0, 30, 60, 120, 200 or 400 mg/kg [ppm] of benzidine hydrochloride (100% pure). Groups of mice were killed after 40, 60 or 80 weeks of treatment. The incidences of LCT (benign and malignant) in the different groups are summarized in Table 4 (Frith *et al.*, 1979, 1980). [The Working Group noted that tumour incidences at the various dose levels could not be estimated.]

Table 4. Incidences of liver-cell tumours (LCT) in mice fed benzidine

Sacrifice period (weeks)	Sex	Controls *Benign* No.	%	*Malignant* No.	%	Treated *Benign* No.	%	*Malignant* No.	%
40	M	0/99	0	0/99	0	4/599	0.7	3/599	0.5
	F	0/96	0	0/96	0	25/594	4.2	15/494	3.0
60	M	0/96	0	1/96	1	27/474	5.7	36/474	7.6
	F	1/96	1	1/96	1	50/566	8.8	201/566	35.5
80	M	2/91	2.2	0/91	0	28/314	8.2	61/341	17.9
	F	0/95	0	0/95	0	26/264	9.8	125/264	47.3

Rat: Two groups, each of 10 female Wistar rats [age not specified], were fed a diet containing 170 mg/kg [ppm] benzidine with casein or a diet containing benzidine with casein hydrolysate-tryptophan. All rats given benzidine plus casein were dead 93-224 days after the start of treatment; and 2/10 had LCT (1 hepatoma seen at 125 days and 1 bile-duct carcinoma at 178 days). Animals fed benzidine-tryptophan survived longer; 3/7 animals examined had LCT (1 carcinoma at 202 days, 1 cholangioma at 236 days and a bile-duct carcinoma at 424 days). None of the animals in the two experimental groups developed bladder tumours (Boyland *et al.*, 1954).

Following preliminary determinations of the maximum tolerated dose, four groups of 10-20 female Sprague-Dawley rats, 40-45 days old, were given total doses of 12, 25, 35 or 50 mg/rat of benzidine by stomach tube. Two control groups, one fed the sesame oil vehicle and the other given 18 mg of dimethylbenz[*a*]anthracene (positive control) were also available. At the end of the nine-month period of observation, when the experiment was terminated, 10/10, 8/10, 0/20 and 4/20 animals were still alive in the four treated groups, respectively. In the positive control group, 19/40 survived, and in the vehicle control group 127/140 were still alive at nine months. Thus, mortality was high in animals fed the two highest doses of benzidine, and only 5 rats (at the highest dose) were autopsied; four of these showed multiple mammary carcinomas. In the groups receiving 12 and 25 mg/rat, 5/10 and 7/9 animals autopsied also showed multiple mammary carcinomas (1 in the group fed 12 mg had a fibroadenoma). All of the positive controls had multiple mammary masses diagnosed as carcinomas, fibroadenomas and hyperplasias; and 5/132 sesame-oil vehicle controls examined also had mammary tumours. In the benzidine-treated groups, the first palpable mammary lesions appeared about 60 days after the first treatment. At this point the mean number of mammary masses per rat showed a dose-response relationship. No effect was reported in organs other than the mammary gland (Griswold *et al.*, 1968). [The Working Group noted that the design of this study was based on the breast tumour induction system of Huggins *et al.* (1959). In this system, benzidine was definitely active in causing mammary cancer.]

It was reported in an abstract that groups of 20 female Fischer 344 rats were given drinking-water containing 100-400 mg/l benzidine dihydrochloride for up to 90 days (estimated daily doses of 10, 21, 28 and 31 mg/kg bw). Rats given the two highest doses

died within nine weeks of exposure. Hepatocellular carcinomas were observed in some rats given 28 mg/kg bw per day (Mennear and Gupta, 1982).

Hamster: Groups of 30 male and 30 female, random-bred Syrian golden hamsters, nine weeks old, were fed diets containing 1000 mg/kg [ppm] benzidine or benzidine dihydrochloride (certified grade) for life. A control group of the same size was also available. No bladder pathology was seen in either the treated or the control group. In the benzidine-treated group, an increased incidence of liver tumours was observed: 19/22 males and 6/26 females developed multiple cholangiomatous tumours, most of which had signs of malignancy; 12 males and 3 females also developed benign and malignant LCT. In the group fed benzidine dihydrochloride, the liver was also the only target organ: 10/20 male and 12/27 female hamsters developed cholangiomas, mostly benign; 7 males and 4 females also developed hepatomas. No liver tumours were seen in females or males of the untreated control group (Saffiotti *et al.*, 1967).

Rabbit: An invasive bladder carcinoma was induced after 2 $\frac{1}{4}$ years in one out of seven animals given oral doses of benzidine (Bonser *et al.*, 1956a).

Dog: Seven dogs were given a total dose of 325 g benzidine over 5 years (200 and then 300 mg per day, on six days a week). Three of the animals developed bladder carcinomas 7, 8 and 10 years after the start of treatment (Spitz *et al.*, 1950; Bonser *et al.*, 1956a).

Frog: A group of five frogs (*Rana temporaria*) received a total oral dose of 60 mg benzidine and were observed for 20 weeks; 1 liver tumour was seen (Khudoley, 1977).

(*b*) *Subcutaneous and/or intramuscular administration*

Mouse: Three groups of 12-24 male, albino Delph mice, 10 weeks of age, were given s.c. injections of 300 mg benzidine base (redistilled) in olive oil three times a week for 45 weeks or received olive oil alone. One group served as untreated controls. The survival rates were good in all groups up to 45 weeks when the experiment was terminated. No changes in the bladder were observed in the benzidine-treated animals. In 2/19 control mice receiving olive oil alone hyperplasia of the bladder was noted. Five of nine mice given benzidine had hepatomas, compared with 3/19 in the olive oil group and 5/17 in the untreated controls (Baker, 1950). [The Working Group noted the short duration of the experiment.] Bonser *et al.* (1956a) obtained similar results.

A group of 54 male and 13 female C3HA mice, 18-20 g, were injected subcutaneously weekly with 6 mg/mouse of benzidine [source and purity not specified] dissolved in 0.2 ml of oil [not specified] over eight months (total dose, 210 mg/mouse). At the appearance of the first tumour (16 months), 22 mice [sex not specified] were still alive. Liver tumours (hepatocellular carcinomas, adenomas and cholangiomas) developed in 13 mice [sex not specified]; and lung adenocarcinomas were found in 2. A further group of 114 females were exposed by the same treatment schedule for 13 months (total dose, 336 mg/mouse). At 16 months, 24 mice were still alive, and 18 developed liver tumours. Hepatomas developed in 1% of historical controls (Prokofjeva, 1971). [The Working Group noted the low survival rates.]

Rat: Groups of Sherman rats, 10 weeks old, average weight 150 g, were administered benzidine (technical and purified grades) or benzidine sulphate (technical grade) subcutaneously once weekly for life. A suitable control group was given the olive oil

vehicle or butyl succinate. The experimental design and data on survival and tumour incidence are summarized in Table 5 (Spitz *et al.*, 1950). [The Working Group noted that the low occurrence of colonic adenocarcinomas was confined to males treated with both grades of benzidine; however, it was impossible to distinguish in which of the two gropus the colonic tumours predominated.]

Table 5. Design and results of experiments in rats given s.c. injections of benzidine and salts

Compound	Average weekly dose (mg)	Total dose (g)	No. of rats at start	No. of rats surviving more than 300 days	Rats with tumours: liver No.	liver %	external auditory canal No.	external auditory canal %	colon No.	colon %
Olive oil	910	92.82	50	28	—	—	—	—	—	—
Technical benzidine	15	1.28	233	36	8	3.4	54	23		
Pure benzidine	15	0.96	152	24	6	3.9	32	21	7	3.8
Benzidine sulphate	15	0.94	153	5	1	0.65	16	10.5		

A group of 25 male and 25 female rats [strain not specified], weighing 100-120 g, were injected subcutaneously with an initial dose of 15 mg benzidine in 0.5 ml of sunflower-seed oil once a week for 14 weeks. Due to severe toxicity, a smaller dose of 10 mg weekly was given for six weeks to each rat, and finally once every 15 days for six weeks. By six months of treatment, each animal had received a total dose of 300 mg benzidine. A group of 50 rats [sex unspecified] served as controls: 25 received s.c. injections of the solvent for six months while the remaining 25 were kept untreated. Of the males surviving at the time of the appearance of the first tumour, 12/15 developed tumours: 2 hepatomas, 4 malignant tumours of the Zymbal gland, 6 sarcomas at the injection site and 2 other sarcomas; 2/5 surviving females developed tumours: 1 malignant tumour of the Zymbal gland and 1 myeloid leukaemia. None of the 25 controls injected with the solvent [sex not specified] developed tumours at the injection site (Pliss, 1964).

A group of 28 rats [from the Rappolovo breeding farm; sex not specified], aged $1\frac{1}{2}$-2 months, were exposed to benzidine [purity, source and vehicle not specified] by weekly s.c. injections of 5 mg/rat for 32-60 weeks (total dose, 170 mg/rat). At 210 days, the time at which the first tumour appeared, 25 rats were still alive. Intestinal tumours developed in four rats between 252 and 318 days (Pliss *et al.*, 1973). [The Working Group noted that no controls were reported.]

Groups of 16 female and 14 male rats [strain and age not specified] were injected subcutaneously with 5 mg/rat benzidine [source and purity not specified] dissolved in 0.5 ml of sunflower oil weekly for about 52 weeks (total dose, 160-260 mg/rat). At 219 days, when the first tumour (a skin epithelioma) was detected, 24 rats [sex not specified] were still alive; all animals were killed at 357 days. Tumours were found in 23 rats (95.8%), with an average latent period of 275 days. Nine rats (39.1%) had multiple primary tumours;

tumours of the Zymbal gland developed in 18 rats (78.3%); 5 had local fibrosarcomas and 1 a local rhabdomyosarcoma (Pliss and Iogannsen, 1974). [The Working Group noted that no untreated or solvent controls were available.]

Groups of 18 male and 16 female ablino non-inbred rats (120-140 g) were injected subcutaneously once a week for about 33 weeks with 5 mg/rat benzidine [source and purity not specified] suspended in 0.5 ml of oil [not specified] (total dose, 170 mg/rat). At 210 days, when the first tumour appeared, 16 males and 12 females were still alive. A total of 26 tumours developed in 14 males; these were 6 local sarcomas, 9 tumours of the Zymbal gland, 9 liver tumours (cystocholangiomas and hepatocellular carcinomas) and 2 intestinal tumours (polyposis and adenocarcinoma). A total of 20 tumours developed in 11 females: 5 local sarcomas, 6 tumours of the Zymbal gland, 4 mammary adenocarcinomas, 1 mammary adenoma, 2 liver tumours (cystocholangioma and hepatocellular carcinoma) and 2 intestinal tumours (Pliss and Volfson, 1974). [The Working Group noted that no untreated or solvent controls were available.]

Groups of 20 female, 100-day-old Sprague-Dawley rats were given weekly s.c. injections of benzidine for 1-24 weeks, at total doses of 150, 250, 450, 625 or 1225 mg/kg bw in sterile saline solution. The length of survival was dose-dependent and ranged from 300 $\pm$ 67 days at the lowest level to 170 days at the highest dose. The total numbers of mammary adenocarcinomas in each of the treated groups were 47, 87, 116, 101 and 66, respectively (Steinhoff, 1974). [The Working Group noted that information on the survival rates in the different groups was not given and that no controls were available.]

Frog: A group of 37 grass frogs (*Rana temporaria*) of both sexes, aged 1-1.5 years, received weekly s.c. injections of 0.2-0.5 ml of a 0.5% solution of benzidine in mineral oil for up to 38 weeks (total dose, 45-114 mg/animal). A group of 120 untreated frogs were observed for 56 weeks (3 of them developed skin cystadenopapillomas); and a further group of 67 frogs were given s.c. injections of 0.2-0.5 ml mineral oil weekly for 42 weeks. Of the treated animals, 6/14 still alive at 16 weeks, when the first tumour appeared, had tumours of the liver and haematopoietic system [not further specified], with an average latent period of 24.8 weeks (Khudoley, 1977).

(*c*) *Intraperitoneal administration*

Rat: Three groups of 30 female CD rats, 4 weeks of age, were given i.p. injections, twice weekly for 4 weeks, of 0, 10 or 30 μmol/kg bw benzidine in trioctanoin suspensions. All survivors were sacrificed 46 weeks after the first injection. No tumours were seen in the kidney or bladder in treated or control groups. In the benzidine-treated groups, a dose-related increase in the incidence of mammary tumours, benign and malignant, was noted: 3/30 in controls, 7/30 in the low-dose group and 12/29 ($p<0.01$, compared with controls) in the high-dose group. Zymbal gland tumours (adenomas or carcinomas) were observed in 1/30 controls, 1/30 low-dose animals and 7/29 ($p<0.05$, compared with controls) high-dose animals. No tumours of the liver were found at sacrifice; however, cellular-altered foci in the liver were observed in 9/30 controls, 14/30 low-dose animals and 20/29 ($p<0.01$) high-dose rats. An increased incidence of neoplastic nodules in the liver noted in the treated animals was not statistically significant compared with that in the controls (Morton *et al.*, 1981).

(*d*) *Inhalation exposure*

Rat: A group of 48 white outbred rats (Rappolovo stock) of both sexes, weighing 100-120 g, were exposed to 10-20 mg/m^3 [1.3-2.7 ppm] benzidine [source and purity not specified] in inhalation chambers on four hours/day, for five days a week over 20 months (total dose, 27 mg/rat). Control rats [number not specified] were kept in inhalation chambers and exposed to air during the same period. Animals were kept until moribund. The first myelogenous leukaemia was found in a treated rat 13 months after the start of the experiment, at which time 28 rats were still alive. By the end of the study (28 months), 5 myeloid leukaemias, 2 breast fibroadenomas, 1 squamous-cell cancer of the Zymbal gland, 1 hepatoma and 1 breast adenocarcinoma were found in 8 animals. Mammary adenomas were found in 2/21 control rats (Zabezhinsky, 1970). [The Working Group noted the lack of information on the size of particles and on survival of controls.]

(*e*) *Other experimental systems*

Oral administration following implantation of glass beads into the bladder: Following the surgical implantation of glass beads into the urinary bladder of 150 ICR strain female *mice* at 5 weeks of age, the animals were divided into three groups: one group (30 mice) served as controls and was fed a commercial basal diet; the second group (60 mice) received a diet containing 2000 mg/kg [ppm] of benzidine; the third group (60 mice) was fed a diet containing a mixture of 2000 mg/kg [ppm] benzidine and 20 000 mg/kg [ppm] DL-tryptophan. The experimental groups received their diets starting at 6 weeks of age for 20 weeks and were then fed the control diet for 40-43 weeks. The experiment was terminated 63 weeks after the start of treatment. Of the group that received benzidine alone, only 19% of the animals were still alive at the end of the experiment; 65.5% of controls and 49.2% of the group treated with benzidine plus tryptophan were still alive at that time. Hepatomas, diagnosed microscopically, were observed in 34/41 (82.9%) mice treated with benzidine and in 24/51 (47.1%) of mice treated with the benzidine-tryptophan mixture; no hepatomas were seen in the controls. No bladder tumour was found in any of the animals; however, the authors reported hyperplasia in all bladders examined (Miyakawa and Yoshida, 1980).

Mixed in diet added to tank water: Benzidine was mixed into a diet and given to a group of 100 *fish* (guppies) of both sexes, 10-12 months old, at a dose of 300 mg/kg dry diet for 56 weeks, at which point the experiment was terminated. The six fish that survived the treatment period had no detectable tumour; however, signs of hepatotoxicity (focal necrosis, fatty dystrophy and diffuse hyperplasia of hepatocytes) were noted. None of the 120 control guppies fed the standard diet developed tumours or preneoplastic changes (Pliss and Khudoley, 1975). [The Working Group noted the high mortality in the treated group.]

(*f*) *Carcinogenicity of metabolites*

Essentially negative results were obtained in early studies in mice which were given bladder implantations of 3-hydroxybenzidine hydrochloride, 4-amino-3-biphenyl sodium sulphate or 4′-nitro-4-amino-3-hydroxybiphenyl hydrochloride (Bonser *et al.*, 1956b, 1963).

In the study by Morton *et al.* (1981) [see section 3.1 (*c*)], two benzidine metabolites, *N*,*N*′-diacetylbenzidine and *N*-hydroxy-*N*,*N*′-diacetylbenzidine, were also given by i.p. injection twice weekly for four weeks, at dose levels of 10 and 30 μmol/kg bw in

trioctanoin suspensions to groups of 30 4-week-old CD rats. A group of 30 solvent-treated controls was also available. The two metabolites produced similar incidences of tumours of the mammary and Zymbal glands and of preneoplastic changes in the liver as the parent compound, benzidine.

3.2 Other relevant biological data

(a) Experimental systems

Toxic effects

The acute oral LD_{50} of benzidine administered as a suspension in water to male Wistar rats was 1.57 g/kg (Marhold *et al.*, 1968).

Few data are available on the toxic effects of benzidine; however, the one consistent finding has been that under experimental conditions it causes liver cirrhosis in rats, rabbits and dogs (reviewed in Haley, 1975).

It was reported in an abstract (Rao *et al.*, 1971) that dietary concentrations of 0.01-0.08% [100-800 ppm] benzidine given to C57BL x C3H F_1 mice [sex unspecified] produced concentration-dependent losses of body weight, cloudy swelling of the liver, vacuolar degeneration of the renal tubules and hyperplasia of myeloid elements in the bone marrow and lymphoid cells in the thymus and spleen. All of the lesions were observed with the lowest dose.

Effects on reproduction and prenatal toxicity

Benzidine has been reported to be teratogenic to chicks when injected into hens' eggs (Noto, 1967).

Absorption, distribution, metabolism and excretion

Little is known about the dermal, pulmonary or gastrointestinal absorption of benzidine in experimental animals. The occurrence of systemic toxic manifestations following dietary administration, however, indicated some absorption from the gut; and varying amounts of benzidine or its metabolites were identified in the urine of dogs treated orally or dermally or by inhalation of a benzidine aerosol (Ghetti, 1960).

Uniformly labelled ^{14}C-benzidine (0.2 mg/kg bw) administered intravenously to male Wistar rats and male beagles exhibited multiphasic blood half-lives. The fourth and final phase, predominant within 24 hours after treatment, had an estimated half-life of 65 hours in the rat and 88 hours in the dog. The ^{14}C label of 0.2 mg/kg bw benzidine administered intravenously to rats, dogs and monkeys was rapidly transferred to the excretory organs: liver, gastroinestinal tract, kidney and bladder; significant amounts were also distributed to the lung. By 7 or 14 days after treatment, residues of radioactivity were detected primarily in liver, bile, intestines and kidney (Kellner *et al.*, 1973).

The major routes of excretion for intraperitoneally or intravenously administered benzidine in experimental animals are urine and faeces (Baker and Deighton, 1953; Kellner *et al.*, 1973). Rats excreted approximately 80% of an i.v. dose of 0.2 mg/kg bw benzidine in faeces within 7 days of treatment, while dogs and monkeys excreted only

30%. Urinary excretion predominated in dogs and monkeys (approximately 67% and 50% in urine, respectively, in 7 days), while rats excreted only 17% by this route. Thus, the rats and dogs cleared virtually all of the i.v. dose of benzidine from the body within 7 days. The recovery of only 80% of the administered dose in the urine and faeces of the monkeys may be a consequence of technical losses of the excreta, primarily faeces (Kellner *et al.*, 1973).

The identities of the faecally-excreted materials have not been ascertained. The materials excreted in the urine are largely metabolites of benzidine involving *N*-acetylation, 3-hydroxylation and sulphate and glucuronide conjugations (Bradshaw and Clayson, 1955; Clayson *et al.*, 1959; Fabre *et al.*, 1960; Haley, 1975; Shriner *et al.*, 1978). An interesting species difference is the apparent inability of dogs to acetylate (or otherwise conjugate) the amino groups of benzidine *in vivo* (Bradshaw and Clayson, 1955; Troll and Nelson, 1958).

In-vitro metabolism and macromolecular binding

Benzidine is metabolized *in vitro* by liver cytosol from rats, mice, hamsters or guinea-pigs to *N*-acetylbenzidine and *N,N'*-diacetylbenzidine. Hepatic microsomal preparations *in vitro* convert synthetic *N,N'*-diacetylbenzidine to *N*-hydroxy-*N,N'*-diacetylbenzidine and 3-hydroxy-*N,N'*-diacetylbenzidine by a NADPH-dependent reaction. Liver cytosol catalysed the binding of synthetic *N*-hydroxy-*N*-acetyl-*N'*-[1-^{14}C]-acetylbenzidine to *t*RNA *in vitro*. In all three reactions, liver preparations from hamsters were more active than those from mice or rats; rodent liver therefore contains the enzymes necessary for metabolizing benzidine to a product capable of binding to nucleic acids (Morton *et al.*, 1979). *N*-Hydroxy-*N,N'*-diacetylbenzidine can also be esterified to an electrophilic reactant by hepatic sulphotransferases in the rat and mouse (Morton *et al.*, 1980).

A microsomal preparation from the inner renal medulla of male New Zealand rabbits metabolized ^{14}C-benzidine to products that covalently bound to exogenous *t*RNA and DNA *in vitro*. The reaction was arachidonic acid-dependent (NADPH-independent) and appeared to be mediated by prostaglandin endoperoxide synthetase. The ratio of trichloroacetic acid-precipitable:non-trichloroacetic acid-precipitable:*t*RNA-bound benzidine products was 10:3:1. The addition of glutathione to the incubation medium decreased the formation of trichloroacetic acid-precipitable and *t*RNA-bound materials, with a concomitant increase in the non-trichloroacetic acid-precipitable materials (Zenser *et al.*, 1979, 1980).

Male Wistar rats treated with 32 mg/kg bw ^{3}H-benzidine (specific radioactivity, 30 mCi/mmol) intraperitoneally incorporated radioactivity into liver RNA, DNA and protein. Incorporation was maximal 24 hours after injection, and greatest in RNA. The majority of the radioactivity associated with DNA was stable for at least four weeks. Enzymatic hydrolysis of the DNA and chromatography on Sephadex LH20 yielded five radioactive peaks, the largest of which (in contrast to total DNA binding) had disappeared from the subsequently isolated liver DNA, probably as a consequence of the transfer of radioactivity to other DNA components by an undefined mechanism. The same pattern of peaks was produced by reaction of ^{3}H-*N*-benzoyloxybenzidine with DNA *in vitro*, suggesting that a nitrenium ion is an electrophilic intermediate (Martin and Ekers, 1980).

Mutagenicity and other short-term tests

Benzidine has been found consistently to be mutagenic to *Salmonella typhimurium* strain TA1538 when tested in the presence of an exogenous metabolic activation system

from rats (see, e.g., Ames *et al.*, 1973; Anderson and Styles, 1978) or humans (see, e.g., Haworth and Lawlor, 1978).

The urine of rats fed benzidine was mutagenic to *S. typhimurium* TA1538, TA98 or TA100 when tested in the presence of a rat liver metabolic activation system or to *S. typhimurium* TA1538 in the presence of a rat liver cytosolic fraction; addition of glucuronidase increased the mutagenic activity in TA1538 (Bos *et al.*, 1980; Tanaka *et al.*, 1980).

N-Acetylbenzidine, a urinary metabolite of benzidine, was mutagenic to *S. typhimurium* strains TA98 and TA100 in the presence of a rat liver metabolic system (Tanaka *et al.*, 1980). *N*-Hydroxy-*N*,*N'*-diacetylbenzidine was mutagenic to *S. typhimurium* TA1538 in the presence of a partially purified *N*,*O*-acyltransferase preparation (Morton *et al.*, 1979).

Benzidine was negative in the *Escherichia coli* pol A test (Fluck *et al.*, 1976) and in the prophage induction test (Speck *et al.*, 1978), when tested either in the presence or absence of a rat liver metabolic activation system.

Mutagenic activity on the X-chromosome recessives (visibles and lethals) and RNA genes of *Drosophila melanogaster* has been reported (Fahmy and Fahmy, 1977).

Benzidine (6×10^{-4} M for 30 min) inhibited DNA synthesis in HeLa cells *in vitro* without activation (Painter, 1978) and *in vivo* in renal and hepatic cells when given intraperitoneally or intragastrically to suckling 14-18-day-old mice in doses of 15-30% of the LD_{50} (Amlacher and Ziebarth, 1979). Unscheduled DNA synthesis was induced by benzidine (active dose range, 10^{-7}-10^{-3} M) in HeLa cells in the presence of a phenobarbital-induced rat liver activation system (Martin *et al.*, 1978) and in rat hepatocytes (Williams, 1978; Brouns *et al.*, 1979). Benzidine, when tested in the presence of a rat liver metabolic activation system, induced DNA strand breaks in Chinese hamster V79 cells (Swenberg *et al.*, 1976). When measured by the alkaline elution assay, there was a dose-related increase in DNA strand breaks in the livers of rats exposed to benzidine *in vivo* (Petzold and Swenberg, 1978). Benzidine (2.5 μg/ml) transformed BHK21 Cl-13 cells in the presence of an Aroclor 1254-induced rat liver metabolic system (Ashby *et al.*, 1978), and was shown to transform Syrian hamster embryo cells (Pienta, 1980).

Conflicting reports exist on the ability of benzidine to induce micronucleated polychromatophilic rat erythrocytes. It was inactive at doses of up to 250 mg/kg (Trzos *et al.*, 1978), but was positive (with no dose response) when tested at comparable doses (100, 200, 300 mg/kg) (Cihak, 1979). It was also reported to be active when given at the high dose of 409.6 mg/kg dermally or subcutaneously (Urwin *et al.*, 1976).

(*b*) *Humans*

Toxic effects

Exposure to benzidine has been shown to produce a spectrum of lesions of the epithelium of the urinary bladder, which may precede appearance of malignancy. These lesions include hyperaemia, inflammation and papillomata (both sessile and pedunculated) (Muller, 1933; DiMaio, 1937; Douillet *et al.*, 1959; Vigliani and Barsotti, 1961). The presence, grossly visible or occult, of blood in the urine or the development of pain or difficulty in urinating may signal the appearance of such lesions.

The occurrence of such bladder lesions in people exposed to benzidine suggests that relatively early detection of premalignant changes may be possible through medical screening procedures. Some authors have relied on periodic cystoscopic examination of exposed workers for such screening. Another, less invasive approach relies on periodic cytological evaluation of bladder epithelial cells shed in urine (Billiard-Duchesne, 1960; Vigliani and Barsotti, 1961). The value of cytological screening has, however, not been established. Screening of the urine for occult blood may provide an effective, noninvasive means for the early detection of bladder lesions.

Absorption, distribution, excretion and metabolism

Benzidine may enter the body by percutaneous absorption, ingestion or inhalation; percutaneous absorption appears to be the primary route of absorption following occupational exposure (Meigs *et al.*, 1951, 1954; von Ehrlicher, 1958).

Following application of 100 mg benzidine to the skin, <0.02 mg benzidine and metabolites were detected in the urine. Concentrations of 0.27 to 1.60 mg/l were measured in the urine of workers in a chemical plant manufacturing benzidine and substituted benzidines (Meigs *et al.*, 1951).

3-Hydroxybenzidine conjugates constituted 80-90% of the urinary constituents in benzidine-exposed workers; another 5-10% was excreted as diacetylbenzidine, 1-5% as monoacetylbenzidine and 4-6% as parent compound (Sciarini and Meigs, 1961). It was reported in an abstract (Troll *et al.*, 1963) that an *N*-acetyl-*N*-hydroxy derivative of benzidine was found in the urine of six people given 200 mg benzidine [route unspecified].

Mutagenicity and chromosomal effects

An increase in the number of sister chromatid exchanges was reported in peripheral blood lymphocytes of 15 subjects occupationally exposed to benzidine (Bassendowska-Karska, 1980). [The effect reported was small, and the Working Group questioned the significance of this observation.]

3.3 Case reports and epidemiological studies of carcinogenicity in humans

An association between industrial exposure to benzidine and cancer of the bladder has been recognized since the early decades of the twentieth century. Several international bodies, including the International Labour Office (1921) and the IARC (1972, 1979), concluded previously that sufficient evidence exists to consider benzidine a carcinogen in man.

Oppenheimer (1927) described 40 cases of tumours of the urinary bladder among workers in the German dyestuffs industry. Six cases occurred in workers whose principal exposure was to benzidine. The mean duration of employment before development of tumour for the entire series was 17 years (range, 2-41 years). Mean induction period was 18.5 years.

Muller (1933) described 19 cases of papilloma of the bladder and 36 of carcinoma among workers in the Swiss dye industry. Eleven of the tumours occurred in workers with principal exposure to benzidine.

DiMaio (1937) performed cystoscopic examination on 86 workers in the benzidine department of two Italian dye factories. Four of the workers were found to have carcinoma of the bladder and another 7 had papillomas.

Goldblatt (1949) studied the incidence of urinary-tract tumours in two British chemical plants. From 1934-1947, 99 tumours of the upper and lower urinary tract occurred, of which 59 were fatal. Six cases occurred in workers exposed to benzidine alone. Comparison of the observed number of deaths in this plant with the number expected for the entire adult male population of England and Wales showed an excess mortality of more than twenty-fold. The mean age at diagnosis of tumour was 50.5 years; mean induction period was 18.9 years.

Barsotti and Vigliani (1949) reevaluated the two Italian plants previously studied by DiMaio (1937). In workers engaged in production and use of benzidine, they found 14 carcinomas of the bladder and 7 papillomas. Duration of employment before appearance of the tumours ranged from 5 to 26 years.

Scott (1952) described 30 cases of bladder tumours (8 fatal) among 284 workers exposed to benzidine in a British dyestuffs manufacturing plant. Mean duration of exposure was 15.9 years (range, 8-32 years); the distribution of induction periods was the same. For workers who began employment before age 30, mean age at death was 44 years; for those beginning employment at 30 to 40 years, mean age at death was 54 years; and for those entering employment after age 40, mean age at death was 66 years. For all adult males in England and Wales, the mean age at death for cancer of the urinary bladder at the time of this study was 67.5 years.

Aboulker and Smagghe (1953) described 21 cases of bladder tumour among workers in a French dyestuffs plant. Two of these cases occurred in workers exposed to benzidine alone, for a mean duration of exposure of 17.5 years.

Uebelin and Pletscher (1954) described 100 cases of urinary-tract tumours among workers in a Swiss dyestuffs factory. Twenty had exposure to benzidine alone, with an average duration of exposure of 11.6 years (range, 1-29 years); mean induction period for workers exposed to benzidine was 14.8 years (range, 5-29 years).

Douillet *et al.* (1959) described 13 cases of urinary-tract tumours among workers in a French plant where benzidine was manufactured. Duration of exposure to benzidine ranged from 2-21 years; the induction periods ranged from 10-26 years (mean, 16 years).

Billiard-Duchesne (1960) reported 12 cases of bladder tumours among workers in a French factory where benzidine was the only aromatic amine manufactured.

Vigliani and Barsotti (1961) reevaluated the workforce in the two Italian factories evaluated by DiMaio (1937) and by Barsotti and Vigliani (1949) and those of four further factories. They found 17 new cases of carcinoma and 11 of papillomas of the bladder in workers exposed to benzidine alone.

Maltoni and Ghetti (1964) reported the occurrence of four cases of upper urinary-tract tumours in workers engaged in the production of benzidine in an Italian dye factory.

Goldwater *et al.* (1965) examined the occurrence of bladder tumours among workers employed from 1912-1962 in a British company where coal-tar dyes were made. Among 76 workers exposed to benzidine alone, 17 developed bladder malignancies (total

incidence, 21.3%). Mean age at diagnosis of tumour was 49.7 years, and the mean induction period was 18.7 years (range, 5-33 years).

Ferber *et al.* (1976) reviewed the incidence of bladder tumours between 1930 and 1975 in workers at a dye plant in the USA. Thirty-six cases of bladder tumour occurred in workers exposed to benzidine alone.

Primary tumours at sites other than the bladder have been noted in several of the series of workers exposed to benzidine (Uebelin and Pletscher, 1954; Mancuso and El-Attar, 1966; Reinl, 1967). Three historical reviews (Hueper, 1942, 1969; Haley, 1975) have reported that the worldwide spread of cancer of the bladder in people exposed to benzidine has followed the international spread of the dyestuffs industry.

Several epidemiological studies of bladder cancer incidence and mortality in people exposed to benzidine have been conducted. An analysis of mortality in a cohort of British chemical workers exposed to benzidine showed 10 deaths certified as being due to bladder tumour; only 0.72 such deaths would have been expected on the basis of rates for the whole male population of England and Wales (standardized mortality ratio, SMR = 1390; $p < 0.001$). An additional 24 nonfatal cases of bladder tumours were noted to have occurred in members of this cohort exposed to benzidine (Case *et al.*, 1954). In a cohort study of a factory population in the US (Ohio) exposed to benzidine, 16 cases of bladder cancer were observed, and the cumulative incidence rate of bladder cancer in workers exposed to benzidine was reported to be 237 per 100 000. The authors calculated that mortality from cancer of the bladder in the exposed workers was 30 times higher than that expected on the basis of Ohio male mortality rates (Mancuso and El-Attar, 1966, 1967).

Zavon *et al.* (1973) conducted an analysis of bladder cancer incidence in 25 workers in the US engaged in benzidine manufacture. Thirteen of them were found to have developed tumours of the bladder (total incidence, 52%), including all 5 workers with more than I5 years' exposure. Mean duration of exposure in workers who developed a tumour was 13.6 years; mean time from onset of exposure to first appearance of tumour was 16.6 years; mean age at first diagnosis of tumour was 45.6 years. Concentrations of airborne benzidine in the plant ranged from less than 0.005 to 17.6 mg/m^3. Three of the original cohort had had about one year of exposure to 2-naphthylamine, and three had had exposure to *ortho*-toluidine. [The Working Group considered that the high incidence of bladder tumour in this cohort was striking evidence of the carcinogenic potency of benzidine.]

An epidemiological analysis of Japanese dyestuffs workers engaged in the production or use of benzidine (Tsuchiya *et al.*, 1975) showed that 72 had developed bladder tumours, 21 of which were fatal. Of these cases, 61 (17.6%) occurred in 346 production workers, and 11 (1.6%) in 669 workers who used benzidine. Mean time from first occupational exposure to benzidine to appearance of tumour was 16.2 years. Mean age at diagnosis of bladder cancer in workers exposed to benzidine was 43.2 years.

The incidence of bladder cancer in workers decreased after a reduction in industrial exposure (Ferber *et al.*, 1976). [The evidence on which this statement is based is incomplete in that no information is provided on the number of workers first exposed after a reduction in the benzidine in the plant.]

4. Summary of Data Reported and Evaluation

4.1 Experimental data

Benzidine and its dihydrochloride were tested in mice, rats and hamsters by oral administration, in mice and rats by subcutaneous administration and in rats by inhalation and intraperitoneally. Following its oral administration to mice of different strains, both sexes, newborn and adult, and following its subcutaneous administration, it significantly increased the incidence of liver-cell tumours (benign and malignant). In female rats, it markedly increased the incidence of mammary tumours; and in male and female hamsters, it increased the incidence of liver tumours following its oral administration. The subcutaneous administration of benzidine or its sulphate to rats produced a high incidence of Zymbal-gland tumours; colonic tumours were also reported. The results of the inhalation study in rats could not be interpreted. The intraperitoneal administration of benzidine to rats resulted in a marked increase in the incidence of mammary and Zymbal-gland tumours. It was also tested in dogs by oral administration, producing bladder carcinomas. Studies in fish, rabbits and frogs could not be evaluated.

The metabolites of benzidine, *N,N'*-diacetylbenzidine and *N*-hydroxy-*N,N'*-diacetylbenzidine, produced mammary and Zymbal-gland tumours in rats following their intraperitoneal injection.

Benzidine and urine from rats fed benzidine are mutagenic to *Salmonella typhimurium* with metabolic activation. Benzidine is mutagenic to *Drosophila melanogaster*. It inhibits DNA synthesis in HeLa cells and in renal and hepatic cells in mice *in vivo*. It induces unscheduled DNA synthesis in HeLa cells and in rat hepatocytes. Benzidine transformed Syrian hamster embryo cells and was positive in the BHK21 clone-13 cell system.

The data were inadequate to assess the teratogenicity of this compound to experimental animals.

4.2 Human data

Occupational exposure to benzidine or its dihydrochloride has and probably still does occur during their manufacture and conversion to derived dyes and during the use of those dyes. When benzidine is used for blood testing or to enhance fingerprints, laboratory or field workers may be exposed. Environmental exposure can occur under certain conditions, when benzidine-based dyes are converted to benzidine in streams into which dye-containing wastes have been discharged.

No data were available to assess the mutagenicity or teratogenicity of benzidine to man.

Occupational exposure to benzidine has been strongly associated with bladder cancer in numerous case reports from many countries. The association has also been observed in several epidemiological studies. In one extreme instance, all five of a group of workers continuously employed in benzidine manufacture for 15 years or more developed bladder cancer. [See also Annex: Some Aspects of Quantitative Cancer Risk Estimation.]

4.3 Evaluation[1]

There is *sufficient evidence* that benzidine is carcinogenic to mice, rats, hamsters and dogs.

There is *sufficient evidence* that benzidine is carcinogenic to man.

[1] This evaluation should be read in conjunction with section 7(*b*)(*c*) of the preamble.

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10. BENZIDINE (Group 1)

Benzidine is carcinogenic in experimental animals after oral and subcutaneous administration, producing liver tumours in rats and hamsters, and bladder cancers in dogs[1].

Case reports and follow-up studies of workers provide sufficient evidence that occupational exposure to benzidine is causally associated with an increased risk of bladder cancer[1]. The causal association is strengthened by data which suggest that the incidence of this cancer in workers decreased after a reduction in industrial exposure[2].

11. BERYLLIUM AND CERTAIN BERYLLIUM COMPOUNDS (Group 2B)

Inhalation of beryllium sulphate, beryl ore, and bertrandite produce lung tumours in rats. Beryllium oxide and beryllium sulphate produce lung tumours in monkeys after intrabronchial implantation or inhalation. Zinc beryllium silicate, beryllium metal, and beryllium phosphate all produce bone tumours in rabbits following intravenous injection[3].

Five early epidemiological studies were considered inadequate to evaluate the carcinogenic effects of beryllium. Three recent epidemiological studies[4-6] concerned men occupationally exposed to beryllium, some of whom developed acute beryllium disease. The populations for these studies come from two beryllium refining and smelting plants, and both show a 1.5 to 2-fold increase in lung cancer mortality. The statistically significant excess of lung cancer mortality was limited to men employed for less than 1 year, and only became apparent after a follow-up of 15 years or more. There was no increase in risk with increased duration of employment. None of the studies adequately consider

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C. Evidence for activity in short-term tests (*limited*)

Benzene was not mutagenic in bacteria, yeast, *Drosophila melanogaster*, mouse lymphoma cells in culture or mammalian cells *in vivo*[2]. It induced chromosomal anomalies in mammalian cells *in vitro*[2] and in mice and rats but not in Chinese hamsters[3] *in vivo*. It did not induce dominant lethal mutations in mice[2]. Benzene induced chromosomal anomalies in occupationally exposed people[2].

	DNA damage	Mutation	Chromosomal anomalies	Other
Prokaryotes		-		
Fungi/Green plants		-		
Insects		-		
Mammalian cells *(in vitro)*		-	+	
Mammals *(in vivo)*		-	+	DL(-)
Humans *(in vivo)*			+	

DL = dominant lethal mutations

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BENZIDINE (Group 1)*

A. Evidence for carcinogenicity to humans (*sufficient*)

Case reports and follow-up studies of workers provide sufficient evidence that occupational exposure to benzidine is causally associated with an increased risk of bladder cancer. The causal association is strengthened by data which suggest that the incidence of this cancer in workers decreased after a reduction in industrial exposure[1].

* Categorized as Group 1 by the earlier Working Group, and data on humans and on animals not reevaluated by the present Group.

B. Evidence for carcinogenicity to animals (*sufficient*)

Benzidine is carcinogenic to experimental animals after its oral and subcutaneous administration, producing liver tumours in rats and hamsters, and bladder cancer in dogs[1].

C. Evidence for activity in short-term tests (*sufficient*)

Benzidine produces indirect evidence of DNA repair in bacteria; there are conflicting reports of its ability to induce DNA repair in mammalian cells *in vitro*[2]. It was mutagenic to bacteria in the presence of an exogenous metabolic activation system[2,3]. There are conflicting reports of its genetic activity: it probably induced mutation, gene conversion and aneuploidy in yeasts[2,3]; one study in *Drosophila melanogaster* was positive for mutations[2] and three others negative[3]. Benzidine induced mutation, sister chromatid exchanges and chromosomal aberrations in mammalian cells treated *in vitro*, in the presence of an exogenous metabolic activation system[3]. There are conflicting reports on its ability to induce chromosomal anomalies in mice treated *in vivo*[3], and it gave inconsistent results in sperm abnormality assays in mice treated *in vivo*[3]. It caused cell transformation (in BHK21 cells)[3]. No data on humans were available.

	DNA damage	Mutation	Chromosomal anomalies	Other
Prokaryotes	+	+		
Fungi/Green plants		+	+	
Insects		?		
Mammalian cells *(in vitro)*	?	+	+	T(+)
Mammals *(in vivo)*			?	SA(?)
Humans *(in vivo)*				

T = cell transformation ; SA = sperm abnormalities

References

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[2] *IARC Monographs, 29*, 151-183, 1982

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BENZIDINE-BASED DYES:

DIRECT BLACK 38 (TECHNICAL-GRADE) (Group 2B)
DIRECT BLUE 6 (TECHNICAL-GRADE) (Group 2B)
DIRECT BROWN 95 (TECHNICAL-GRADE) (Group 2B)

A. Evidence for carcinogenicity to humans (*inadequate* for Direct Black 38, Direct Blue 6 and Direct Brown 95)

The epidemiological data were inadequate to evaluate the carcinogenicity to man of the three benzidine-based dyes, Direct Black 38, Direct Blue 6 and Direct Brown 95. However, a study of silk dyers and painters who had had multiple exposure to benzidine-based and other dyes indicated that those exposures were strongly associated with the occurrence of bladder cancer. Benzidine has been detected in the urine of workers exposed to direct azo dyes[1].

B. Evidence for carcinogenicity to animals (*sufficient* for Direct Black 38 and Direct Blue 6, *limited* for Direct Brown 95)

Commercial *Direct Black 38* is carcinogenic to experimental animals after oral exposure: administration to mice in drinking-water produced liver and mammary tumours; administration to rats in the diet produced hepatocellular carcinomas within 13 weeks. In another study in rats, in which the dye was administered in drinking-water, small numbers of carcinomas were found in the urinary bladder, liver and colon. Commercial material may contain small quantities of two other animal carcinogens, 4-aminobiphenyl and 2,4-diaminobenzene[1].

In a single study, *Direct Blue 6* produced hepatocellular carcinomas in rats within 13 weeks after its oral administration. The commercial product contains small amounts of benzidine[1].

Direct Brown 95 produced neoplastic nodules in the liver and one hepatocellular carcinoma in 10 female rats after its oral administration, in a single study terminated after 13 weeks. The finding of preneoplastic lesions after such a short exposure period indicates a carcinogenic effect similar to that of Direct Black 38 and Direct Blue6[1]. In rats, mice and monkeys, oral administration of Direct Brown 95 is followed by excretion of benzidine in the urine.

C. Evidence for activity in short-term tests (*inadequate* for Direct Black 38, *no data* for Direct Blue 6 and Direct Brown 95)

Direct Black 38 was mutagenic to *Salmonella typhimurium* in the presence of an exogenous metabolic activation system[1]. No data on the mutagenicity of Direct Blue 6 or Direct Brown 95 were available to the Working Group. No data on humans were available.

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Benzidine and Benzidine-Based Dyes
pp. 123-126

BENZIDINE (Group 1)

A. Evidence for carcinogenicity to humans (*sufficient*)

Case reports and follow-up studies of workers in many countries have demonstrated that occupational exposure to benzidine is causally associated with an increased risk of bladder cancer. In one extreme instance, all five of a group of workers continuously employed in the manufacture of benzidine for 15 years or more developed bladder cancer[1]. Earlier data suggesting that the incidence of this cancer in workers decreased after a reduction in industrial exposure[1] have been supported by a study of a cohort of workers at a US benzidine-manufacturing facility, in which major preventive measures were instituted in 1950 to minimize worker exposure. The study period covered 1945-1979, and, overall, there was a clearly significant excess of bladder cancer incidence, which, however, declined in those first employed after 1950[2]. Although a longer follow-up is required to evaluate fully the effect of preventive measures on cancer risks, the causal association is strengthened by these two independent observations. Few other epidemiological studies have examined the cancer risk associated with exposure to benzidine alone. In a study at a dyestuffs factory in Italy, it was possible to distinguish a very high bladder cancer risk (5 deaths observed, 0.06 expected) associated with benzidine production[3]. The study was extended and updated, but the role of exposure to benzidine alone in the dramatically increased bladder cancer risk could not be examined further[4]. Of 25 benzidine 'operators' at a plant in the USA, 13 developed bladder cancer; all cases had been exposed for six years or more[5]. A surveillance programme of 179 active and 65 retired workers in a dyestuffs manufacturing plant in Japan revealed nine cases of bladder cancer that occurred between 1968 and 1981; all of the cases had been engaged in benzidine production[6].

Other investigations have shown high incidences of cancer of the bladder and urinary tract after concomitant exposure to benzidine and 2-naphthylamine (see p. 261) [7,8]. Exposure to these two compounds was also associated with an increase in the occurrence of second primary cancers at sites other than the bladder, including the liver[9].

Among 1601 workers in the chemical-dye industry in China who were exposed to benzidine, methylnaphthylamine and dianisidine (see p. 198), 21 cases of bladder carcinoma were found. All had a history of exposure to benzidine, while no carcinoma was found among workers exposed to methylnaphthylamine or dianisidine. Suggestions of a dose-response relationship were provided by analysis according to length of exposure[10].

Bladder cancer was also found to be increased in ecological studies of areas where benzidine (as well as 2-naphthylamine and other compounds) was used, manufactured or stored[11,12].

B. Evidence for carcinogenicity to animals (*sufficient*)

Benzidine and/or its salts were tested for carcinogenicity by oral administration in mice, rats, hamsters and dogs and by subcutaneous and intraperitoneal injection and inhalation in rats. Following oral administration of benzidine and its hydrochloride, significant increases in the incidences of benign and malignant liver neoplasms were observed in mice and

hamsters[1,13-17] and of mammary cancer in rats; benzidine induced bladder carcinomas in dogs. Following subcutaneous administration of benzidine and its sulphate to rats, a high incidence of Zymbal-gland tumours was observed. After intraperitoneal administration of benzidine to rats, a marked increase in the incidence of mammary-gland and Zymbal-gland neoplasms was observed. The results of one study in rats by inhalation could not be evaluated[1].

Two metabolites of benzidine, *N,N'*-diacetylbenzidine and *N*-hydroxy-*N,N'*-diacetylbenzidine, produced mammary-gland and Zymbal-gland tumours in rats following their intraperitoneal injection[1].

C. Other relevant data

No data were available on the genetic and related effects of benzidine in humans.

Covalent binding products of benzidine with DNA have been described in the liver of mice and rats treated *in vivo*. Benzidine induced micronuclei, sister chromatid exchanges, DNA strand breaks and unscheduled DNA synthesis in cells of rodents treated *in vivo*. It induced unscheduled DNA synthesis in humans cells *in vitro*. It caused transformation of Syrian hamster embryo and BALB/c 3T3 cells and induced chromosomal aberrations, sister chromatid exchanges, unscheduled DNA synthesis and DNA strand breaks in rodent cells *in vitro*; conflicting results were obtained for mutation. Benzidine induced aneuploidy, gene conversion and DNA damage in yeast, but not mutation. It was mutagenic to plants and bacteria[18].

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BENZIDINE-BASED DYES (Group 2A)

A. Evidence for carcinogenicity to humans (*inadequate*)

The epidemiological data were inadequate to evaluate the carcinogenicity of three benzidine-based dyes, Direct Black 38, Direct Blue 6 and Direct Brown 95, to humans. However, a study of silk dyers and painters who had had multiple exposure to benzidine-based and other dyes indicated that those exposures were strongly associated with the occurrence of bladder cancer[1].

B. Evidence for carcinogenicity to animals (*sufficient* for technical-grade Direct Black 38, technical-grade Direct Blue 6 and technical-grade Direct Brown 95)

Direct Black 38 was tested for carcinogenicity in mice by administration in drinking-water, producing liver and mammary tumours. Commercial Direct Black 38 produced hepatocellular carcinomas within 13 weeks after administration in the diet to rats and small numbers of carcinomas in the urinary bladder, liver and colon after administration to rats in drinking-water[1].

In a single study, commercial Direct Blue 6 produced hepatocellular carcinomas in rats within 13 weeks after its oral administration.

Commercial Direct Brown 95 produced neoplastic nodules in the livers of 4/8 female rats, and a hepatocellular carcinoma in one, after its oral administration in a single study

terminated after 13 weeks. The finding of preneoplastic lesions after such a short exposure prior indicates a carcinogenic effect similar to that of Direct Black 38 and Direct Blue 6[1].

C. Other relevant data

Benzidine-based dyes are structurally related to benzidine, exposure to which is causally associated with cancer in humans (see p. 123), and commercial material may contain small amounts of benzidine. Commercial Direct Black 38 may contain small quantities of 4-aminobiphenyl (see p. 91) and 2,4-diaminobenzene (the hydrochloride of which is chrysoidine [see p. 169])[1].

Benzidine has been detected in the urine of workers exposed to benzidine-based azo dyes. No data were available on the genetic and related effects of Direct Black 38, Direct Blue 6 or Direct Brown 95, in humans[1].

In experimental animals, Direct Black 38, Direct Blue 6 and Direct Brown 95 undergo reduction of the azo bonds with the appearance in the urine of benzidine and monoacetylbenzidine. The reductive cleavage of the azo bond has been attributed to the activities of intestinal microflora and/or liver azoreductases[2]

Direct Black 38 was mutagenic to bacteria. Urine from rodents treated with Direct Black 38 was mutagenic to bacteria in the presence of an exogenous metabolic system, and human intestinal microflora metabolized Direct Black 38 to highly mutagenic metabolites[2].

DNA adducts (including covalent binding products of benzidine) have been described in the livers of rats treated with Direct Blue 6 *in vivo*. Direct Blue 6 is mutagenic to bacteria only in the presence of an exogenous metabolic system and the cofactor flavine mononucleotide[2].

Direct Brown 95 induced unscheduled DNA synthesis in rat hepatocytes in an in-vivo/in-vitro assay but not in hepatocytes *in vitro*. It was mutagenic to bacteria in the presence of an exogenous metabolic system; this activity was enhanced by the cofactor flavine mononucleotide. The urine from rats treated with Direct Brown 95 was mutagenic to bacteria in the presence of an exogenous metabolic system[2].

References

[1] *IARC Monographs, 29*, 295-310, 311-320, 321-330, 1982

[2] *IARC Monographs, Suppl. 6*, 275-281, 1987

BENZOYL CHLORIDE (Group 3)

A. Evidence for carcinogenicity to humans (*inadequate*)

Six cases of respiratory cancer were reported among workers in two small factories where benzoyl chloride and its chlorinated precursors were produced[1].

APPENDIX B

Excerpts from the IARC Monograph on the
Evaluation of the Carcinogenic Risk of Chemicals to Humans
Volume 29 (Some Industrial Chemicals and Dyestuffs) 1982
Direct Black 28, pp. 295-310
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DIRECT BLACK 38

The commercial material is a reaction product and is not to be regarded as a single substance; therefore, the evaluation of this compound was based on experience with mixtures of substances that varied in composition.

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 1937-37-7

Chem. Abstr. Name: 2,7-Naphthalenedisulfonic acid, 4-amino-3-{[4'-((2,4-diaminophenyl)azo)(1,1'-biphenyl)-4-yl]azo}-5-hydroxy-6-(phenylazo)-, disodium salt

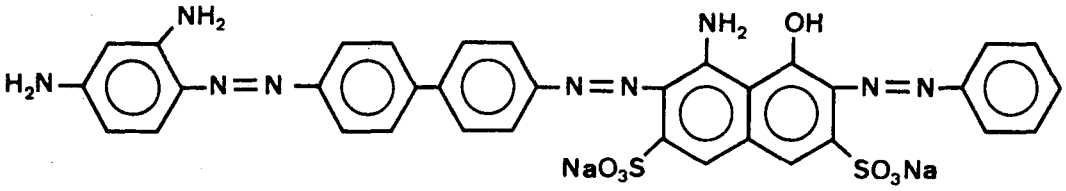
IUPAC Systematic Name: Disodium 4-amino-3-{[4'-((2,4-diaminophenyl)azo)-4-biphenyl]azo}-5-hydroxy-6-(phenylazo)-2,7-, naphthalene disulfonate

Synonyms: C.I. 30235; C.I. Direct Black 38, disodium salt

Trade names: Ahco Direct Black GX; Airedale Black ED; Aizen Direct Deep Black EH; Aizen Direct Deep Black GH; Aizen Direct Deep Black RH; Amanil Black GL; Amanil Black WD; Apomine Black GX; Atlantic Black BD; Atlantic Black C; Atlantic Black E; Atlantic Black EA; Atlantic Black GAC; Atlantic Black GG; Atlantic Black GXCW; Atlantic Black GXOO; Atlantic Black SD; Atul Direct Black E; Azine Deep Black EW; Azocard Black EW; Azomine Black EWO; Belamine Black GX; Bencidal Black E; Benzanil Black E; Benzo Deep Black E; Benzoform Black BCN-CF; Benzo Leather Black E; Black 2EMBL; Black 4EMBL; Brasilamina Black GN; Brilliant Chrome Leather Black H; C.I. 30235; C.I. Direct Black 38; Calcomine Black; Calcomine Black EXL; Carbide Black E; Chloramine Black C; Chloramine Black EC; Chloramine Black ERT; Chloramine Black EX; Chloramine Black EXR; Chloramine Black XO; Chloramine Carbon Black S; Chloramine Carbon Black SJ; Chloramine Carbon Black SN; Chlorazol Black E; Chlorazol Black EA; Chlorazol Black E (Biological Stain); Chlorazol Black EN; Chlorazol Burl Black E; Chlorazol Leather Black ENP; Chlorazol Silk Black G; Chrome Leather Black E; Chrome Leather Black EC; Chrome Leather Black EM; Chrome Leather Black G; Chrome Leather Brilliant Black ER; Coir Deep Black C; Columbia Black EP; Diacotton Deep Black; Diacotton Deep Black RX; Diamine Deep Black EC; Diamine Direct Black E; Diaphtamine Black V; Diazine Black E; Diazine Direct Black E; Diazine Direct Black G; Diazol Black 2V; Diphenyl Deep

Black G; Direct Black 3; Direct Black A; Direct Black BRN; Direct Black CX; Direct Black CXR; Direct Black E; Direct Black EW; Direct Black EX; Direct Black FR; Direct Black GAC; Direct Black GW; Direct Black GX; Direct Black GXR; Direct Black JET; Direct Black Meta; Direct Black Methyl; Direct Black N; Direct Black RX; Direct Black SD; Direct Black WS; Direct Black Z; Direct Deep Black E; Direct Deep Black EAC; Direct Deep Black EA-CF; Direct Deep Black E Extra; Direct Deep Black EW; Direct Deep Black EX; Enianil Black CN; Erie Black B; Erie Black BF; Erie Black GAC; Erie Black GXOO; Erie Black JET; Erie Black NUG; Erie Black RXOO; Erie Brilliant Black S; Erie Direct Black G Extra; Erie Fibre Black VP; Fenamin Black E; Fibre Black VF; Fixanol Black E; Formaline Black C; Formic Black C; Formic Black CW; Formic Black EA; Formic Black MTG; Formic Black TG; Hispamin Black EF; Interchem Direct Black Z; Kayaku Direct Deep Black EX; Kayaku Direct Deep Black GX; Kayaku Direct Deep Black S; Kayaku Direct Leather Black EX; Kayaku Direct Special Black AAX; Lurazol Black BA; Meta Black; Mitsui Direct Black EX; Mitsui Direct Black GX; Nippon Deep Black; Nippon Deep Black GX; Paper Black BA; Paper Black T; Paper Deep Black C; Paramine Black B; Paramine Black E; Peeramine Black E; Peeramine Black GXOO; Phenamine Black BCN-CF; Phenamine Black CL; Phenamine Black E; Phenamine Black E 200; Pheno Black EP; Pheno Black SGN; Pontamine Black E; Pontamine Black EBN; Sandopel Black EX; Seristan Black B; Telon Fast Black E; Tetrazo Deep Black G; Tetrodirect Black E; Tetrodirect Black EFD; Union Black EM; Vondacel Black N

1.2 Structural and molecular formulae and molecular weight of the principal ingredient



$C_{34}H_{25}N_9Na_2O_7S_2$ Mol. wt: 783.7

1.3 Chemical and physical properties

(*a*) *Description*: Grey-black powder (Richter, 1951)

(*b*) *Solubility*: Soluble in water; moderately soluble in ethanol and ethylene glycol monoethyl ether; insoluble in other organic solvents (The Society of Dyers and Colourists, 1971a)

(*c*) *Conversion factor*: ppm = 0.0312 x mg/m^3

1.4 Technical products and impurities

The benzidine content of six US-produced samples of Direct Black 38 ranged from 2-20 mg/kg and that of seven samples imported from Egypt, India, The Netherlands and Poland ranged from 2-1254 mg/kg (Boeniger, 1980). In another study, the benzidine content of a Direct Black 38 sample was found to be <0.1 mg/kg, but 150 mg/kg 4-aminobiphenyl [see IARC, 1972] and 9200 mg/kg 2,4-diaminoazobenzene (the hydrochloride of which is chrysoidine [see IARC, 1975]) were present (Nony and Bowman, 1980).

The manufacture and testing of Direct Black 38 do not conform to rigid chemical specifications, and its composition varies in order to meet the shade and intensity requirements of each customer.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(a) Production

Direct Black 38 was first synthesized in 1898 (The Society of Dyers and Colourists, 1971a). It is prepared commercially by: (1) coupling diazotized benzidine with 1 mol H-acid (8-amino-1-naphthol-3,6-disulphonic acid) under acid conditions, (2) reacting the resulting product with 1 mol diazotized aniline under alkaline conditions, (3) coupling this intermediate with *meta*-phenylenediamine, and (4) neutralization with sodium hydroxide.

Direct Black 38 was first produced in commercial quantities in the US in 1914 (US Tariff Commission, 1922). In 1976, US production of Direct Black 38 by five companies amounted to 1.71 million kg (US International Trade Commission, 1977), down sharply from the 3.1 million kg produced in 1973 (US International Trade Commission, 1975). It is presently produced commercially by only one US company, whose production in 1978 amounted to 374 thousand kg (National Institute for Occupational Safety and Health, 1980). It was the benzidine-based dye made in the largest quantity in the US in that year.

US imports of Direct Black 38 through the principal customs districts in 1980 amounted to nearly 95 thousand kg (US International Trade Commission, 1981), down from the 149 thousand kg imported in 1979 (US International Trade Commission, 1980).

This compound is believed to be produced commercially by one company in France. It may be imported into other western European countries from India or from eastern Europe.

In 1980, Japan imported an estimated 250 thousand kg of Direct Black 28, principally from South Korea but also from Taiwan.

Benzidine-based dyes are also believed to be produced commercially in Argentina, Brazil, India, Mexico, the People's Republic of China, Poland, Romania and the USSR, but whether Direct Black 38 is one of the dyes produced is not known.

(b) Use

Direct Black 38 can be used to: (1) dye cellulose, wool, silk, bast and hog's hair; (2) print cellulose, wool and silk; (3) dye leather, plastics, vegetable-ivory buttons and wood flour used as a resin filler; (4) stain wool, silk, acetate, nylon, wood and biological materials; and (5) produce aqueous inks (The Society of Dyers and Colourists, 1971b). It has reportedly been used in hair dyes (National Cancer Institute, 1978) [see IARC, 1982].

Two US companies developed a non-benzidine-dye substitute for Direct Black 38 for use on cellulosic fibres (Auerbach Associates, Inc., 1978); however, in 1980 (Boeniger, 1980), Direct Black 38 was being used commercially to dye textiles, leather and paper.

In Japan, 60% of the Direct Black used is for dyeing fibres, 20% for dyeing paper and 20% for dyeing leather.

The US Occupational Safety and Health Administration has issued guidelines that its field personnel 'use the general duty clause of existing occupational safety laws to control worker exposure' (Anon., 1980a) and has issued a health hazard alert to employers (Anon., 1980b), although no specific regulations limiting occupational exposure to Direct Black 38 have been established.

2.2 Occurrence

(a) Natural occurrence

Direct Black 38 has not been reported to occur as such in Nature.

(b) Occupational exposure

Direct Black 38 has been detected in the workplace air of a paper-dyeing facility, at total airborne particulate levels of 1.6-5.1 mg/m^3 [0.05-0.16 ppm], and of a textile-dyeing operation, at unspecified levels (Boeniger, 1980).

The National Institute for Occupational Safety and Health (1980) estimated that approximately 13 000 US workers are exposed to Direct Black 38.

Direct Black 38 has been reported to be among the dyes used for painting kimonos in Japan, and occupational exposure to such dyes has resulted from the painters' practice of moistening their brushes with their tongues (Yoshida and Miyakawa, 1972; US Environmental Protection Agency, 1979).

(c) Other

The Working Group had no information on the extent of exposure, if any, of the general population to Direct Black 38 as a result of its presence in consumer products.

2.3 Analysis

Direct dyes such as Direct Black 38 are reportedly distinguished from basic and disperse dyes by their pH-dependent chromatographic behaviour on cellulose acetate (Schlegelmilch and Khodadadian, 1973). Typical methods for the analysis of Direct Black 38 in various matrices are summarized in Table 1.

Table 1. Methods for the analysis of Direct Black 38

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Air	Collect sample with glass-fibre filter (method detects all dye particles)	Gravimetric	not given	Boeniger *et al.* (1980)
	Extract filter with appropriate solvent; scan from 400-700 nm and compare with scans of bulk dye sample solutions for quasi-specific identification	S	not given	
Bulk or mixtures	Elute solution of dye in distilled water on Silica Gel G (4:1 phenol:water)	TLC	not given	Mashruwala and Mehta (1979)
	Analyse distilled-water dye solutions or extracts from dyed yarn hanks (in colourless dimethylformamide) to detect most direct dyes (probably not dye-specific)	S/R	not given	

a Abbreviations: S, spectrometry; TLC, thin-layer chromatography; S/R, spectrometry/reflectance

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(*a*) *Oral administration*

Mouse: A group of 60 ICR mice [sex unspecified], 14 weeks old, received 3000 mg/l Direct Black 38 in their drinking-water for 55-60 weeks, at which time the 59 surviving animals were killed. Hepatocellular carcinomas were found in 46/59 mice, and mammary carcinomas in 20/59; 9 animals developed both types of tumours. A further 40 mice were given the same concentration of Direct Black 38 in drinking-water, and 2 mice were killed every two weeks starting from the 16th week of treatment. The first liver tumour occurred in a mouse killed 20 weeks after the start of treatment. No liver or mammary tumour was reported to occur in a group of 20 untreated controls (Asada *et al.*, 1981).

Rat: Groups of 10 male and 10 female Fischer 344 rats, 6 weeks old, were fed a diet containing 0, 190, 375, 750, 1500 or 3000 mg/kg [ppm] Direct Black 38 and 1.3% corn oil. (The compound was determined by high-performance liquid chromatography to be 87.1 ± 3.4% pure, with the following impurities: water, 7.13 ± 0.54%; NaCl, 7.9%; benzidine, <0.004%; and traces of at least eight other impurities. The infra-red spectrum was as expected.) Surviving rats were killed at 13 weeks. All male and female animals administered 3000 mg/kg Direct Black 38 died prior to termination of the experiment: male rats survived for less than 5 weeks and female rats less than 12 weeks. Of the 9 surviving males that received 1500 mg/kg, 4 had hepatocellular carcinomas and 5, neoplastic nodules. No male receiving another dose exhibited a tumour, although 7 of 10 male animals given 375 mg/kg, 9 of 10 males given 750 mg/kg, and 5 of 9 males given 1500 mg/kg had foci of cellular alteration or basophilic foci in the liver. Of the female animals, 5 of 10 given 1500 mg/kg exhibited neoplastic nodules in the liver at the termination of the experiment; and all females administered 750 or 1500 mg/kg had foci of cellular alteration in the liver. In the same bioassay, no increased incidence of tumours, compared with that in controls, was found in groups of 10 male and 10 female $B6C3F_1$ mice fed diets containing 750, 1500, 3000, 6000 or 12 500 mg/kg [ppm] of Direct Black 38 and killed 13 weeks later (National Cancer Institute, 1978; Robens *et al.*, 1980). [The Working Group noted the short duration of the experiment, the limited number of animals tested, and the impurity of the compound used. Other samples of the compound may have different impurities.]

A group of 12 Wistar rats were administered 100 mg/l commercial Direct Black 38 (Direct Deep Black EX; benzidine-free, as shown by high-performance liquid chromatography) in their drinking-water. When the 8 rats still alive at 60 weeks were killed, no tumour was observed. Of 15 rats administered 500 mg/l Direct Black 38, 13 survived until 60 weeks; and 2 papillomas and 3 carcinomas of the urinary bladder, 3 carcinomas of the liver and 2 adenocarcinomas of the colon were observed in 6 animals. In addition, 9 survivors developed hyperplasia of the bladder mucosa, and 8, hyperplasia of the liver. No tumour was observed in a control group of 9 rats (Okajima *et al.*, 1975).

A group of 20 male and 25 female rats were given 400 mg/l Direct Black 38 [source and characteristics unspecified] in their drinking-water (0.04%) for 14 months, at which time 4 males and 2 females were still alive. One of the females had 'breast cancer' [pathological designation not specified]; no other neoplasm was noted (Niitsu, 1973). [The Working Group noted the poor survival of the animals and the short duration of the experiment; in addition, the number of control animals was not specified.]

(*b*) *Other experimental systems*

Bladder implantation: Two groups of 50 female dd *mice* (20 g) received either a paraffin wax pellet (20 mg) containing 10% Direct Black 38 or a wax pellet alone implanted in the bladder. After 40 weeks, when the surviving animals were killed, one bladder carcinoma was observed among the 21 mice still alive. In the control group, one bladder carcinoma was observed in 36 surviving mice (Niitsu, 1973). [The Working Group noted the short duration of the experiment.]

3.2 Other relevant biological data

(*a*) *Experimental systems*

Toxic effects

Studies in which rats were fed diets containing 190-3000 mg/kg [ppm] Direct Black 38 and mice 750-12 500 mg/kg for 13 weeks resulted in a series of dose- and substance-

related changes, seen when all animals were killed at the end of treatment. In rats, portal fibrosis and multifocal necrosis of the liver were observed; lymphoid depletion in spleen, and thymus and myeloid depletion of the bone marrow were also seen. Other effects included haemosiderosis of the spleen, and interstitial haemorrhage and seminiferous tubular degeneration in the testis. Biliary hyperplasia was seen with doses of 750 mg/kg and above. In mice, diffuse hepatocellular degeneration, biliary hyperplasia and pigment deposition in the liver, haemosiderosis of the spleen and kidney, and pigment deposition in the thyroid were observed (National Cancer Institute, 1978).

Effects on reproduction and prenatal toxicity

Wistar rats were injected subcutaneously on days 7, 8 and 9 of pregnancy with 10 mg (about 40 mg/kg bw per day) Direct Black 38 (Chlorazol black E, biological stain quality). Three of the 16 dams died and 4 resorbed completely, but no malformations were observed in the 70 fetuses that survived to term (Wilson, 1955).

Absorption, distribution, excretion and metabolism

Benzidine and monoacetylbenzidine were found in the urine of rats and mice in the course of 13-week subchronic toxicity studies with Direct Black 38. Analysis of 24-hour urine samples 4 and 12 weeks (rats) and 3 and 11 weeks (mice) later showed a clear-cut relationship between the concentration of dyestuff in the food consumed and the amount of benzidine excreted. Mice biotransformed considerably more dye to benzidine than did rats. However, it is not clear whether and to what extent the presence of other metabolites, or impurities in the dyestuff fed, influenced the results, since the method of determination used was not specific for benzidine (National Cancer Institute, 1978).

Rhesus monkeys excreted an average of 1.25% benzidine plus monoacetylbenzidine of the benzidine moiety in Direct Black 38 in the urine after receiving two different doses by gavage, whereas gavage with pure benzidine yielded 1.45%. The authors thus postulated a nearly complete metabolic conversion of Direct Black 38 to benzidine (Rinde and Troll, 1975). This conclusion has been questioned, since the amount of dye (given in dimethyl sulphoxide solution) absorbed may be different from that of benzidine (National Institute for Occupational Safety and Health, 1980). [In the absence of more detailed metabolic studies, it cannot be concluded that Direct Black 38 is completely converted to benzidine.]

Following oral administration of a single dose of 10 mg/kg bw Direct Black 38 to Syrian golden hamsters, 10.7 μg benzidine, 535 μg monoacetylbenzidine, 27.6 μg diacetylbenzidine [see IARC, 1978], 11.5 μg 4-aminobiphenyl [see IARC, 1972] and, as alkaline hydrolysable conjugates, 328.5 μg benzidine and 6.3 μg 4-aminobiphenyl, were identified in the urine by parallel electron capture gas chromatography and high-performance liquid chromatography. Peak excretion occurred between 0-8 and 8-16 hours. These results indicate that a total of 10% of the dye is metabolized to benzidine and its metabolic follow-up products (National Center for Toxicological Research, 1979; Nony *et al.*, 1980).

Direct Black 38 is rapidly metabolized to benzidine by 14 common bacterial species (Dieckhues, 1960). Preparations of rat and mouse intestine *in vitro* have also been shown to convert Direct Black 38 to benzidine (Niitsu, 1973; Yoshida and Miyakawa, 1973). After increasing microbial activity in rats by feeding a meat-based diet, the azo-reductase level was enhanced (Goldin *et al.*, 1978).

Mutagenicity and other short-term tests

Direct Black 38 was mutagenic in *Salmonella typhimurium* strains TA98 and TA100 when tested in the presence of a mouse liver metabolic activation system; no mutagenicity was observed in the absence of activation (Lazear *et al.*, 1979). Monoacetylbenzidine, a major metabolite of benzidine (see above), and urine from hamsters given Direct Black 38 (100 mg/kg bw) were mutagenic for *S. typhimurium* strain TA1538, but only when tested in the presence of metabolic activation (Lazear *et al.*, 1979; Nony *et al.*, 1980).

Urine from rats given 500 mg/kg bw Direct Black 38 was also mutagenic for *S. typhimurium* strains TA98 and TA100 in the presence of metabolic activation (Tanaka, 1980).

(*b*) *Humans*

Toxic effects

No data were available to the Working Group.

Effects on reproduction and prenatal toxicity

No data were available to the Working Group.

Absorption, distribution, excretion and metabolism

Benzidine-derived azo dyes may be degraded metabolically in the gut or liver in man to free benzidine or monoacetylbenzidine (Walker, 1970).

Genin (1977) analysed the urine of 22 workers who had potential long-term exposure to benzidine-based dyes during the manufacture of Direct Black 38 and other direct azo dyes. Traces to 300 ppb benzidine were detected in the urine of eight workers, and dianisidine [see IARC, 1974] in three.

Using immunological methods, Korosteleva *et al.* (1974, 1977) identified a benzidine-albumin complex in the serum of female textile-mill workers. The amount present depended on the extent and duration of exposure to direct dyestuffs in the work place; and the complex was found only in workers exposed to direct azo dyes and not in those exposed to non-direct dyes or in controls.

Environmental and urine samples were collected at six factories where workers were potentially exposed to benzidine-based dyes (two benzidine-based dye manufacturers, two textile-dyeing plants, a leather-tanning and dyeing plant and a mill where paper was dyed). Monoacetylbenzidine was detected in the urine of 2/8 workers at one of the dye-manufacturing plants at levels of 3 and 7 ppb. At the second factory, 4 workers exposed to average levels of 7.9, 5.2, 11.7 and 17.4 mg total particulate/m^3 had corresponding urinary concentrations of 52, 11, 10 and 112 ppb benzidine; 590, 248 and 22 ppb monoacetylbenzidine were detected in urine samples containing 112, 52 and 11 ppb benzidine. Traces of diacetylbenzidine, *ortho*-tolidine [see IARC, 1972] and *ortho*-dianisidine were also detected. Benzidine (0-39 ppb) and/or monoacetylbenzidine was detected in the urine of workers in one textile-dyeing factory where Direct Black 38 and Direct Blue 2 were being used. The total level of airborne particulates (measured gravimetrically) was 1-4 mg/m^3. Benzidine was not detected in the urine of workers from

the other facilities (Boeniger, 1980; Lowry *et al.*, 1980). Minute levels of impurities in the dyestuffs could not account for the quantity of benzidine and its derivatives that were found in the urine samples (National Institute for Occupational Safety and Health, 1980).

Mutagenicity and chromosomal effects

No data were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity in humans

No study specific to Direct Black 38 was available to the Working Group. In the following reports, workers were exposed to dyestuffs which may have included Direct Black 38 (and/or Direct Blue 6 and/or Direct Brown 95).

Numerous case studies [described in more detail in section 3.3 of the monograph on benzidine] describe the occurrence of bladder tumours among workers in dye manufacture. Such reports include those of Oppenheimer (1927), Muller (1933), DiMaio (1937), Barsotti and Vigliani (1949), Goldblatt (1949), Scott (1952), Aboulker and Smagghe (1953), Uebelin and Pletscher (1954), Vigliani and Barsotti (1961), Maltoni and Ghetti (1964), Goldwater *et al.* (1965) and Ferber *et al.* (1976). Epidemiological studies, including those of Case *et al.* (1954) and of Tsuchiya *et al.* (1975), have also indicated increased incidences and mortality from cancer of the bladder among workers in dye manufacture. Three historical reviews (Hueper, 1942, 1969; Haley, 1975) described the international spread of cancer of the bladder among dye workers concomitantly with the spread of the industry.

Several epidemiological studies of dye users suggest that there may be excess mortality from bladder cancer in people possibly exposed occupationally to benzidine-based dyes. Such occupations include shoe and leather workers, tailors, textile workers and hairdressers (Wynder *et al.*, 1963; Anthony and Thomas, 1970; Cole *et al.*, 1972; Anthony, 1974; Viadana *et al.*, 1976). A proportional mortality study of 1429 bleachers and dyers in the UK showed no excess deaths from cancer of the bladder (Newhouse, 1978). [That study was limited in that no certificates of deaths occurring in the first 20 years after start of exposure were available, and only approximately one-third of the workers included in the analysis had actually been exposed to dyes.]

A hospital-based case-control study of 200 male bladder cancer cases and 148 male controls of the same age range with urinary disorders in Kyoto, Japan, showed that 17 (8.5%) of the cases and 2 (1.4%) of the controls had worked in the silk-dyeing industry. The relative risk for employment in the silk-dyeing industry was 6.8 ($p = 0.002$). At least 7 of the 17 patients with bladder cancer who had worked in the dyeing industry were kimono painters, some of whom may have ingested dyes by holding brushes or spatulas in their mouths while working. Among the dyes in wide use in Japan in the 1970s were the benzidine-based compounds, Direct Red 28, Direct Red 17, Direct Green 1 and Direct Black 38 (Yoshida *et al.*, 1971; Yoshida and Miyakawa, 1972). [The Working Group noted that the cases and the controls were recruited from different populations, a procedure which might introduce bias. No data on potential confounding factors were provided.]

In a metabolic evaluation of 22 workers engaged in drying and grinding benzidine-based azo dyes (Direct Black 38 and Direct Blue 2) and dyes based on *ortho*-dianisidine

(Direct Blue 15 and Direct Blue 218) [see section 3.2 (*b*)], benzidine was found in the urine of 8 and dianisidine in that of 3. A retrospective search of plant records showed 5 cases of bladder cancer in dryers and grinders (Genin, 1977).

4. Summary of Data Reported and Evaluation

4.1 Experimental data

Direct Black 38 was tested by oral administration in mice and rats and by bladder implantation in mice. In one study in mice, the compound produced hepatocellular carcinomas and mammary carcinomas following its administration in drinking-water. The other study in mice was inadequate for evaluation. Oral administration to rats of one commercial sample of Direct Black 38 resulted in hepatocellular carcinomas in males and neoplastic nodules in males and females sacrificed 13 weeks after start of exposure to the highest dose at which animals survived. Lower doses produced only liver-cell changes such as foci of cellular alteration. In another study in rats, sacrificed after 60 weeks' exposure to the dye in drinking-water, mucosal hyperplasia and carcinoma of the bladder and carcinomas of the liver and colon were seen.

One study has shown that Direct Black 38 and the urine of hamsters given this compound are mutagenic to *Salmonella typhimurium* with metabolic activation.

One limited study in rats has shown it to be embryolethal but not teratogenic.

4.2 Human data

Occupational exposure to Direct Black 38 has and probably still does occur during its production and its use for the dyeing of textiles, leather and paper. Benzidine and its metabolic derivatives have been detected in the urine of workers exposed to direct azo dyes.

No data were available to assess the mutagenicity or chromosomal effects of this compound to man.

No study of exposure to Direct Black 38 alone was available to the Working Group. An epidemiological study of silk dyers and painters who had multiple exposure to benzidine-based and other dyes indicated that those exposures were strongly associated with the occurrence of bladder cancer. Case reports and other epidemiological studies support the existence of such a relationship.

4.3 Evaluation[1]

There is *sufficient evidence* that commercial Direct Black 38 is carcinogenic to rats.

Although the epidemiological data were inadequate to evaluate the carcinogenicity in man of Direct Black 38 alone, they, together with the presence of benzidine in the urine of exposed workers, provide *sufficient evidence* that occupational exposure to benzidine-based dyes represents a carcinogenic risk to man.

[1] This evaluation should be read in conjunction with section 7(*b*)(*c*) of the preamble.

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DIRECT BLUE 6

The commercial material is a reaction product and is not to be regarded as a single substance; therefore, the evaluation of this compound was based on experience with mixtures of substances that varied in composition.

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 2602-46-2

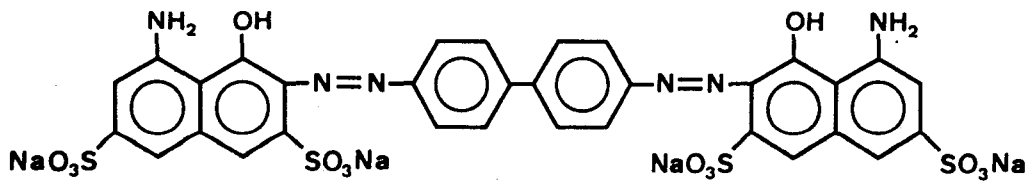
Chem. Abstr. Name: 2,7-Naphthalenedisulfonic acid, 3,3′-[(1,1′-biphenyl)-4,4′-diylbis(azo)]bis(5-amino-4-hydroxy)-, tetrasodium salt

IUPAC Systematic Name: Tetrasodium 3,3′-[4,4-biphenylenebis(azo)] bis(5-amino-4-hydroxy)-2,7-naphthalenedisulfonate

Synonyms: C.I. 22610; C.I. Direct Blue 6, tetrasodium salt; sodium diphenyl-4,4′-bis-azo-2"-8"-amino-1"-naphthol-3",6"-disulphonate

Trade Names: Airedale Blue 2BD; Aizen Direct Blue 2BH; Amanil Blue 2BX; Atlantic Blue 2B; Atul Direct Blue 2B; Azocard Blue 2B; Azomine Blue 2B; Belamine Blue 2B; Bencidal Blue 2B; Benzanil Blue 2B; Benzo Blue BBA-CF; Benzo Blue BBN-CF; Benzo Blue GS; Blue 2B; Blue 2B Salt; Brasilamina Blue 2B; Calcomine Blue 2B; Chloramine Blue 2B; Chlorazol Blue B; Chlorazol Blue BP; Chrome Leather Blue 2B; CI 22610; C.I. 22610; C.I. Direct Blue 6; C.I. Direct Blue 6, Tetrasodium Salt; Cresotine Blue 2B; Diacotton Blue BB; Diamine Blue 2B; Diamine Blue BB; Diaphtamine Blue BB; Diazine Blue 2B; Diazol Blue 2B; Diphenyl Blue 2B; Diphenyl Blue KF; Diphenyl Blue M2B; Direct Blue A; Direct Blue 2B; Direct Blue BB; Direct Blue GS; Direct Blue K; Direct Blue M2B; Direct Blue WBB; Enianil Blue 2BN; Fenamin Blue 2B; Fixanol Blue 2B; Hispamin Blue 2B; Indigo Blue 2B; Kayaku Direct; Kayaku Direct Blue BB; Mitsui Direct Blue 2BN; Naphtamine Blue 2B; Niagara Blue B; Niagara Blue 2B; Nippon Blue BB; Paramine Blue 2B; Phenamine Blue BB; Pheno Blue 2B; Pontamine Blue BB; Tertrodirect Blue 2B; Vondacel Blue 2B

1.2 Structural and molecular formulae and molecular weight of the principal ingredient



$C_{32}H_{20}N_6Na_4O_{14}S_4$ Mol. wt: 936.8

1.3 Chemical and physical properties

(*a*) *Description*: Blue-violet solid (Richter, 1951)

(*b*) *Solubility*: Soluble in water; slightly soluble in ethanol and ethylene glycol monoethyl ether; insoluble in other organic solvents (The Society of Dyers and Colourists, 1971a)

(*c*) *Conversion factor*: ppm = 0.0261 x mg/m^3

1.4 Technical products and impurities

The benzidine content of two US-produced Direct Blue 6 samples were 4 and 12 mg/kg, that of a sample imported from Belgium was 6.6 mg/kg and that of a sample imported from India was 10 mg/kg (Boeniger, 1980). The manufacture and testing of Direct Blue 6 do not conform to rigid chemical specifications, and its composition varies in order to meet the shade and intensity requirements of each customer.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(*a*) *Production*

Direct Blue 6 was first synthesized in 1890 (The Society of Dyers and Colourists, 1971a). It is prepared commercially by coupling diazotized benzidine with 2 mol H-acid (8-amino-1-naphthol-3,6-disulphonic acid) under alkaline conditions (Richter, 1951).

Direct Blue 6 was first produced in commercial quantities in the US in 1914 (US Tariff Commission, 1922). In both 1973 and 1976, US sales amounted to 148 thousand kg (US International Trade Commission, 1975; National Institute for Occupational Safety and Health, 1980). It is presently produced commercially by only one US company, whose production in 1978 totalled 28 thousand kg (National Institute for Occupational Safety and Health, 1980), making it the benzidine-based dye produced in the seventh largest volume in the US in that year.

US imports of Direct Blue 6 through the principal customs districts were last reported in 1978, when they totalled 2.0 thousand kg (US International Trade Commission, 1979).

This compound is believed to be produced commercially by one company in France. It may be imported into other western European countries from India or from eastern Europe.

Direct Blue 6 is not produced commercially in Japan; imports from South Korea and Taiwan, are estimated to have been less than 1 thousand kg per year in recent years.

Benzidine-based dyes are also believed to be produced commercially in Argentina, Brazil, the People's Republic of China, India, Mexico, Poland, Romania and the USSR, but whether Direct Blue 6 is one of the dyes produced is not known.

(*b*) *Use*

Direct Blue 6 can be used to: (1) dye cellulose and silk; (2) stain silk, wool and nylon fibres; (3) print cellulose fabrics; (4) dye leather and paper; (5) stain biological materials; and (6) produce aqueous writing inks (The Society of Dyers and Colourists, 1971b; Boeniger, 1980). Direct Blue 6 has reportedly been used in hair dyes (National Cancer Institute, 1978) [see IARC, 1982].

The US Occupational Safety and Health Administration has issued guidelines that its field personnel 'use the general duty portion of existing occupational safety laws to control worker exposure' to Direct Blue 6 (Anon. 1980a) and has issued a health hazard alert to employers (Anon., 1980b), although no specific regulations limiting occupational exposure to Direct Blue 6 have been established.

2.2 Occurrence

(*a*) *Natural occurrence*

Direct Blue 6 has not been reported to occur as such in Nature.

(*b*) *Occupational exposure*

Direct Blue 6 has been detected in the workplace air of a textile-dyeing operation, at total airborne particulate levels of 1.20-3.94 mg/m^3 (Boeniger, 1980).

The National Institute for Occupational Safety and Health (1980) estimated that approximately 800 US workers are exposed to Direct Blue 6.

(*c*) *Other*

The Working Group had no information on the extent of exposure, if any, of the general population to Direct Blue 6 as a result of its presence in consumer products.

2.3 Analysis

Direct dyes such as Direct Blue 6 are reportedly distinguished from basic and disperse dyes by their pH-dependent chromatographic behaviour on cellulose acetate, and are separated from each other by chromatography on silica gel (Schlegelmilch and Khodadadian, 1973). However, several eluent systems were tried without success in an effort to move Direct Blue 6 during thin-layer chromatographic separation of dye mixtures (Mashruwala and Mehta, 1979).

Typical methods for the analysis of Direct Blue 6 in various matrices are summarized in Table 1.

Table 1. Methods for the analysis of Direct Blue 6

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Air	Collect sample on glass-fibre filter (method detects all dye particles)	Gravimetric	not given	Boeniger *et al.* (1980)
	Extract filter with appropriate solvent; scan from 400-700 nm and compare with scans of bulk dye sample solutions for quasi-specific identification	S	not given	
Bulk or mixtures	Analyse distilled-water-dye solutions or extracts of dye-yarn hanks (in colourless dimethylformamide) to detect most direct dyes (probably not dye-specific)	S/R	not given	Mashruwala and Mehta (1979)

a Abbreviations: S, spectrometry; S/R, spectrometry and reflectance

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(*a*) *Oral administration*

Rat: Groups of 10 male and 10 female Fischer 344 rats, 6 weeks old, were fed a diet containing 0, 190, 375, 750, 1500 or 3000 mg/kg [ppm] Direct Blue 6 and 1.3% corn oil. (The compound was determined by high-performance liquid chromatography to be 59.9 $\pm$ 1.9% pure, with the following impurities: water, 9.18 $\pm$ 0.51%; NaCl, 20.8%; benzidine, $<$ 0.004%; and traces of at least eight other impurities.) Survivors were killed at 13 weeks. All male and female animals administered 3000 mg/kg Direct Blue 6 and 1 male administered 1500 mg/kg diet of the dye died prior to termination of the study; all males given the highest dose died before 5 weeks on the study, and all females at that dose were dead by 10 weeks on test. Liver-cell tumours were seen in 8 of 10 males given 1500 mg/kg; 2 were hepatocellular carcinomas and 6, neoplastic nodules. Of animals given 3000 mg/kg, 1 of 9 males and 7 out of 9 females were found to have liver-cell tumours at autopsy prior to the termination of the experiment; 4 of the tumours in females were

hepatocellular carcinomas and 3 were neoplastic nodules. No neoplastic lesion was seen in animals of either sex given lower doses. The first tumours appeared after 4 weeks of feeding in the males and after 5 weeks of feeding in the females. Almost all animals fed 750 or 1500 mg/kg exhibited foci of cellular alterations in the liver and some basophilic foci were seen in the livers of animals receiving 3000 mg/kg. In the same bioassay, no increased incidence of tumours, compared with that in controls, was found in groups of 10 male and 10 female B6C3F$_1$ mice fed diets containing 750, 1500, 3000, 6000 or 12 500 mg/kg [ppm] of Direct Blue 6 and killed 13 weeks later (National Cancer Institute, 1978; Robens *et al.*, 1980). [The Working Group noted the short duration of the experiment, the limited number of animals tested, and the impurity of the compound used. Other samples of the compound may have different impurities.]

Twenty female Wistar rats [age unspecified] were given 400 mg/l Direct Blue 6 [purity unspecified] in their drinking-water (0.04%) for 14 months. At 12 months, 12 animals were still alive, and 1 exhibited a 'glandular tumour' of the outer ear. No other neoplasm was found (Niitsu, 1973). [The Working Group noted the small number of animals and lack of a control group.]

(*b*) *Subcutaneous and/or intramuscular administration*

The results of a study by Fujita *et al.* (1957) in rats were inconclusive because of poor survival and lack of proper controls.

(*c*) *Other experimental systems*

Bladder implantation: A group of 50 female dd *mice* (20 g) received either a paraffin wax pellet (20 mg) containing 10% Direct Blue 6 or a wax pellet alone implanted in the bladder. After 40 weeks, when the surviving animals were killed, bladder carcinomas were found in 3 of 21 treated mice and in 1 of 36 controls still alive at that time (Niitsu, 1973). [The Working Group noted that the difference from controls was not significant ($p = 0.13$) by the Fisher exact test.]

3.2 Other relevant biological data

(*a*) *Experimental systems*

Toxic effects

Studies in which rats were fed diets containing 190-3000 mg/kg [ppm] Direct Blue 6 and mice 750-12 500 mg/kg for 13 weeks resulted in a series of dose-related changes seen when all animals were killed at the end of treatment. In rats, hepatocellular degeneration, cholangiofibrosis and portal fibrosis were observed in the liver. Lymphoid depletion of the spleen, thymus and lymph nodes, and myeloid depletion of the bone marrow were also seen. Other effects included oedema of the large intestine and interstitial haemorrhage of the testes. Biliary hyperplasia was seen in animals fed 750 mg/kg and more. In mice, pigment deposition in the liver and haemosiderosis of the kidneys were observed in the groups that received the highest dose, and haemosiderosis of the spleen in all groups that received 1 500 mg/kg or more; extramedullary haematopoiesis was also seen in females (National Cancer Institute, 1978).

Effects on reproduction and prenatal toxicity

In Wistar rats, s.c. administration of 150 mg/kg Direct Blue 6 (as Niagara Blue 2B) on day 8.5 of pregnancy was highly teratogenic (25% of fetuses were malformed) (Beck and Lloyd, 1966; Lloyd and Beck, 1966). I.p. administration of 140 or 200 mg/kg on day 8 of pregnancy to Wistar rats caused 29 or 15% maternal mortality and was teratogenic (2.5 or 11% of fetuses malformed). Hydrocephalus and eye defects were the commonest malformations observed (Beaudoin, 1968). It seems likely that the teratogenic action is an indirect one on the yolk sac epithelium (Beck and Lloyd, 1966; Jensh and Brent, 1972).

Absorption, distribution, excretion and metabolism

Benzidine and monoacetylbenzidine were found in the urine of rats and mice in the course of 13-week subchronic toxicity studies with Direct Blue 6. Analysis of 24-hour urine samples 4 and 12 weeks (rats) and 3 and 11 weeks (mice) later showed a clear-cut relationship between the concentration of dyestuff in the food consumed and the amount of benzidine excreted. Mice biotransformed considerably more dye to benzidine than did rats. However, it is not clear whether and to what extent the presence of other metabolites, or impurities in the dyestuff fed, influenced the results, since the method of determination used was not specific for benzidine (National Cancer Institute, 1978).

Rhesus monkeys excreted an average of 1.25% benzidine plus monoacetylbenzidine of the benzidine moiety in Direct Blue 6 in the urine after receiving two different doses by gavage, whereas gavage with pure benzidine yielded 1.45%. The authors thus postulated a nearly complete metabolic conversion of Direct Blue 6 to benzidine (Rinde and Troll, 1975). This conclusion has been questioned, since the amount of dye (given in dimethyl sulphoxide solution) absorbed may be different from that of benzidine (National Institute for Occupational Safety and Health, 1980). [In the absence of more detailed metabolic studies it cannot be concluded that Direct Blue 6 is completely converted to benzidine.]

Mutagenicity and other short-term tests

No data were available to the Working Group.

(*b*) *Humans*

Toxic effects

No data were available to the Working Group.

Effects on reproduction and prenatal toxicity

No data were available to the Working Group.

Absorption, distribution, excretion and metabolism

Benzidine-derived azo dyes may be degraded metabolically in the gut or liver in man to free benzidine or monoacetylbenzidine (Walker, 1970).

Using immunological methods, Korosteleva *et al.* (1974, 1977) identified a benzidine-albumin complex in the serum of female textile-mill workers. The amount present depended on the extent and duration of exposure to direct dyestuffs in the work place; and the complex was found only in workers exposed to direct azo dyes and not in those exposed to non-direct dyes or in controls.

Environmental and urine samples were collected at six factories where workers were potentially exposed to benzidine-based dyes (two benzidine-based dye manufacturers, two textile-dyeing plants, a leather dyeing plant and a mill where paper was dyed). Monoacetylbenzidine was detected in the urine of 2/8 workers at one of the dye-manufacturing plants at levels of 3 and 7 ppb. At the second factory, 4 workers exposed to average levels of 7.9, 5.2, 11.7 and 17.4 mg total particulate/m^3 had corresponding urinary concentrations of 52, 11, 10 and 112 ppb benzidine; 590, 248 and 22 ppb monoacetylbenzidine were detected in urine samples containing 112, 52 and 11 ppb benzidine. Traces of diacetylbenzidine, *ortho*-tolidine [see IARC, 1972] and *ortho*-dianisidine [see IARC, 1974] were also detected. Benzidine (0-39 ppb) and/or monoacetyl-benzidine was detected in the urine of workers in one textile-dyeing factory where Direct Black 38 and Direct Blue 2 were being used. The total level of airborne particulates (measured gravimetrically) was 1-4 mg/m^3. Benzidine was not detected in the urine of workers from the other facilities (Boeniger, 1980; Lowry *et al.*, 1980). Minute levels of impurities in the dyestuffs could not account for the quantity of benzidine and its derivatives that were found in the urine samples (National Institute for Occupational Safety and Health, 1980).

Mutagenicity and chromosomal effects

No data were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity in humans

No study specific to Direct Blue 6 was available to the Working Group. Studies of exposures to benzidine-based dyes are summarized in the monograph on Direct Black 38.

4. Summary of Data Reported and Evaluation

4.1 Experimental data

Oral administration to rats of one commercial sample of Direct Blue 6 resulted in hepatocellular carcinomas and neoplastic nodules in males and females sacrificed 13 weeks after start of exposure to the highest dose at which animals survived. Lower doses produced only liver-cell changes such as foci of cellular alteration.

Direct Blue 6 is teratogenic in rats only when injected during the first half of pregnancy.

No data were available to assess the mutagenicity of this compound.

4.2 Human data

Occupational exposure to Direct Blue 6 has and probably still does occur during its production and its use for the dyeing of textiles, leather and paper. Benzidine and its metabolic derivatives have been detected in the urine of workers exposed to direct azo dyes.

No data were available to assess the mutagenicity or chromosomal effects of this compound to man.

No study of exposure to Direct Blue 6 was available to the Working Group. An epidemiological study of silk dyers and painters who had multiple exposure to benzidine-based and other dyes indicated that those exposures were strongly associated with the occurrence of bladder cancer. Case reports and other epidemiological studies support the existence of such a relationship.

4.3 Evaluation[1]

There is *sufficient evidence* that commercial Direct Blue 6 is carcinogenic to rats.

Although the epidemiological data were inadequate to evaluate the carcinogenicity to man of Direct Blue 6 alone, they, together with the presence of benzidine in the urine of exposed workers, provide *sufficient evidence* that occupational exposure to benzidine-based dyes represents a carcinogenic risk to man.

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[1] This evaluation should be read in conjunction with section 7(*b*)(*c*) of the preamble.

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DIRECT BROWN 95

The commercial material is a reaction product and is not to be regarded as a single substance; therefore, the evaluation of this compound was based on experience with mixtures of substances that varied in composition.

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 16071-86-6

Chem. Abstr. Name: Cuprate (2-), (5-[(4'-((2,6-dihydroxy-3-((2-hydroxy-5-sulfophenyl)azo)phenyl)azo)(1,1'-biphenyl)-4-yl)azo]- 2-hydroxy benzoato(4-))-, disodium salt

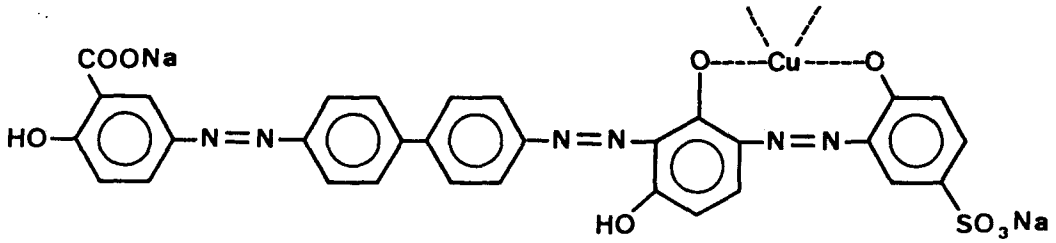
IUPAC Systematic Name: Disodium (5-[(4'-((2,6-dihydroxy-3-((2-hydroxy-5-sulfophenyl)azo)phenyl)azo)-4-biphenyl)azo]salicylato (4))cuprate(2-)

Synonyms: C.I. 30145; copper, dihydrogen(5-[(4'-((2,6-dihydroxy-3-((2-hydroxy-5-sulphophenyl)azo)phenyl)azo)-4-biphenylazo]salicyla to(2-))-, disodium salt

Trade Names: Aizen Primula Brown BRLH; Aizen Primula Brown PLH; Amanil Fast Brown BRL; Amanil Supra Brown LBL; Atlantic Fast Brown BRL; Atlantic Resin Fast Brown BRL; Belamine Fast Brown BRLL; Benzanil Supra Brown BRLL; Benzanil Supra Brown BRLN; Brown 4EMBL; C.I. 30145; C.I. Direct Brown; Calcodur Brown BRL; Chloramine Fast Brown BRL; Chloramine Fast Cutch Brown PL; Chlorantine Fast Brown BRLL; Chrome Leather Brown BRLL; Chrome Leather Brown BRSL; Cuprofix Brown GL; Derma Fast Brown W-GL; Dermafix Brown PL; Dialuminous Brown BRS; Diaphtamine Light Brown BRLL; Diazine Fast Brown RSL; Diazol Light Brown BRN; Dicorel Brown LMR; Diphenyl Fast Brown BRL; Direct Brown BRL; Direct Fast Brown BRL; Direct Fast Brown LMR; Direct Light Brown BRS; Direct Supra Light Brown ML; Durazol Brown BR; Durofast Brown BRL; Eliamina Light Brown BRL; Enianil Light Brown BRL; Fastolite Brown BRL; Fastusol Brown LBRSA; Fastusol Brown LBRSN; Fenaluz Brown BRL; Helion Brown BRSL; Hispaluz Brown BRL; KCA Light Fast Brown BR; Kayarus Supra Brown BRS; Paranol Fast Brown BRL; Peeramine Fast Brown BRL; Pontamine Fast Brown BRL; Pontamine Fast Brown NP; Pyrazol Fast Brown BRL; Pyrazoline Brown BRL; Saturn Brown LBR; Sirius Supra Brown BRL; Sirius Supra Brown BRS; Solantine Brown BRL; Solar Brown PL; Solex Brown R; Solius Light Brown BRLL; Solius Light Brown BRS; Sumilight Supra Brown BRS;

Suprazo Brown BRL; Suprexcel Brown BRL; Tertrodirect Fast Brown BR; Tetramine Fast Brown BRDN Extra; Tetramine Fast Brown BRP; Tetramine Fast Brown BRS; Triantine Brown BRS; Triantine Fast Brown OG; Triantine Fast Brown OR; Triantine Light Brown BRS; Triantine Light Brown OG

1.2 Structural and molecular formulae and molecular weight of the principal ingredient



$C_{31}H_{18}CuN_6Na_2O_9S$ Mol. wt: 760.1

1.3 Chemical and physical properties

(*a*) *Solubility*: Soluble in water; slightly soluble in ethanol; insoluble in acetone (The Society of Dyers and Colourists, 1971a)

(*b*) *Conversion factor*: ppm = 0.0322 x mg/m^3

1.4 Technical products and impurities

The benzidine contents of two US-produced Direct Brown 95 samples were 19 and 270 mg/kg (Boeniger, 1980). The manufacture and testing of Direct Brown 95 do not conform to rigid chemical specifications, and its composition varies in order to meet the shade and intensity requirements of each customer.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(*a*) *Production*

Direct Brown 95 was first synthesized in 1931. It is prepared commercially by: (1) coupling diazotized 2-amino-1-phenol-4-sulphonic acid with resorcinol; (2) coupling 1

mol of the resulting intermediate with 1 mol diazotized benzidine; (3) coupling the resulting intermediate with salicylic acid; and (4) forming a copper complex with a copper salt. In one product (Sirius Supra Brown BRLN), 20% of the salicylic acid is replaced by 2,3-cresotic acid (The Society of Dyers and Colourists, 1971a).

Direct Brown 95 was first produced in commercial quantities in the US in 1937 (US Tariff Commission, 1938). In 1976, US production of Direct Brown 95 by four companies totalled 270 thousand kg (US International Trade Commission, 1977), up somewhat from the 257 000 kg produced in 1973 (US International Trade Commission, 1975). It is presently produced commercially by one US company, whose production in 1978 amounted to 34.5 thousand kg (National Institute for Occupational Safety and Health, 1980), making it the benzidine-based dye produced in the fifth largest volume in the US in that year.

US imports of Direct Brown 95 through the principal customs districts in 1980 were 10.9 thousand kg (US International Trade Commission, 1981).

This compound is believed to be produced commercially by one company in France. It may be imported into other western European countries from India or eastern Europe.

Direct Brown 95 is not produced commercially in Japan. Imports in 1980 are estimated to have been 14 thousand kg, all from South Korea and Taiwan.

Benzidine-based dyes are also believed to be produced commercially in Argentina, Brazil, the People's Republic of China, India, Mexico, Poland, Romania and the USSR, but whether Direct Brown 95 is one of the dyes produced is not known.

(b) Use

Direct Brown 95 can be used to: (1) dye cellulose and silk fibres; (2) stain wool, acetate and nylon fibres; (3) print cellulose and silk fabrics; (4) dye leather, paper and casein-formaldehyde plastics; and (5) produce its heavy metal salts which can be used as pigments (The Society of Dyers and Colourists, 1971b; Boeniger, 1980).

In Japan, 70% of the Direct Brown used is for dyeing leather and 30% for dyeing paper.

The US Occupational Safety and Health Administration has issued guidelines that its field personnel 'use the general duty portion of existing occupational safety laws to control worker exposure to Direct Brown 95' (Anon. 1980a) and has issued a health hazard alert to employers (Anon., 1980b), although no specific regulations limiting occupational exposure to this compound have been established.

2.2 Occurrence

(a) Natural occurrence

Direct Brown 95 has not been reported to occur as such in Nature.

(b) Occupational exposure

Total airborne particulate levels of Direct Brown 95 detected in the workplace air of a textile-dyeing operation were 1.3-1.54 mg/m^3; those in a leather-dyeing operation, 1.12-14.72 mg/m^3; and those in a paper-dyeing facility, 0.17-3.30 mg/m^3 (Boeniger, 1980).

The National Institute for Occupational Safety and Health (1980) estimated that approximately 700 US workers are exposed to Direct Brown 95.

(c) Other

The Working Group had no information on the extent of exposure, if any, of the general population to Direct Brown 95 as a result of its presence in consumer products.

2.3 Analysis

Direct dyes such as Direct Brown 95 are reportedly distinguished from basic and disperse dyes by their pH-dependent chromatographic behaviour on cellulose acetate, and separated from each other by chromatography on silica gel (Schlegelmilch and Khodadadian, 1973).

Direct dyes can be collected from air with a glass-fibre filter and analysed gravimetrically. (This method will detect all dye particles.) For more specific identification, the filter may be extracted with an appropriate solvent and scanned in a spectrometer from 400-700 nm for comparison with scans of bulk dye sample solutions (Boeniger *et al.*, 1980).

Direct Brown 95 may be detected as an impurity in reactive dyes by thin-layer chromatography. Achwal and Abhyankar (1979) studied several eluent systems for separating Direct Brown 95 from Reactive Brown 9 by this method; the most successful were 6:9:5 *n*-butyl acetate:pyridine:water and 4:2:1:3 *n*-propanol:isobutanol:ethyl acetate:water.

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(a) Oral administration

Rat: Groups of 10 male and 10 female Fischer 344 rats, 6 weeks old, were fed a diet containing 0, 190, 375, 750, 1500 or 3000 mg/kg [ppm] Direct Brown 95 and 1.3% corn oil. [The compound was determined by high-performance liquid chromatography to be 72.2 $\pm$ 7.0% pure, with the following impurities: water, 4.99 $\pm$ 0.22%; NaCl, 14.9%; benzidine, $<$ 0.004%; and traces of at least eight other impurities.] Surviving rats were

killed at 13 weeks. All male and female animals administered 1500 or 3000 mg/kg Direct Brown 95 died prior to termination of the studies; male rats survived for less than 5 weeks, females given the high dose less than 6 weeks on the study, and females fed 1500 mg/kg about 12 weeks; 2 males receiving 750 mg/kg Direct Brown 95 also died prior to the end of the study. Among male rats, basophilic foci or foci of cellular alteration were seen in 2/9 animals given 3000 mg/kg, in 7/8 given 1500 mg/kg and in 8/10 given 750 mg/kg. In female animals, 4/8 given the 1500 mg/kg dose exhibited neoplastic nodules, and 1 of these showed a hepatocellular carcinoma; basophilic foci or foci of cellular alteration in the liver were seen in 3/8 females given 3000 mg/kg, 6/8 given 1500 mg/kg and 3/10 given 750 mg/kg. No other relevant findings in relation to neoplastic development were seen in these animals. In the same bioassay, groups of 10 male $B6C3F_1$ mice, 6-7 weeks of age, were fed a diet containing 750, 1500, 3000, 6000 or 12 500 mg/kg [ppm] Direct Brown 95 and 1.3% corn oil. Groups of 10 female $B6C3F_1$ mice, 6-7 weeks of age, were fed similar diets containing 350, 750, 1500, 3000 or 6000 mg/kg of the dye. Control diets contained corn oil in amounts equal to that in the diets of groups given the highest doses. The compound was administered for 13 weeks, when all animals were killed. The only suggestive neoplastic lesion observed was foci of basophilic cellular alteration in one male mouse administered 12 500 mg/kg Direct Brown 95 (National Cancer Institute, 1978; Robens *et al.*, 1980). [The Working Group noted the short duration of the experiment, the limited number of animals tested, and the impurity of the compound used. Other samples of the compound may have different impurities.]

3.2 Other relevant biological data

(a) Experimental systems

Toxic effects

Studies in which rats were fed diets containing 190-3000 mg/kg [ppm] Direct Brown 95 and mice 375-12 500 mg/kg for 13 weeks resulted in a series of dose- and substance-related changes seen when all animals were killed at the end of treatment. In rats, biliary hyperplasia and portal fibrosis were observed in the liver; lymphoid depletion in spleen and thymus, lymphoid necrosis in lymph nodes and myeloid depletion in the bone marrow were also seen. Other effects included subacute glomerulonephritis, interstitial haemorrhage and degeneration of germinal epithelium of the testes, and some extramedullary haematopoiesis in the liver. Biliary hyperplasia was seen in all animals given 375 mg/kg or more. In mice, pigment deposition in the liver and haemosiderosis of the spleen were observed (National Cancer Institute, 1978).

Effects on reproduction and prenatal toxicity

No data were available to the Working Group.

Absorption, distribution, excretion and metabolism

Benzidine and monoacetylbenzidine were found in the urine of rats and mice in the course of 13-week subchronic toxicity studies with Direct Brown 95. Analysis of 24-hour urine samples 4 and 12 weeks (rats) and 3 and 11 weeks (mice) later showed a clear-cut relationship between the concentration of dyestuff in the food consumed and the amount

of benzidine excreted. Mice biotransformed considerably more dye to benzidine than did rats. However, it is not clear whether and to what extent the presence of other metabolites, or impurities in the dyestuff fed, influenced the results, since the method of determination used was not specific for benzidine (National Cancer Institute, 1978).

Rhesus monkeys excreted an average of 1.25% benzidine plus monoacetylbenzidine of the benzidine moiety in Direct Brown 95 in the urine after receiving two different doses by gavage, whereas gavage with pure benzidine yielded 1.45%. The authors thus postulated a nearly complete metabolic conversion of Direct Brown 95 to benzidine (Rinde and Troll, 1975). This conclusion has been questioned, since the amount of dye (given in dimethyl sulphoxide solution) absorbed may be different from that of benzidine (National Institute for Occupational Safety and Health, 1980). [In the absence of more detailed metabolic studies, it cannot be concluded that Direct Brown 95 is completely converted to benzidine.]

Mutagenicity and other related short-term tests

No data were available to the Working Group.

(*b*) *Humans*

Toxic effects

No data were available to the Working Group.

Effects on reproduction and prenatal toxicity

No data were available to the Working Group.

Absorption, distribution, excretion and metabolism

Benzidine-derived azo dyes may be degraded metabolically in the gut or liver in man to free benzidine or monoacetylbenzidine (Walker, 1970).

Using immunological methods, Korosteleva *et al.* (1974, 1977) identified a benzidine-albumin complex in the serum of female textile-mill workers. The amount present depended on the extent and duration of exposure to direct dyestuffs in the work place; and the complex was found only in workers exposed to direct azo dyes and not in those exposed to non-direct dyes or in controls.

Environmental and urine samples were collected at six factories where workers were potentially exposed to benzidine-based dyes (two benzidine-based dye manufacturers, two textile-dyeing plants, a leather-tanning and dyeing plant and a mill where paper was dyed). Monoacetylbenzidine was detected in the urine of 2/8 workers at one of the dye-manufacturing plants at levels of 3 and 7 ppb. At the second factory, 4 workers exposed to average levels of 7.9, 5.2, 11.7 and 17.4 mg total particulate/m^3 had corresponding urinary concentrations of 52, 11, 10 and 112 ppb benzidine; 590, 248 and 22 ppb monoacetylbenzidine were detected in urine samples containing 112, 52 and 11 ppb benzidine. Traces of diacetylbenzidine, *ortho*-tolidine [see IARC, 1972] and *ortho*-dianisidine [see IARC, 1974] were also detected. Benzidine was not detected in the urine of workers from the other facilities (Boeniger, 1980; Lowry *et al.*, 1980). Minute levels of

impurities in the dyestuffs could not account for the quantity of benzidine and its derivatives that were found in the urine samples (National Institute for Occupational Safety and Health, 1980).

Mutagenicity and chromosomal effects

No data were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity in humans

No study specific to Direct Brown 95 was available to the Working Group. Studies of exposures to benzidine-based dyes are summarized in the monograph on Direct Black 38.

4. Summary of Data Reported and Evaluation

4.1 Experimental data

Oral administration to rats of one commercial sample of Direct Brown 95 resulted in one hepatocellular carcinoma and several neoplastic nodules in females sacrificed 13 weeks after start of exposure to the highest dose at which animals survived. The study in mice was inadequate for evaluation.

No data were available to assess the mutagenicity or teratogenicity of Direct Brown 95.

4.2 Human data

Occupational exposure to Direct Brown 95 has and probably still does occur during its production and its use for the dyeing of textiles, leather and paper. Benzidine and its metabolic derivatives have been detected in the urine of workers exposed to direct azo dyes.

No data were available to assess the mutagenicity or teratogenicity of Direct Brown 95 to man.

No study of exposure to Direct Brown 95 alone was available to the Working Group. An epidemiological study of silk dyers and painters who had multiple exposure to benzidine-based and other dyes indicated that those exposures were strongly associated with the occurrence of bladder cancer. Case reports and other epidemiological studies support the existence of such a relationship.

4.3 Evaluation[1]

The number of preneoplastic lesions in rats and the precocity of their onset indicate a carcinogenic effect similar to that of Direct Black 38. The present data, however, provide only *limited evidence* that commercial Direct Brown 95 is carcinogenic to rats.

Although the epidemiological data were inadequate to evaluate the carcinogenicity to man of Direct Brown 95 alone, they, together with the presence of benzidine in the urine of exposed workers, provide *sufficient evidence* that occupational exposure to benzidine-based dyes represents a carcinogenic risk to man.

[1]This evaluation should be read in conjunction with section 7(*b*)(*c*) of the preamble.

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APPENDIX C

Excerpts from the NCI Technical Report
13-Week Subchronic Toxicity Studies of
Direct Blue 6, Direct Black 38, and Direct Brown 95 Dyes
NCI No. 108, 1978

SUMMARY

Thirteen-week subchronic toxicity studies of direct blue 6, direct black 38, and direct brown 95 dyes were conducted by administering the test chemicals in feed to Fischer 344 rats and B6C3F1 mice.

Groups of 10 rats and 10 mice of each sex were administered one of the three dyes at one of five concentrations for 13 weeks and then necropsied, beginning the second day after the end of the dosing period. The concentrations used for the rats were 190, 375, 750, 1,500, and 3,000 ppm. The concentrations used for the mice were 750, 1,500, 3,000, 6,000, and 12,500 ppm, except for the female mice administered direct brown 95 dye, which were given concentrations of 375, 750, 1,500, 3,000, and 6,000 ppm. Matched controls consisted of groups of 10 untreated rats and 10 untreated mice of each sex.

Mean body weights of the male and female rats administered the two or three highest doses of any one of the test dyes were lower than mean body weights of the corresponding controls, and the depressions in mean body weight were dose related. Mean body weights of the male and female mice administered the highest dose of any one of the test dyes were slightly lower than mean body weights of the corresponding controls; mean body weights of mice administered lower doses were generally unaffected.

All male and female rats administered 3,000 ppm of any one of the dyes or 1,500 ppm of direct brown 95 dye died before the end of the studies. One male administered 1,500 ppm direct blue 6 dye, six males administered 1,500 ppm direct black 38 dye, and two males administered 750 ppm direct brown 95 dye also died by the end of the studies. No deaths occurred in any other dosed group or in any control group of rats. All male and female mice administered the test dyes survived to the end of the studies, except for one male whose death was attributed to bacterial infection.

Benzidine and monoacetyl benzidine were detected in the urine of male and female rats and mice administered the test dyes, but neither compound was detected in the urine of control rats and mice. Determinations of methemoglobin in control and dosed rats showed no differences.

In rats, neoplastic lesions occurred only in dosed groups and consisted of hepatocellular carcinomas and neoplastic nodules of the liver. The incidences of hepatocellular carcinomas in female rats administered 3,000 ppm direct blue 6 dye (4/9) and male rats administered 1,500 ppm direct black 38 dye (4/9) were significant ($P = 0.033$) when related to the incidences of the tumors in the corresponding controls (0/10); hepatocellular carcinomas were also observed in two male rats administered 1,500 ppm direct blue 6 dye and in one female rat administered 1,500 ppm direct brown 95 dye. No control rats from any of the three studies developed hepatocellular carcinomas.

When incidences of neoplastic nodules were combined with those of hepatocellular carcinomas, the significance increased to $P < 0.001$ for male rats administered 1,500 ppm direct blue 6 dye, $P = 0.001$ for females administered 3,000 ppm direct blue 6 dye, $P < 0.001$ for males administered 1,500 ppm direct black 38 dye, and $P = 0.007$ for females administered 1,500 ppm direct brown 95 dye. No controls developed neoplastic nodules. Female rats administered direct black 38 dye developed no hepatocellular carcinomas, but had an incidence of neoplastic nodules of 5/10, with a significance of $P = 0.016$. Male rats administered direct brown 95 dye developed neither hepatocellular carcinomas nor neoplastic nodules, but as indicated below, had significant incidences of preneoplastic lesions. The failure of groups of rats administered 3,000 ppm dye to develop tumors when other groups administered 1,500 ppm did develop tumors may be due to earlier deaths at the higher dose.

Preneoplastic hepatic lesions (basophilic foci) occurred only in dosed rats and did not occur in the controls. The incidences of the basophilic foci were significant ($P \leq 0.033$) in male (4/9) and female (7/9) rats administered 3,000 ppm direct blue 6 dye and in male rats (7/8) administered 1,500 ppm direct brown 95 dye. Basophilic foci also occurred, at lower incidences, in

males (1/10) administered 1,500 ppm direct blue 6 dye, in males (3/9) administered 1,500 ppm direct black 38 dye, in females (1/8) administered 3,000 ppm direct black 38 dye, in males administered 750 ppm (3/10) or 3,000 ppm (2/9) direct brown 95 dye, and in females administered 1,500 ppm (3/8) or 3,000 ppm (3/8) direct brown 95 dye. When incidences of foci of cellular alteration, a possible preneoplastic lesion, were added to those of basophilic foci, significance occurred in additional dosed groups.

In mice, no neoplastic lesions occurred in the liver or other tissues of groups administered the different dyes. However, three mice administered 12,500 ppm direct black 38 dye and one mouse administered 12,500 ppm direct brown 95 dye had foci of cellular alteration, in which the cells were basophilic when compared with surrounding normal cells.

It is concluded that under the conditions of these 13-week subchronic toxicity studies, direct blue 6 and direct black 38 dyes were carcinogenic in male and female Fischer 344 rats and direct brown 95 was carcinogenic in female rats; all three dyes induced hepatocellular carcinomas and neoplastic nodules in the liver. The test dyes were not carcinogenic for B6C3F1 mice in the 13-week subchronic toxicity studies.

I. INTRODUCTION

Direct blue 6 (CAS 2602-46-2; NCI C54579), direct black 38 (CAS 1937-37-7; NCI C54557), and direct brown 95 (CAS 16071-86-6; NCI C54568) are azo dyes used on textiles such as cotton, silk, wool, nylon, and acetate. All three dyes also have commercial use on leather. In addition to use as textile dyes, direct blue 6 and direct black 38 are used in aqueous printing inks and as biological stains, and one or another of these dyes has been used in plastics (direct black 38, direct brown 95), paper (direct blue 6, direct brown 95), wood stains (direct black 38), and wood flour (direct black 38) (Society of Dyers and Colourists, 1971).

Two of these dyes, direct blue 6 and direct black 38, have been used in hair dyes (Comptroller General of the U.S., 1977).

The United States International Trade Commission (1977a) reports that 70,753 pounds of direct black 38 and 8,205 pounds of direct brown 95 were imported for use in the United States in 1976. In the same year, U.S. manufacturers produced 3,759,000 pounds of direct black 38, and 595,000 pounds of direct brown 95. Similar data are not available for direct blue 6, although its production is believed to be greater than 5,000 pounds annually (USITC, 1977a and 1977b).

These three dyes were selected for study in the Carcinogenesis

Testing Program because they are derived from benzidine, which is known to be carcinogenic in animals and man (IARC, 1972), because large quantities were used industrially, and because of the potential for long-term human exposure both through industrial use and through contact with products containing the dyes.

II. MATERIALS AND METHODS

A. Chemicals

The chemicals used were technical-grade factory-strength (unformulated) dyes, manufactured by GAF Corporation (New York, N.Y.). Direct blue 6 (Phenamin Blue BB-FS) was obtained in one batch (Lot No. 43762), direct black 38 (Phenamin Black E-FS) in one batch (Lot No. 43761), and direct brown 95 (Fastusal Brown LBRS-FS) in one batch (Lot No. 43763). The molecular structures of these test dyes are given in Appendix E and show the occurrence of the benzidine moiety in each structure. The identity and purity of each chemical were determined by analyses at Midwest Research Institute. According to the manufacturer, the purities by dyestuff assay of direct blue 6, direct black 38, and direct brown 95 were 66%, 86%, and 79%, respectively; according to analyses performed at Midwest Research Institute, the corresponding purities by titration of azo groups with titanous chloride were 59.9 $\pm$ 1.9%, 87.1 $\pm$ 3.4%, and 72.2 $\pm$ 7.0%. Elemental analyses (for all elements except oxygen) were reasonably consistent with the molecular formulas of direct blue 6 ($C_{32}H_{20}O_{14}N_6Na_4S_4$), direct black 38 ($C_{34}H_{25}N_9O_7S_2Na_2$), and direct brown 95 ($C_{31}H_{20}N_6O_9SNa_2 \cdot Cu$), after correction for the percent dye determined by titanous chloride titration, the water content, and sodium chloride (estimated from analyses for Na and

Cl); direct blue 6 and direct brown 95 were somewhat high in C, H, and Na. Water analyses (Karl Fischer) were 9.18 ± 0.51%, 7.13 ± 0.54%, and 4.99 ± 0.22%, respectively, and NaCl concentrations were estimated at 20.8%, 7.9%, and 14.9%. The infrared spectra of direct black 38 and direct brown 95 were consistent with those in the literature (Sadtler, 1960); the infrared spectrum for direct blue 6 was not consistent with that in the literature and could not be taken as assurance of identity. Thin-layer chromatographic analyses using two different solvent systems showed 8-15 minor or trace impurities. No attempt was made to identify or quantitate these impurities. High-pressure liquid chromatography showed several small impurity peaks in each of the dyes, but no benzidine (detection limit, 0.004%). The methodology used would have detected total amounts of benzidine — that is, both benzidine salts and free benzidine.

Mazola® corn oil (Best Foods, Division of CPC International, Inc., Englewood Cliffs, N. J.) was added to the dyes as a dust suppressant. The concentration of corn oil in the dye was 1.3%. Bulk dyes containing the corn oil were stored at 5°C.

B. Dietary Preparation

A 1-week supply of each diet was formulated 1 or 2 days before use by mixing Purina® Laboratory Chow® animal meal (Ralston

Purina Co., St. Louis, Mo.) and dye containing 1.3% corn oil. Weighed amounts of animal meal were combined with weighed amounts of dye containing the corn oil and mixed in a Patterson-Kelly twin shell blender for 15 minutes to assure homogeneity. Formulated diets were stored at 23°C until used. The control diets contained corn oil in amounts equal to that in the highest dose groups for each species; i.e., 39 ppm for rats and 163 ppm for mice. Corn oil was present in the diets containing the dyes at 3 to 39 ppm for rats and 5 to 163 ppm for mice.

Stability of diets formulated with 10% of the bulk dyes containing 1.3% corn oil was determined by analyses performed after storage for 2 weeks at -20°, 5°, 25°, or 45°C. Spectrophotometric analysis of extracts of the diets showed that each of the dyes was stable in feed for 2 weeks at all temperatures tested. Analyses for benzidine were not performed.

As a quality control test on the accuracy of diet preparations, the concentration of dye in one sample at each dose level was determined for each dye during the studies and verified to be within $\pm$ 10% of the required concentration.

C. Animals

Fischer 344 rats and B6C3F1 mice of each sex were obtained from Frederick Cancer Research Center, Frederick, Maryland, through

contracts with the Division of Cancer Treatment, National Cancer Institute. On arrival at the laboratory, the rats were 4 weeks of age and the mice were 4-5 weeks of age. All animals were quarantined (rats for 12 days, mice for 13 days) prior to the start of the studies. During the quarantine periods, all animals of each species and sex were examined, and several were necropsied to detect observable disease. For the study of each chemical, rats and mice of each sex were randomized into dosed or control groups from the quarantine pool by tables of random numbers, and marked to assure individual identification.

D. Animal Maintenance

All animals were housed in temperature- and humidity-controlled rooms. The temperature range was 21-23°C, and the relative humidity was maintained at 40-60%. The air in each room was filtered with high-efficiency particulate air (HEPA) filters and changed 20-25 times per hour. Fluorescent light provided 12 hours of illumination per day. Tap water was available ad libitum, and diets were replenished as necessary, usually at 2-day intervals. Fresh control and test diets were provided every week.

Both rats and mice were housed five per cage in solid polycarbonate cages (Lab Products, Inc., Garfield, N.J.) suspended from

stainless steel racks. Rack shelves were covered with spun-bonded polyester filters (Dupont 2024). Absorb-Dri® hardwood chip bedding (Lab Products, Inc., Garfield, N.J.) was used for all cages and was changed two times per week. All rat and mouse cages were changed two times per week and were mechanically washed at temperatures not less than 82°C using Exceed® detergent (Economics Labs, Inc., Osborn Building, St. Paul, Minn.). All feed hoppers were changed once per week. Automatic watering systems provided water for both the rats and the mice.

All rats were housed in one room, and the mice were housed in another room. No animals administered any other test compounds were housed in these rooms. Neither cage positions within the racks nor rack positions within the rooms were rotated.

E. Two-Week Toxicity Tests

Two-week toxicity tests were conducted with Fischer 344 rats and B6C3F1 mice to estimate the toxicity of each of the test dyes; on the basis of these tests various concentrations were selected for use in 13-week studies. In the 2-week tests, the dyes were administered in the feed at concentrations of 3,000, 6,000, 12,500, 25,000, and 50,000 ppm. Five males and five females of each species were administered each dose, and five males and five females of each species were given basal diets. After the

administration of the dyes for 2 weeks, all animals were killed and necropsied.

Rats administered the dyes had severe dose-related decreases in food consumption and dose-related depressions in mean body weight. The effects appeared even at the lowest concentrations administered. In rats administered direct blue 6 dye, one male given 50,000 ppm died on day 6; in rats administered direct black 38 dye, all males given 50,000 ppm except one and all females given 50,000 ppm except one died by day 9; and in rats administered direct brown 95 dye, one male given 50,000 ppm died on day 12 and all females given 12,500 ppm died by day 11. All other animals survived to the end of the tests. Gross observations of rats administered the different dyes included thymic atrophy, splenic enlargement, and darkening of the spleen and kidneys. In rats administered the direct black 38, pigmentation of the liver also was noted. The pigmentation of the spleen and kidneys was dose related; the thymic atrophy was attributed to the low consumption of food. Methemoglobin was measured in four animals from tests using each of the dyes and was found to be elevated.

Mice administered the dyes had depressions in mean body weights in all groups except the males administered direct blue 6 dye. The effects were generally dose related and extended in most

cases to all but the lowest doses administered. In mice administered direct blue 6 or direct brown 95 dyes, no deaths occurred; in mice administered direct black 38 dye, three males given 25,000 ppm died by day 10 and two females given 50,000 ppm died by day 12. All other animals survived to the end of the tests. Hunched appearance and lethargic body movement were noted in mice at the higher concentrations. Gross observations of mice administered the different dyes consisted primarily of pigmentation of spleen and kidneys in mice at the higher doses of the dyes, related directly to the dye. The brown-colored viscera and blood of mice administered direct black 38 dye was attributed to methemoglobin, although tests for concentration of methemoglobin in the blood were not performed for this species.

Concentrations for the 13-week subchronic toxicity studies were selected mainly on the basis of the effects of the dyes on mean body weight. Because of generally excessive weight losses in the male and female rats at 6,000 ppm or higher, the concentrations set for the rats were 190, 375, 750, 1,500, and 3,000 ppm; similarly, because of generally excessive weight losses in the male and female mice at 25,000 ppm or higher, the concentrations set for the mice were 750, 1,500, 3,000, 6,000, and 12,500 ppm, except for the females administered the direct brown 95 dye, for which the highest concentration was set at 6,000 ppm.

F. Thirteen-Week Subchronic Toxicity Studies

The test groups, doses administered, and times on study of the 13-week subchronic toxicity studies are shown in tables 1 and 2. These studies were conducted as a part of the bioassay protocol to establish concentrations for use in the 2-year bioassays of the test chemicals in both rats and mice.

G. Clinical and Pathologic Examinations

Inspections for mortality and morbidity were carried out twice daily. Clinical observations were recorded daily. Body weights of individual animals were determined weekly. Tests for benzidine in the urine were performed at weeks 4 and 12 of the studies for the rats and at weeks 3 and 11 for the mice. Tests for methemoglobin of the rats were performed at the end of the studies.

Moribund animals and those animals that survived to the end of the studies were killed using CO_2 anesthesia and necropsied. Necropsies were also performed on all animals found dead, except one that was cannibalized. The tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues were examined microscopically in all control rats and in rats administered 1,500 or 3,000 ppm of each dye and 750 ppm of direct brown 95

Table 1. Thirteen-Week Subchronic Toxicity Studies of Direct Blue 6, Direct Black 38,and Direct Brown 95 Dyes Administered in Feed to Rats

Sex and Test Group[a]	Initial No. of Animals[b]	Time on Study	
		Dosed (days)	Observed (days)[c]
Male			
Matched-Control[d]	10		93
190 ppm	10	91	2
375 ppm	10	91	2
750 ppm	10	91	2
1,500 ppm	10	91	2
3,000 ppm	10	91	2
Female			
Matched-Control[d]	10		93
190 ppm	10	91	2
375 ppm	10	91	2
750 ppm	10	91	2
1,500 ppm	10	91	2
3,000 ppm	10	91	2

[a]Each dye was mixed with animal meal to give the concentrations indicated. Corn oil was also present in the various dosed diets, with concentrations ranging from 3 to 39 ppm.

[b]Male and female rats were 6 weeks of age when placed on study.

[c]Surviving animals were necropsied beginning the second day after the end of the dosing period.

[d]Matched-control rats were fed animal meal containing 39 ppm corn oil.

Table 2. Thirteen-Week Subchronic Toxicity Studies of Direct Blue 6, Direct Black 38, and Direct Brown 95 Dyes Administered in Feed to Mice

Sex and Test Group[a]	Initial No. of Animals[b]	Time on Study	
		Dosed (days)	Observed (days)[c]
Male			
Matched-Control[d]	10		93
750 ppm	10	91	2
1,500 ppm	10	91	2
3,000 ppm	10	91	2
6,000 ppm	10	91	2
12,500 ppm	10	91	2
Female			
Matched-Control[d]	10		93
750 ppm	10	91	2
1,500 ppm	10	91	2
3,000 ppm	10	91	2
6,000 ppm	10	91	2
12,500 ppm	10	91	2

[a]Each dye was mixed with animal meal to give the concentration indicated. The concentrations given the female mice administered diet containing direct brown 95 dye were 375 to 6,000 ppm instead of the concentrations indicated. Corn oil was also present in the various dosed diets, with concentrations ranging from 5 to 163 ppm.

[b]Male and female mice were 6-7 weeks of age when placed on study.

Table 2. Thirteen-Week Subchronic Toxicity Studies of Direct Blue 6, Direct Black 38, and Direct Brown Dyes Administered in Feed to Mice

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[c]Surviving animals were necropsied beginning the second day after the end of the dosing period.

[d]Matched-control mice were fed animal meal containing 163 ppm corn oil.

dye: skin, lung, bone marrow, spleen, mandibular lymph node, mesenteric lymph node, thymus, heart, salivary gland, liver, pancreas, stomach, small intestine, colon, kidney, bladder, adrenals, thyroids, testes, and epididymis. Microscopic examination was also performed on the above tissues (plus bile duct) of control mice, males and females administered 12,500 ppm of each dye, females administered 6,000 ppm of direct brown 95 dye, and a male mouse that was administered 750 ppm of direct brown 95 dye and that died early. In addition, certain tissues were examined in rats and mice administered lower doses, as indicated in Appendix A, tables A1-A6 and Appendix B, tables B1-B7.

A few tissues from some animals were not examined. Thus, the number of animals from which particular organs or tissues were examined microscopically varies, and does not necessarily represent the number of animals that were placed on study in each group.

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design,

clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

Data on the incidences of neoplastic and nonneoplastic lesions were analyzed using the statistical techniques described in this section. Those analyses of the experimental results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site is examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control

animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of dosed animals at each dose level. When results for a number of dosed groups (k) are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966) requires that the P value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P values are shown.

The approximate 95 percent confidence interval for the relative risk of each dosed group compared to its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a dosed group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a dosed group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the dosed group than in the control.

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95% of a large number of identical experiments, the true ratio of the risk in a dosed group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result ($P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity, but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

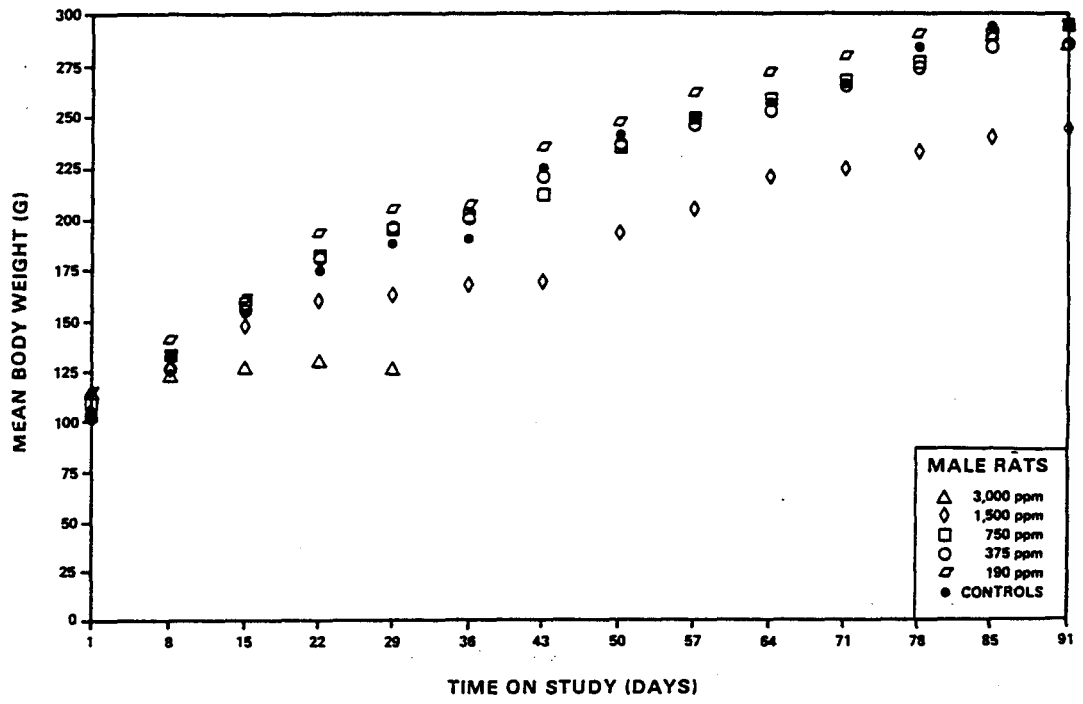
III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

Mean body weights of the male and female rats dosed with the two or three highest doses of any one of the test dyes were lower than mean body weights of the corresponding controls, and the depressions in mean body weight were dose related (figures 1, 2, and 3). No other clinical signs related to administration of the dyes were reported.

B. Benzidine and Methemoglobin Studies (Rats)

Urine collected over a 24-hour period during weeks 4 and 12 of the subchronic toxicity studies from male and female rats receiving each of three respective test dyes contained benzidine and monoacetyl benzidine, while specimens of urine taken from corresponding controls contained neither compound. The benzidine and monoacetyl benzidine were identified by thin-layer chromatography and mass spectroscopy. Quantities excreted in the urine were determined by combined extraction and spectrometric procedures. In most tests, the amounts excreted were dose related for each of the dyes administered. No consistent differences in results were found between males and females. Details of the methods and results are given in Appendix D.



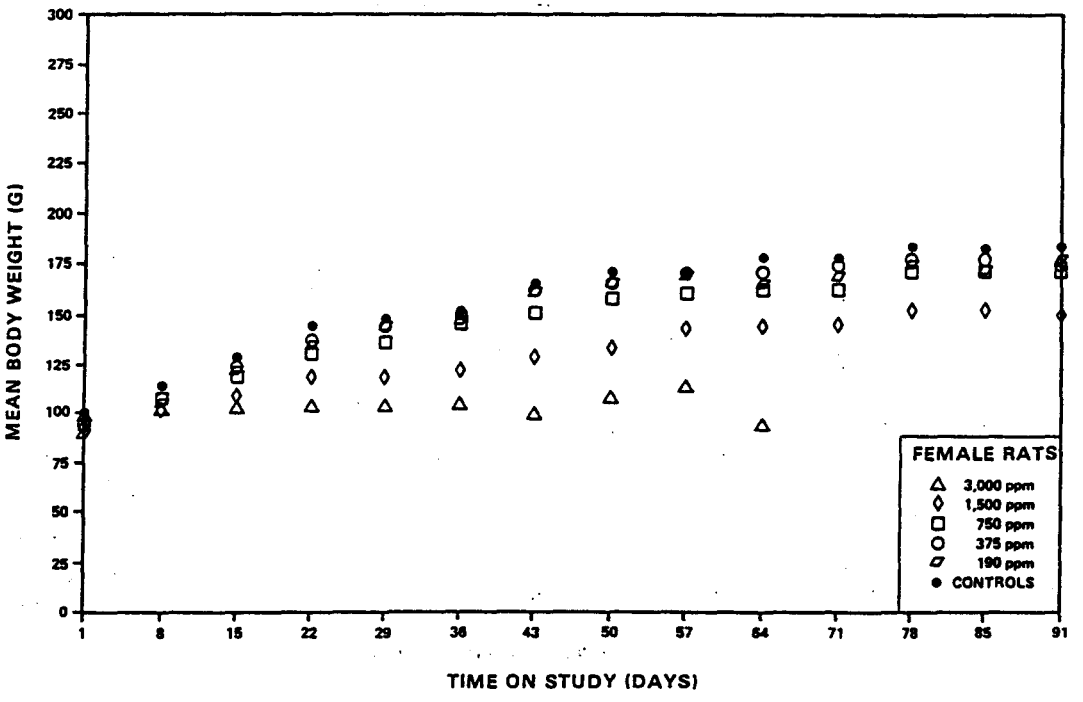
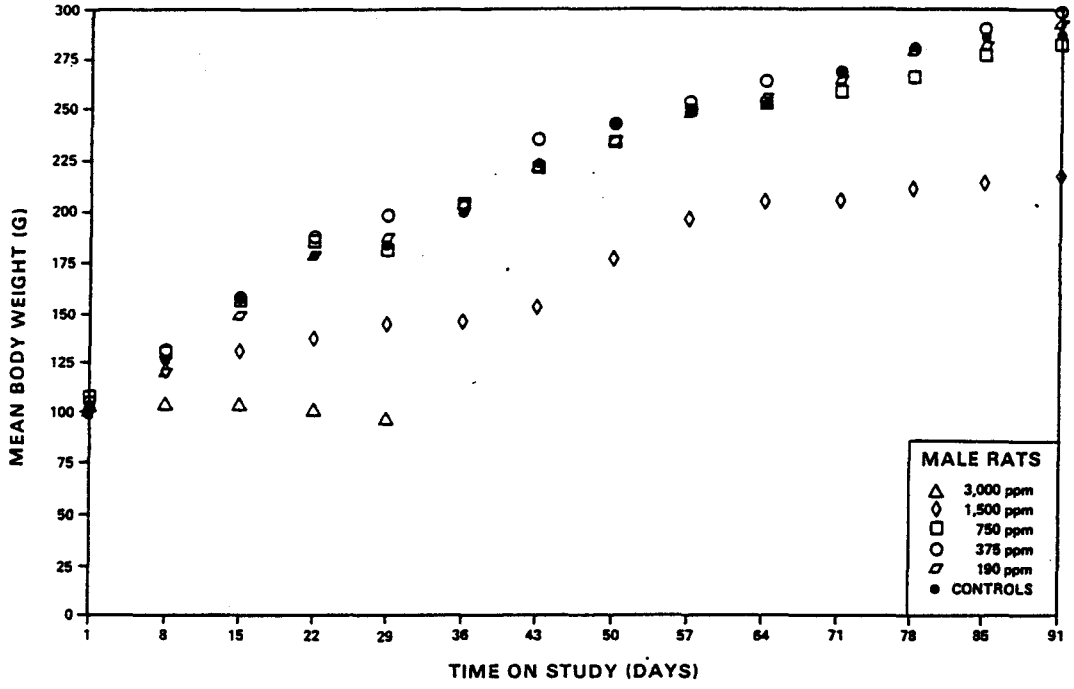


Figure 1. Growth Curves for Rats Administered Direct Blue 6 in the Diet



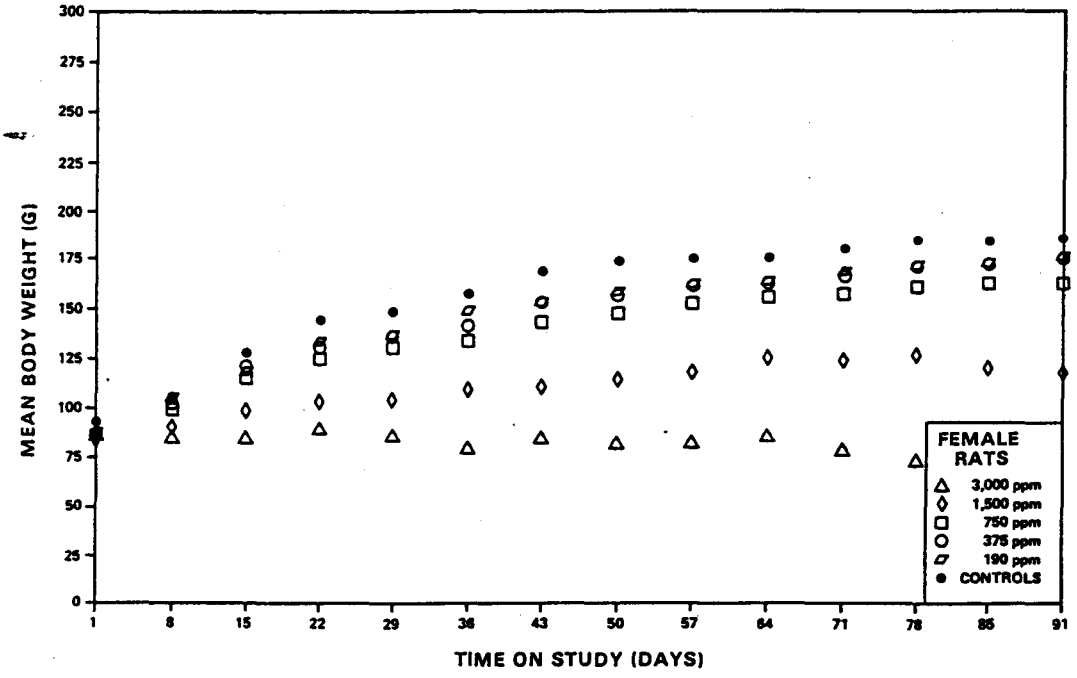
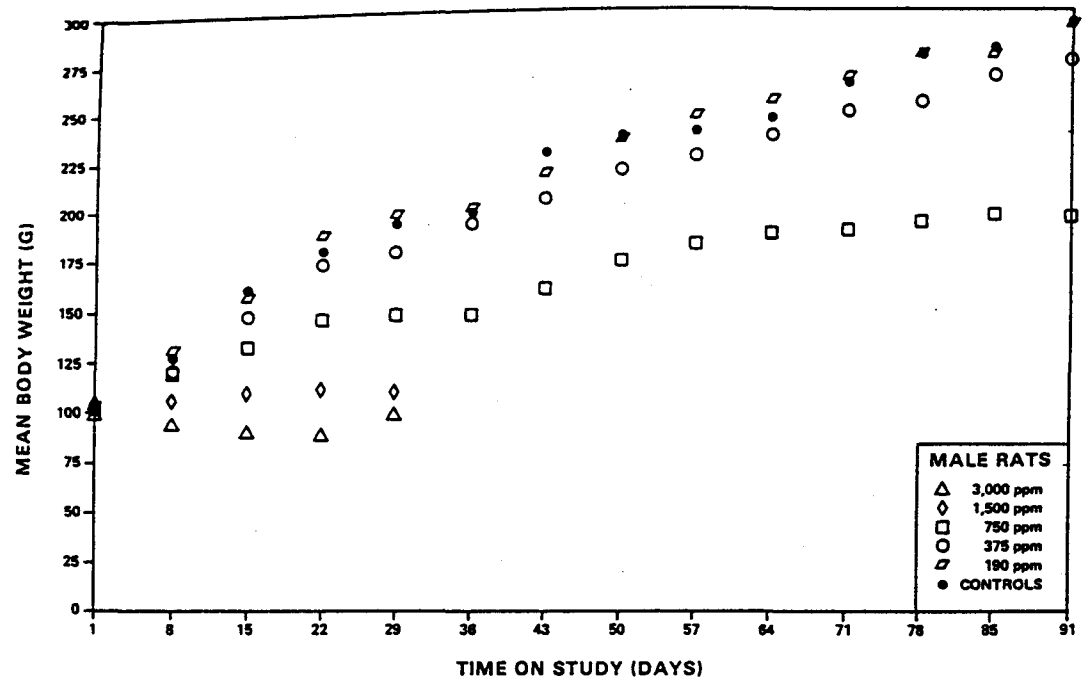


Figure 2. Growth Curves for Rats Administered Direct Black 38 in the Diet



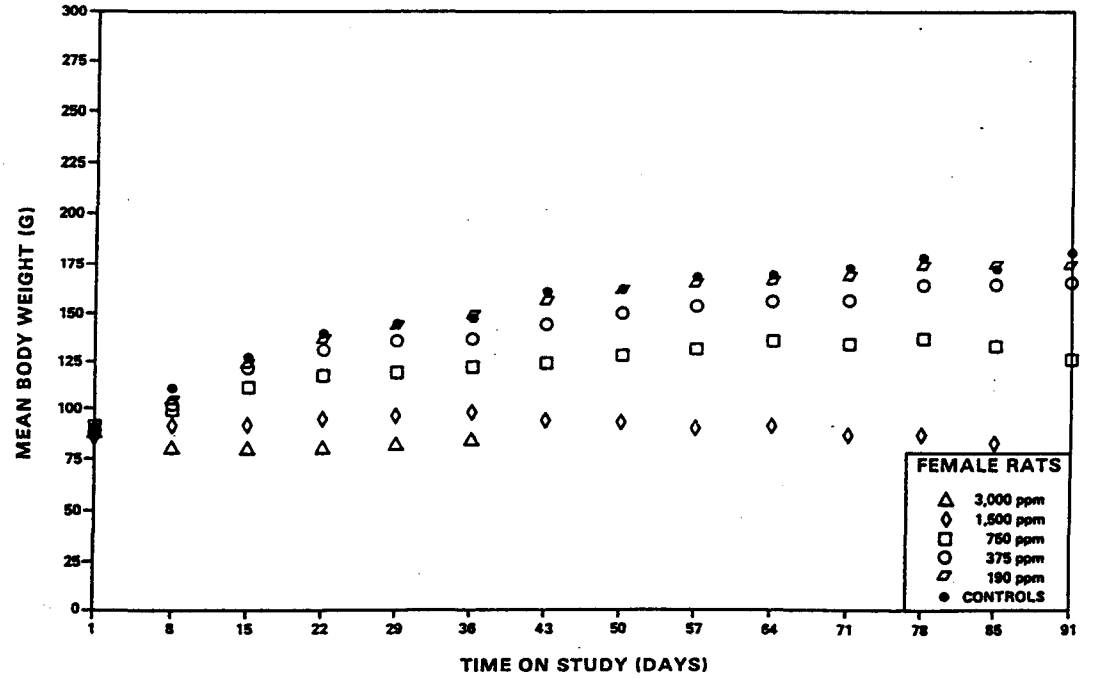
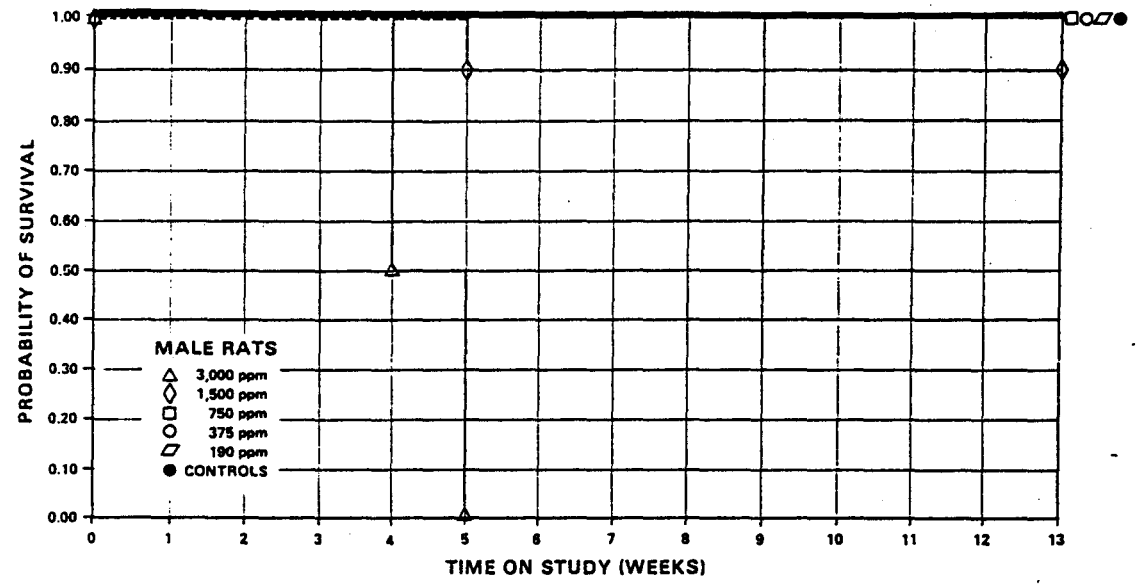


Figure 3. Growth Curves for Rats Administered Direct Brown 95 in the Diet

Concentrations of methemoglobin (Evelyn and Malloy, 1938) were measured because methemoglobin was elevated in selected rats at the higher dose concentration in the 2-week toxicity test. However, the dye concentrations administered in the 13-week studies were much lower, and determinations of methemoglobin in rats administered various doses of each of the dyes were not different from those of control rats.

C. Survival (Rats)

Curves of survival of control rats and of rats dosed with each of the test dyes are shown in figures 4, 5, and 6. All male and female rats administered 3,000 ppm of any one of the dyes and all male and female rats administered 1,500 ppm direct brown 95 dye died before the termination of the studies. One male administered 1,500 ppm direct blue 6 dye, six males administered 1,500 ppm direct black 38 dye, and two males administered 750 ppm direct brown 95 dye also died before the termination of the studies. No deaths occurred in any other dosed group or in any control group. The mortality was dose related, and, based on times and incidences of deaths, direct brown 95 dye was most toxic, followed in order by direct black 38 dye, then direct blue 6 dye.



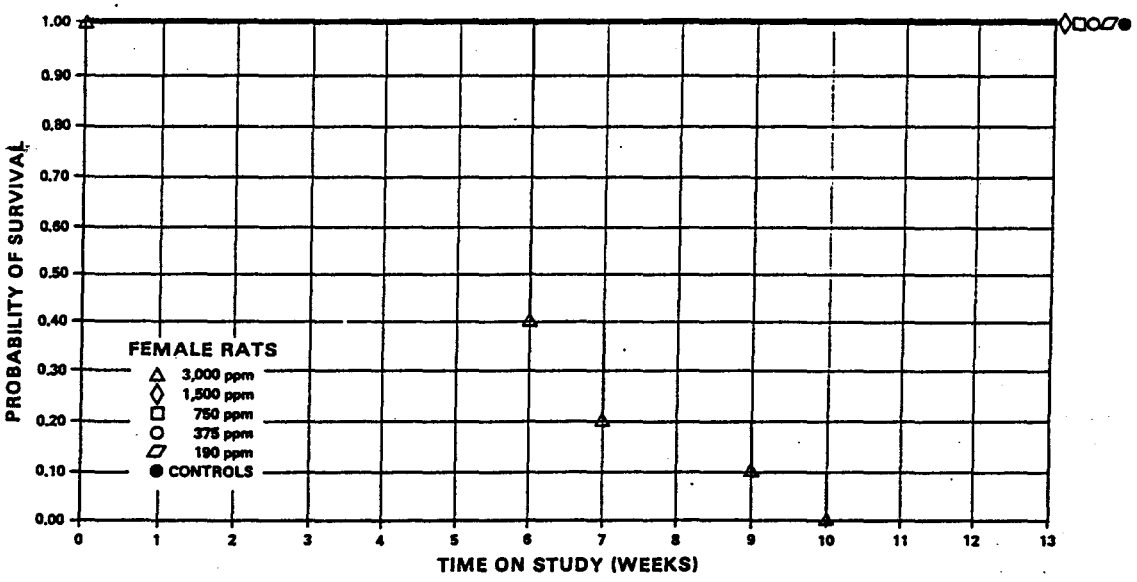
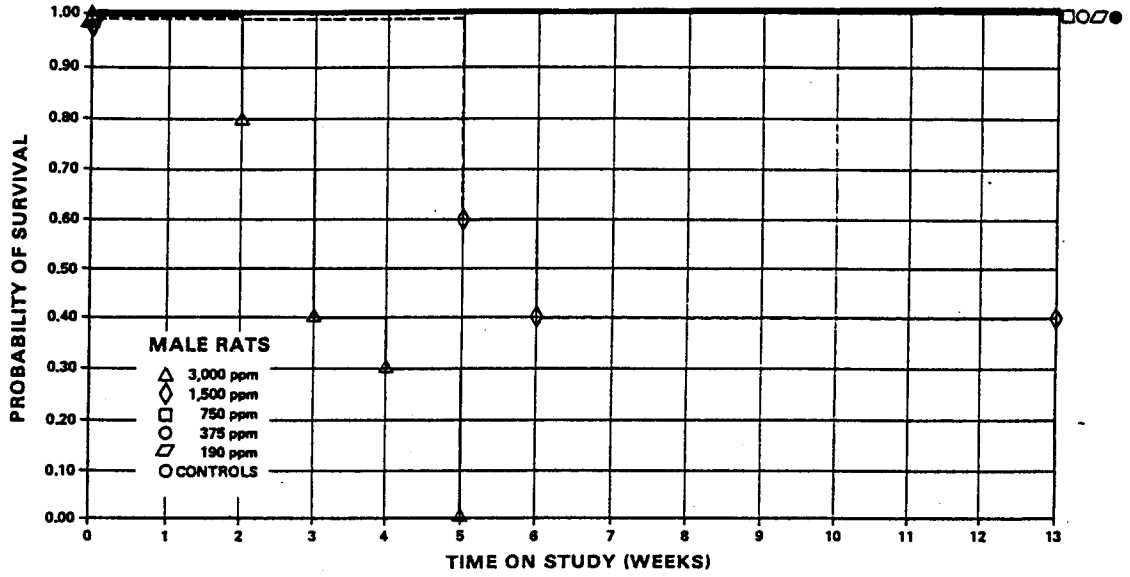


Figure 4. Survival Curves for Rats Administered Direct Blue 6 in the Diet



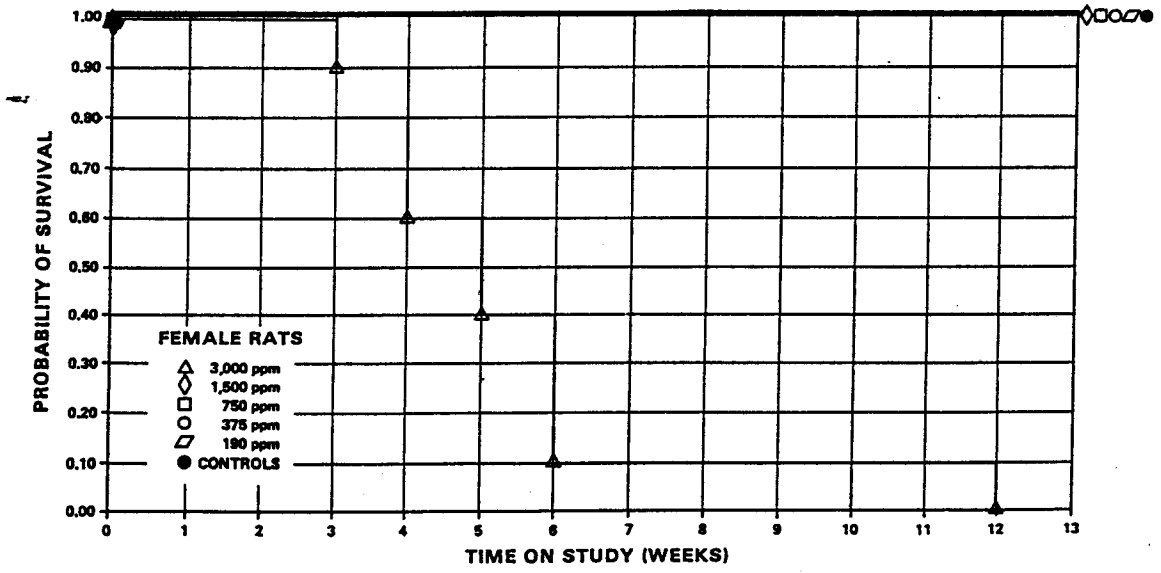
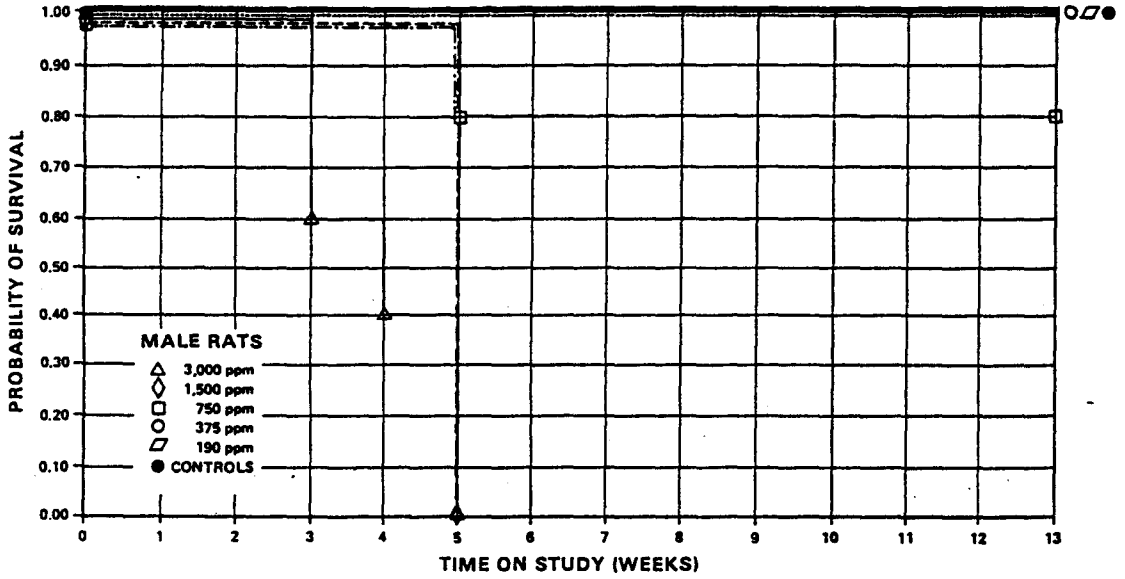


Figure 5. Survival Curves for Rats Administered Direct Black 38 in the Diet



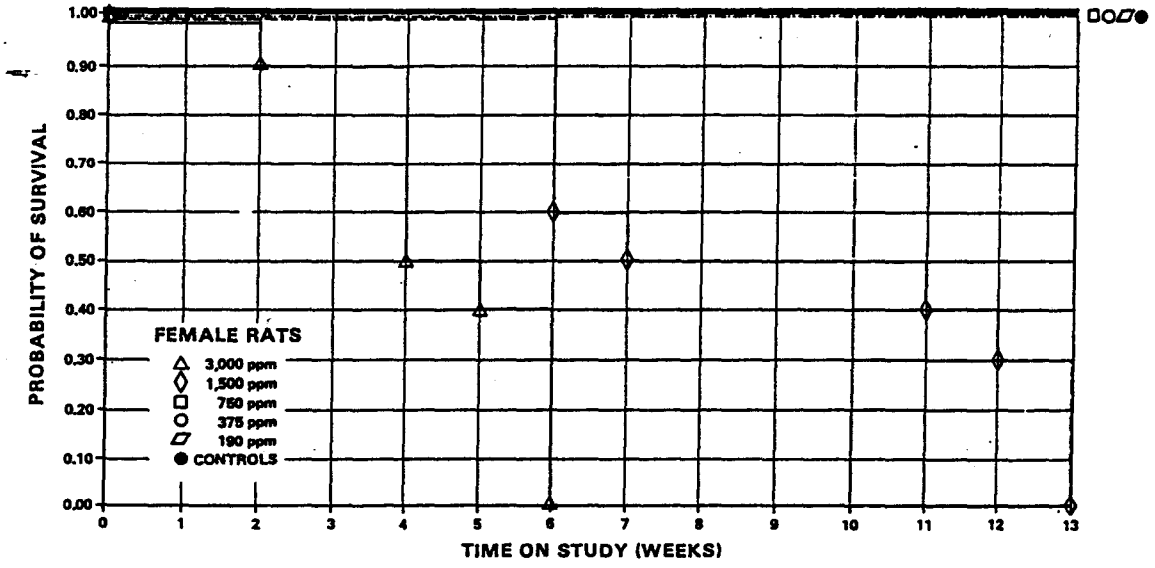


Figure 6. Survival Curves for Rats Administered Direct Brown 95 in the Diet

D. Pathology (Rats)

Gross Lesions. Gross lesions that were related to administration of any one of the dyes varied, depending on the dose and the length of time the animals survived. Livers of the rats given the highest doses and dying first (generally the males) were pale, yellow, or tan; these animals had ascites, hydrothorax, and edema of subcutaneous tissues and intestinal submucosa. Rats surviving longer but not to the end of the studies had, in addition, roughened surfaces on their livers. Rats given the highest doses at which survival was complete had livers with more severely roughened surfaces, due to random, multiple, pale, 2- to 3-mm spherical nodules that were scattered throughout the hepatic parenchyma and that elevated the capsule.

Histopathologic Lesions. Histopathologic lesions observed in control and dosed rats are summarized in Appendix A. Tables A1, A3, and A5 list those lesions that were observed only in rats administered dye in the diet; tables A2, A4, and A6 list other lesions. The histopathologic lesions varied, depending largely on the time of death. The terms "basophilic foci," "foci of cellular alteration," "neoplastic nodule," "hepatocellular carcinoma," and "cholangiofibrosis," applying to lesions of the liver, are used as defined by Squire and Levitt (1975). The term "basophilic foci" is used separately from the term "foci of

cellular alteration," because of the possible greater significance of the basophilic lesion. The term "nodular regeneration" refers to lobules containing hepatocytes that appear (1) to be normal in arrangement, size, shape, and tinctorial quality, but often lacking in central veins or portal areas or both, (2) to be pushing against adjacent areas, and (3) to be larger than normal lobules. They are delineated by focal biliary hyperplasia and fibrosis.

The first animals to die during administration of each of the dyes had varying degrees of biliary hyperplasia, lymphoid depletion of the spleen and the thymus, and myeloid depletion of the bone marrow. Animals that survived longer had more numerous proliferative changes of the liver, including biliary hyperplasia, cholangiofibrosis, nodular regeneration, foci of cellular alteration, neoplastic nodules, and hepatocellular carcinomas. Some of the rats had histopathologic evidence of bacterial septicemia just prior to death.

The most severely affected livers were usually of one of two types: (1) a liver with severe oval cell (biliary, cholangiolar) hyperplasia, multiple foci of cellular alteration, and nodules or (2) a liver with cirrhosis and nodules. In the first type (oval cell), hyperplasia started as a mild increase in periportal oval cells and progressed to large numbers of these cells,

proliferating along the sinusoids in such a fashion as to almost obscure the hepatocytes throughout the lobule. The initial proliferative hepatocellular lesions seen were multiple foci consisting of 10 to 20 or more cells that were larger and much more basophilic than normal hepatocytes. These cells also had larger, more vesicular nuclei, and some mitotic figures were seen. The basophilic foci appeared to progress to neoplastic nodules with basophilic hepatocytes, compressing adjacent parenchyma. The larger nodules with foci of prominent trabecular formation and acini were diagnosed as hepatocellular carcinomas. At least one carcinoma invaded the wall of a vein. None metastasized. The other foci of cellular alteration of hepatocytes included cells with clear cytoplasm, with cytoplasm containing eosinophilic droplets, or with cytoplasm having an eosinophilic, ground-glass appearance.

The second type of severely affected liver occurred in those rats that survived to the end of the studies after receiving the highest dose. These rats had cirrhosis (nodular regeneration and biliary hyperplasia, focal) characterized by multifocal, roughly spherical, nodular aggregations of hepatocytes; the hepatocytes generally appeared normal, although the lobules sometimes lacked central veins. Oval cells and connective tissue separated the regenerative nodules from each other. These livers contained

neoplastic nodules composed of large hepatocytes with eosinophilic cytoplasm. Hepatocellular carcinomas were diagnosed when neoplastic nodules contained foci of basophilic hepatocytes forming prominent trabeculae. Bizarre mitotic figures were occasionally noted. Cholangiofibrosis was commonly seen in livers of rats with neoplastic nodules and hepatocellular carcinomas. The severity of the proliferative changes of the liver decreased as the doses decreased; rats administered 190 or 375 ppm direct blue 6 dye and rats administered 190 ppm direct black 38 dye had essentially normal livers.

Lesions of the spleen, thymus, and bone marrow were characterized by a marked decrease in the number of mature lymphocytes in the white pulp of the spleen and in the cortex of the thymus and of the myeloid elements in the bone marrow.

Those rats receiving 1,500 ppm direct black 38 dye or 750 ppm direct brown 95 dye and surviving to the end of the studies had subacute glomerulonephropathy characterized by an eosinophilic amorphous material in Bowman's space and in the lumen of adjacent tubules. Some affected glomeruli had parietal epithelial cells in Bowman's capsule. There was some thickening of glomerular basement membranes. These kidney lesions were not observed in the rats administered direct blue 6 dye.

All females administered 375 ppm direct brown 95 dye had a degenerative change in pancreatic acinar epithelial cells. Individual cells had separated from the basement membrane and were rounded, with pyknotic nuclei.

Other lesions were considered incidental and not related to administration of the test dyes.

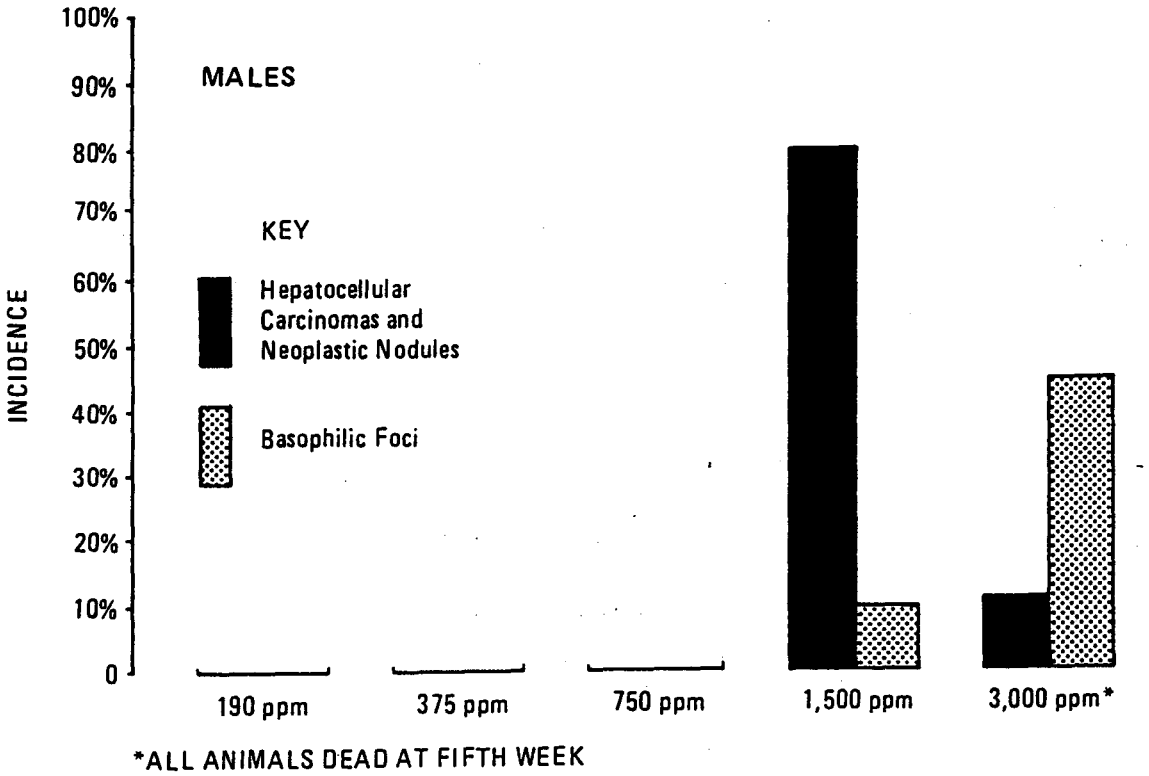
Based on the histopathologic examination, it was concluded that proliferative and neoplastic lesions were induced in the livers of Fischer 344 rats by each of the three test dyes administered for 13 weeks.

E. Statistical Analyses of Results (Rats)

Tables C1-C6 in Appendix C contain the statistical analyses of liver tumors which, along with other morphology concerning changes in liver cells, were observed in the studies of the three chemicals. Since each group for each chemical contained a maximum of 10 animals, the power of the Fisher exact test to determine significance of results is low; for example, with 0/10 incidence of a lesion in the controls, a significant result of $P \leq 0.05$ is not seen until the incidence of the lesion in a dosed group is over 4/10 (40%), at which incidence $P = 0.043$. The higher dosed groups developed neoplastic or nonneoplastic lesions that did not appear in the controls or in lower dose groups. In

some instances, mostly those involving nonneoplastic morphology, Fisher exact test results have P values lower than the 0.01 level required for an overall 0.05 significance level, taking into account the criterion for multiple comparisons of five dosed groups with a single control.

In male rats, liver tumors were observed in the 1,500 ppm- (8/10, 80%; $P < 0.001$) and the 3,000 ppm- (1/9, 11%; P is not significant) dose groups fed direct blue 6 as well as in the 1,500 ppm- (9/9, 100%; $P < 0.001$) dose group fed direct black 38 (see figures 7, 8, and 9). No incidence of these tumors appeared in any of the three control groups or in any dosed group fed direct brown 95. Foci of cellular alteration or basophilic foci were observed in significant incidences ($P < 0.01$) in the 750 ppm- and 1,500 ppm-dose groups fed direct blue 6, in the 375 ppm- and 750 ppm-dose groups fed direct black 38, and in the 375 ppm- and 1,500 ppm-dose groups fed direct brown 95, when compared with corresponding control groups. Incidences of these foci were also observed in the 3,000 ppm-dose group fed direct blue 6, the 1,500 ppm-dose group fed direct black 38, and in the 750 ppm- and 3,000 ppm-dose groups fed direct brown 95. These observed incidences are in contrast to the absence of such incidences in any of the control and 190 ppm-dose groups. In some of the higher dosed groups, occurrences of either neoplastic nodules or cell changes



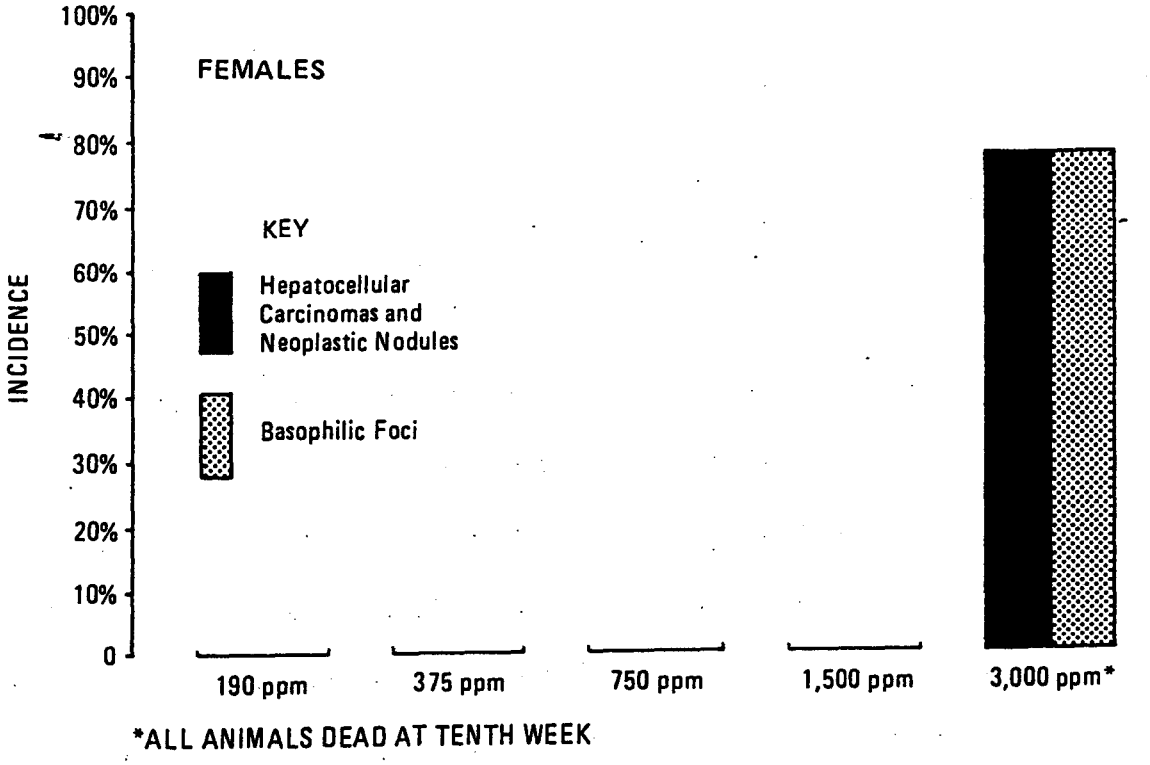
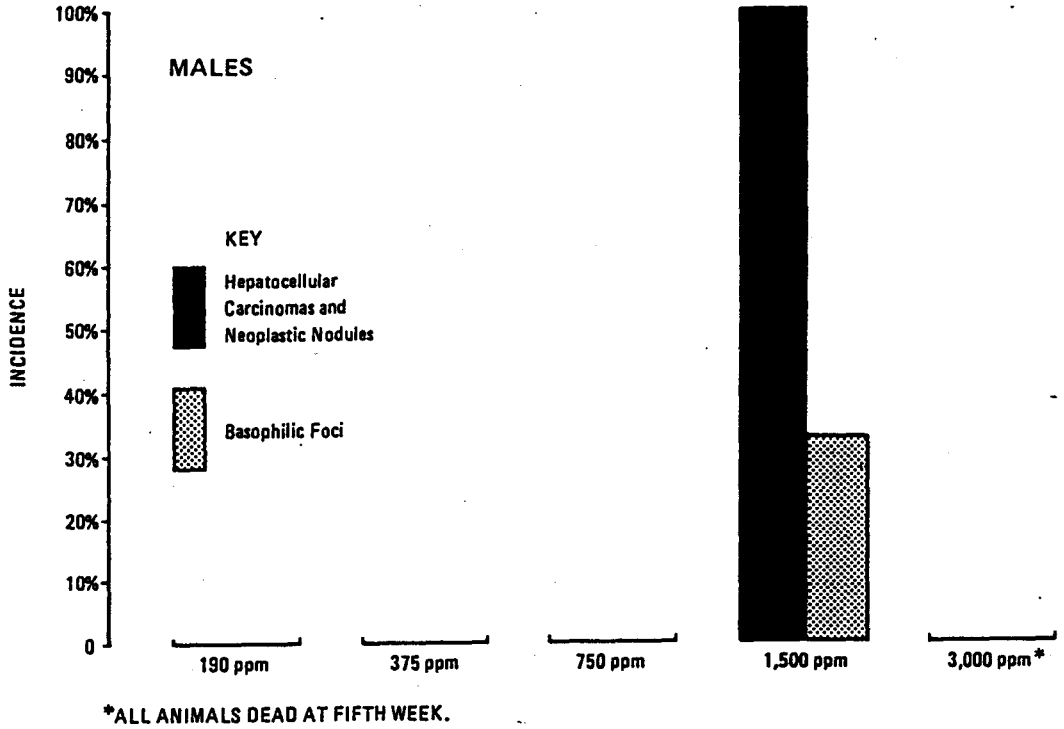
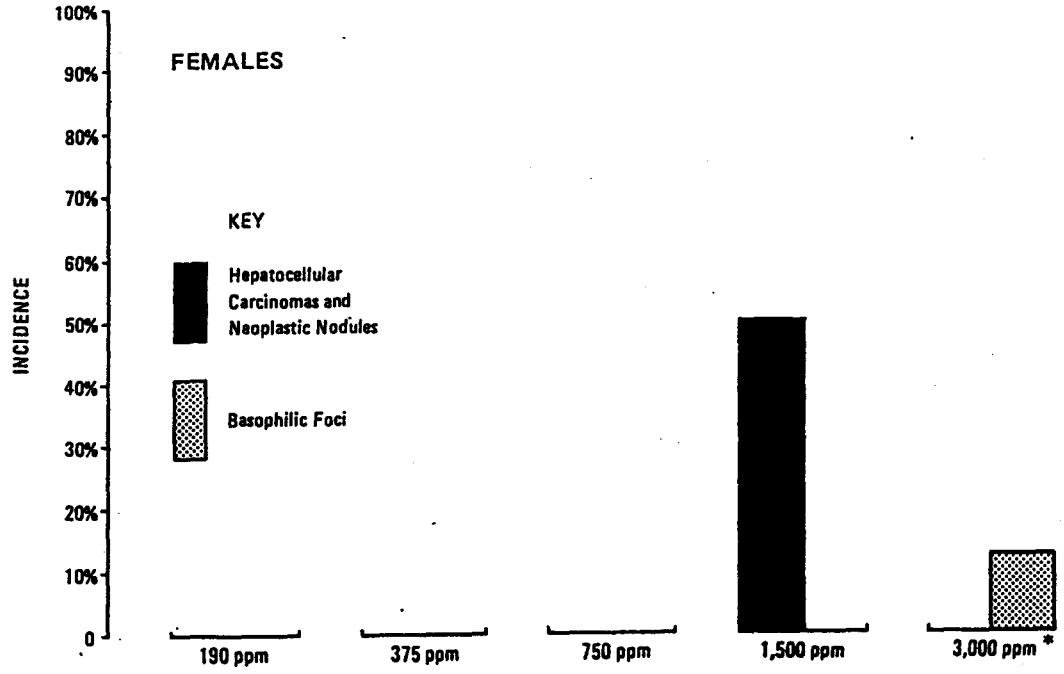


Figure 7. Hepatic Lesions Observed in Rats Administered Direct Blue 6 in the Diet

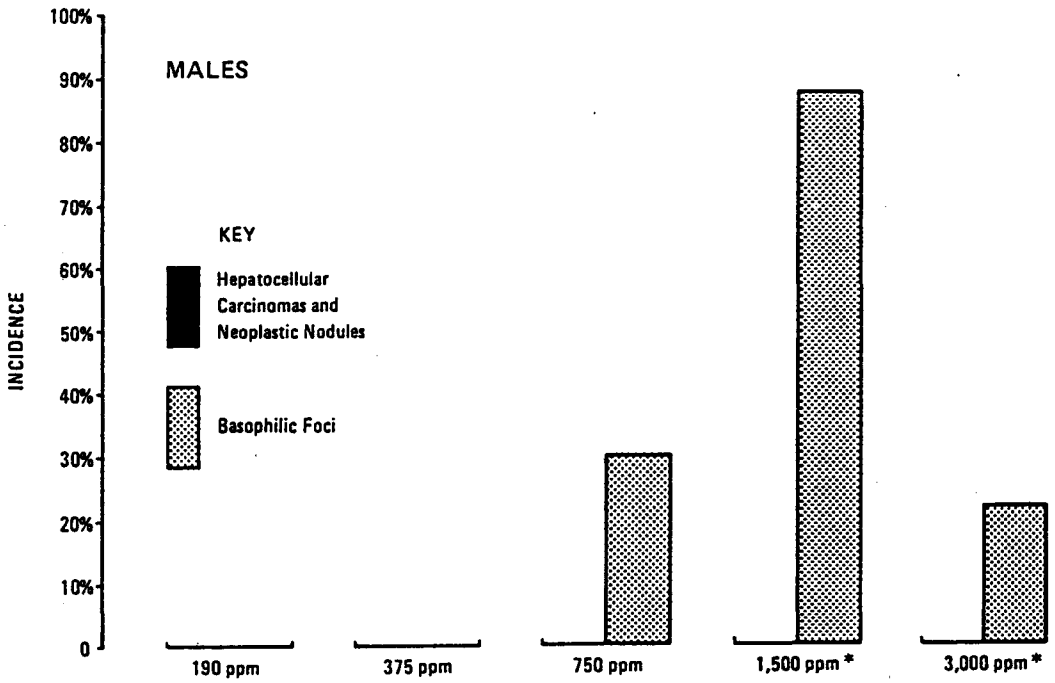


*ALL ANIMALS DEAD AT FIFTH WEEK.

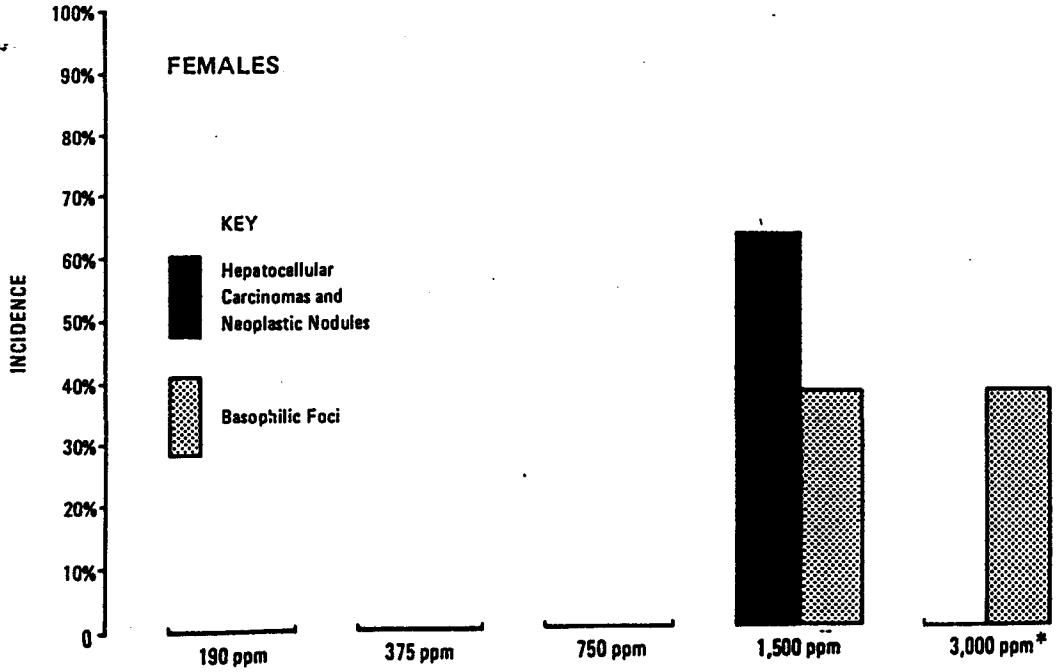


*ALL ANIMALS DEAD AT TWELFTH WEEK.

Figure 8. Hepatic Lesions Observed in Rats Administered Direct Black 38 in the Diet



*ALL ANIMALS DEAD AT FIFTH WEEK.



*ALL ANIMALS DEAD AT SIXTH WEEK.

Figure 9. Hepatic Lesions Observed in Rats Administered Direct Brown 95 in the Diet

were observed as early as week 4 on study. The data on control male Fisher 344 rats, compiled to date from 2-year studies performed by all laboratories in the Carcinogenesis Testing Program, indicate an incidence of neoplastic nodules or hepatocellular adenomas or carcinomas of 32/1,806 (1.8%). There were no such tumors in the 220 male rats that died before week 78.

In female rats, liver tumors were observed in the 3,000 ppm-dose group (7/9, 77%; P = 0.001) fed direct blue 6, in the 1,500 ppm-dose group (5/10, 50%; P = 0.016) fed direct black 38, and in the 1,500 ppm-dose group (5/8, 63%; P = 0.007) fed direct brown 95, but in none of the controls or the three lower dosed groups of each study in the females. Foci of cellular alteration or basophilic foci occurred in significant incidences ($P < 0.01$) in the 750 ppm-, 1,500 ppm-, and 3,000 ppm-dose groups fed direct blue 6, in the 750 ppm- and 1,500 ppm-dose groups fed direct black 38, and in the 1,500 ppm-dose group fed direct brown 95. Some incidences of these foci were observed in the 375 ppm- and 3,000 ppm-dose groups fed direct black 38 and in the 375 ppm-, 750 ppm-, and 3,000 ppm-dose groups fed direct brown 95. Historical records from 2-year studies indicate that in control animals the incidence of neoplastic nodules or hepatocellular adenomas or carcinomas was 55/1,765 (3.1%). There were two such

tumors in the 182 female rats that died prior to week 78 on study.

In summary, the occurrence of lesions of the liver at statistically significant levels in dosed rats when compared with controls as well as the comparison of incidences of the lesions in these present 13-week subchronic toxicity studies with those in historical records indicate that the observed hepatocellular carcinomas, neoplastic nodules, and related proliferative lesions are associated with the administration of the test dyes.

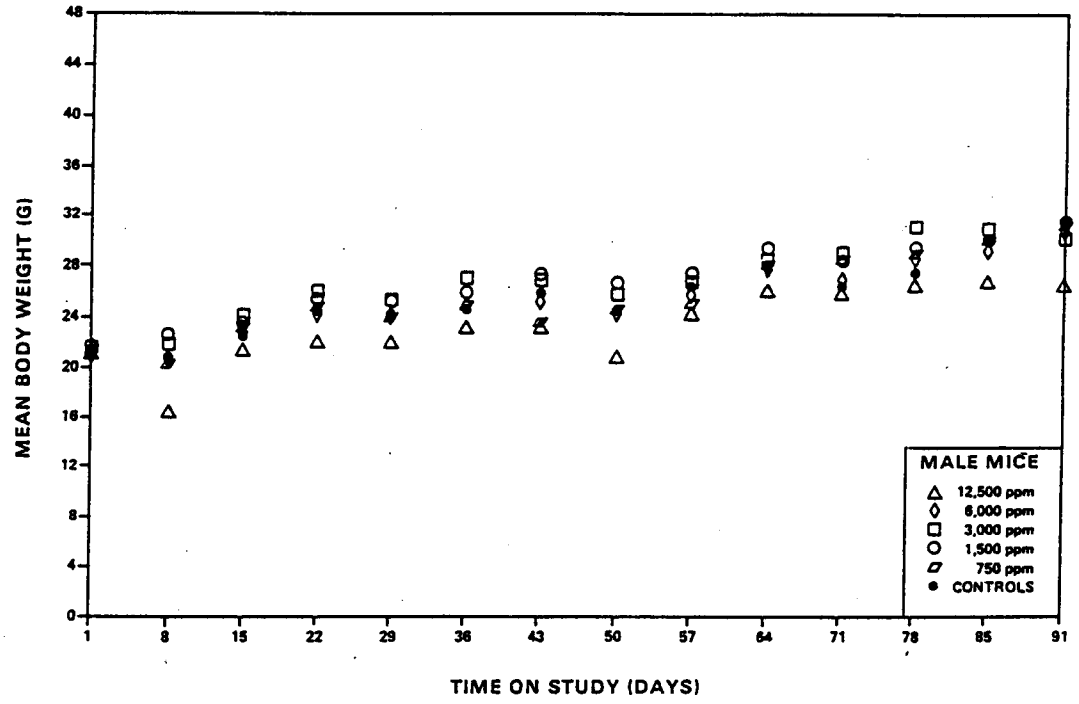
IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of the male and female mice administered the highest dose of any one of the test dyes were slightly lower than mean body weights of the corresponding controls; mean body weights of mice administered lower doses were generally unaffected (figures 10, 11, and 12). No other clinical signs related to administration of the dyes were reported.

B. Benzidine Studies (Mice)

Urine collected over a 24-hour period during weeks 3 and 11 of the subchronic toxicity studies from male and female mice dosed with any one of the test dyes was found to contain benzidine and monoacetyl benzidine, while specimens of urine taken from corresponding control groups contained neither compound. The benzidine and monoacetyl benzidine were identified by thin-layer chromatography and mass spectroscopy. Quantities excreted in the urine were determined by combined extraction and spectrometric procedures. In most tests, the amounts excreted were dose related for each of the dyes administered. No consistent differences in results were found between males and females. Details of the methods and results are given in Appendix D.



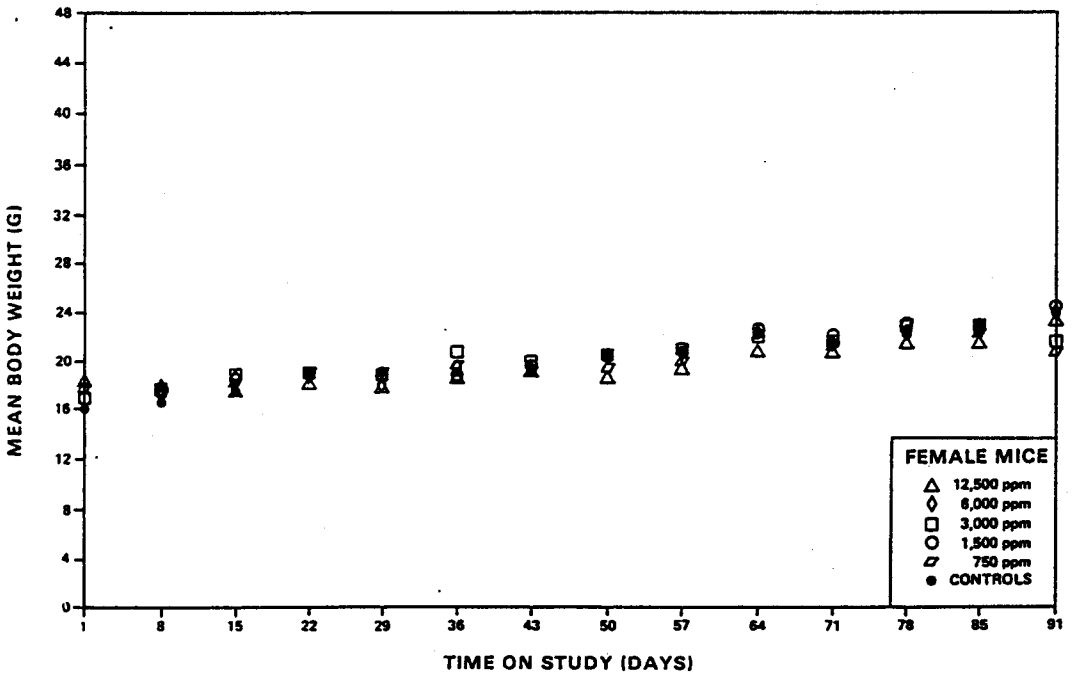
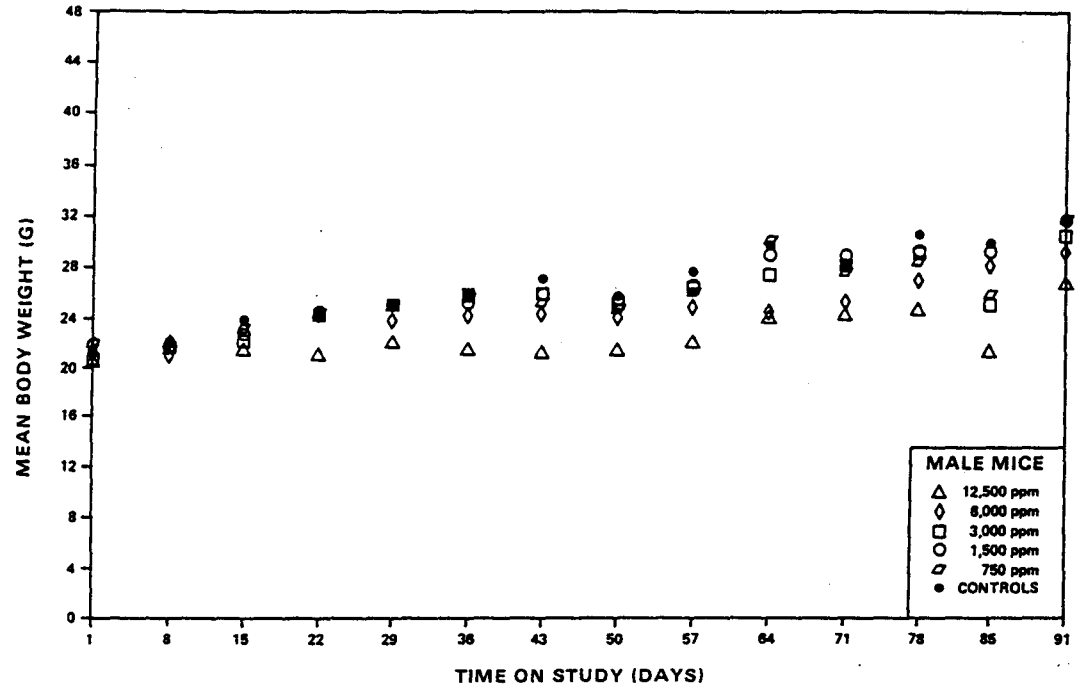


Figure 10. Growth Curves for Mice Administered Direct Blue 6 in the Diet



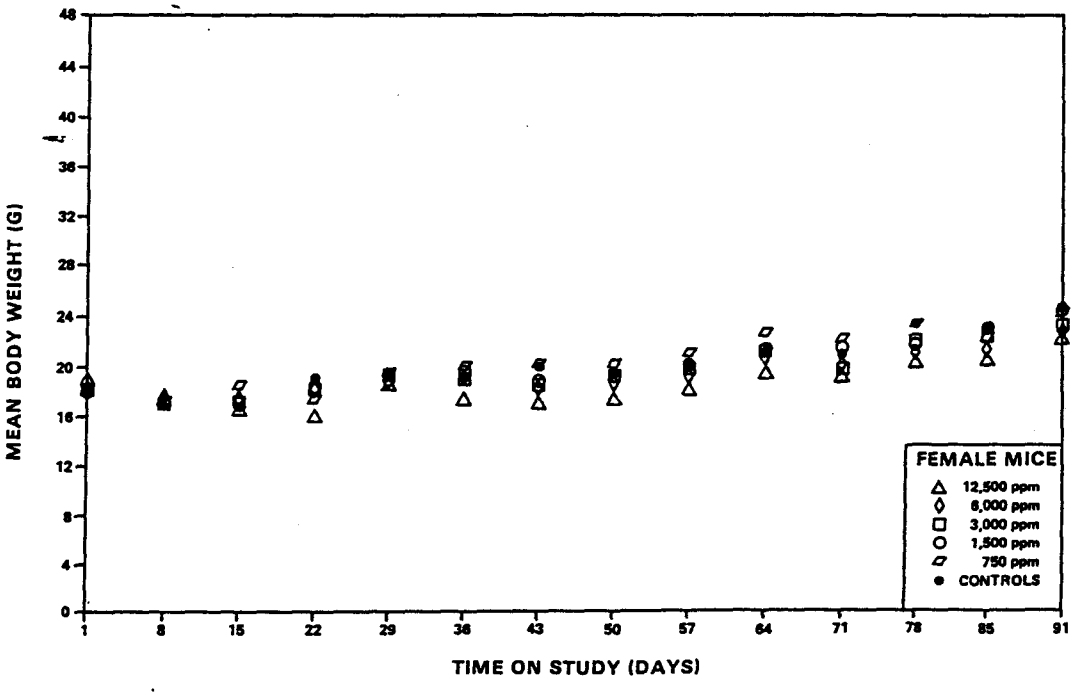
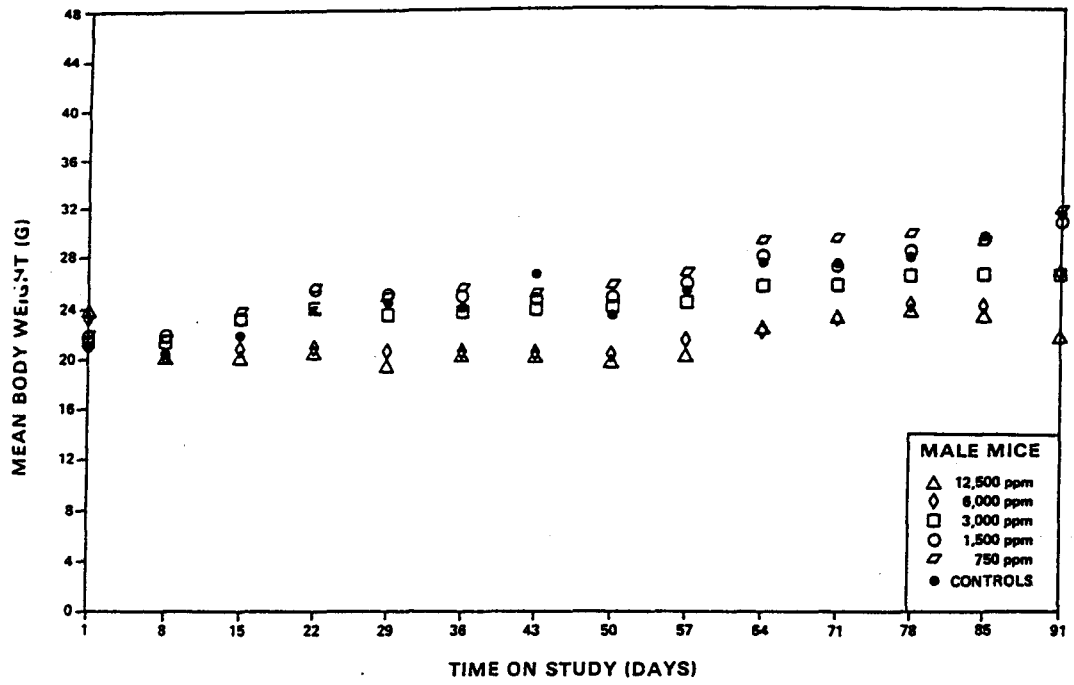


Figure 11. Growth Curves for Mice Administered Direct Black 38 in the Diet



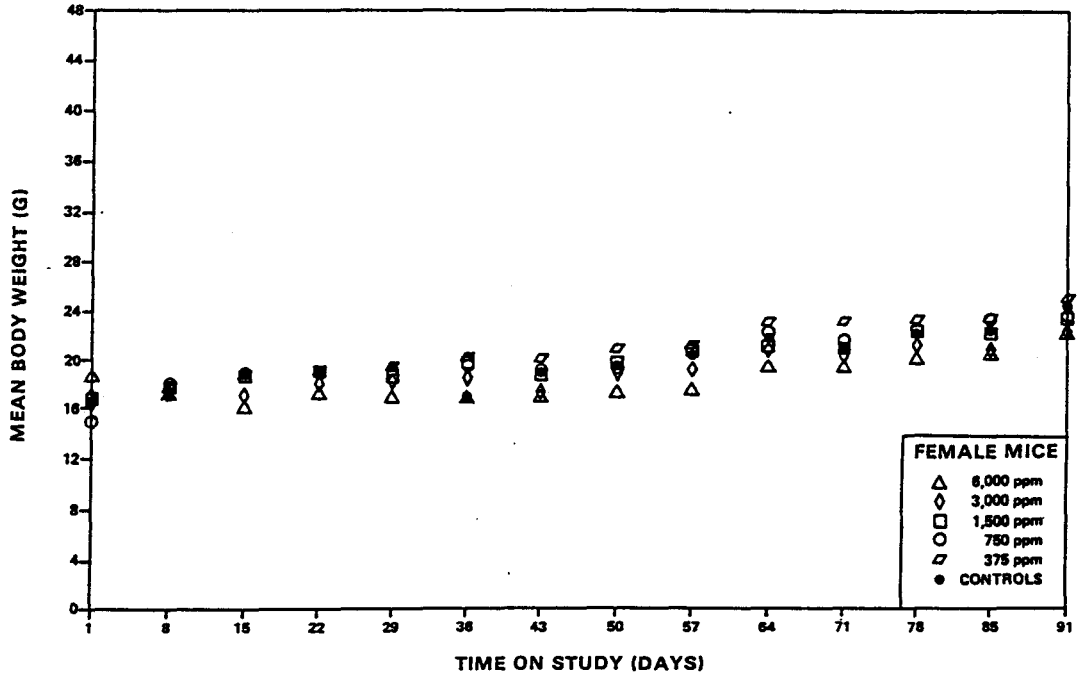


Figure 12. Growth Curves for Mice Administered Direct Brown 95 in the Diet

C. Survival (Mice)

All male and female mice administered any one of the doses of the dyes survived to the end of the subchronic toxicity studies, except for one male administered 750 ppm direct brown 95 dye. The death of this animal was attributed, however, to bacterial infection, and was not related to administration of the dye.

D. Pathology (Mice)

Gross Lesions. Gross lesions observed in mice administered direct blue 6 at doses of 6,000 or 12,500 ppm consisted of bluish-black and slightly enlarged spleens. Those observed in mice administered direct black 38 dye at 12,500 ppm consisted of slightly darkened livers and of enlarged and darkened spleens; those observed in the mice administered 6,000 ppm consisted of slightly enlarged and darkened spleens. Similar lesions were not reported in mice administered direct brown 95 dye.

Histopathologic Lesions. Histopathologic lesions observed in control and dosed mice are summarized in Appendix B. Tables B1, B3, and B5 list those lesions that were observed only in mice administered dye in the diet; tables B2, B4, B6, and B7 list other lesions. The findings observed in animals administered any one of the test dyes consisted mainly of hemosiderosis of the kidney and pigmentation of the liver at the highest doses and of

hemosiderosis of the spleen at low as well as high doses. The splenic hemosiderosis was characterized by an increase in the golden brown, iron-positive pigment that is normally seen in macrophages in the red pulp. The kidney hemosiderosis consisted of a finely granular, iron-positive pigment in epithelial cells of the proximal convoluted tubules; the pigment was difficult to detect without use of the Prussian-blue reaction. The hepatic pigment was yellow to yellowish-green and occasionally iron-positive; it occurred in occasional macrophages lining the sinusoids. Pigment of the thyroid, observed only in mice administered the two highest doses of direct black 38 dye, was finely granular, yellow, iron-negative, and located largely in the follicular cells.

Biliary hyperplasia was observed in the mice administered the highest doses of direct black 38 and direct brown 95 dyes, but not in mice administered direct blue 6 dye. The hyperplasia consisted of a modest increase in the number of biliary cells adjacent to most portal areas. Other hepatic lesions were observed in mice administered direct black 38 dye or direct brown 95 dye, but not in mice administered direct blue 6 dye. Hepatocellular degeneration, observed in 9/10-10/10 male and female mice administered 3,000, 6,000, or 12,500 ppm direct black 38 dye and in 10/10 male mice administered 12,500 ppm direct brown 95

dye, was characterized by pleomorphic nuclei, cytoplasmic vacuolization, eosinophilic droplet formation, and hydropic change, and, in the mice administered the direct black 38 dye, also an increased mitotic index. Three mice administered the highest dose of direct black 38 dye and one mouse administered the highest dose of direct brown 95 dye had foci of cellular alteration in their livers; in these instances, the hepatocytes were distinctly basophilic when compared with surrounding normal cells.

Based on the histopathologic examination, hemosiderosis of the spleen and kidney and pigmentation of the liver were the principal lesions occurring in B6C3F1 mice administered any one of the test dyes. The hepatocellular degeneration found in large numbers of mice given high doses of direct black 38 and direct brown 95 dyes also was related to administration of dye. No hepatic lesions occurred in mice administered direct blue 6 dye.

E. Statistical Analyses of Results (Mice)

No neoplasms occurred in the mice administered any one of the test dyes.

V. DISCUSSION

These subchronic toxicity studies of direct blue 6 dye, direct black 38 dye, and direct brown 95 dye were conducted as a part of the bioassay protocol for testing for possible carcinogenicity. Thirteen-week studies are conducted to establish respective doses of test chemicals to use in 2-year studies with both rats and mice.

In these feeding studies of the three dyes, mean body weights of the male and female rats administered the two or three highest concentrations of the test dyes were markedly lower than mean body weights of the corresponding controls throughout the studies, and the depressions in mean body weight were dose related. Mean body weights of the male and female mice administered the highest dose of any one of the test dyes were slightly lower than mean body weights of the corresponding controls; mean body weights of mice administered lower doses were generally unaffected.

All male and female rats administered 3,000 ppm of any one of the dyes or 1,500 ppm direct brown 95 dye died before the end of the studies. One male rat administered 1,500 ppm direct blue 6 dye, six males administered 1,500 ppm direct black 38 dye, and two males administered 750 ppm direct brown 95 dye also died by the

end of the subchronic toxicity studies. No deaths occurred in any other dosed group or in any control group of rats. Mortality in the rats was dose related, and based on times and incidences of deaths, direct brown 95 dye was most toxic, followed in order by direct black 38 dye, then direct blue 6 dye. All male and female mice administered the test dyes survived to the end of the studies, except for one male whose death was attributed to bacterial infection.

In rats, neoplastic lesions occurred only in dosed groups and consisted of hepatocellular carcinomas and neoplastic nodules of the liver. The time to onset of the tumors was remarkably short. The incidences of the hepatocellular carcinomas in female rats administered 3,000 ppm direct blue 6 dye (4/9) and male rats administered 1,500 ppm direct black 38 dye (4/9) were significant ($P = 0.033$) when related to the incidences of the tumors in the corresponding controls (0/10); hepatocellular carcinomas were also observed in two male rats administered 1,500 ppm direct blue 6 dye and in one female rat administered 1,500 ppm direct brown 95 dye. No control rats from any of the three studies developed hepatocellular carcinomas.

When incidences of neoplastic nodules were combined with those of hepatocellular carcinomas, the significance increased to $P < 0.001$ for male rats administered 1,500 ppm direct blue 6 dye, P =

0.001 for females administered 3,000 ppm direct blue 6 dye, $P < 0.001$ for males administered 1,500 ppm direct black 38 dye, and $P = 0.007$ for females administered 1,500 ppm direct brown 95 dye. No controls developed neoplastic nodules. Female rats administered direct black 38 dye developed no hepatocellular carcinomas, but had an incidence of neoplastic nodules of 5/10, with a significance of $P = 0.016$. Male rats administered direct brown 95 dye developed neither hepatocellular carcinomas nor neoplastic nodules, but as indicated below, had significant incidences of preneoplastic lesions. The failure of groups of rats administered 3,000 ppm dye to develop tumors when other groups administered 1,500 ppm did develop tumors may be due to earlier deaths at the higher dose.

Preneoplastic hepatic lesions (basophilic foci as described by Squire and Levitt, 1975) occurred only in dosed rats and did not occur in controls. The incidences of the basophilic foci were significant ($P \leq 0.033$) in male (4/9) and female (7/9) rats administered 3,000 ppm direct blue 6 dye and in male rats (7/8) administered 1,500 ppm direct brown 95 dye. Basophilic foci also occurred, at lower incidences, in males (1/10) administered 1,500 ppm direct blue 6 dye, in males (3/9) administered 1,500 ppm direct black 38 dye, in females (1/8) administered 3,000 ppm direct black 38 dye, in males administered 750 ppm (3/10) or

3,000 ppm (2/9) direct brown 95 dye, and in females administered 1,500 ppm (3/8) or 3,000 ppm (3/8) direct brown 95 dye. When incidences of foci of cellular alteration, a possible preneoplastic lesion, were added to those of basophilic foci, significance occurred in additional dosed groups.

In mice, no neoplastic lesions occurred in the liver or other tissues of groups administered the different dyes. The principal nonneoplastic lesions found in mice consisted of hemosiderosis of the kidney and pigmentation of the liver at doses of 6,000 or 12,500 ppm and of hemosiderosis of the spleen at low as well as high doses. Other nonneoplastic lesions in the dosed mice involved the liver. Both biliary hyperplasia and hepatocellular degeneration occurred in mice given high doses of direct black 38 dye or direct brown 95 dye. In addition, three mice administered 12,500 ppm direct black 38 dye and one mouse administered 12,500 ppm direct brown 95 dye had foci of cellular alteration, in which the cells were basophilic when compared with surrounding normal cells. No mice administered direct blue 6 dye had these lesions of the liver.

In previous work, Rinde and Troll (1975) reported that when azo dyes direct blue 6, direct black 38, direct brown 95, or an additional azo dye (direct red 28) were administered by gavage to rhesus monkeys, benzidine appeared in the urine in yields that

approximated those of animals administered equivalent amounts of free benzidine. In Wistar rats, the metabolic breakdown of benzidine-derived azo dyes to free benzidine has been demonstrated in incubation mixtures of such dyes with intestine (Miyakawa et al., 1973). In the present studies, benzidine and monoacetyl benzidine were detected in the urine of male and female rats and mice administered the test dyes, but neither compound was detected in the urine of control rats and mice.

The biliary (oval cell) lesions observed in Fischer 344 rats in the present studies have been previously reported to be induced in SHR, Wistar, Sprague-Dawley, and Buffalo rats by several chemicals that cause hepatocellular carcinoma (Ito et al., 1973) and by benzidine itself in Sherman rats (Spitz et al., 1950). The foci of cellular alteration, nodules, and carcinomas are identical to those caused by benzidine in Sherman rats (Spitz et al., 1950). In addition, the administration of benzidine in the diet of Wistar rats and hamsters has been reported to induce cholangiomas and hepatocytic tumors (Boyland et al., 1954; Saffiotti et al., 1967). Direct blue 6 dye and direct black 38 dye were reported not to induce tumors in female mice when the dyes were administered by implantation in the bladder in wax pellets (Niitsu, 1973); however, foci of alteration as well as hepatocellular carcinomas have been described in both male and

female mice given benzidine (Frith and Dooley, 1976). The failure of the dyes tested in the present studies to induce tumors in mice may have been due, however, to the short period of administration and/or observation. Papillomas and carcinomas of the bladder were found to develop in 3/7 dogs administered benzidine orally by capsule (Bonser et al., 1956; Spitz et al., 1950), although no control dogs were tested at the same time. Humans exposed to benzidine during its manufacture or industrial use have a significantly high incidence of cancer of the bladder (Case et al., 1954; Goldwater et al., 1965; Hueper, 1969; Mancuso and El-Attar, 1967; Scott, 1952; Uebelin and Pletscher, 1954).

The presence of benzidine in the urine of rats and mice and of liver lesions in rats and mice identical to those caused by benzidine alone suggests that the benzidine released from the metabolism of the dyes may be responsible for the liver lesions. The failure of male rats receiving direct brown 95 dye to develop hepatocellular carcinomas or neoplastic nodules may be due to the toxicity of the chemical, which resulted in deaths of all animals in the highest two dose groups by week 5 of the study. Two-year studies of these three dyes in mice were not conducted, since benzidine was detected in the urine of mice in these studies, and since there is evidence from prior studies that benzidine can produce hepatocellular carcinomas in mice (Frith and Dooley, 1976).

It is concluded that under the conditions of these 13-week subchronic toxicity studies, direct blue 6 and direct black 38 dyes were carcinogenic in male and female Fischer 344 rats and direct brown 95 was carcinogenic in female rats; all three dyes induced hepatocellular carcinomas and neoplastic nodules in the liver. The test dyes were not carcinogenic for B6C3F1 mice in the 13-week subchronic toxicity studies.

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APPENDIX D

SUMMARY FROM THE REPORT ON CARCINOGENS, EIGHTH EDITION (1998)

Benzidine
Direct Black 38
Direct Blue 6

BENZIDINE
CAS No. 92-87-5
First Listed in the *First Annual Report on Carcinogens*

CARCINOGENICITY

There is sufficient evidence for the carcinogenicity of benzidine in experimental animals (IARC V.29, 1982; IARC S.4, 1982). When administered in the diet, benzidine induced urinary bladder carcinomas in dogs and increased the incidence of benign and malignant cholangiomatous tumors and hepatocellular tumors in hamsters of both sexes. When administered by gavage, benzidine induced multiple mammary carcinomas in female rats. When administered by subcutaneous injection, benzidine induced hepatocellular carcinomas and adenomas and cholangiomas in mice of both sexes. When administered by subcutaneous injection, benzidine induced hepatomas, cystocholangiomas, or hepatocellular carcinomas, tumors of the Zymbal gland, and local sarcomas in rats of both sexes. In another study, subcutaneous injection induced mammary adenocarcinomas in female rats. When administered by intraperitoneal injection, benzidine induced a dose-related increase in the incidence of benign and malignant mammary tumors and adenomas and carcinomas of the Zymbal gland in female rats.

An IARC Working Group reported that there is sufficient evidence for the carcinogenicity of benzidine in humans (IARC V.29, 1982; IARC S.4, 1982). Case reports and follow-up studies of workers provide sufficient evidence that occupational exposure to benzidine is strongly associated with an increased risk of bladder cancer. The association is strengthened by data that suggest that the incidence of this cancer in workers decreased after a reduction in industrial exposure.

PROPERTIES

Benzidine occurs as a grayish-yellow, white, or reddish-grey crystalline powder. It is slightly soluble in hot water, boiling ethanol and diethyl ether. When heated to decomposition, it emits highly toxic fumes of nitrogen oxides (NO_x).

USE

Benzidine, an industrial chemical, has been used for more than 60 years as an intermediate in the production of azo dyes, sulfur dyes, fast color salts, naphthols, and other dyeing compounds. More than 250 benzidine-based dyes have been reported (IARC V.29, 1982). Benzidine-based dyes are used primarily for dyeing textiles, paper, and leather products. There are approximately 550 dye applications. Approximately 50% of the dyes are applied to textiles, 45% to paper, and 5% to leather (NCI DCCR, 1975). In recent years, general use of benzidine has fallen dramatically because of its potential carcinogenicity (IARC V.29, 1982).

PRODUCTION

The Chem Sources International directory identified one high volume and four bulk suppliers of the ten overall listed suppliers of benzidine in 1990 (Chem. Sources International, 1991). The Chem Sources International directory identified two domestic suppliers of benzidine in 1988 (Chem. Sources International, 1988). Benzidine is no longer manufactured for commercial sale in the United States (IARC, V.29, 1982; SRIa, 1986; USITC, 1988; ATSDR, 1995c). All benzidine production is for captive consumption and it must be maintained in closed systems under stringent workplace controls (ATSDR, 1995c). An estimated production of only 227 kg (500 lbs) is given for 1983 though this may omit some captive production (ATSDR, 1995c). The 1979 TSCA Inventory identified one company producing 500 lb of benzidine in 1977 (TSCA, 1979). Prior to 1977, U.S. production of benzidine amounted to many millions of lb per year (IARC V.29, 1982). No data on imports or exports were available.

EXPOSURE

The primary routes of potential human exposure to benzidine are inhalation, ingestion, and dermal contact. Benzidine may get into the respiratory tract from accidental releases into the air; into the gastrointestinal tract from contaminated fingers, cigarettes, or food; and onto the skin directly or from contaminated clothing and gloves (NCI DCCR, 1975). Before 1974, benzidine and its derivatives were manufactured and used in open systems that permitted atmospheric releases at the workplace. Under OSHA regulations adopted in 1974, only closed systems were permitted. Although atmospheric emissions were expected to be reduced because of these regulations, there were no data available that reflected current concentrations of benzidine in air (ATSDR, 1995c).

The major release routes of benzidine to the environment appear to be by wastewaters and sludges, and by solid wastes generated by the use of benzidine and production of benzidine-based dyes. The median concentrations of benzidine in waste effluents, ground water, surface water, and soils appear to be low probably because significant levels are associated with localized areas of contamination. The production and utilization of benzidine-based dyes has decreased in the last 30 years, and environmental and health regulations have been implemented to reduce release of benzidine to the environment (ATSDR, 1995c).

In most cases, benzidine is a hazard only in the vicinity of dye and pigment plants where wastes may escape or be discharged. Potential health risks exist for workers in the production of benzidine and its congeners and their conversion to azo dyes and for workers in the garment, leather, printing, paper, and homecraft industries where benzidine-based dyes are used (ATSDR, 1995c). The National Occupational Exposure Survey (1981-1983) indicated that 15,554 workers, including 426 women, potentially were exposed to benzidine (NIOSH, 1984). The NIOSH numbers were not based on actual measurements. Workers in the United States routinely wear protective equipment to eliminate inhalation and skin contact (ATSDR, 1995c). No TLV has been assigned for benzidine because it is a recognized human carcinogen (ACGIH, 1996). All exposures to benzidine should be kept to an absolute minimum.

REGULATIONS

In 1980, CPSC collected economic and toxicological data to propose a ban on the use of benzidine-based dyes in direct consumer dye products. CPSC also completed studies on the dermal penetration of two benzidine congener dyes with negative results. The use of benzidine congener dyes in consumer products and commercial textile applications has been decreased voluntarily. Therefore, CPSC voted to deny the petition that requested a ban of these consumer dye products. Educational materials have been developed to warn artists of the potential hazard of these dyes. EPA regulates benzidine under the Clean Water Act (CWA), the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), the Resource Conservation and Recovery Act (RCRA), the Superfund Amendments and Reauthorization Act (SARA), and the Toxic Substances Control Act (TSCA). Effluent discharge guidelines have been set under CWA, and benzidine is subject to reporting rules under CWA, SARA, and TSCA. A reportable quantity (RQ) of 1 lb has been proposed for benzidine under CERCLA. It is regulated as a hazardous constituent of waste under RCRA. FDA also regulates, under the Food, Drug, and Cosmetic Act (FD&CA), The amount of benzidine in various color additives for use in food, drugs, and cosmetics. NIOSH (1994) has recommended that exposure to benzidine be minimized. OSHA has established protective standards for occupational exposure to benzidine. OSHA also regulates benzidine under the Hazard Communication Standard and as a chemical hazard in laboratories.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 63-- PART 63--NATIONAL EMISSION STANDARDS FOR HAZARDOUS AIR POLLUTANTS FOR SOURCE CATEGORIES. Promulgated: 57 FR 61992, 12/29/92. U.S. Code: 7401 et seq.; CAA. Standards that regulate specific categories of stationary sources that emit (or have potential to emit) one or more hazardous air pollutants are listed in this part pursuant to section 112(b) of the CAA.	

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 63.70-- Subpart D--Regulations Governing Compliance Extensions for Early Reductions of Hazardous Air Pollutants.	The provisions of this subpart apply to an owner/operator of an existing source wishing to obtain a compliance extension from a standard issued under section 112(d) of the CAA.
	40 CFR 63.800ff.-- Subpart JJ--National Emission Standards for Wood Furniture Manufacturing Operations. Promulgated: 60 FR 62936, 12/07/95. Emission limitations for existing sources presented in Table 3 of this subpart shall be met using any of the compliance methods in 40 CFR 63.804.	Specific limitations apply to limiting VHAP emissions from contact adhesives. Specific work and compliance requirements apply. The provisions of this subpart apply to each facility that is engaged in the manufacture of wood furniture or wood furniture components and that is a major source as defined in 40 CFR 63.2.
	40 CFR 79-- PART 79--REGISTRATION OF FUELS AND FUEL ADDITIVES. Promulgated: 40 FR 52011, 11/07/75 U.S. Code: 42 U.S.C. 7414, 7524, 7545, and 7601.	The regulations of this part apply to the registration of fuel additives designated by the Administrator, pursuant to section 211 of the CAA.
	40 CFR 122-- PART 122--EPA ADMINISTERED PERMIT PROGRAMS: THE NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM (NPDES). Promulgated: 48 FR 14153, April 1, 1983. U.S. Code: 33 U.S.C. 1251 et seq., CWA.	Regulations cover basic EPA permitting requirements for effluent discharges from point sources to waters of the United States. Appendix D lists pollutants that must be identified by dischargers if expected to be present.
	40 CFR 122.64-- Sec. 122.64 Termination of permits (applicable to State programs, see Sec. 123.25). Promulgated: 48 FR 14153, 04/01/1983, as amended through 54 FR 18784, 05/02/1989.	Termination of a NPDES permit may occur due to noncompliance by the permittee with any condition of the permit; the permittee's misrepresentation of any relevant facts at any time; or, the permitted activity endangers human health or the environment.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 129-- PART 129--TOXIC POLLUTANT EFFLUENT STANDARDS. Promulgated: 42 FR 2613, 01/12/1977. U.S. Code: 33 U.S.C. 1251 et seq.	
	40 CFR 129-- Subpart A--Toxic Pollutant Effluent Standards and Prohibitions.	The provisions of this subpart apply to owners/operators of specified facilities discharging into navigable waters. Establishes effluent standards and prohibitions for toxic pollutants that may be incorporated into any NPDES permit.
	40 CFR 129.4-- Sec. 129.4 Toxic pollutants. Promulgated: 42 FR 2613, 01/12/1977, as amended through 42 FR 6555, 02/02/1977.	
	40 CFR 129.104--Sec. 129.104 Benzidine. Promulgated: 42 FR 2620, 01/12/1977.	Ambient water criterion for benzidine in navigable waters: 0.1 μg/L. Effluent standards: Existing sources of discharges shall not contain benzidine concentrations. exceeding 10 Mg/L/day, a monthly average daily loading of 0.130 kg/kkg benzidine produced, and shall not exceed 50 Mg/L in a sample(s) representing any working day. The same standards apply to new sources. Manufacture of benzidine has dropped considerably since promulgation.
	40 CFR 136-- PART 136--GUIDELINES ESTABLISHING TEST PROCEDURES FOR THE ANALYSIS OF POLLUTANTS. U.S. Code: 33 U.S.C. 1251, et seq.	The procedures described in this part shall be used whenever the waste constituent specified is required to be measured for section 402 of the CWA and/or reports required to be submitted under NPDES permits.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 136.3-- Sec. 136.3 Identification of test procedures. 40 CFR 136.5-- Sec. 136.5 Approval of alternate test procedures.	
	40 CFR 192-- PART 192--HEALTH AND ENVIRONMENTAL PROTECTION STANDARDS FOR URANIUM AND THORIUM MILL TAILINGS. Promulgated: 48 FR 602, 01/05/1983. U.S. Code: 42 U.S.C. 2022, as added by the Uranium Mill Tailings Radiation Control Act of 1978.	The provisions of this part control the residual radioactive material at designated processing or depository sites under section 108 of the Uranium Mill Tailings Radiation Control Act of 1978, and applies to the restoration of such sites following any use of the subsurface minerals under section 104(h) of the Uranium Mill Tailings Radiation Control Act of 1978.
	40 CFR 192-- Subpart E--Standards for Management of Thorium Byproduct Materials Pursuant to Section 84 of the Atomic Energy Act of 1954, as Amended by RCRA.	Appendix VIII hazardous waste constituents are regulated by reference in this part.
	40 CFR 261--Identification and Listing of Hazardous Waste, Appendix VIII--Hazardous Constituents. Promulgated: 45 FR 33119, May 19, 1980; 53 FR 13388, April 22, 1988. U.S. Code: 42 U.S.C. 6905, 6912(a), 6921, 6922, and 6938. Hazardous waste number is U021.	Appendix VIII is a consolidated list of hazardous constituents identified in this part. Solid wastes containing these constituents are subject to notification requirements of RCRA section 3010 and must be disposed of in RCRA-permitted facilities.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 261.11, 261.33. Promulgated 5/19/80. RCRA 3001-3004: Subjects waste products, off-specification batches, and spill residues in excess of 1,000 kg to handling and report/recordkeeping requirements. Also designates benzidine as a hazardous constituent of waste and subjects wastes known to contain it to the same requirements.	Based on toxic effects other than acute. EPA Carcinogen Assessment Group has included this chemical on its list of potential carcinogens. As a result of this listing, benzidine is regulated under the hazardous waste disposal rule of RCRA.
	40 CFR 266.100--Subpart H--Hazardous Waste Burned in Boilers and Industrial Furnaces.	Appendix V to Part 266 lists a risk specific dose (for carcinogenicity; 10^{-5}) of 1.5 x 10^{-4} $\mu g/M^3$ for benzidine: The sum for all compounds of the ratios of the actual ground level concentration to the level established in Appendix V cannot exceed 1.0.
	40 CFR 302-- Part 302--Designation, Reportable Quantities, And Notification. Promulgated: 50 FR 13474, 04/04/85. U.S. Code: 42 U.S.C. 9602, 9603, and 9604; 33 U.S.C. 1321 and 1361.	This part designates under section 102(a) of CERCLA 1980 those substances in the statutes referred to in section 101(14) of CERCLA, identifies reportable quantities for these substances, and sets forth the notification requirements for releases of these substances. This part also sets forth reportable quantities for hazardous substances designated under section 311(b)(2)(A) of the CWA.
	40 CFR 302.4-- Sec. 302.4 Designation of hazardous substances. Superfund (CERCLA, SARA) reportable quantity (RQ) is 1 lb (0.454 kg).	EPA designated as hazardous those substances that when released into the environment may present substantial danger to the public health or welfare or the environment. Notification of EPA is required if the RQ is released to the environment.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 372-- PART 372--TOXIC CHEMICAL RELEASE REPORTING: COMMUNITY RIGHT-TO-KNOW. Promulgated: 53 FR 4525, 02/16/88. U.S. Code: 42 U.S.C. 11013, 11028. This part sets forth requirements for the submission of information relating to the release of toxic chemicals under section 313 of Title III of SARA (1986). Details reporting and notification requirements for handlers of hazardous materials. General threshold amounts set at 10,000 lb/yr for toxic chemicals used at a facility and 25,000 lb/yr if manufactured or processed at a facility. 40 CFR 372-- Subpart D--Specific Toxic Chemical Listings. 40 CFR 372.65-- Sec. 372.65 Chemicals and chemical categories to which this part applies.	Information collected under this part is intended to inform the general public and the communities surrounding covered facilities about releases of toxic chemicals, to assist research, to aid in the development of regulations, guidelines, and standards.
	40 CFR 401-- PART 401--GENERAL PROVISIONS. Promulgated: 39 FR 4532, 02/01/74, as amended at 47 FR 24537, 06/04/82. U.S. Code: 33 U.S.C. 1251 et seq. 40 CFR 401.15-- Sec. 401.15 Toxic pollutants. Promulgated: 39 FR 4532 Feb. 1, 1974; 44 FR 44502 July 30, 1979. U.S. Code: FWPCA section 307(a)(1). Concentration limits are established for particular point sources described in other parts of 40 CFR.	The provisions of this part set forth the legal authority and general definitions which will apply to all regulations issued concerning specific classes and categories of point sources of industrial effluents under parts 402 through 699.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 403-- PART 403--GENERAL PRETREATMENT REGULATIONS FOR EXISTING AND NEW SOURCES OF POLLUTION. Promulgated: 46 FR 9439, January 28, 1981. U.S. Code: Several sections of the FWPCA and the CWA of 1977 (Public Law 95-217).	Establishes responsibilities of federal, state, and local government; industry; and the public to implement National Pretreatment Standards to control pollutants that pass through POTWs and contaminate sewage sludge or interfere with treatment processes.
	40 CFR 403.18-- Sec. 403.18 Modification of POTW Pretreatment Programs. Promulgated: 53 FR 40615, October 17, 1988. Appendices following 403.18 list 65 Toxic Pollutants (51 FR 20431. June 4, 1986) and industrial categories subject to National Categorical Pretreatment Standards (51 FR 20429, June 4, 1986).	
	40 CFR 716-- PART 716--HEALTH AND SAFETY DATA REPORTING. Promulgated: 51 FR 32726, 09/15/86 U.S. Code: 15 U.S.C. 2607(d) .	The provisions of this part require the submission of lists and copies of health and safety studies on chemical substances and mixtures selected for priority consideration for testing rules under section 4(a) of TSCA and on other chemicals for which EPA requires health and safety information in fulfilling the purposes of TSCA.
	40 CFR 716.105ff-- Subpart B gives specific chemical listings, and gives substances and listed mixtures to which this subpart applies.	Identifies uses of chemical substances which EPA has determined are significant new uses under the authority section 5(a)(2) of TSCA, and specifies procedures for manufacturers, importers, and processors to report on those significant new uses.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 721-- PART 721-- SIGNIFICANT NEW USES OF CHEMICAL SUBSTANCES. U.S. Code: 15 U.S.C. 2604, 2607, and 2625(c).	
	40 CFR 721-- Subpart E--Significant New Uses for Specific Chemical Substances.	
	40 CFR 798-- Subpart F--Genetic Toxicity.	
	40 CFR 798.5395-- Sec. 798.5395.	In vivo mammalian bone marrow. cytogenetics tests: Micronucleus assay.
F D A	21 CFR 74-- PART 74--LISTING OF COLOR ADDITIVES SUBJECT TO CERTIFICATION. Promulgated: 42 FR 15654, 03/22/77. U.S. Code: 21 U.S.C. 321, 341, 342, 343, 348, 351, 352, 355, 361, 362, 371, 379e.	This part lists color additives that are subject to certification in drugs, cosmetics and medical devices.
	21 CFR 74.101 ff-- Subpart A--Foods.	
	21 CFR 74.705-- Sec. 74.705 FD&C Yellow No. 5.	The color additive FD&C yellow no. 5 shall not contain more than 1 ppb benzidine.
	21 CFR 74.706-- Sec. 74.706 FD&C Yellow No. 6.	The color additive FD&C yellow no. 6 shall not contain more than 1 ppb benzidine.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
F D A	21 CFR 74.1101 ff-- Subpart B--Drugs.	The color additives listed may be safely used for some externally applied drugs and for some ingested drugs in amounts consistent with current good manufacturing practice.
	21 CFR 74.1333-- Sec. 74.1333 D&C Red No. 33.	The color additive D&C red no. 33 shall not contain more than 20 ppb benzidine.
	21 CFR 74.2101 ff-- Subpart C-- Cosmetics.	The color additives listed may be safely used for coloring externally applied cosmetics including some intended for use in the area of the eye, in amounts consistent with good manufacturing practice.
	21 CFR 74.2705-- Sec. 74.2705 FD&C Yellow No. 5.	The color additive FD&C yellow no. 5 shall not contain more than 1 ppb benzidine.
	21CFR81-- PART 81--GENERAL SPECIFICATIONS AND GENERAL RESTRICTIONS FOR PROVISIONAL COLOR ADDITIVES FOR USE IN FOODS, DRUGS, AND COSMETICS. U.S. Code: 21 U.S.C. 371, 379e, 379e note	Certain color additives used in foods, drugs, and cosmetics are listed as provisional. Termination of provisional listings and cancellation of certificates of various color additives are also included along with limitation of certain certificates.
	21CFR81.10-- Sec. 81.10 Termination of provisional listings of color additives.	D&C yellow no. 1 cannot be produced with any reasonable assurance that it will not yield benzidine from the decomposition of a subsidiary reaction product that might be present in the color.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
N I O S H	9/14/73. Statement on proposed permanent standard for certain carcinogens, at OSHA hearing before administrative law Judge Burton Sternberg	Summary of current NIOSH recommendation for benzidine and benzidine-based dyes: exposure limit - Ca, lowest feasible concentration.
	4/17/78. Current Intelligence Bulletin #24 -- Direct Blue 6, Direct Black 38, Direct Brown 95, Benzidine Derived Dyes.	
	1/80. Special Occupational Hazard Review for Benzidine - Based Dyes.	
	12/80. OSHA/NIOSH Health Hazard Alert: Benzidine, *o*-Toluidine and *o*-Dianisidine - Based Dyes.	NIOSH believes that the dyes themselves are potential carcinogens.
	1/83. NIOSH recommended that exposure to benzidine-based dyes be reduced to the lowest level feasible, preferably by substituting safer dyes for benzidine-based dyes.	
	1/83. Preventing Health Hazards from Exposure to Benzidine Congener Dyes.	

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
O S H A	29 CFR 1910.11 ff--Subpart B--Adoption and Extension of Established Federal Standards. Effective 1972. OSH Act: Adopts and extends application of established federal standards in effect on 4/28/71 to Construction, Ship Repairing, Ship Building, and Long-shoring and Marine Terminals.	Regulations based on high incidence of cancer, either in humans or in animals.
	29 CFR 1910.1010--Sec. 1910.1010 Benzidine. Promulgated: 61 FR 9245, 03/07/96. OSH Act: Regulations based on high incidence of cancer, either in humans or in animals.	Regulates protective clothing, respirator, hygiene, training and medical surveillance requirements for workers; exhaust fan requirements; open vessel operations prohibited; contamination control requirements; sign requirements for regulated areas; labeling requirements for containers.
	29 CFR 1910.1200--Sec. 1910.1200 Hazard Communication. Promulgated: 61 FR 9245, 03/07/96. OSH Act: Hazard Communication.	Requires chemical manufacturers and importers and all employers to assess chemical hazards and to provide information to employees. Hazard Communication Program to include labels, material safety data sheets, and worker training.
	29 CFR 1910.1450--Sec. 1910.1450 Occupational Exposure to Hazardous Chemicals in Laboratories. Promulgated: 61 FR 5508, 02/13/96. OSH Act: Final rule for occupational exposure to hazardous chemicals in laboratories.	As a select carcinogen (IARC Group 1 and NTP known carcinogen), benzidine is included as a chemical hazard in laboratories. Employers are required to provide employee information, training and a Chemical Hygiene Plan.

[a] The regulations in this table have been updated through the 1996 Code of Federal Regulations: 40 CFR, July 1, 1996; 21 CFR, April 1, 1996; 29 CFR, July 1, 1996.

DIRECT BLACK 38
CAS No. 1937-37-7
First Listed in the *Third Annual Report on Carcinogens*

CARCINOGENICITY

There is sufficient evidence for the carcinogenicity of commercial Direct Black 38 in experimental animals (IARC V.29, 1982; IARC S.4, 1982; IARC S.7, 1987). When administered in the diet, Direct Black 38 induced hepatocellular carcinomas in male rats and neoplastic nodules of the liver in rats of both sexes. When administered in the drinking water, Direct Black 38 induced papillomas and carcinomas of the urinary bladder, carcinomas of the liver, and adenocarcinomas of the colon in rats. There was no evidence that the compound was carcinogenic in mice when administered by these routes (NCI 108, 1978; IARC V.29, 1982).

There is inadequate evidence for the carcinogenicity of Direct Black 38 in humans. Several epidemiological studies of dye users suggest that there may be excess mortality from bladder cancer in workers possibly exposed occupationally to benzidine-based dyes. However, there are no studies available to assess the carcinogenicity of Direct Black 38 alone. In a recent occupational hazard review, it was concluded that all benzidine-based dyes, including Direct Black 38, regardless of their physical state or proportion in the mixture, should be recognized as potential human carcinogens (NIOSH Review, 1980; IARC V.29, 1982; IARC S.4, 1982; IARC S.7, 1987).

PROPERTIES

Direct Black 38 is a gray-black powder. It is soluble in water, moderately soluble in ethanol and ethylene glycol monoethyl ether, and insoluble in other organic solvents. When heated to decomposition, Direct Black 38 emits toxic fumes of nitrogen oxides (NO_x) and sulfur oxides (SO_x). The benzidine content of domestically produced Direct Black 38 has been found to range from 2 to 20 mg/kg; benzidine content of imported samples ranged from 2 to 1254 mg/kg. Another product was found to contain < 0.1 mg/kg benzidine, 150 mg/kg 4-aminobiphenyl, and 9,200 mg/kg 2,4-diaminoazobenzene. The composition of commercial Direct Black 38 varies in order to meet individual shade and intensity requirements.

USE

Direct Black 38 is possibly being used to dye fabric, leather, cotton, cellulosic materials, and paper, which then are used in consumer products. According to CPSC and EPA, artists also may use the chemical. FDA indicated that Direct Black 38 is identified in the literature as a hair dye component, but it is not presently used by the cosmetic industry. After a health hazard alert issued by OSHA cautioning workers and employers about the carcinogenic effect of benzidine-derived Direct Black 38, researchers developed new non-benzidine Direct Black dyes. The paper and leather industries recently have used these new dyes with success in commercial applications. The nonbenzidine dyes were developed with the prospect of replacing benzidine-based dyes throughout the industry. Direct Black 38 can also be used to print cellulose, wool, and silk; to dye plastics, vegetable-ivory buttons, and wood flour used as a resin filler; to stain textiles, typewriter ribbon, wood, and biological materials; and to produce aqueous inks (NIOSH 24, 1978).

PRODUCTION

Direct Black 38 is not currently produced in the United States. The 1984 Chem Sources USA directory identified only one supplier of Direct Black 38 (Chem Sources, 1984). The U.S. imported 147,800 lb in 1983, although U.S. manufacturers have stated that they have discontinued the use of benzidine-based dyes (USITCa, 1984). The USITC last identified a single producer of Direct Black 38 in 1981, but no production volume was reported (USITC, 1982). In 1978, one manufacturer produced more than 824,000 lb of Direct Black 38 (NIOSH Review, 1980). The 1979 TSCA Inventory identified two companies producing 6 million lb of Direct Black 38 and four companies importing 555,000 lb in 1977. The CBI Aggregate was between 1 million and 10 million lb (TSCA, 1979). No data on exports were available. Direct Black 38 was first produced in commercial quantities in the United States in 1914 (IARC V.29, 1982).

EXPOSURE

The primary routes of potential human exposure to Direct Black 38 are inhalation, ingestion, and dermal contact. Consumer exposure to Direct Black 38 depends upon the ability of the dye to migrate out of consumer products and either penetrate the skin or to degrade prior to penetrating the skin. No data quantifying the rate of migration or degradation of this dye are currently available. In the general population, unspecified exposure levels may possibly occur through the use of dyed textile products and of retail packaged dyes for home dyeing and school use. The National Occupational Hazard Survey, conducted by NIOSH from 1972 to 1974, estimated that about 16,000 workers were possibly exposed to Direct Black 38 in the workplace, including dyers of leather, plastics, cotton, wool, and silks, along with makers of aqueous inks, biological stains, typewriter ribbons, and wood stains (NIOSH, 1976). In a more recent occupational hazard review, NIOSH estimated that approximately 13,000 workers were potentially exposed to Direct Black 38 in the workplace (NIOSH Review, 1980). Direct Black 38 has been detected in the workplace air of a paper dyeing facility, at total airborne particulate levels of 1.6-5.1 mg/m^3 (0.05-0.16 ppm), and of a textile dyeing facility, at unspecified concentrations (IARC V.29, 1982).

REGULATIONS

In 1980, CPSC collected scientific and economic data to propose a ban on the use of all benzidine congener dyes in consumer dye products. CPSC also completed studies on the dermal penetration of two of these dyes, and noted no dermal penetration. The use of benzidine congener dyes in consumer dyeing products and commercial textile application has been voluntarily decreased. Therefore, CPSC voted to deny the petition that requested a ban of these consumer dye products. Educational materials have been developed to warn artists of the potential hazard of benzidine congener dyes.

EPA subjects Direct Black 38 to reporting requirements under the Superfund Amendments and Reauthorization Act (SARA) and the Toxic Substances Control Act (TSCA). FDA does not regulate cosmetic use of Direct Black 38, but was petitioned to approve Direct Black 38 for use as an indirect food additive (e.g., as a dye for paper and paperboard products); in 1979, the petition was withdrawn. OSHA regulates Direct Black 38 under the Hazard Communication Standard and as a chemical hazard in laboratories.

REGULATIONS

	Regulatory Action	Effect of Regulation/Other Comments
EPA	40 CFR 372. Promulgated 2/16/88. Toxic Chemical Release Reporting: Community Right-to-Know. SARA 313: Establishes list of toxic chemicals and groups of chemicals subject to reporting requirements.	Details reporting and notification requirements for handlers of hazardous materials. General threshold amounts set at 10,000 lb/yr for toxic chemicals used at a facility and 25,000 lb/yr if manufactured or processed at a facility.
	40 CFR 712. Promulgated 6/22/82. TSCA 8(a): Final rule to require production and use data.	Based on TSCA 8(e) submission. Would provide added means to estimate exposure potential.
	40 CFR 716.17(a)(1). Promulgated 10/4/82. TSCA 4(a): Final rule to require past, current, and prospective manufacturers, importers, and processors to submit unpublished health and safety studies.	
NIOSH	4/27/78. Current Intelligence Bulletin #24 - Direct Blue 6, Direct Black 38, Direct Brown 95, Benzidine Derived Dyes.	Summary of current NIOSH recommendation: exposure limit - Ca, lowest feasible concentration.
	1/80. Special Occupational Hazard Review for Benzidine-Based Dyes.	
	9/80. OSHA/NIOSH Health Hazard Alert; Benzidine-, o-Tolidine and o-Dianisidine-Based Dyes.	
OSHA	29 CFR 1910.1200. Promulgated 11/25/83. OSH Act: Hazard Communication.	Requires chemical manufacturers and importers and all employers to assess chemical hazards and to provide information to employees. Hazard Communication Program to include labels, materials safety data sheets, and worker training.

REGULATIONS

	Regulatory Action	Effect of Regulation/Other Comments
OSHA	29 CFR 1910.1450. Promulgated 1/31/90. OSH Act: Final rule for occupational exposure to hazardous chemicals in laboratories.	As a select carcinogen (IARC Group 2A), Direct Black 38 is included as a chemical hazard in laboratories. Employers required to provide employee information and training and to provide Chemical Hygiene Plan.

DIRECT BLUE 6
CAS No. 2602-46-2

First Listed in the *Third Annual Report on Carcinogens*

CARCINOGENICITY

There is sufficient evidence for the carcinogenicity of Direct Blue 6 (technical grade) in experimental animals. In a single study, administration of technical-grade Direct Blue 6 in the diet induced liver hepatocellular carcinomas and neoplastic nodules in rats of both sexes. There is no evidence that technical grade Direct Blue 6 was carcinogenic to mice under the same conditions (IARC V.29, 1982; IARC S.4, 1982; IARC S.7, 1987).

An IARC Working Group reported that there is inadequate evidence for the carcinogenicity of Direct Blue 6 in humans. There is no case report or epidemiological study available involving exposure to Direct Blue 6. IARC has determined that there is sufficient evidence that occupational exposure to benzidine-based dyes represents a carcinogenic risk to humans (IARC V.29, 1982; IARC S.4, 1982; IARC S.7, 1987). In a recent occupational hazard review, NIOSH concluded that all benzidine-based dyes, including Direct Blue 6, regardless of their physical state or concentration in the mixture, should be recognized as potential human carcinogens (NIOSH Review, 1980).

PROPERTIES

Direct Blue 6 occurs as a blue-violet solid. It is soluble in water, slightly soluble in ethanol and ethylene glycol monoethyl ether, and insoluble in other organic solvents. When heated to decomposition, Direct Blue 6 emits toxic fumes of nitrogen oxides (NO_x) and sulfur oxides (SO_x). The benzidine content of domestically produced Direct Blue 6 has been measured as being between 4 and 12 mg/kg. The composition of commercial Direct Blue 6 varies in order to meet individual shade and intensity requirements.

USE

Direct Blue 6 is possibly used to dye fabric, leather, silk, wool, cotton, cellulosic materials, and paper, to stain biological materials, and to produce aqueous inks (IARC V.29, 1982; NIOSH 24, 1978). CPSC and EPA indicated that artists also may use the chemical. Direct Blue 6 has been used as a hair dye component, but FDA indicated that it presently is not used by the cosmetic industry (NIOSH 24, 1978).

PRODUCTION

Current production data for Direct Blue 6 are not available. Chem Sources identified two suppliers of Direct Blue 6 in 1990 (Chem Sources, 1991). The USITC identified one producer of Direct Blue 6 in 1979 and 1982, with implied annual production volumes of > 5,000 lb (USITC, 1983). In 1978, domestic companies produced nearly 62,000 lb of Direct Blue 6 and imported 4,400 lb (IARC V.29, 1982; NIOSH Review, 1980). The 1979 TSCA Inventory identified three companies producing 110,500 lb and two companies importing 5,500 lb in 1977. The CBI Aggregate was less than 1 million lb (TSCA, 1979). No data on exports of Direct Blue 6 were available. Direct Blue 6 was first produced in commercial quantities in the U.S. in 1914 (IARC V.29, 1982).

EXPOSURE

The primary routes of potential human exposure to Direct Blue 6 are inhalation, ingestion, and dermal contact. According to CPSC, the use of benzidine congener dyes in consumer dyeing products and commercial textile applications has been voluntarily decreased. The primary source for potential exposure to Direct Blue 6 is at the production site. The initial production step is in a closed system, but other production operations (for example, filtering, drying, and blending) may be performed in the open and therefore may afford greater potential for worker exposure. The National Occupational Hazard Survey, conducted by NIOSH from 1972 to 1974, estimated that 1,300 workers were possibly exposed to Direct Blue 6 (NIOSH, 1976). In 1980, NIOSH estimated that about 500 workers were potentially exposed to the dye in the workplace (NIOSH Review, 1980). Occupational exposure to Direct Blue 6 may occur for workers in a variety of industries identified by NIOSH, including paper and allied products, petroleum and related industries, rubber and plastic products, leather and leather products, instrumentation and measuring devices, and banking. In addition, the textile industry accounts for substantial risk for occupational exposure. Direct Blue 6 has been detected in the workplace air of a textile dyeing operation, at total airborne particulate concentrations of 1.20-3.94 mg/m^3. It has been estimated that 25% of the benzidine-derived azo dyes are applied to textiles, 40% to paper, 15% to leather, and the remainder to other diverse applications (NIOSH 24, 1978; IARC V.29, 1982).

The general population may possibly be exposed to Direct Blue 6 through the use of retail packaged dyes containing the benzidine-based dye. Potential consumer exposure to Direct Blue 6 depends upon the ability of the dye to migrate out of the consumer product and either penetrate the skin or break down prior to penetrating the skin. A risk of potential exposure to Direct Blue 6 may have existed for people using hair dyes which contained the compound. In addition, ingestion of Direct Blue 6 may occur if food is eaten which contained residues from packaging in which the dye was used.

REGULATIONS

In late 1980, CPSC collected scientific and economic data to propose a ban on the use of all benzidine congener dyes in consumer products. CPSC also completed studies on the dermal penetration of two of these dyes, and noted no dermal penetration. The use of benzidine congener dyes in retail packaged dyes for home and school use has been voluntarily decreased. Therefore, CPSC voted to deny the petition that requested a ban of these consumer dye products. Educational materials have been developed and are available to warn artists of the potential danger of benzidine congener dyes. EPA regulates Direct Blue 6 under the Superfund Amendments and Reauthorization Act (SARA), and the Toxic Substances Control Act (TSCA), subjecting it to reporting requirements. FDA does not regulate the cosmetic use of Direct Blue 6, but was petitioned to approve the chemical for use as an indirect food additive (e.g., as a dye for paper and paperboard products). In 1979, the petition was withdrawn. OSHA regulates Direct Blue 6 under the Hazard Communication Standard and as a chemical hazard in laboratories.

REGULATIONS

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 372. Promulgated 2/16/88. Toxic Chemical Release Reporting: Community Right-to-Know. SARA 313: Establishes list of toxic chemicals subject to reporting requirements.	Details reporting and notification requirements for handlers of hazardous materials. General threshold amounts set at 10,000 lb/yr for toxic chemicals used at a facility and 25,000 lb/yr if manufactured or processed at a facility.
	40 CFR 712. Promulgated 6/22/82. TSCA 8(a): Final rule to require production and use data.	Based on ITC testing recommendation. Would provide added means to estimate exposure potential.
	40 CFR 716.17(a)(1). Promulgated 9/2/82. TSCA 4(a): Health and safety study reporting rule.	Requires past, current, and prospective manufacturers, importers, and processors to submit unpublished health and safety studies.
N I O S H	1/80. Special Occupational Hazard Review for Benzidine Based Dyes.	
	9/80. OSHA/NIOSH Health Hazard Alert; Benzidine-, o-Toluidine- and o-Dianisidine Based Dyes.	
	4/27/88. Current Intelligence Bulletin #24 - Direct Blue 6, Direct Black 38, Direct Brown 95, Benzidine Derived Dyes.	Summary of current NIOSH recommendation: exposure limit - Ca, lowest feasible concentration.
O S H A	29 CFR 1910.1200. Promulgated 11/25/83. OSH Act: Hazard Communication.	Requires chemical manufacturers and importers and all employers to assess chemical hazards and to provide information to employees. Hazard Communication Program to include labels, material safety data sheets, and worker training.
	29 CFR 1910.1450. Promulgated 1/31/90. OSH Act: Final rule for occupational exposure to hazardous chemicals in laboratories.	As a select carcinogen (IARC Group 2A), Direct Blue 6 is included as a chemical hazard in laboratories. Employers required to provide employee information and training and to provide Chemical Hygiene Plan.

APPENDIX E

DESCRIPTION OF ONLINE SEARCHES FOR BENZIDINE DYE CLASS

DESCRIPTION OF ONLINE SEARCHES FOR BENZIDINE DYE CLASS

Searches were limited to 1993 [the year before the ATSDR (1994) Toxicological Profile] through July 1997.

Online searches for benzidine dyes were performed in databases on the systems of STN International, DIALOG, NLM's TOXNET, and the Chemical Information System from 1993 to date. Toxicology information was sought in the EMIC, EMICBACK, TSCATS (epidemiology, chromosomal aberration, gene toxicity, mutagenicity), the Toxic Chemicals Release Inventory 1995 (online availability 1997), TOXLINE (reviews as well as MESH heading for all neoplasms). Occupational safety and health information was obtained from NIOSHTIC. The Chemical Abstracts files were searched by appropriate section codes (59, air pollution and industrial hygiene; 60, waste treatment and disposal; 61, water). The Chemical Abstracts Service Registry file and SANSS provided chemical identification information.

APPENDIX F

NAME AND *COLOUR INDEX* NUMBER OF SOME DIRECT DYES CONTAINING THE BENZIDINE MOIETY

APPENDIX F: 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

APPENDIX F (Continued)

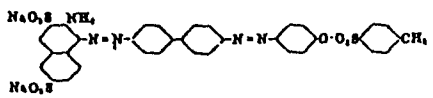
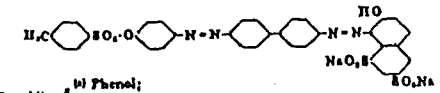
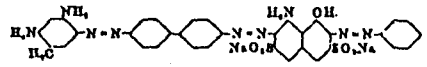
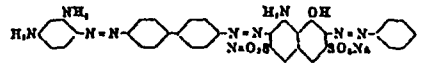
107.	Direct Dye	22640
108.	Direct Brown 7	30035
109.	Direct Brown 171	30040
110.	Direct Brown 1	30045
111.	Direct Brown 79	30050
112.	Direct Brown 61	30055
113.	Direct Brown 20	30060
114.	Direct Dye	30065
115.	Direct Brown 158	30070
116.	Direct Dye	30075
117.	Direct Dye	30080
118.	Direct Dye	30085
119.	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*.
Reprinted from 1980 NIOSH Benzidine-Based Dyes (Special Hazard Review), DHEW (NIOSH) Publication No. 80-109.

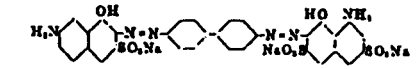
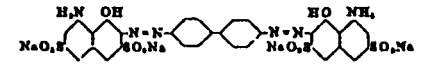
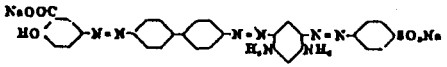
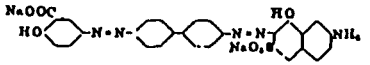
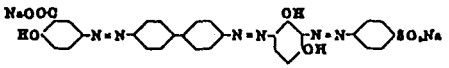
APPENDIX G

BENZIDINE-BASED DYES REPORTED TO BE COMMERCIALLY AVAILABLE IN THE UNITED STATES [ca. 1977-1979]
(Reprinted from 1980 NIOSH Benzidine-Based Dyes. Special Hazard Review)

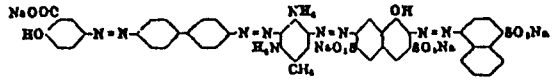
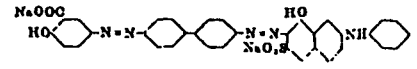
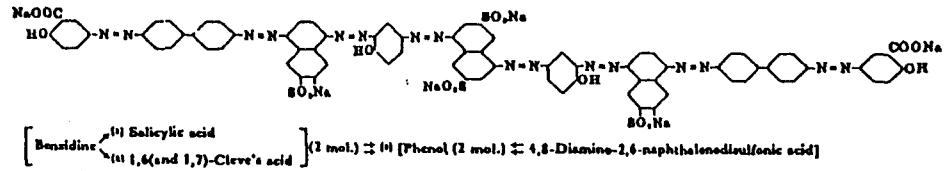
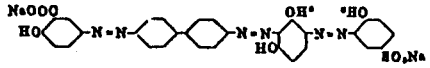
APPENDIX G: BENZIDINE-BASED DYES REPORTED TO BE COMMERCIALLY AVAILABLE IN THE UNITED STATES

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 → 1-Amino-2,7-naphthalenedisulfonic acid 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 → (b) Phenol; (t) 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 → (t) Toluene-2,4-diamine (t) (acid) H acid (alk.) (t) ← 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 → (t) m-Phenylenediamine (t) (acid) H acid (alk.) (t) ← 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

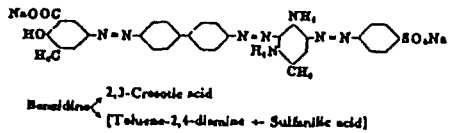
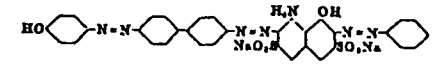
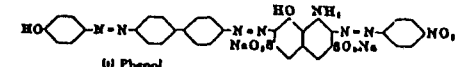
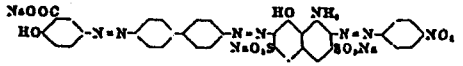
APPENDIX G (Continued)

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 Blue 2 (Dull Blue)  Benzidine → (a) (alk.) Gamma acid; (b) (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.)	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
Aqueous solution + HCl conc. — navy blue, ppt; + NaOH conc. — dark violet, ppt.						
C.I. Direct Brown 1 (Brown)  Benzidine → (a) Salicylic acid; (b) [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 → (a) Salicylic acid; (b) (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 → (a) Salicylic acid; (b) Resorcinol (a) ← Sulfanilic acid	30140	NA	8,563(1978)	Not listed	Dyeing of leather, paper, silk, wool, and cotton	Unknown
This sequence of operations, which is that recorded for Congo Brown G, differs slightly from C.I. 1st Edition 398						

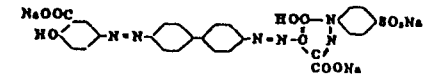
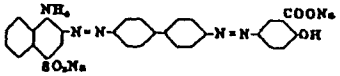
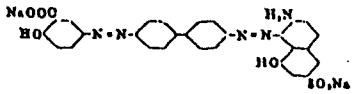
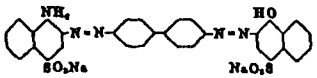
APPENDIX G (Continued)

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 31 (Reddish brown)  Benzidine → (1) Salicylic acid; (2) (Toluene-2,4-diamine (acid) ← *O*-Phenylsulfonyl 1R 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.35450)	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) (*alA.*) *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]	36300	NA	32,414(1978)	"	Dyeing of cotton, wool, silk, leather, chrome tannage (occasional)	"
HNO_3 conc. — dull red solution, turns yellow brown. Aqueous solution + HCl conc. — brownish yellow to olive ppt; + NaOH conc. — orange brown						
C.I. Direct Brown 95 (Reddish brown)  Copper complex derived from Benzidine → (1) Salicylic acid; (2) [Copper complex formed at * from 1-Amino-1-phenol-4-sulfonic acid → Resorcinol]. In Sirius Supra Brown BRLN 20% of the salicylic acid is replaced by 2,1-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

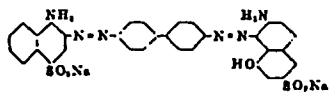
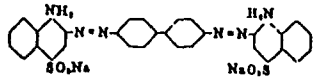
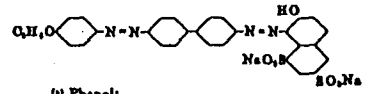
APPENDIX G (Continued)

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: (o) Phenol (i) (*acid*) H acid (*alk.*) (o) ← 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: (o) Phenol (i) (*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: (o) Salicylic acid (i) (*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

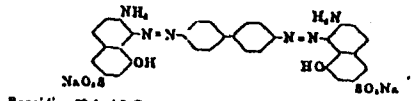
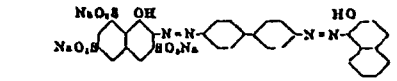
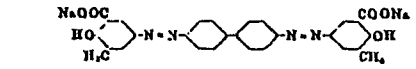
APPENDIX G (Continued)

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 + 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 + [a] Naphthionic acid + [b] 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 + [a] Salicylic acid + [b] (*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 + [a] Naphthionic acid + [b] Neville 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

APPENDIX G (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 Red 13 (Bordeaux)  Benzidine → (1) Naphthionic acid; (1) (*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; (1) 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 G (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.

REFERENCES CITED IN APPENDIX G

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

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

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

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

46. Kasprzak S. J. 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.

APPENDIX H

REPORT ON CARCINOGENS (RoC), 9th EDITION
REVIEW SUMMARY

Report on Carcinogens (RoC), 9th Edition
Review Summary

Dyes Metabolized to Benzidine (Benzidine Dyes as a Class)

NOMINATION
Review based on knowledge that Benzidine-Based Dyes are metabolized to Benzidine, which is listed in the RoC as a *known to be human carcinogen.*

DISCUSSION
Benzidine-based dyes are used primarily for dyeing textiles, paper, and leather products. More than 250 benzidine-based dyes have been reported by the Society of Dyers and Colorists. All studied Benzidine Dyes are metabolized to an amount of free benzidine equal to that observed from an equimolar dose of benzidine in dye workers exposure studies and experimental animal studies. This review is the first example in which mechanistic considerations play a major role in listing members of an entire chemical class. Epidemiological evidence for the carcinogenicity of benzidine-based dyes has been difficult to obtain because exposure is almost always associated with co-exposure to benzidine. The recommendations from the three NTP reviews of this nomination are as follows:

Review Committee	Recommendation	Vote
NIEHS (RG1)	list as known human carcinogen	7 yes/1 no
NTP EC Working Group (RG2)	list as known human carcinogen	8 yes/0 no
NTP Board RoC Subcommittee	list as known human carcinogen	7 yes/0 no

Public Comments Received
No comments were received concerning the listing of Dyes Metabolized to Benzidine (Benzidine Dyes as a Class) in the RoC.