BENZIDINE

Ambient Water Quality Criteria

Criteria and Standards Division
Office of Water Planning and Standards
U.S. Environmental Protection Agency
Washington, D.C.

CRITERION DOCUMENT

BENZIDINE

CRITERIA

Aquatic Life

For freshwater aquatic life, no criterion for benzidine can be derived using the Guidelines, and there are insufficient data to estimate a criterion using other procedures.

For saltwater aquatic life, no criterion for benzidine can be derived using the Guidelines, and there are insufficient data to estimate a criterion using other procedures.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to benzidine through ingestion of water and contaminated aquatic organisms, the ambient water concentration is zero. Concentrations of benzidine estimated to result in additional lifetime cancer risks ranging from no additional risk to an additional risk of 1 in 100,000 are presented in the Criterion Formulation section of this document. The Agency is considering setting criteria at an interim target risk level in the range of 10^{-5}, 10^{-6}, or 10^{-7} with corresponding criteria of 1.67×10^{-3} µg/l, 1.67×10^{-4} µg/l, and 1.67×10^{-5} µg/l, respectively.

Introduction

Benzidine (4,4'-diaminobiphenyl) is an aromatic amine. A proven human carcinogen, its primary site of tumor induction is the urinary bladder. It is also mutagenic.

The incidence of bladder tumors in humans resulting from occupational exposure to aromatic amines (benzidine) was first researched in Germany in 1895. The first cases of this condition in the United States were diagnosed in 1931 and reported in 1934.

Several studies implicating the high risk of bladder tumors in workers exposed to benzidine and other aromatic amines are well documented.

Adversary proceedings under section 307(a) of the Federal Water Pollution Control Act resulted in the promulgation of a toxic pollutant effluent standard for benzidine. The ambient water criterion upon which the standard was based was 0.1 µg/l (42 FR 2588, January 12, 1977).

Benzidine is an aromatic amine with a molecular weight of 184.24 (Weast, 1972). Existing as a grayish-yellow, white, or reddish-gray crystalline powder (melting point 128^{o}C; boiling point 400^{0}C (Standen, 1972)), benzidine's solubility increases as water temperature rises. One gram of benzidine will dissolve in 2.5 liters of cold water. Solubility is greatly enhanced with dissolution into organic solvents (Stecher, 1968). Benzidine is easily converted to and from its salt (Morrison and Boyd, 1972).

Diazotization reactions involving benzidine will result in colored compounds (color will vary with molecular structure). Because of their color, azo compounds are important as dyes for industrial use (Morrison and Boyd, 1972). The pKa values for the amino groups in benzidine were reported to be 4.66 and 3.57 (Weast, 1972).

REFERENCES

Morrison, R.T., and R.M. Boyd., eds. 1972. Organic chemistry. 2nd ed. Allyn and Bacon, Inc., Boston.

Standen, A., ed. 1972. Kirk-Othmer Encyclopedia of Chemical Technology. Interscience Publishers, John Wiley and Sons, Inc., New York.

Stecher, P.G., ed. 1968. The Merck Index. 8th ed. Merck and Co., Inc., Rahway, N.J.

Weast, R.C., ed. 1972. Handbook of chemistry and physics 53rd ed. CRC Press, Cleveland, Ohio.

AQUATIC LIFE TOXICOLOGY

No appropriate data are available for freshwater or saltwater organisms and benzidine.

CRITERION FORMULATION

Aquatic Life

No freshwater or saltwater criterion can be derived for benzidine using the Guidelines because no Final Chronic Value for either fish or invertebrate species or a good substitute for either value is available, and there are insufficient data to estimate a criterion using other procedures.

Mammalian Toxicology and Human Health Effects

EXPOSURE

Introduction

In general, exposure to benzidine compounds occurs in factories that synthesize benzidine and its congeners and convert them to dyes. It is also probable that some exposure occurs when the closed system used in synthesis is cleaned (Haley, 1975). Exposure also occurs from breathing contaminated air, ingesting contaminated food, and wearing contaminated clothing (Meigs, et al. 1951). Pointing of brushes by Japanese kimono painters results in the ingestion of benzidine dyes (Yoshida and Miyakawa, 1973), although ingestion is not generally an important source of exposure.

Ingestion from Water

Water could be contaminated with benzidine and its derivatives and dyes if plant water is discharged into water supplies serving a residential community. However, as of this time no reports of such contamination have appeared in the literature.

Ingestion from Food

While it is possible for food to become contaminated with benzidine and its derivatives under poor industrial hygienic conditions, ingestion of contaminated food is not a real contributor to the overall problem of benzidine toxicity.

A bioconcentration factor (BCF) relates the concentration of a chemical in water to the concentration in aquatic organisms, but BCF's are not available for the edible portions of all four major groups of aquatic organisms consumed in the United States. Since data indicate that the BCF for lipid-soluble compounds is proportional to percent lipids, BCF's can be adjusted to edible portions using data on percent lipids and the amounts of various species consumed by Americans. A recent survey on fish and shellfish consumption in the United States (Cordle, et al. 1978) found that the per capita consumption is 18.7 g/day. From the data on the 19 major species identified in the survey and data on the fat content of the edible portion of these species (Sidwell, et al. 1974), the relative consumption of the four major groups and the weighted average percent lipids for each group can be calculated:

Group	Consumption (Percent)	Weighted Average Percent Lipids
Freshwater fishes	12	4.8
Saltwater fishes	61	2.3
Saltwater molluscs	9	1.2
Saltwater decapods	18	1.2

Using the percentages for consumption and lipids for each of these groups, the weighted average percent lipids is 2.3 for consumed fish and shellfish.

No measured steady-state bioconcentration factor (BCF) is available for benzidine. A weighted average BCF of 1,150 is available for 3,3'-dichlorobenzidine and the calculated octanol-water partition coefficients for the two compounds are 35.5 and 2,190, respectively. The proportionality (Veith, et al. Manuscript) BCF/BCF = Antilog (0.76 log (P/P)) can be used to calculate a weighted average bioconcentration factor of 50 for benzidine for the edible portion of all aquatic organisms consumed by Americans.

Inhalation

In the early phases of the chemical and dye industries, the lack of good industrial hygienic practices and the use of open systems made inhalation one of the principal routes of entry of benzidine and its derivatives into the body. Similar inhalation exposures can occur at the present time unless workers wear respirators and protective clothing while cleaning the equipment (Haley, 1975).

Dermal

Skin absorption is the most important path of entry into the body. Intact skin is readily penetrated by benzidine and 3,3'-dimethylbenzidine (Meigs, et al. 1951). 3,3'-Dichlorobenzidine, because of its nonvolatility and large particle size, presents less of an inhalation and skin penetration hazard than benzidine (Gerarde and Gerarde, 1974; Rye, et al. 1970). It is the light, fluffy, powdery nature of benzidine base that poses the tumorigenic hazard to benzidine workers from skin absorption (Barsotti and Vigliani, 1952). The ease of skin penetration determines the following order of decreasing toxicity from these chemicals: benzidine, 3,3'-dimethoxybenzidine, and 3,3'-dichlorobenzidine (Rye, et al. 1970).

Environmental conditions of high air temperature and humidity increase skin absorption of benzidine, 3,3'-dimethoxybenzidine, 3, '-dichlorobenzidine, and 3,3'-dimethylbenzidine. Higher amounts of benzidine are found in the urine of workers who perspire freely and have a wet skin (Meigs, et al. 1954). Urinary benzidine measurements indicate that benzidine does not accumulate in body tissues, but no direct human tissue determinations have been performed to absolutely establish this concept (Meigs, et al. 1951).

PHARMACOKINETICS

Absorption and Distribution

Benzidine is rapidly absorbed after intravenous injection into rats with maximum concentrations of free and bound benzidine being found at two and three hours, respectively. The highest concentrations were found in the blood, followed by liver, kidney, spleen, heart, and lung (Soloimskaya, 1968). Body distribution of benzidine in various tissues and urine 4 and 12 hours after intraperitoneal injection of 100 mg/kg was as follows: high concentrations in the stomach, stomach contents, and small intestine at 4 hours, and in the small intestine and its contents at 12 hours (Baker and Deighton, 1953). The amine content of the erythrocytes was low at both time intervals. Conjugated material, indicative of metabolites, was high in tissues and urine at 12 hours. Benzidine concentrations in the liver, the target organ for toxicity in rats, were relatively high and constant over the 12-hour period. When rats were given 20 mg of 3,3'-dimethylbenzidine subcutaneously once a week for eight weeks, the highest amine content was found in the Zymbal's gland followed by the kidney, omentum, spleen, and liver (Pliss and Zabezhinsky, 1970).

Metabolism and Excretion

A pharmacokinetic study of benzidine uniformly labeled with ^{14}C and dichlorobenzidine labeled in the 3,3' positions indicated that substitution in the 3,3' positions of the benzidine molecule significantly affects the routes of metabolism and excretion. The blood half-life for benzidine

was 68 hours in the rat and 88 hours in the dog. The weekly excretions of a dose of 0.2 mg/kg of benzidine in the rat, dog, and monkey were 97, 96, and 83 percent respectively. The excretion values for the dichloro compound were 98, 97, and 88.5 percent, respectively. Biliary excretion appears to be the main route of excretion of the dichloro compound in all three species. The dog and monkey excrete free benzidine with the urine, while the rat uses the biliary route. The urinary bladder of the dog had a high content of benzidine, suggesting that this is the reason for urinary bladder cancer in this species (Kellner, et al. 1973).

The various metabolites reported for benzidine and its congeners are given in Table 1. It can be seen that various species handle these chemicals in different ways and that the animal metabolites differ considerably from those excreted by humans. The improvements in analytical techniques have made identification of differences more positive. Of greatest interest are the human studies which will now be discussed.

A single oral dose of 100 mg of benzidine to a human resulted in the excretion of free benzidine and its mono- and diacetylated conversion products in the urine. The entire dose was not recovered indicating that fecal excretion probably occurred. This cannot be proven because the feces were not analyzed (Engelbertz and Babel, 1953). After ingesting 200 mg of benzidine, persons excreted free benzidine and N-hydroxyacetylamino benzidine in their urine (Troll, et al. 1963). In plant workers exposed to benzidine in

TABLE 1

Metabolites Formed by Biotransformation of Benzidine and Benzidine Derivatives in Animals

Compound	Species	Metabolites	Reference
Benzidine	Human	Acetyl N-hydroxy compound	Troll, et al. 1963
	Human	N-Hydroxy acetylaminobenzidine	Haley, 1975
	Human	Monoacetylbenzidine and diacetylbenzidine	Haley, 1975
	Human	3-Hydroxybenzidine	Haley, 1975
	Human	3,3'-Dihydroxybenzidine	Haley, 1975
	Monkey	Monoacetylbenzidin·	Rinde and Troll, 1975
	Dog	3-Hydroxybenzidine and glucuronide	Troll and Nelson, 1958
	Dog	3-Hydroxybenzidine hydrogen sulfate	Sciarini and Meigs, 1958
	Dog	3-Hydroxybenzidine	Bradshaw and Clayson, 1955 Sciarini, 1957
	Dog	4,4'-Diamino-3-diphenyl hydrogen sulfate	Clayson, et al. 1959
	Dog	4-Amino-4-hydroxybiphenyl	Haley, 1975
	Dog	Monoacetylbenzidine and diacetylbendizine	Haley, 1975
	Guinea pig	4'-Acetamido-4-aminodiphenyl	Clayson, et al. 1959
	Guinea pig	4'-Acetamido-4-amino-3-diphenyl hydrogen sulfate	Clayson, et al. 1959
	Guinea pig	4'-Amino-4-diphenyl sulfamic acid	Clayson, et al. 1959
	Guinea pig	N-Glucuronides	Clayson, et al. 1959
	Guinea pig	4'-Acetamido-4-diphenyl sulfamic acid	Clayson, et al. 1959

TABLE 1 (Cont'd)

Compound	Species	Metabolites	Reference
	Rabbit	3-Hydroxybenzidine sulfate and glucuronide	Troll and Nelson, 1958
	Rabbit	4'-Acetamido-4-aminodiphenyl	Clayson, et al. 1959
	Rabbit	3-Hydroxybenzidine	Clayson, et al. 1959
	Rabbit	4'-Acetamido-4-amino-3-diphenylyl hydrogen sulfate	Clayson, et al. 1959
	Rabbit	4'-Amino-4-diphenylyl sulfamic acid	Clayson, et al. 1959
	Rabbit	4'-Acetamido-4-diphenylyl sulfamic acid	Clayson, et al. 1959
	Rabbit	N-Glucuronides	Clayson, et al. 1959
	Rat	3,3'-Dihydroxybenzidine	Haley, 1975
	Rat	N-Glucuronides	Elson, et al. 1958 Clayson, et al. 1959
	Rat	4'-Acetamido-4-Aminodiphenyl	Clayson, et al. 1959
	Rat	3-Hydroxybenzidine	Clayson, et al. 1959
	Rat	4,4'-Diamino-3-diphenyl hydrogen sulfate	Clayson, et al. 1959
	Rat	4'-Acetamido-4-amino-3-diphenylyl hydrogen sulfate	Clayson, et al. 1959
	Rat	4'-Amino-4-diphenylyl sulfamic acid	Clayson, et al. 1959
	Rat	4'-Acetamido-4-diphenylyl sulfamic acid	Clayson, et al. 1959
	Mouse	Monoacetylbenzidine and diacetylbenzidine	Sciarini and Meigs, 1961a
	Mouse	Monoacetylated 3-hydroxybenzidine glucuronide and/or ethereal sulfate	Sciarini and Meigs, 1961a
	Mouse	N-Hydrogen sulfate and/or glucuronide	Sciarini and Meigs, 1961a
	Mouse	3-Hydroxybenzidine glucuronide	Sciarini and Meigs, 1961a

TABLE 1 (Cont'd)

Compound	Species	Metabolites	Reference
	Mouse	4'-Acetamido-4-amino-diphenyl	Clayson, et al. 1959
	Mouse	4,4'-Diamino-3-diphenyl hydrogen sulfate	Clayson, et al. 1959
	Mouse	4'-Acetamido-4-amino-3-diphenylyl hydrogen sulfate	Clayson, et al. 1959
	Mouse	N-Glucuronides	Clayson, et al. 1959
3,3'-dimethyl-benzidine (orthotolidine)	Human	Diacetyl-o-tolidine	Dieteren, 1966
	Human	5-Hydroxy-o-tolidine	Dieteren, 1966
	Human	Monoacetyl-o-tolidine	Dieteren, 1966
	Dog	5-Ethereal sulfate of o-tolidine	Sciarini and Meigs, 1961b
3,3'-Dimethoxy-benzidine (dianisidine)	Dog	Unidentified diamine metabolite	Sciarini and Meigs, 1961b
3-Methoxybenzidine (monosubstituted dianisidine)	Rat	4-Amino-4'-acetamido-3-methoxybiphenyl	Laham, 1971

unknown quantities, free benzidine, its mono-and diacetylated derivatives, and 3-hydroxybenzidine were identified in the urine. The latter compound comprised 78.5 to 89.7 percent of the total (Sciarini and Meigs, 1961a). This work was a repeat of an earlier study by Meigs, et al. (1954), and confirmed the previous findings. It has been suggested that an 8-hour exposure to an air concentration of 0.018 mg/m^3 of benzidine would result in a urinary excretion of not more than 0.026 mg/l of diamines. Thus an air exposure to 0.02 mg/m^3 or less of benzidine would be safe (Meigs, et al. 1954).

Dyestuff factory workers exposed to benzidine excreted free benzidine, 4-amino-4-oxybiphenyl, and monoacetylbenzidine in their urine (Vigliani and Barsotti, 1962).

Exposure to 3,3'-dimethylbenzidine results in urinary excretion of free 3,3'-dimethylbenzidine, its diacetyl derivative, and 5-hydroxy-3,3'-dimethylbenzidine. Although the monoacetylated derivative was not detected, there is a probability of its formation because 3,3'-dimethylbenzidine appears to be metabolized similarly to benzidine (Dieteren, 1966).

3,3'-Dichlorobenzidine has been identified in the urine of workers handling benzidine yellow. This establishes the weakness of the azo linkage in dyes made from this compound (Akiyama, 1970).

It is questionable how comparable animal data are to human data, and whether the former allow predictions to be made concerning the metabolic conversion of chemicals in various species. This is taken into consideration in

the following discussion of the animal data in Table 1 and their relevance to the human situation. Intraperitoneal injection of 100 mg/kg of benzidine in mice produced free benzidine, mono-and diacetylated derivatives as well as the ethereal sulfates and glucuronates of 3-hydroxybenzidine (Sciarini and Meigs, 1961a). The same dose of benzidine in dogs caused the excretion of free benzidine and conjugates of 3-hydroxybenzidine but no acetylated derivatives, because the dog lacks this biotransformation mechanism (Sciarini, 1957). The ethereal sulfate of 3-hydroxy-benzidine has been identified in dog urine and constitutes 25 to 50 percent of the administered dose (Sciarini and Meigs, 1958). The ethereal sulfate and glucuronide were the only metabolites found in dogs given 1 g of benzidine or rabbits given 100 to 300 mg of this chemical (Troll and Nelson, 1958).

The differences in the biotransformation of benzidine by the rat, mouse, rabbit, guinea pig, and dog are related to the presence or absence of specific enzymatic pathways. For example, the dog cannot acetylate benzidine. The rat, rabbit, and guinea pig can produce 4'-amino- and 4'-acetamido--4-diphenylyl sulfamic acid whereas the mouse and dog cannot. Other metabolites found were 4'-acetamido-4-aminodiphenyl, 3-hydroxybenzidine, 4,4'-diamino-3-diphenylyl hydrogen sulfate, and 4'-acetamido-4-amino-3-diphenylyl hydrogen sulfate in the rat; and 4'-acetamido-4-aminodiphenyl, 4,4'-diamino-3-diphenyl hydrogen sulfate, and 4'-acetamido-4-amino-3-diphenylyl hydrogen sulfate in the mouse. 4,4'-Diamino-3-diphenylyl hydrogen sulfate was absent from rabbit and

guinea pig urine, although the other metabolites were present. 3-Hydroxybenzidine and 4,4'-diamino-3-diphenylyl hydrogen sulfate were present in dog urine. In all cases, N-glucuronides were present (Clayson, et al. 1959). Metabolite differences occur when different routes of elimination are considered. Dogs excrete the same benzidine metabolites in urine and bile but their feces have no 3-hydroxybenzidine or N-glucuronides (Clayson, et al. 1959). Comparison of routes of excretion of benzidine and its dichloro derivative in rats, dogs, and monkeys showed that the rat eliminated both compounds in greater quantities in the feces than in the urine; whereas the dog eliminated the dichloro compound to a greater extent in the feces. Neither route was decisive in the monkey, but more of both compounds did appear in the urine (Kellner, et al. 1973). Previously it had been shown that dog fecal excretion of dichlorobenzidine was ten times greater than urinary excretion (Sciarini and Meigs, 1961b), while the opposite was true for benzidine (Sciarini and Meigs, 1958).

When benzidine-based azo dyes were fed to monkeys, benzidine and monoacetylbenzidine were found in the urine (Rinde and Troll, 1975). This shows that the monkey, like man, can reductively cleave the azo linkage (Akiyama, 1970).

Intraperitoneal injection of dimethylbenzidine, dimethoxybenzidine and dichlorobenzidine in dogs resulted in recovery of part of these chemicals in nonmetabolized form. The dichloro compound was not metabolized whereas the other two derivatives of benzidine were recovered from urine as

unidentified conjugated ethereal sulfates (Sciarini and Meigs, 1961b).

EFFECTS

Acute, Subacute, and Chronic Toxicity

In vitro studies have shown that benzidine, 3,3'-dimethylbenzidine, and 3,3'-dimethoxybenzidine are moderate reducers of cytochrome c. 3,3'-Diaminobenzidine is a strong reducer, whereas 3,3'-dichlorobenzidine is an ineffective reducer. It has been suggested that there is a relationship between carcinogenic potential and the reduction of cytochrome c (Hirai and Yasuhira, 1972; Cammer and Moore, 1973).

There is a significant increase in urinary B -glucuronidase activity in workers exposed to benzidine. The elevated activity, although decreased by removal from benzidine exposure, does not return to normal levels (Kleinbauer, et al. 1969; Popler, et al. 1964).

While 3,3'-dimethylbenzidine administered subcutaneously to rabbits had no effect on blood phenolase activity, benzidine decreased the activity of this enzyme (Nakajima, 1955). Rats injected with benzidine showed reduced catalase and peroxidase activity as well as a reduction in erythrocytes and thrombocytes and an increase in leucocytes (Soloimskaya, 1968). An intraperitoneal dose of 12.7 mg/kg of benzidine in rats increased liver glutathione from 182 mg/100 g to 272 mg/100 g in 24 hours (Neish, 1967).

Dermatitis has been reported in workers in the benzidine dyestuff industry, involving both benzidine and its dimethyl derivative. Individual sensitivity plays a promi-

nent role in this condition (Schwartz, et al. 1947).

Glomerulonephritis and nephrotic syndrome have been produced in Sprague-Dawley rats fed 0.043 percent N,N'-diacetylbenzidine. Both sexes developed proteinuria in 3 to 4 weeks. After 2 months the females were excreting 0.1 g of protein per 24 hours. The females developed severe anemia which was rarely seen in the males. The former also had a hypoproteinemia, hyperlipemia, and generalized edema. Glomerular lesions in the females consisted of florid epithelial crescents, progressive sclerosis, and glomerular obliteration. In the males, the lesions were slower in developing and less extensive, but all males showing the nephrotic syndrome also developed testicular atrophy. There were morphological similarties between the human nephrotic syndrome and that induced by N,N'-diacetylbenzidine in rats, including extracapillary cell proliferation, formation of luxuriant crescents in 80 percent of the glomeruli, intact glomerular tufts, and the presence of normal glomeruli in the advanced stages of the syndrome (Harman, et al. 1952; Harman, 1971).

Rats fed N,N'-diacetylbenzidine or 4,4,4',4'-tetramethylbenzidine developed glomerular lesions with fat-filled spaces in the glomerular tuft from 2 to 4.5 months of treatment (Dunn, et al. 1956). Severe glomerulonephritis developed in rats receiving N,N'-diacetylbenzidine by subcutaneous (100 mg) or intraperitoneal (100 or 200 mg) injections. These lesions were dose related (Bremner and Tange, 1966). A similar low grade glomerulonephritis has been produced

in rats fed benzidine (Christopher and Jairam, 1970).

Mice fed 0.01 and 0.08 percent benzidine dihydrochloride developed the following toxic symptoms: decreased carcass, liver, and kidney weights; increased spleen and thymus weights; cloudy swelling of the liver; vacuolar degeneration of the renal tubules; and hyperplasia of the myeloid elements in the bone marrow and of the lymphoid cells in the spleen and thymic cortex. There was a dose dependent body weight loss of 20 percent in males and 7 percent in females. Moreover, male mice were more sensitive to benzidine than female mice (Rao, et al. 1971). This disagrees with Harman's (1971) findings in rats, but it may only be a species difference in response.

Synergism and/or Antagonism

No available data.

Teratogenicity

Embryonic mouse kidney cultures have an increased survival time but show hyperplastic epithelial changes in the presence of 3,3'-dimethylbenzidine (Golub, 1969; Shabad, et al. 1972). Administration of 8 to 10 mg of 3,3'-dimethylbenzidine to mice during the last week of pregnancy resulted in lung adenomas and mammary gland tumors in their progeny. These tumors could have resulted from transplacental transmission of the chemical or from its presence in the milk (Golub, et al. 1974). No teratogenic effects of benzidine derivatives in humans have been reported.

Mutagenicity

The results of the Ames assay on the mutagenicity of benzidine are positive (Ames, et al. 1973; McCann, et al. 1975; Garner, et al. 1975). With metabolic activation, benzidine causes an increase in the recovery of histidine revertants in Salmonella typhimurium strain TA 1537 and TA 1538, both sensitive to frameshift mutagens. The greatest increase was seen with TA 1538.

Another more recently developed assay, used to screen for putative mutagenic/carcinogenic compounds, has been used to test benzidine. This assay detects the inhibition of DNA synthesis in HeLa cells by test compounds (Painter and Howard, 1978). The concentration of a compound that is required to inhibit DNA synthesis by 40 percent corresponds with its mutagenic effects in Salmonella typhimurium. Benzidine has been shown to be positive in this DNA synthesis inhibition test (Painter and Howard, 1978).

Results of a Salmonella mutagenesis assay indicate that benzidine causes a significant increase in the reversion index of tester strains TA 98 and TA 1538 when the compound is activated by the addition of human liver microsomes (U.S. EPA, 1978).

Carcinogenicity

Benzidine and its derivatives are carcinogenic in both experimental animals and humans. In the latter these chemicals have been shown to produce bladder cancer after a long latent period (Clayson, 1976). Additionally, these compounds produce dermatitis, cystitis, and hematuria in humans, indi-

TABLE 2

Effects of Benzidine, Its Congeners, and Metabolites
On Various Animal Species
(Adapted from Haley, 1975)

Species	Carcinogen	Effect
Mouse	Benzidine	Hepatoma, lymphoma, bile duct proliferation
	3,3'-Dihydroxybenzidine	Hepatoma, lymphoma, bile duct proliferation, benign bladder papilloma
Rat	Benzidine and its sulfate	Cirrhosis of liver, hepatomas, carcinoma of Zymbal's gland, adenomacarcinoma, degeneration of bile ducts, sarcoma, mammary gland carcinoma
	3,3'-Dihydroxybenzidine	Hepatoma, adenocarcinoma of colon, carcinoma of forestomach, Zymbal's gland carcinoma, bladder carcinoma
	Dianisidine[a]	Zymbal's gland carcinoma, ovarian tumor
	o-Ditoluidine[b]	Papilloma of stomach, Zymbal's gland carcinoma, mammary tumor, leukemia
	3,3'-Benzidinedioxyacetic acid	Papilloma of bladder, hepatic sarcoma
	3,3'-Dichlorobenzidine	Extensive cancer
	N,N'-Diacetylbenzidine	Chronic glomerulonephritis
Hamster	Benzidine	Hepatoma, liver carcinoma, cholangiomas
	o-Ditoluidine[b]	Bladder cancer
Rabbit	Benzidine	Proteinuria, hematuria, liver cirrhosis, myocardial atrophy, bladder tumor, gall bladder tumor
Dog	Benzidine	Recurrent cystitis, bladder tumor, convulsions, liver cirrhosis, hematuria
Monkey	Benzidine	No pathological changes
Human	Benzidine	Bladder tumor, papilloma, chronic cystitis, hematuria

[a] 3,3'-Dimethoxybenzidine.
[b] 3,3'-Dimethylbenzidine.

cating an early attack on the urinary bladder and presenting a sign that unless exposure is stopped, cancer may result (Haley, 1975). Table 2 gives various animal species and the type of cancer induced in them by benzidine and its congeners. It should be noted that only the dog gets urinary bladder cancer similar to that seen in humans after exposure to benzidine. The animal cancers in general differ significantly in their locations. This may be related to differences in specific target tissues or to differences in excretory pathways. In some cases, excessive dosage may cause death due to toxicity, thus preventing the development of bladder cancer (Haley, 1975).

Benzidine and many other aromatic amines attack the urinary bladder and other organs (Hueper, 1954). However, it is the metabolites of these compounds that are considered to be the proximate carcinogens (Clayson, 1969). These aromatic amines are ring hydroxylated, converted to N-hydroxylated, acylated and deacylated derivatives, and conjugated with sulfate and glucuronide (Haley, 1975). It has been suggested that the conjugated N-hydroxy compounds are the active carcinogens in vivo. Bladder cancer has been induced in rabbits and dogs fed benzidine, but these findings are controversial (Haley, 1975). Spitz, et al. (1950) induced papillary carcinoma in one of seven dogs fed benzidine for 5 years, but the cancer only appeared 7.5 years after the beginning of the experiment. Orally administered benzidine did not produce urinary bladder cancer in dogs (Marhold, et al. 1967). No tumors were found in female beagle dogs fed 1 mg/kg 5 days a week for 3 years (Deichmann, et al.

1965). The lack of a carcinogenic effect in dogs in these latter two studies is probably related to the known long latency for benzidine cancer induction and the shortness of both studies.

Extensive bile duct proliferations and cysts appeared along with cholangiofibrosis, hepatomas, and liver cell carcinoma but no urinary bladder tumors were found in hamsters fed benzidine at 0.1 percent of the diet throughout their life spans (Saffiotti, et al. 1967).

Benzidine administered subcutaneously to rats at a rate of 15 mg/week produced liver injury, cirrhosis, hepatomas, sebaceous gland carcinomas, and adenocarcinomas of the rectum but no bladder tumors (Spitz, et al. 1950). Rats fed 0.125 percent of dihydroxybenzidine in the diet developed liver cirrhosis, hepatomas, adenocarcinomas of the colon, Zymbal's gland carcinoma, and squamous cell carcinomas of the stomach. One sessile papilloma and two keratinized squamous cell carcinomas were found in the bladder wall (Baker, 1953). Intraperitoneal or subcutaneous injection of N,N'-diacetylbenzidine in Wistar rats induced tumors of Zymbal's gland and of the mammary glands 6 to 15 months later. Glomerulonephritis was also reported and appeared to be dose related. Female Sprague-Dawley rats given 12 to 50 mg/rat orally developed mammary gland carcinomas (Griswold, et al. 1968).

Early cirrhosis occurred in rats given benzidine by subcutaneous injection for 6 months (Pliss, 1963). Injection site sarcomas, hepatomas, and Zymbal gland tumors were also found, and constituted 70 percent of the tumors in

these rats (Pliss, 1964). Benzidine was more toxic to the females. Tumors of Zymbal's gland and the liver were induced by 3,3'-benzidine dicarboxylic acid within 1 year (Pliss, 1969). Benzidine, in 5 mg weekly doses, produced intestinal tumors in rats (Pliss, et al. 1973). A cumulative dose of 0.75 mg/kg of benzidine for 15 days produced tumors in 20 of 22 rats, including 19 hepatomas, 18 cholangiomas, 7 intestinal tumors and 4 sebaceous gland carcinomas. Subcutaneous tetramethylbenzidine doses of from 4.15 to 8.3 g/kg produced benign tumors at the injection site (Holland, et al. 1974).

Female Wistar rats given a single intraperitoneal injection of 100 or 200 mg of N,N'-diacetylbenzidine subcutaneously developed Zymbal gland and mammary gland tumors after 6 to 15 months. The 100 mg intraperitoneal injection produced tumors in 11 out of 18 rats while the 200 mg dose gave no tumors (Bremner and Tange, 1966).

Hepatomas, bile duct proliferation, and benign papillomas of the urinary bladder were found in Delph albino mice injected subcutaneously with 300 mg of benzidine or dihydroxybenzidine. Only the latter chemical caused the bladder changes (Baker, 1950).

Benzidine or 3,3-dihydroxybenzidine administered subcutaneously at 6 mg weekly for 52 weeks produced tumors in exposed mice in 70 weeks. Benzidine induced hepatomas and lymphomas while the 3,3-dihydroxy derivative induced lymphomas and benign intestinal polyps. The significance of the lymphomas is obscure because one-third of the controls developed this condition spontaneously (Bonser, et al. 1956). Subcuta-

neous administration of 3,3-dihydroxybenzidine in mice caused tumors of the liver and mammary glands as well as leucosis (Pliss, 1961). Inner organ tumors developed after skin application of the chemical. Subcutaneous weekly doses of 6 mg of benzidine to C3HA mice induced hepatomas in 31 of 46 animals after 15 to 16 months. One animal developed a pulmonary adenocarcinoma (Prokofjeva, 1971).

3,3-Dimethylbenzidine in a cumulative dose of 5.4 g/kg for 241 days induced 11 gastrointestinal tract tumors, 7 hepatomas, 7 bone tumors and 4 Zymbal's gland carcinomas in rats. Total oral doses of 500 mg in Sprague-Dawley rats produced 4 mammary carcinomas in 9 months in 3 of 16 surviving animals (Griswold, et al. 1968). Subcutaneous injection of 3,3'-dimethylbenzidine in rats caused skin tumors, large sebaceous gland tumors, and mammary tumors in 60 to 70 percent of the animals. When 20 mg of the chemical was implanted subcutaneously, hepatocellular carcinomas and subcutaneous sarcomas were produced (Pliss and Zabezhinsky, 1970).

3,3'-Dimethoxybenzidine given subcutaneously to rats induced Zymbal gland tumors in two animals and an ovarian tumor and a fibroadenoma of the mammary gland in another one (Pliss, 1963). Both male and female Fischer strain rats developed tumors of the gastrointestinal tract, skin, breast, and ear duct after receiving 260 oral 10 mg doses of 3,3'-dimethoxybenzidine. The latency was 293 days (Weisburger, et al. 1967).

Subcutaneous administration of 3,3'-dichlorobenzidine to rats induced tumors in 74 percent of the animals (Pliss, 1963). Tumors appeared in the skin, sebaceous and mammary

glands, intestines, bones, and urinary bladder. Dichlorobenzidine given by ingestion or injection into the underlying fat produced sarcomas at the injection site, an adenocarcinoma in the intestine, papillomas in the urinary bladder, and tumors in the sebaceous and mammary glands (Pliss, 1959). Total doses of 300 mg/rat orally of dichlorobenzidine produced no tumors (Griswold, et al. 1968). Rats fed 1,000 mg/kg in the diet developed mammary gland tumors in both sexes and Zymbal's gland and hematopoietic tumors in males (Stula, et al. 1971, Stula, et al. 1975). Progeny of BALB/c mice given total subcutaneous doses of 8 to 10 mg of dichlorobenzidine had a significant increase in tumor incidence. Tumors developed in 13 of 24 mice; with 4 adenocarcinomas of the mammary gland, 5 lung adenomas and 7 cases of lymphatic leukemia (Golub, et al. 1974).

The carcinogencity risk for workers exposed to benzidine is 14 times higher than for the unexposed population (Case, et al. 1954). In the American dyestuff industry, 24 cases of bladder carcinomas were found in workers exposed to aromatic amines including benzidine. The latency for tumor development was 12 years (Gehrman, 1936). In England the tumor induction time averaged 16 years but one case occurred in 2 years (Case, et al. 1954). In 30 cases of bladder tumors the induction period varied from 8 to 32 years, with an average of 15.9 years. The concentration of benzidine in the exposure appeared to be the main factor in early tumor induction. Benzidine manufacturing was associated with 14 papillomas, 7 carcinomas and 2 cases in which

the papillomas were converted to carcinomas (Scott, 1952). Only a few weeks of exposure followed by a latent period of several years can produce bladder tumors (Deichmann and Gerarde, 1969). A latent period of 18.6 years has also been reported (Hamblin, 1963). Initial exposure concentration, exposure duration and years of survival following exposure as well as work habits and personal hygiene are involved in the development of carcinomas where benzidine appears to be implicated (Rye, et al. 1970). There is little doubt that benzidine exposure is associated with an increase in the occurrence of bladder cancer (Int. Agency Res. Cancer, 1972; Riches, 1972; Sax, 1975). However, there is a lack of information on the exact concentrations of benzidine to which workers have been exposed.

Long exposure to benzidine produced bladder tumors in 13 out of 25 men (Zavon, et al. 1973). Comparison of the two groups showed that the tumor group was exposed to benzidine for an average of 13 years while the non-tumor group was exposed for an average of less than 9 years. Observations were carried out for approximately 12 years following exposure. Ambient air benzidine in the plant varied from 0.005 to 0.415 mg/m^3 with one area giving a value of 17.6 mg/m^3 (Wendel, et al. 1974). Death records of 171 workers showed that 18 were due to bladder and kidney cancers and that there was a higher rate of neoplasms of the digestive system. It appeared that there could have been a synergistic effect between benzidine and β-naphthyla-

mine, since these workers were exposed to both chemicals (Mancuso and El-Attar, 1966, 1967).

When benzidine dyestuff manufacturing begins in any country the incidence of bladder tumors among exposed workers increases. Table 3 shows the times of discovery of aromatic amine bladder cancer in a number of countries. Urinary system tumors occurred in 17 percent of the workers in one benzidine plant. The highest rate of tumors was in the group exposed for 6 to 10 years (Kuzelova, et al. 1969). Men working in a French aromatic amine plant developed bladder tumors. One Normandy factory had 54 cases, with 17 occurring prior to 1947 and 34 subsequent to 1947. Symptoms of hematuria and stranguria were found in 18 cases (Billiard-Duchesne, 1960).

In Italy, 24 cancers were found in workers exposed to benzidine or benzidine-β-naphthylamine (Vigliani and Barsotti, 1962). Italian benzidine workers were found to have developed 47 cases of bladder cancer during the period from 1931 to 1960. There were 21 carcinomas and 16 papillomas. During the period from 1931 to 1948, 13 of 83 workers developed bladder carcinomas from benzidine (Barsotti and Vigliani, 1952). The greatest exposure occurred in workers in filtration, pressing, drying, and milling of benzidine. Maximum latency for benzidine tumors was 16 years from the cessation of exposure. Ten papillomas and seven carcinomas were found in a cohort of 858 benzidine dyestuff workers (Forni, et al. 1972).

TABLE 3

Time of Discovery of Aromatic Amine Bladder Cancer by Country (Haley, 1975)

Country	Year
Germany	1895
Switzerland	1905
United Kingdom	1918
U.S.S.R.	1926
United States	1931
Austria	1932
Italy	1936
Japan	1940
France	1946

Studies in dyestuff plants in Japan showed 100 cases of bladder cancer during the period 1949 to 1970. Benzidine production workers accounted for 11.25 percent of the cases and benzidine users for 1.45 percent. Eight cases developed cancer of the upper urinary tract and not the bladder. There was a long latent period of 16.25 years (Tsuchiya, et al. 1975). The silk kimono painters are the highest risk bladder cancer group in Japan because they point their brushes, thereby ingesting benzidine dyes (Yoshida and Miyakawa, 1973).

There was a high incidence of bladder tumors, (21.3 percent) in benzidine workers in a coal tar dye factory. The latent period was 18.4 years for papillomas and 18.7 for carcinomas (Goldwater, et al. 1965). A further study showed that the combined exposure to benzidine plus β-naphthylamine increased the bladder cancer rate to 45.5 percent (Kleinfeld, et al. 1966). Occupational bladder cancers are morphologically similar to spontaneous bladder tumors found in the general population. Both have a tendency for high recurrence after treatment.

At the present time there is no evidence that 3,3'-dimethylbenzidine, 3,3'-dimethyoxybenzidine, or 3,3'dichlorobenzidine are human bladder carcinogens (Rye, et al. 1970). However, future epidemiological study may show them to be carcinogenic agents. No bladder neoplasms related to exposure to 3,3'-dichlorobenzidine over a 35-year period were found. However, the following neoplasms were reported in 17 workers: two lung cancers, one bone marrow cancer, six lipomas, three

rectal papillomas, two sigmoid colon carcinomas, one prostate carcinoma, one breast muscle myoblastoma, and one basal cell epithelioma (Gerarde and Gerarde, 1974). No bladder tumors were found in British workers handling this chemical but the worker exposure time of less than 16 years could account for these findings (MacIntyre, 1975). It is possible that the latent period for bladder tumors is longer for 3,3'-dichlorobenzidine since workers exposed to benzidine plus dichlorobenzidine developed such tumors while those exposed to the latter compound alone did not (Gadian, 1975).

CRITERION FORMULATION

Existing Guidelines and Standards

In 1973 the Environmental Protection Agency proposed but did not promulgate a toxic pollutant standard for benzidine (30 FR 35388).

The industrial standards instituted by the Occupational Safety and Health Administration in 1974 excluded from regulation any compounds containing less than 0.1 percent benzidine. These standards did not recognize a safe level of water contamination and provided no provisions for environmental monitoring.

New standards for benzidine discharges have been proposed (41 FR 27012) based upon information on the toxicological and environmental effects and the fate of benzidine. These standards, promulgated in 1977, established an ambient water criterion for benzidine of 0.1 µg/l. Effluent standards were set at 10 µg/l (daily average) with a maximum for any single day of 50 µg/l. Based on a monthly average, daily loading was limited to 0.13 kg/1000 kg of benzidine produced. The standards set for users of benzidine-based dyes were the same except that the maximum daily effluent concentration of benzidine was limited to 25 µg/l (42 FR 2617).

Current Levels of Exposure

It is essential that consideration be given to the manner in which benzidine and its congeners and the dyes derived from them contaminate water supplies. In most cases these chemicals are a hazard only in the vicinity of dye and pigment plants where wastes escape or are discharged.

A field survey of the Buffalo and Niagara river areas using the chloramine-T method, with a sensitivity of 0.2 ug/l, showed no benzidine in the samples. However, this method of analysis is photosensitive and leads to low estimates of benzidine. Moreover, the samples may have been below the level of detectability or oxidative degradation may have converted the benzidine compounds to materials not detectable by the analytical method used (Howard and Saxena, 1976). A Japanese survey of the Sumida River area detected 0.082, 0.140, and 0.233 mg/l of benzidine in the water. The authors believed that the benzidine came from azo dyes by H_2S or SO_2 reduction (Takemura, et al. 1965).

Information on 3,3'-dimethylbenzidine, 3,3'-dimethoxybenzidine, and 3,3'-dichlorobenzidine and their dye derivatives as water contaminants is non-existent and research should be instituted to correct this deficiency.

It has been stated that benzidine resists physical and biological degradation (Lutin, et al. 1965; Malaney, et al. 1967; Radding, et al. 1975). Benzidine in water is oxidatively degraded by free radical, enzymatic or photochemical processes (Radding, et al. 1975). Its half-life in water has been estimated to be 100 days. Air oxidation of benzidine in water seems to occur readily (Howard and Saxena, 1976).

Humic material seems to bind 3,3'-dichlorobenzidine tightly and its degradation appears to be slower than benzidine, but the half-lives of the two compounds are the same (Radding, et al. 1975). There is no information available

on the dimethyl- and dimethoxy derivatives. This deficiency must be corrected.

Benzidine is converted to a chloramine type compound during water chlorination processes (Jenkins and Baird, 1975). Soil and intestinal bacteria reduce benzidine azo dyes to free benzidine (Yoshida and Miyakawa, 1973), and although aquatic organisms might also cause this same transformation, no data are available to prove this point. It should be remembered that the hydrochlorides of benzidine are much more soluble in water than the free amines and are more resistant to degradation than the latter (Bowman, et al. 1976).

Special Groups at Risk

A potential health hazard exists in the production of benzidine and its congeners and their conversion to azo dyes. There is no maximum permissible level of contamination in the industrial environment although there are specific regulations governing the manufacture of benzidine and its congeners (39 FR 3756). These standards have reduced the risks to benzidine workers.

The use of benzidine and its congeners poses a potential risk to workers in biochemical, chemical, and microbiological laboratories where these chemicals are used as analytical reagents (Collier, 1974; Veys, 1972; Wood and Spencer, 1972). The greatest risk occurs in laboratories working with known carcinogens when good laboratory practices are not enforced. No epidemiological evidence is available to determine the exact extent of the problem.

The risk to the general population from benzidine, its congeners, and their dyes is unknown, but contamination of water supplies, which is known to occur in Japan (Takemura, et al. 1965), poses a yet to be determined risk. There also is a potential risk for workers in the garment, leather, and homecraft industries where the benzidine dyes are used.

Basis and Derivation of Criteria

The available data concerning the carcinogenicity of benzidine in experimental animals are severely limited. It is extremely difficult to extrapolate the experimental results to man because, with the possible exception of the dog and the rabbit, the target organs are different. Moreover, the metabolites produced by the various species, in general, differ significantly from those produced by man (Haley, 1975), although 3-hydroxybenzidine and its conjugation products are common to both man and animals.

Despite the limitations of the available data, a suggested criterion for benzidine was calculated using the linear non-threshold model described in the appendix. The calculation assumes a risk of 1 in 100,000 of developing cancer as a result of daily consumption of 2 liters of benzidine contaminated water and the daily consumption of 18.7 g of benzidine contaminated aquatic organisms. Based on the data of Zavon, et al. (1973), a benzidine criterion of 1.67×10^{-3} ($\mu g/l$) is suggested to be adequate to protect the population consuming the water.

Epidemiological data indicate that exposure to benzidine is associated with an increase in bladder cancer in man.

The possibility that benzidine may be found in wastewater may also pose a problem. In order to determine the extent of the potential problem, measurements must be made of wastewater not only for benzidine but also for its congeners. Moreover, further evaluation must be made on these chemicals and their azo dye derivatives to determine their stability to microbiological degradation. It is essential that studies of their carcinogenicity in experimental animals be made at doses which produce a bare minimum of liver pathology. A detailed pharmacokinetic study should be undertaken to establish routes of absorption, body transport, storage and excretion of benzidine, its congeners, and the azo dyes synthetized from them. Programs covering both industrial hygienic and epidemiologic aspects of exposure to benzidine and its congeners to establish the degree of dermal and pulmonary absorption are a necessity if we are to prevent this chemically induced cancer from occurring.

Under the Consent Decree in NRDC vs. Train, criteria are to state "recommended maximum permissible concentrations (including where appropriate, zero) consistent with the protection of aquatic organisms, human health, and recreational activities." Benzidine is suspected of being a human carcinogen. Because there is no recognized safe concentration for a human carcinogen, the recommended concentration of benzidine in water for maximum protection of human health is zero.

Because attaining a zero concentration level may be infeasible in some cases and in order to assist the Agency

and States in the possible future development of water quality regulations, the concentrations of benzidine corresponding to several incremental lifetime cancer risk levels have been estimated. A cancer risk level provides an estimate of the additional incidence of cancer that may be expected in an exposed population. A risk of 10^{-5} for example, indicates a probability of one additional case of cancer for every 100,000 people exposed, a risk of 10^{-6} indicates one additional case of cancer for every million people exposed, and so forth.

In the Federal Register notice of availability of draft ambient water quality criteria, EPA stated that it is considering setting criteria at an interim target risk level of 10^{-5}, 10^{-6} or 10^{-7} as shown in the table below.

Exposure Assumptions	Risk Levels and Corresponding Criteria(1)			
	0	10^{-7}	10^{-6}	10^{-5}
2 liters of drinking water and consumption of 18.7 grams of fish and shellfish (2)	0	1.67×10^{-5} µg/l	1.67×10^{-4} µg/l	1.67×10^{-3} µg/l
Consumption of fish and shellfish only.	0	5.24×10^{-5} µg/l	5.24×10^{-4} µg/l	5.24×10^{-3} µg/l

(1) Calculated by applying a modified "one hit" extrapolation model described in the FR 15926, 1979. Appropriate bioassay data used in the calculation of the model are presented in Appendix I. Since the extrapolation model is linear to low doses, the additional lifetime risk is directly proportional to the water concentration. Therefore, water concen-

trations corresponding to other risk levels can be derived by multiplying or dividing one of the risk levels and corresponding water concentrations shown in the table by factors such as 10, 100, 1,000, and so forth.

(2) Thirty-two percent of benzidine exposure results from the consumption of aquatic organisms which exhibit an average bioconcentration potential of 50 fold. The remaining 68 percent of benzidine exposure results from drinking water.

Concentration levels were derived assuming a lifetime exposure to various amounts of benzidine, (1) occurring from the consumption of both drinking water and aquatic life grown in water containing the corresponding benzidine concentrations and, (2) occurring solely from the consumption of aquatic life grown in the waters containing the corresponding benzidine concentrations.

Although total exposure information for benzidine is discussed and an estimate of the contributions from other sources of exposure can be made, this data will not be factored into the ambient water quality criteria formulation because of the tenuous estimates. The criteria presented, therefore, assume an incremental risk from ambient water exposure only.

REFERENCES

Akiyama, T. 1970. The investigation on the manufacturing plant of organic pigment. Jikeikai Med. Jour. (Japan) 17: 1.

Ames, B. et al. 1973. Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. Proc. Natl. Acad. Sci. 70: 2281.

Baker, R.K. 1950. The carcinogenic activity of dihydroxy benzidine (3,3'-dihydroxy 4,4'-diamino diphenyl). Acta Unio Int. Contra Cancrum (Belgium) 7: 46.

Baker, R.K. 1953. The carcinogenic activity of dihydroxy-benzidine, further investigations. Cancer Res. 13: 137.

Baker, R.K., and J.G. Deighton. 1953. The metabolism of benzidine in the rat. Cancer Res. 13: 529.

Barsotti, M., and E.C. Vigliani. 1952. Bladder lesions from aromatic amines. Arch. Ind. Hyg. Occup. Med. 5: 234.

Billiard-Duchesne, J.L. 1960. Cas Francais de tumeurs professionelles de la vessie. Acta Unio Int. Contra Cancrum (Belgium) 16: 284.

Bonser, G.M., et al. 1956. The induction of tumours of the subcutaneous tissues, liver and intestine in the mouse by certain dyestuffs and their intermediates. Br. Jour. Cancer 10: 653.

Bowman, M.C., et al. 1976. Benzidine and congeners: Analytical chemical properties and trace analysis in five substrates. Int. Jour. Environ. Anal. Chem. 4: 205.

Bradshaw, L., and D.B. Clayson. 1955. Metabolism of two aromatic amines in the dog. Nature (Great Br.) 176: 974.

Bremner, D.A., and J.D. Tange. 1966. Renal and neoplastic lesions after injection of N,N'-diacetylbenzidine. Arch. Pathol. 81: 146.

Cammer, W., and C.L. Moore. 1973. Oxidation of 3,3'-diaminobenzidine by rat liver mitochondria. Biochemistry 12: 2502.

Case, R.A.M., et al. 1954. Tumours of the urinary bladder in workmen engaged in the manufacture and use of certain dyestuff intermediates in the British chemical industry: Part I. The role of aniline, benzidine, alpha-naphthylamine and beta-naphthylamine. Br. Jour. Ind. Med. 11: 75.

Christopher, K.J., and B.T. Jairam. 1970. Benzidine (H_2NC_6-$H_4C_6H_4NH_2$) poisoning in white rats. Sci. Cult. (India) 36: 511.

Clayson, D.B. 1969. Some problems in bladder carcinogenesis. Page 284 in E.D. Bergmann and B. Pullman, eds. Physicochemical mechanisms of carcinogenesis. The Israel Acad. Sci. Human., Jerusalem.

Clayson, D.B. 1976. Case Study 2: Benzidine and 2-naphthylamine - voluntary substituion or technological alternatives. Ann. N.Y. Acad. Sci. 271: 170.

Clayson, D.B., et al. 1959. The fate of benzidine in various species. Acta Unio Int. Contra Cancrum (Belgium) 15: 581.

Collier, H.B. 1974. Are orthotolidine and dianisidine health hazards to laboratory workers? Clin. Biochem. (Can.) 7: 3.

Cordle, F., et al. 1978. Human exposure to polychlorinated biphenyls and polybrominated biphenyls. Environ. Health Perspect. 24: 157.

Deichmann, W.B., and H.W. Gerarde. 1969. Toxicology of drugs and chemicals. Academic Press, New York.

Deichmann, W.B., et al. 1965. Synergism among oral carcinogens. III. Simultaneously feeding four bladder carcinogens to dogs. Ind. Med. Surg. 34: 640.

Dieteren, H.M.L. 1966. The biotransformation of o-tolidine. Arch. Environ. Health 12: 30.

Dunn, T.B., et al. 1956. Lipemia and glomerular lesions in rats fed diets containing N,N'-diacetylbenzidine and 4,4,4',4'-tetramethylbenzidine. Proc. Soc. Exp. Biol. Med. 91: 105.

Elson, L.A., et al. 1958. The metabolism of aromatic amines in relation to carcinogenesis. Br. Jour. Cancer 12: 108.

Englebertz, P., and E. Babel. 1953. Nachweis von benzidin und seinen umwand lungs produkten im harn und in organteilen. Zentr. Arbeitsmed. Arbeitsschutz 3: 161.

Forni, A., et al. 1972. Urinary cytology in workers exposed to carcinogenic aromatic amines: A six-year study. Acta Cytol. 16: 142.

Gadian, T. 1975. Carcinogens in industry, with special reference to dichlorobenzidine. Chem. Ind. (Great Br.) 19: 821.

Garner, et al. 1975. Testing of some benzidine anologies for microsomal activation to bacterial mutagens. Cancer Let. 1: 39.

Gehrman, G.H. 1936. Papilloma and carcinoma of the bladder in dye workers. Jour. Am. Med. Assoc. 107: 1436.

Gerarde, H.W., and D.F. Gerarde. 1974. Industrial experience with 3,3'-dichlorobenzidine: An epidemiological study of a chemical manufacturing plant. Jour. Occup. Med. 16: 322.

Goldwater, L.J., et al. 1965. Bladder tumors in a coal tar dye plant. Arch. Environ. Health 11: 814.

Golub, N.I. 1969. Transplacental action of 3,3'-dichlorobenzidine and orthotoludine on organ cultures of embryonic mouse kidney tissue. Bull. Exp. Biol. Med. (USSR) 68: 1280.

Golub, N.I., et al. 1974. Oncogenic action of some nitrogen compounds on the progeny of experimental mice. Bull. Exp. Biol. Med. (USSR) 78: 1402.

Griswold, D.P. Jr., et al. 1968. The carcinogenicity of multiple intragastric doses of aromatic and heterocyclic nitro or amino derivatives in young female Sprague-Dawley rats. Cancer Res. 28: 924.

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

Hamblin, D.O. 1963. Aromatic nitro and amino compounds. Page 2105 in D.W. Fassett and D.D. Irish, eds. Industrial hygiene and toxicology. Vol. II. Interscience Publishers, New York.

Harman, J.W. 1971. Chronic glomerulonephritis and the nephrotic syndrome induced in rats with N,N'-diacetylbenzidine. Jour. Pathol. (Scotland) 104: 119.

Harman, J.W., et al. 1952. Chronic glomerulonephritis and nephrotic syndrome induced in rats by N,N'-diacetylbenzidine. Am. Jour. Pathol. 28: 529.

Hirai, K., and K. Yasuhira. 1972. Mitochondrial oxidation of 3,3'-diaminobenzidine and related compounds, and their possible relation to carcinogenesis. Gann (Japan) 63: 665.

Holland, V.R., et al. 1974. A safer substitute for benzidine in the detection of blood. Tetrahedron (Great Br.) 30: 3299.

Howard, P.H., and J. Saxena. 1976. Persistence and degradability testing of benzidine and other carcinogenic compounds. EPA-560/5-76-005. U.S. Environ. Prot. Agency, Washington, D.C.

Hueper, W.C. 1954. Recent developments in environmental cancer. Arch. Pathol. 58: 475.

International Agency for Research on Cancer. 1972. IARC monographs on the evaluation of carcinogenic risk of chemicals to man. Vol. I. Lyon, France.

Jenkins, R.L., and R.B. Baird. 1975. The determination of benzidine in wastewaters. Bull. Environ. Contam. Toxicol. 13: 436.

Kellner, H.M., et al. 1973. Animal studies on the kinetics of benzidine and 3,3'-dichlorobenzidine. Arch. Toxicol. (West Germany) 31: 61.

Kleinbauer, V., et al. 1969. Sledovani expozice zamestnancu pri vyrobe benzidinu. Cesk. Hyg. (Czechoslovakia) 14: 150.

Kleinfeld, M., et al. 1966. Bladder tumors in a coal tar dye plant. Ind. Med. Surg. 35: 570.

Kuzelova, M., et al. 1969. Sledovani pracovniku zamestnanych pri vyrobe benzidinu. Prac. Lek. (Czechoslovika) 21: 310.

Laham, S. 1971. Metabolism of a new carcinogen related to benzidine. Toxicol. Appl. Pharmacol. 19: 368.

Lutin, P.A., et al. 1965. Oxidation of selective carcinogenic compounds by activated sludge. Proc. Ind. Waste Conf. 20: 131.

MacIntyre, I. 1975. Experience of tumors in a British plant handling 3,3'-dichlorobenzidine. Jour. Occup. Med. 17: 23.

Malaney, G.W., et al. 1967. Resistance of carcinogenic organic compounds to oxidation by activated sludge. Jour. Water Pollut. Control Fed. 39: 2020.

Mancuso, T.F., and A.A. El-Attar. 1966. Cohort studies of workers exposed to betanaphthylamine and benzidine. Ind. Med. Surg. 35: 571.

Mancuso, T.F., and A.A. El-Attar. 1967. Cohort study of workers exposed to betanaphthylamine and benzidine. Jour. Occup. Med. 9: 277.

Marhold, J., et al. 1967. Possible complicity of diphenyline in the origin of tumors in the manufacture of benzidine. Toxicol. Appl. Pharmacol. 10: 397.

McCann, J., et al. 1975. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc. Natl. Acad. Sci. 72: 5135.

Meigs, J.W., et al. 1951. A study of exposure to benzidine and substituted benzidines in a chemical plant. Arch. Ind. Hyg. Occup. Med. 4: 533.

Meigs, J.W., et al. 1954. Skin penetration by diamines of the benzidine group. Arch. Ind. Hyg. Occup. Med. 9: 122.

Nakajima, T. 1955. On the influence of the aromatic nitro- and amino-derivatives on the blood phenolase activity. Rodo Kagaku Kiho (Japan) 4: 22.

Neish, W.J.P. 1967. Liver glutathione and polyamines in hepatocarcinogen-treated rats. Biochem. Pharmacol. (Great Br.) 16: 163.

Pliss, G.G. 1959. The blastomogenic action of dichloro-benzidine. Vopr. Onkol. (USSR) 5: 524.

Pliss, G.B. 1961. On the cancerogenic action of 3,3'-dioxy-benzidine (Is 3,3'-dioxybenzidine a basic cancerogenic metabolite of benzidine?) Vopr. Onkol. (USSR) 7: 33.

Pliss, G.B. 1963. On some regular relationships between carcinogenicity of aminodiphenyl derivatives and the structure of substance. Acta Unio Int. Contra Cancrum (Belgium) 19: 499.

Pliss, G.B. 1964. On the cancerogenic properties of benzidine. Vopr. Onkol. (USSR) 10: 50.

Pliss, G.B. 1969. On peculiarities of carcinogenic effect of 3,3'-benzidine bicarboxylic acid. Vopr. Onkol. (USSR) 15: 60.

Pliss, G.B., and M.A. Zabezhinsky. 1970. Carcinogenic properties of orthotolidine (3,3'-dimethylbenzidine). Jour. Natl. Cancer Inst. 45: 283.

Pliss, G.B., et al. 1973. On intestinal tumors induced by benzidine in rats. Vopr. Onkol. (USSR) 19: 75.

Popler, A., et al. 1964. Follow-up of exposure in people working in the production of benzidine. Prac. Lek. (Czechoslovakia) 16: 147.

Prokofjeva, O.G. 1971. Induction of hepatic tumors in mice by benzidine. Vopr. Onkol. (USSR) 17: 61.

Radding, S.B., et al. 1975. Review of the environmental fate of selected chemicals. EPA 560/5-75-001. U.S. Environ. Prot. Agency, Washington, D.C.

Rao, K.V.N., et al. 1971. Subacute toxicity of benzidine in the young adult mice. Fed. Proc. Am. Soc. Exp. Biol. 30: 344.

Riches, E. 1972. Industrial cancers. Nurs. Mirror (Great Br.) 134: 21.

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

Rye, W.A., et al. 1970. Facts and myths concerning aromatic diamine curing agents. Jour. Occup. Med. 12: 211.

Saffiotti, U., et al. 1967. Induction of bladder cancer in hamsters fed aromatic amines. Page 129 in W. Deichmann and K.F. Lampe, eds. Bladder cancer; a symposium. Aesculapis, Birmingham, Ala.

Sax, N.I. 1975. Dangerous properties of industrial materials. 4th ed. Van Nostrand Reinhold Co., New York.

Schwartz, L., et al. 1947. Dermatitis in synthetic dye manufacture. Page 268 in Occupational diseases of the skin. Lea and Febiger, Philadelphia, Pa.

Sciarini, L.J. 1957. 3-Hydroxybenzidine, a metabolite of benzidine. Arch. Biochem. Biophys. 71: 437.

Sciarini, L.J., and J.W. Meigs. 1958. The biotransformation of benzidine (4,4'-diaminobiphenyl), an industrial carcinogen in the dog. I. Am. Med. Assoc. Arch. Ind. Health 18: 521.

Sciarini, L.J., and J.W. Meigs. 1961a. The biotransformation of benzidine. II. Studies in mouse and man. Arch. Environ. Health 2: 423.

Sciarini, L.J., and J.W. Meigs. 1961b. Biotransformation of the benzidines: III. Studies on diorthotoludine, dianisidine and dichlorobenzidine: 3,3'-disubstituted congeners of benzidine (4,4'-diaminobiphenyl). Arch. Environ. Health 2: 584.

Scott, T.S. 1952. The incidence of bladder tumours in a dyestuffs factory. Br. Jour. Ind. Med. 9: 127.

Shabad, L.M., et al. 1972. Transplacental effect of some chemical compounds on organ cultures of embryonic kidney tissue. Cancer Res. 32: 617.

Sidwell, V.D., et al. 1974. Composition of the edible portion of raw (fresh or frozen) crustaceans, finfish, and mollusks. I. Protein, fat, moisture, ash, carbohydrate, energy value, and cholesterol. Mar. Fish. Rev. 36: 21.

Soloimskaya, E.A. 1968. The distribution of benzidine in rat organs and its effect on the peripheral blood. Vopr. Onkol. (USSR) 14: 51.

Spitz, S., et al. 1950. The carcinogenic action of benzidine. Cancer 3: 789.

Stula, E.F., et al. 1971. Experimental neoplasia in ChR-CD rats with the oral administration of 3,3'-dichlorobenzidine, 4,4'-methylenebis (2-chloroaniline) and 4,4'-methylenebis (2-methylaniline). Toxicol. Appl. Pharmacol. 19: 380.

Stula, E.F., et al. 1975. Experimental neoplasia in rats from oral administration of 3,3'-dichlorobenzidine, 4,4'-methylenebis (2-chloroaniline) and 4,4'-methylene-bis (2-methylaniline). Toxicol. Appl. Pharmacol. 31: 159.

Takemura, N., et al. 1965. A survey of the pollution of the Sumida River, especially on the aromatic amines in the water. Int. Jour. Air Water Pollut. 9: 665.

Troll, W., and N. Nelson. 1958. Studies on aromatic amines: I. Preliminary observations on benzidine metabolism. Am. Ind. Hyg. Assoc. Jour. 19: 499.

Troll, W., et al. 1963. N-hydroxy acetyl amino compounds, urinary metabolites of aromatic amines in man. Proc. Am. Assoc. Cancer Res. 4: 68.

Tsuchiya, K., et al. 1975. An epidemiological study of occupational bladder tumours in the dye industry of Japan. Br. Jour. Ind. Med. 32: 203.

U.S. EPA. 1978. In-depth studies on health and environmental impacts of selected water pollutants. Contract No. 68-01-4646. U.S. Environ. Prot. Agency. Washington, D.C.

Veith, G.D., et al. An evaluation of using partition coefficients and water solubility to estimate bioconcentration factors for organic chemicals in fish. (Manuscript.)

Veys, C.A. 1972. Aromatic amines: The present status of the problem. Ann. Occup. Hyg. 15: 11. (Great Br.)

Vigliani, E.C., and M. Barsotti. 1962. Environmental tumors of the bladder in some Italian dyestuff factories. Acta Unio Int. Contra Cancrum (Belgium) 18: 669.

Weisburger, J.H., et al. 1967. New carcinogenic naphthalene and biphenyl derivatives. Nature 213: 930. (Great Br.)

Wendel, R.G., et al. 1974. Benzidine: A bladder carcinogen. Jour. Urol. 111: 607.

Wood, J.M., and R. Spencer. 1972. Carcinogenic hazards in the microbiological laboratory. Page 185 in D.A. Shapton and R.G. Board, eds. Safety in microbiology. Academic Press, London.

Yoshida, O., and M. Miyakawa. 1973. Etiology of bladder cancer "metabolic" aspects. Page 31 in W. Nakahara, et al. eds. Analytical and experimental epidemiology of cancer. University Park Press, Baltimore.

Zavon, M.R., et al. 1973. Benzidine exposure as a cause of bladder tumors. Arch. Environ. Health 27: 1.

APPENDIX I

Summary and Conclusions Regarding the Carcinogenicity of Benzidine*

Benzidine ((1,1'-Biphenyl)-4,4'-diamine) is used in the manufacture of dyes, as a reagent for detection of H_2O_2 in milk and as a reagent for hemoglobin.

It appears that the greatest hazard to exposure from benzidine occurs during its manufacture. Absorption through the skin is the primary route of entry into the body, although other routes of exposure such as inhalation and ingestion also exist. Exposure to benzidine, its derivatives, and other chemicals involved in the manufacture of dyes has long been known to be associated with an elevated incidence of bladder cancer in workers in Germany, England, Italy, France, Switzerland, Japan, and the United States (see Haley, et al. 1975, Zavon, et al. 1973, Clayson, 1976).

Epidemiological data clearly demonstrate that benzidine is a bladder carcinogen in humans and experimental evidence indicates that it can induce cancer in a variety of organs in several species of animals. Several animal studies have reported carcinogenic effects of benzidine in hamsters (liver), rats (liver and Zymbal glands), and mice (liver). Dogs have been reported to develop urinary bladder tumors following chronic exposure to large doses of benzidine (Spitz, et

*This summary has been prepared and approved by the Carcinogens Assessment Group, EPA, on July 15, 1979.

al. 1950; Bonser, et al. 1956). However, the small numbers of animals involved make the significance of these findings questionable.

The difference in organotropic properties of benzidine among the different species is probably due to both its route of excretion and metabolism. For example, in humans and dogs, benzidine or its metabolites are largely excreted through the urine, whereas, in mice and rats, excretion is largely through the bile. In man, 70 to 90 percent of benzidine is excreted in the urine in the form of 3-hydroxybenzidine; in rats, it is questionable whether this metabolite is even formed, but it is formed in the dog and rabbit.

Three studies have reported mutagenic activity of benzidine towards Salmonella typhimurium (TA 1537 and TA 1538) in the presence of a rat liver mixed function oxidase system (Ames, et al. 1973; McCann, et al. 1975).

The carcinogenic and mutagenic activities of benzidine in animal systems clearly substantiate the epidemiological findings that show benzidine to be carcinogenic in humans. In a recent report, The National Academy of Science (NAS, 1976) calculated an estimate of the total benzidine exposure of occupationally exposed humans on the basis of the urinary levels. The NAS report presented a table comparing tumor incidence and total accumulated dose in humans and two species of laboratory animals.

Table 1 contains data from the NAS (1975) report as well as additional animal data.

TABLE 1

Degree of Exposure and Reported Cancer Frequencies for Agents Carcinogenic to Man and Laboratory Animals

	Conditions of Exposure	Total Accumulated dose (mg/kg)	Cancer	Reference
Man	13.6 yr; occupational	200	52% bladder (13/25)	Zavon, et al. 197
Mouse	1/wk; 32 - 52 wks S.C. injection	10,000	67% liver (31/46)	Prokofjevea, 1971
Mouse	1/wk; 52 wks S.C. injection	10,400	12% liver (7/60)	Bonser, et al. 19
Rat	1/wk; 64 wks S.C. injection	3,200	4% liver (6/152)	Spitz, et al. 195
Rat	1/3 days for 30 days gastric intubation	100 50 control	78% mammary (7/9) 50% mammary (5/10) 4% mammary (5/132)	Griswold, et al. 1968

*dose calculated on basis of an average rat weight of .25 kg, from NAS, 1975.

On the basis of the data presented in this table, it is apparent that in animal studies where benzidine was injected and where liver tumors were induced, much higher doses of benzidine were required than in the sensitive mammary tumor rat model system and in the doses estimated to give a high bladder cancer incidence in man.

The data from the human epidemiology study of Zavon, et al. 1973 was used to estimate the concentration of benzidine in water calculated to keep the lifetime cancer risk below 10^{-5}. In this study 25 workers in a benzidine manufacture plant, were observed for the appearance of bladder cancer over a period of 13 years. In this series 13 of 25 men developed bladder tumors after a mean exposure period

of 13.61 years, their average age at the end of exposure was 44 years and at the end of a 13 year observation was 57 years. The men not showing evidence of cancer had a mean exposure period of 8.91 years, their average age at the end of exposure was 43 years and at the end of observation 56 years. The estimated total accumulated dose of 200 mg/kg was estimated from average urinary levels of benzidine in these workers at the end of a workshift (see Table 1 and Zavon, et al. 1973). From this data the concentration of benzidine in water calculated to keep lifetime cancer risk below 10^{-5} is 1.67×10^{-3} µg/l.

Four animal studies shown in Table 1 were considered for possible use in the calculation of the water quality criterion. The most sensitive response occurred in the Griswold study, where 10 to 20 female Sprague-Dawley rats per treatment group were administered benzidine by gastric intubation in ten equal doses at three-day intervals over a 30-day period and observed for nine months. Total doses of 25 and 12 mg/l benzidine/rat induced carcinomas in 7/9 and 5/10 animals, respectively, compared to 5/132 animals in the control group. All tumor-bearing rats had multiple carcinomas and one had a fibroadenoma. Based on these data, the concentration of benzidine in water, calculated to keep the lifetime cancer risk below 10^{-5}, is 8.5×10^{-4} µg/l.

Although the criterion value derived from human exposure data is higher than that calculated from the most sensitive animal system, it seems reasonable that human epidemiological data are most appropriate for estimating human risks. The

study of Zavon, et al. (1973) was selected as the data base for deriving the water quality criterion. Based on these data, the concentration of benzidine in water calculated to keep the lifetime cancer risk below 10^{-5} is 1.67×10^{-3} µg/l.

National Academy of Science. 1975. Pest control: An assessment of present and alternative technologies. Vol. 1: Contemporary pest control practices and prospects: The report of the Executive Committee. Washington, D.C. 20418.

Summary of Pertinent Data

The data from the human epidemiology study of Zavon, et al. 1973 was used to estimate the concentration of benzidine in water calculated to keep the lifetime cancer risk below 10^{-5}. In this study 25 workers in a benzidine manufacturing plant were observed for the appearance of bladder tumors after a mean exposure period of 13.61 years, their average age at the end of exposure was 44 years and at the end of a 13 year observation was 57 years. The men not showing evidence of cancer had a mean exposure period of 8.91 years, their average age at the end or exposure was 43 years and at the end of observation 56 years. The estimated total accumulated dose of 200 mg/kg was estimated from average urinary levels of benzidine in these workers at the end of a workshift (see Table 1 and Zavon, et al. 1973). The criterion was calculated from the following parameters:

Average weight of man = 70 kg

Observed incidence of bladder cancer = 13/25 (52 percent)

Accumulated dose = 200 mg/kg

Bioconcentration factor of benzidine = 50

X = average daily exposure producing lifetime risk of 10^{-5}

B* = potency factor, which is an estimate of the linear dependency of cancer rates on lifetime average dose

C = concentration of benzidine in water, calculated to produce a lifetime risk of 10^{-5}, assuming a daily ingestion of 2 liters of water and 0.0187 kg fish.

Workers were assumed to have received 200 mg/kg of benzidine in a lifetime. At the end of a 13-year observation period, the average age of the workers was 57 years. Therefore, benzidine exposure on a mg/day basis amounts to:

$$\frac{200 \times 70}{365 \times 57} = .673 \text{ mg/day}$$

This gives a response at 57 years of 52% so that:

$$.52 = 1 - e^{-B(.673)}$$
$$B = \frac{.734}{.673} = 1.091$$

$$B^* = B\left(\frac{tf}{\overline{tf}}\right)^3 = 1.091 \left(\frac{70}{57}\right)^3 = 2.021$$

$$(2.021)\ (X) = 10^{-5}$$
$$X = 4.9 \times 10^{-6} \text{ mg/day to obtain a rate of } 10^{-5} \text{ or } 4.9 \times 10^{-3} \text{ µg/day}$$

Therefore:

$$C(2 + 50 \times .0187) = 4.9 \times 10^{-3}$$
$$C = 1.67 \times 10^{-3} \text{ µg/l}$$

From this data the concentration of benzidine in water calculated to keep lifetime cancer risk below 10^{-5} is 1.67×10^{-3} µg/l.