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Ambient Water Quality Criteria for Dichlorobenzidine

AMBIENT WATER QUALITY CRITERIA FOR

DICHLOROBENZIDINE

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U.S. ENVIRONMENTAL PROTECTION AGENCY

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FOREWORD

Section 304 (a)(1) of the Clean Water Act of 1977 (P.L. 95-217), requires the Administrator of the Environmental Protection Agency to publish criteria for water quality accurately reflecting the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare which may be expected from the presence of pollutants in any body of water, including ground water. Proposed water quality criteria for the 65 toxic pollutants listed under section 307 (a)(1) of the Clean Water Act were developed and a notice of their availability was published for public comment on March 15, 1979 (44 FR 15926), July 25, 1979 (44 FR 43660), and October 1, 1979 (44 FR 56628). This document is a revision of those proposed criteria based upon a consideration of comments received from other Federal Agencies, State agencies, special interest groups, and individual scientists. The criteria contained in this document replace any previously published EPA criteria for the 65 pollutants. This criterion document is also published in satisifaction of paragraph 11 of the Settlement Agreement in Natural Resources Defense Council, et. al. vs. Train, 8 ERC 2120 (D.D.C. 1976), modified, 12 ERC 1833 (D.D.C. 1979).

The term "water quality criteria" is used in two sections of the Clean Water Act, section 304 (a)(1) and section 303 (c)(2). The term has a different program impact in each section. In section 304, the term represents a non-regulatory, scientific assessment of ecological effects. The criteria presented in this publication are such scientific assessments. Such water quality criteria associated with specific stream uses when adopted as State water quality standards under section 303 become enforceable maximum acceptable levels of a pollutant in ambient waters. The water quality criteria adopted in the State water quality standards could have the same numerical limits as the criteria developed under section 304. However, in many situations States may want to adjust water quality criteria developed under section 304 to reflect local environmental conditions and human exposure patterns before incorporation into water quality standards. It is not until their adoption as part of the State water quality standards that the criteria become regulatory.

Guidelines to assist the States in the modification of criteria presented in this document, in the development of water quality standards, and in other water-related programs of this Agency, are being developed by EPA.

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DICHLOROBENZIDINE

CRITERIA

Aquatic Life

The data base available for dichlorobenzidines and freshwater organisms is limited to one test on bioconcentration of 3,3'-dichlorobenzidine, and no statement can be made concerning acute or chronic toxicity.

No saltwater organisms have been tested with any dichlorobenzidine, and no statement can be made concerning acute or chronic toxicity.

Human Health

For the maximum protection of human health from the potential carcinogenic effects due to exposure of dichlorobenzidine through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero based on the non-threshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 0.103 µg/l, 0.010 µg/l, and 0.001 µg/l, respectively. If the above estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 0.204 µg/l, 0.020 µg/l, and 0.002 µg/l, respectively.

INTRODUCTION

Dichlorobenzidine (4,4'-diamino-3,3'-dichlorobiphenyl or 3,3'-dichlorobenzidine) (DCB) is used in the production of dyes and pigments and as a curing agent for polyurethanes. The molecular formula of dichlorobenzidine is $C_{12}H_{10}Cl_2N_2$ and the molecular weight is 253.13 (Stecher, 1968).

DCB forms brownish needles with a melting point of 132 to 133°C (Pollock and Stevens, 1965). It is readily soluble in alcohol, benzene, and glacial acetic acid (Stecher, 1968), slightly soluble in HCl (Radding, et al. 1975), and sparingly soluble in water (0.7 g/l at 15°C) (Stecher, 1968). When combined with ferric chloride or bleaching powder, a green color is produced (Pollock and Stevens, 1965).

The affinity of DCB for suspended particulates in water is not clear; its basic nature suggests that it may be fairly tightly bound to humic materials in soils (Radding, et al. 1975). Soils may be moderate to long term reservoirs.

Pyrolysis of DCB will most likely lead to the release of HCl. Because of the halogen substitution, DCB compunds probably biodegrade at a slower rate than benzidine alone. The photochemistry of DCB is not completely known. DCB may photodegrade to benzidine (Sikka, et al. 1978).

Assuming the clean air concentrations of ozone (2×10^{-9} M) and an average atmospheric concentration of hydroxyl radicals (3×10^{-15} M), the half-life for oxidation of DCB by either of these chemical species is on the order of one and one to 10 days, respectively. Furthermore, assuming a representative concentration of 10^{-10} M for peroxy radicals in sunlit oxygenated water, the half-life for oxidation by these species is approximately 100 days, given the variability of environmental conditons (Radding, et al. 1975).

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Aquatic Life Toxicology*

INTRODUCTION

The data base for dichlorobenzidines and freshwater and saltwater organisms is limited to a bioconcentration and depuration study with the bluegill and 3,3'-dichlorobenzidine (Appleton and Sikka, 1980).

EFFECTS

Residues

The apparent equilibrium bioconcentration factors for the bluegill during tests of from 96 to 168 hours were from 114 to 170 for edible flesh and 495 to 507 for whole body (Table 1). An initial rapid rate of elimination was followed by a low or negligible rate, with appreciable residues remaining after 14 days in clean water.

CRITERIA

The data base available for dichlorobenzidines and freshwater organisms is limited to one test on bioconcentration of 3,3'-dichlorobenzidine and no statement can be made concerning acute or chronic toxicity.

No saltwater organisms have been tested with any dichlorobenzidine and no statement can be made concerning acute or chronic toxicity.

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*The reader is referred to the Guidelines for Deriving Water Quality Criteria for the Protection of Aquatic Life and Its Uses in order to better understand the following discussion and recommendation. The following tables contain the appropriate data that were found in the literature, and at the bottom of each table are calculations for deriving various measures of toxicity as described in the Guidelines.

Table 1. Residues for dichlorobenzidine (Appleton & Sikka, 1980)

Species	Tissue	Chemical	Bioconcentration Factor	Duration (days)
		FRESHWATER SPECIES		
Bluegill, Lepomis macrochirus	whole body	3,3'-dichloro-benzidine	495-507	4-7
Bluegill, Lepomis macrochirus	edible flesh	3,3'-dichloro-benzidine	114-170	4-7

REFERENCES

Appleton, H.T. and H.C. Sikka, 1980. Accumulation, elimination, and metabolism of dichlorobenzidine in the bluegill sunfish. Environ. Sci. Technol. 14: 50.

Mammalian Toxicology and Human Health Effects

EXPOSURE

Ingestion from Water

To date, few systematic measurements of DCB in water supplies have been undertaken. In one instance, analysis of purge wells and seepage water near a waste disposal lagoon receiving DCB-manufacture wastes showed levels of DCB ranging from 0.13 to 0.27 mg/l. High levels of benzidine (up to 2.5 mg/l) were also seen, which may have arisen from photodegradation of DCB (Sikka, et al. 1978), since benzidine is no longer manufactured in the U.S. Several other dichlorobenzidine isomers were also detected at levels from 1 to 8 mg/l. The use of lagoons to handle DCB-containing wastes might lead to contamination of ground water and pose a threat to persons relying on nearby wells for drinking water.

Takemura, et al. (1965) analyzed the water of the Sumida River in Tokyo during 1964. This river receives the waste effluents of several dye and pigment factories. The presence of DCB was demonstrated by thin layer chromatography. Although levels of DCB itself were not quantified, colorimetric analysis revealed that total aromatic amine content of the water (including benzidine, dichlorobenzidine, α-naphthylamine, and β-naphthylamine) reached levels up to 0.562 mg/l. The authors suggested that the presence of the free amines might be due to chemical reduction of the azo-dyes by the high levels of H_2S and SO_2 in the river.

Ingestion from Food

Few studies have attempted to identify DCB as a contaminant of human food. Since DCB has never had an application as an agricul-

tural or food chemical, the most likely source of dietary DCB would be through consumption of contaminated fish.

A bioconcentration factor (BCF) relates the concentration of a chemical in aquatic animals to the concentration in the water in which they live. The steady-state BCFs for a lipid-soluble compound in the tissues of various aquatic animals seem to be proportional to the percent lipid in the tissue. Thus, the per capita ingestion of a lipid-soluble chemical can be estimated from the per capita consumption of fish and shellfish, the weighted average percent lipids of consumed fish and shellfish, and a steady-state BCF for the chemical.

Data from a recent survey on fish and shellfish consumption in the United States were analyzed by SRI International (U.S. EPA, 1980). These data were used to estimate that the per capita consumption of freshwater and estuarine fish and shellfish in the United States is 6.5 g/day (Stephan, 1980). In addition, these data were used with data on the fat content of the edible portion of the same species to estimate that the weighted average percent lipids for consumed freshwater and estuarine fish and shellfish is 3.0 percent.

A measured steady-state bioconcentration factor of 500 was obtained for 3,3'-dichlorobenzidine using bluegills (Appleton and Sikka, 1980). Since bluegills from another source contained an average of 4.8 percent lipids (Johnson, 1980), these bluegills probably contained about the same percent lipids. An adjustment factor of 3.0/4.8 = 0.625 can be used to adjust the measured BCF from the 4.8 percent lipids of the bluegill to the 3.0 percent

lipids that is the weighted average for consumed fish and shellfish. Thus, the weighted average bioconcentration factor for 3,3'-dichlorobenzidine and the edible portions of all freshwater and estuarine aquatic organisms consumed by Americans is calculated to be 500 x 0.625 = 312.

No DCB was detected in fish sampled from the vicinity of a DCB-contaminated waste lagoon using analytical methods with sensitivity of 10 to 100 µg/kg (G. Diachenko, personal communication).

Inhalation

The physical properties of DCB (low volatility, large crystal structure) probably minimize the risk of exposure of general populations to DCB through inhalation of air contaminated through industrial processes. However, inhalation might represent a major source of occupational exposure under sub-optimum working conditions. Akiyama (1970) examined the exposure of workers to DCB in a pigment plant in Japan and determined that during the addition of DCB to reaction vessels for synthesis of DCB pigments, the concentration of DCB in air reached 2.5 mg/100 m^3 in 10 minutes of charging of reaction vessels and decreased to 0.2 mg/100 m^3 within 20 minutes. The distance of the sampling device from the operation was not specified. Also, the amount of total aromatic amines was elevated in exposed personnel (presumably due to the presence of DCB). The mean urinary concentrations of aromatic amines in process workers charging the reaction vessels with DCB and plant laboratory workers were 20.1 ppm and 21.1 ppm, respectively. Levels were only 14.5 ppm in workers who dried and cracked the pigments, 12.7 ppm in office clerks, and 13.6 ppm in controls (medical students). Al-

though the concentrations detected were highly variable (i.e., the mean 20.1 ppm from the charging personnel was derived from data ranging from 48.5 to 10 ppm), it is possible that the elevated levels result from DCB exposure, since Akiyama claims that few or no precautions were taken to prevent exposure, particularly on hot days. It is uncertain whether the amines entered the body through respiration or through dermal absorption.

Gerarde and Gerarde (1974) reported on an industrial process in which both DCB and the DCB diarylide pigments were manufactured. Most steps in the process were performed in closed systems, and the DCB was handled in a salt form in a slurry (ca. 80 percent water content). DCB dust was said not to be a problem. The possibility that DCB contamination exposure could, however, occur is indicated by the statement that "...the floor and accessible surfaces contaminated with the slurry were usually hosed down to prevent accumulation of dried material...." Also, an outbreak of dermatitis in the plant was attributed to a process change in DCB production. In utilizing DCB in pigment production, the major sources of potential exposure are listed as the weighing process and charging of the tanks. Prior to May 1973, operators wore gloves and goggles but not dust face masks. DCB was manufactured in this plant from 1938 to 1957. Thereafter, DCB was purchased from an outside supplier. On-site inspection of three DCB utilizing plants showed that two of the plants posed relatively low exposure potential which was due to use of metal reactors and protective arrangements at the point of tank charging. However, in the third plant, chemicals were dumped into open reaction vessels from an elevated platform, posing an

enhanced potential for exposure. Therefore, a great deal of variability concerning the exposure of individuals to DCB may exist among various operations.

Dermal

Because of large particle size and increased usage of closed systems and protective clothing, dermal absorption of DCB probably represents a relatively minor route of DCB exposure in humans at present. However, Meigs, et al. (1954) presented some experimental evidence that under certain environmental conditions favoring moist skin conditions, such as high relative humidity and high air temperature, the dermal absorption by humans of benzidine and possibly other congeners such as DCB may be enhanced.

PHARMACOKINETICS

Absorption

Virtually no information exists that quantifies the degree and rate of absorption of DCB in experimental animals or in humans, although Meigs, et al. (1954) detected DCB in the urine of DCB processing and manufacturing workers.

Distribution

A detailed distribution study of DCB in rats, monkeys, and dogs given 0.2 mg/kg of ^{14}C-DCB by intravenous injection was reported by Kellner, et al. (1973). The results indicate a rather general distribution within the body after a 14-day observation period with highest levels found in the livers of all three species. The bile of monkeys and the lungs of dogs showed significant levels of radio-activity.

Metabolism

DCB metabolites have not been detected in the urine of dogs administered DCB orally or by intraperitoneal injection (Sciarini and Meigs, 1961; Gerarde and Gerarde, 1974).

Kellner, et al. (1973) examined the urine of a Rhesus monkey given 0.2 mg/kg ^{14}C-DCB intravenously and found that in the first four hours following injection, about one-third of the urinary ^{14}C was unchanged DCB, with another third identified as mono-N-acetyl DCB, based on chromatographic properties. The remainder of the urinary ^{14}C was not recoverable via ether extraction at pH 11. At later intervals, mostly metabolites were excreted, with nonextractable ^{14}C comprising the majority of this material.

No ortho-hydroxy metabolites of DCB were detected in the urine of human subjects after oral dosing (Gerarde and Gerarde, 1974).

Aksamitnaia (1959) reported that prolonged ingestion (7.5 to 8.5 months) of small doses or a single large dose of DCB in rats led to the appearance of four transformation products, including benzidine and possibly glucuronide conjugates. This conclusion may be tenuous because analysis was done by paper chromatography (one solvent system) without benefit of radiotracer techniques, and the products were not quantified or further characterized. DCB was never detected in the urine in any of the experiments; by-products were seen only after seven months of chronic DCB ingestion.

In a study of the bioconcentration of DCB in bluegill sunfish, over one-half of the DCB residues in the fish were in the form of a conjugate which, under very mildly acidic conditions, hydrolyzed to reform free DCB (Sikka, et al. 1978).

Hirai and Yasuhira (1972) noted that DCB was not oxidized by cytochrome c, whereas benzidine and other derivatives were oxidized.

The majority of information available at present suggests that DCB is resistant to metabolism, with the exception of certain conjugative mechanisms and possibly certain bioactivation steps. Ring chlorination of benzidine probably blocks ring hydroxylation reactions of DCB for both electronic and steric reasons (Shriner, et al. 1978).

Excretion

The excretion of DCB and metabolites following a 0.2 mg/kg intravenous dose of ^{14}C-DCB was studied by Kellner, et al. (1973) in rats, dogs, and monkeys. With all species, measurable elimination had ceased within seven days of administration (Table 1). Fecal excretion was the predominant route of elimination in rats and dogs, and possibly in monkeys.

Sciarini and Meigs (1961) also noted a preponderance of fecal elimination of DCB in dogs. Finally, Gerarde and Gerarde (1974) cite an unpublished study utilizing human volunteers which concluded that DCB is excreted largely by the fecal route in man as well as in dogs.

Insufficient data is available to assess the ability of the body to accumulate significant burdens of DCB through repeated exposures.

TABLE 1

Excretion of ^{14}C-DCB in Rats, Dogs and Monkeys Administered ^{14}C-DCB*

Species	Interval (day)	Elimination of Total Dose Administered					
		Urine	Feces		Feed	Balance	Residues
		%	%	t_{50} Phase IV (hr)	%	0-7 day	%
Rat	0-6	18 ± 4	79 ± 12	45	1	98 ± 12	2
Dog	0-7	8 ± 6	84 ± 11	--	5	97 ± 8	3
Monkey 1	0-7	27	46	--	21	94	
Monkey 2	0-7	37	26	--	20	83	

*Source: Kellner, et al. 1973

EFFECTS

Acute, Subacute, and Chronic Toxicity

Gaines and Nelson (1977) reported the acute oral toxicity of DCB to male and female mice. The LD_{50} (mg/kg/day) for DCB given daily for seven days was 352 for female mice (slope = 27.39) and 386 for male mice (slope = 23.15). The single dose LD_{50} (mg/kg) was 488 for females and 676 for males.

Gerarde and Gerarde (1974) listed results of several toxicological studies with DCB. DCB-dihydrochloride failed to produce skin irritation in rabbits at an unspecified dose. An intradermal dose of 700 mg/kg also gave a negative reaction. One hundred mg of DCB-free amine placed in the conjunctival sac of the eye of a rabbit gave a negative reaction, while 20 mg of DCB dihydrochloride produced erythema, pus, and opacity of the eye, giving a score of 84 of a possible 110 in one hour according to the method of Draize. The oral LD_{50} was given as 7.07 g/kg in albino rats for DCB free amine, and 3.82 g/kg in male and female Sprague-Dawley rats for DCB dihydrochloride. For topical application to skin, an LD_{50} of 8 g/kg in male and female rats was seen. Pliss (1959) noted that rats given 120 mg of DCB subcutaneously exhibited a state of excitation with short-lived convulsions.

No human fatalities resulting from exposure to DCB have been reported.

Ten rats exposed to a concentrated atmospheric dust of DCB dihydrochloride for 14 days showed, upon autopsy, slight to moderate pulmonary congestion and one pulmonary abcess (Gerarde and Gerarde, 1974). An irritant effect from HCl cannot be discounted in the study.

Freeman, et al. (1973) noted that DCB was cytotoxic to embryonic rat cells in culture at concentrations of 5 ppm or greater.

No mortalities were obtained in inhalation studies where rats were exposed to a concentrated atmosphere of concentrated DCB dihydrochloride dust for 14 days, or to 355 mg DCB free amine for 2 hours daily for 7 days (Gerarde and Gerarde, 1974).

Gerarde and Gerarde (1974) listed the principal reasons for visits to a company medical clinic by employees working with DCB. These were as follows: (1) gastro-intestinal upset, (2) upper respiratory infection, (3) sore throat, (4) caustic burns, (5) headache, (6) dizziness, and (7) dermatitis. The only illness apparently directly related to DCB was dermatitis. An outbreak of dermatitis was attributed to a manufacturing process change which led to small amounts of DCB-free base in the isolated DCB sulfate salt. Two cases of acute cystitis were found in the medical record review of the workers. One was of infectious origin and the other related to the presence of renal calculi. Cystoscopic examination of three other workers with urinary system symptoms revealed two had renal calculi, and another had cystitis cystica.

Synergistic and/or Antagonistic Compounds

No data are available concerning compounds which synergize or antagonize the toxicity of DCB.

Teratogenicity

No information is available defining the teratogenic potential of DCB. While perhaps not directly relevant to the question of DCB-induced teratogenesis, several studies summarized in the following discussion show that DCB can cross the placental barrier and can also affect developmental systems.

DCB has been demonstrated to significantly increase the incidence of leukemia in the offspring of pregnant female mice given comparatively low doses (ca. 8-10 mg) of DCB by subcutaneous injection in the last week of gestation (Golub, et al. 1974). This could have been due to postnatal transfer of DCB to the young through lactation. However, transplacental effects of DCB have also been observed. Shabad, et al. (1972) and Golub (1969) noted that kidney tissue taken from embryos of pregnant female mice treated with DCB exhibited altered behavior in organ culture, including increased survival and hyperplastic changes in epithelium not seen in controls.

The degree of exposure of pregnant women to DCB is probably low. The work force involved in the manufacture and utilization of DCB is predominantly or totally male. MacIntyre (1975) lists five women, all between the ages of 20 and 34 years, as having been DCB service or production workers in a plant in Great Britain. The same area of the plant employed 217 men.

Mutagenicity

Garner, et al. (1975) compared the relative mutagenicity of benzidine, DCB, and other analogs in the bacterial mutagenesis system developed by Ames, et al. (1973), utilizing the Salmonella typhimurium tester strain TA1538, an indicator of frameshift mutagenesis. The relevant data are summarized in Table 2. These results show that DCB is considerably more potent as a frameshift mutagen in this system than is benzidine. Also, a low degree of mutation is elicited by DCB but not by benzidine in the absence of the S-9 activation enzyme system.

TABLE 2

Mutagenicity of DCB in the Ames Assay*

Compound	µg Chemical/ Plate	S-9**	Revertants/ Plate
3,3'-Dichlorobenzidine	50	+	3,360
	100	+	7,520
	50	-	114
	100	-	131
3,3'-Dichlorobenzidine	50	+	5,490
Sulfate salt,	100	+	8,350
technical grade	50	-	127
	100	-	129
Benzidine	50	+	430
	100	+	640
	50	-	5
	100	-	15
Dimethyl sulfoxide		+	16
(control)		-	8

*Source: Garner, et al. 1975

**S-9 is the NADPH-fortified rat liver activation enzyme preparation. + signifies preparation present; -, preparation absent.

Similar observations were made by Lazear and Louis (1977), utilizing an enzyme activation system obtained from the livers of male mice and Ames tester strain TA98 (an indicator of frameshift mutation). As before, DCB was much more mutagenic than benzidine and, unlike benzidine, retained an appreciable mutagenic activity without the liver enzymes. DCB was also slightly mutagenic towards tester strain TA100, indicating base-pair substitution mutation.

Carcinogenicity

Stula, et al. (1975) maintained 50 male and 50 female rats on a dietary level of DCB of 1,000 mg/kg. The average 50 percent survival was 356 days, with average days on the test of 349 days for females and 353 days for males. The range of days on the test was 118-486 days for males and 143-488 days for females. The rats were 38 days old at the start of the assay and were apparently autopsied at time of death or after 486-488 days (not specified). The results of this study are listed in Table 3.

In addition to the cancers listed in Table 3, the occurrence of malignant lymphoma was elevated over controls but not at statistically significant ($p < 0.05$) levels. No bladder cancer was noted.

In a recent study, Stula, et al. (1978) reported on the induction of both papillary transitional cell carcinomas of the urinary bladder and hepatic carcinomas in female beagle dogs. An oral dose of 100 mg DCB was administered to the experimental animals, three times per week for six weeks, then five times per week continuously for periods up to 7.1 years. DCB was found to be carcinogenic at statistically significant levels ($p < .025$). The incidences of hepatic carcinomas were 4/5 and 0/6 in DCB-treated and control

TABLE 3

Induction of Cancer in Male and Female Rats
by 1,000 ppm Dietary DCB[a]

Type of Cancer	No. of Cancers			
	Male[c] DCB	Vehicle control	Female[c] DCB	Vehicle control
Mammary adenocarcinoma	7[b]	0	26[b]	3
Granulocytic leukemia	9[b]	2	0	0
Zymbal's gland carcinoma	8[b]	0	1	0

[a]Source: Stula, et al. 1975

[b]Significantly greater than controls at $p < 0.05$

[c]The number of animals examined histologically was 44 each for male and female.

groups, respectively. The incidences of urinary bladder carcinomas were 5/5 and 0/6, respectively (Table 4).

For 12 months, 6 times weekly, Pliss (1959) added 0.5 to 1.0 ml of a 4.4 percent suspension of DCB to the feed of rats of both sexes of a strain assumed by Pliss to have a low spontaneous tumor rate. Each rat received a total dose of 4.53 g. Neoplasms were detected in 22 of 29 (75.8 percent) surviving animals. Tumors, primarily carcinomas, were observed in a broad spectrum of organs including mammary gland, Zymbal's gland (sebaceous gland of the external auditory meatus), bladder, skin, small intestine, liver, thyroid gland, kidney, hematopoietic (lymphatic) system, and salivary glands.

An assay of DCB carcinogenicity was also done with mice (Pliss, 1959). The mice received 0.1 ml of a 1.1 percent DCB suspension in their food for 10 months, receiving a total dose of 127.5 to 135 mg DCB. Hepatic tumors were found in 4 of 18 mice surviving after 18.5 months (22.2 percent). A sebaceous gland carcinoma and a lung adenoma were also seen.

The Pliss studies show that DCB may possess carcinogenic activity in both rats and mice. However, the massive and apparently acutely-toxic dose levels employed, the uncertain purity of the commercial product used, the virtual lack of dose-response data, and the lack of adequate controls limit the studies' utility for assessing human health hazards.

Carcinogenicity assays were also performed using rats and mice which received DCB by subcutaneous injection (Pliss, 1959, 1963). However, these studies are not considered here because of the

Table 4. Summary of Gross Pathology and Microscopic Pathology

Dog Number and Group	E - Sacrificed in extremis S - Sacrificed	Years on test	GROSS PATHOLOGY	HISTOPATHOLOGY	Urinary bladder: follicular cystitis	Glomerulo-nephritis	Cholangio-fibrosis	Liver: focal cell alteration	Liver: nodular hyperplasia
3,3'-DICHLOROBENZIDINE									
584	E	3.5	Liver: pale fatty appearance	Liver: fatty change +++; kidney: parasitic granulomas; fatty change +	-	-	+	+	-
652	E	6.6	Numerous gray firm nodules, up to 2.5 cm, in liver, lung, urinary bladder, kidney, heart, lymph nodes, temporal muscles, uterus, and gall bladder.	Urinary bladder: papillary transitional cell carcinoma; liver: undifferentiated carcinoma metastatic to many organs; uterus: endometrial cysts; liver: bile duct hyperplasia	+	-	+	+	-
587	S	7.1	One pink raised irregular nodule on urothelial surface of urinary bladder, 4.0 mm; numerous pale brown irregular foci, up to 2.5 cm, in liver.	Urinary bladder: papillary transitional cell carcinoma; liver: hepatocellular carcinoma; bile duct hyperplasia	+	+	+	+	-
586	S	7.1	Six raised gray nodules, up to 2.5 cm, on urothelial surface of urinary bladder; numerous pale brown foci, up to 2.5 cm, in liver.	Urinary bladder: papillary transitional cell carcinoma; uterus: endometrial cysts	+	+	+	+	+++
597	S	7.1	Several raised gray nodules, up to 4.0 mm, on urothelial surface of urinary bladder. Numerous nodules, up to 7.0 cm, in liver with adhesions to gall bladder and pancreas.	Urinary bladder; papillary transitional cell carcinoma; liver: hepatocellular carcinoma; anterior pituitary: adenoma; intestine: lymphoid hyperplasia; liver: bile duct hyperplasia	+	-	++	+	-
591	S	7.1	Six raised gray nodules, up to 4.0 mm, on urothelial surface of urinary bladder. Numerous gray, hemorrhagic, cystic nodules, up to 2.5 cm, in liver.	Urinary bladder: papillary transitional cell carcinoma; liver: hepatocellular carcinoma; thyroid: lymphocytic thyroiditis; liver: bile duct hyperplasia	+	-	+	+	-
CONTROL									
653	S	8.3	Mammary: 2 nodules (3 cm); liver: pale nodule (2 mm); lung: multiple nodule (1 cm); spleen: 2 nodules (8 mm)	Mammary: carcinoma, solid simple type, metastatic to lungs; spleen: nodular hyperplasia; liver: cholangiitis; uterus: cystic hyperplasia of endometrium	-	+	-	++	-
654	S	8.0	Mammary: nodule (2 cm); adrenal cortex: nodule (6 mm); liver: 2 pale nodules (12 mm)	Mammary: adenocarcinoma, tubular complex type; liver: fatty change and hematopoiesis; adrenal cortex: adenoma; anterior pituitary: adenoma	-	-	-	++	-
600	S	9.0	Mammary: nodule (6 mm); spleen: irregular gray foci on surface (5 mm); liver: pale firm streaks, nodular	Lung: subpleural fibrosis and osseous metaplasia; retina: focal atrophy; mammary: carcinosarcoma; spleen: focal subcapsular fibrosis	-	+	+++	+	-
651	S	9.0	All mammary glands enlarged, contained milk; spleen: nodule (10 mm); vagina: one raised nodule (2 mm); liver: multiple pale nodules (1.5 cm)	Adrenal cortex: nodular hyperplasia and hematopoiesis; anterior pituitary: focal hyperplasia; mammary: diffuse hyperplasia in all glands; vagina: fibrous polyp	-	-	-	+++	-
650	S	9.0	Mammary: nodule (5 mm); vagina: several firm nodules (2 mm)	Lung: subpleural fibrosis; adrenal cortex: hematopoiesis; liver: fatty change and hematopoiesis; mammary: carcinosarcoma; vagina: fibrous polyps	-	+	-	+++	-
649	S	9.0	Mammary: 2 nodules (8 mm) firm; kidney: several gray nodules in cortex (2 mm); lung: diffuse areas of firmness; liver: several gray nodules (5 mm)	Mammary: focal hyperplasia; lung: subpleural fibrosis; kidney: parasitic granulomata	-	-	+	++	-

Code + = Slight degree of lesion present. + + = Moderate degree + + + = Marked degree. − = Lesion was not found

Note 1 All dogs had periodontal disease with loss of some teeth

2 The organs examined histologically included: brain, spinal cord, heart, aorta, lung, trachea, ovary, uterus, vagina, esophagus, stomach, small intestine, cecum, large intestine, bone marrow, spleen, thymus, liver, pancreas, salivary gland, pituitary, thyroid, parathyroid, adrenal, kidney, eye, urinary bladder and all gross lesions.

Source: Stula, et al. 1978.

irrelevancy of the subcutaneous route of administration to human exposure.

Griswold, et al. (1968) examined the potency of cancer induction by DCB, benzidine, and other compounds, using induction of mammary cancer in young female Sprague-Dawley rats as the major index. Forty-day-old female Sprague-Dawley rats were given 30 mg of DCB every three days for 30 days by gavage and were then observed for nine months. Under the conditions of this assay, DCB was ineffective as a mammary carcinogen but benzidine was highly effective at lower doses.

Sellakumar, et al. (1969) maintained male and female hamsters for an unspecified length of time on a diet containing 0.1 percent (1,000 ppm) of DCB. With 30 animals of each sex, no cancer was observed. However, at 0.3 percent dietary DCB, four transitional cell bladder carcinomas, some liver tumors, and diffuse chronic intrahepatic obstructing cholangitus were seen. At 0.1 percent in the diet of benzidine, many liver tumors were obtained but no bladder cancer was found.

DCB was also found to produce transformation in cultured rat embryo cells infected with Rauscher leukemia virus (Freeman, et al. 1973). The index of transformation was the development of macroscopic foci of spindle cells, lacking polar orientation and contact inhibition. Cells from typical foci were tumorigenic when transplanted into newborn Fisher rats, although this transplantability was not quantitated. DCB-induced transformation was seen at a concentration of 5 ppm in the medium, but not at 1 ppm. Levels of 10 ppm or higher were cytotoxic. This in vitro test system detected

transformation-activity in 6 of 7 aromatic amines characterized as active *in vivo* carcinogens, 1 of 2 aromatic amines classed as weak *in vivo* carcinogens, and 0 of 3 aromatic amines classed as non-carcinogenic *in vivo*. DCB was classed by the authors as a weak carcinogen.

The history of human industrial experience with DCB has been summarized and analyzed by Gerarde and Gerarde (1974) and Rye, et al. (1970) in the United States; by MacIntyre (1975) and Gadian (1975) in Great Britain; and by Akiyama (1970) in Japan. The concensus of these authors, achieved through epidemiological studies, is that there is no evidence that DCB itself has induced bladder cancer, the characteristic lesion induced by benzidine, naphthylamine, and other carcinogenic aromatic amines used in the dye and pigment industry. The case for DCB carcinogenicity has been made largely on the basis of its structural similarity to benzidine and its tumorigenicity in several species of animals (MacIntyre, 1975). One problem associated with epidemiological studies of DCB effects in humans is that the population which has been exposed only to DCB is small. Many workers have also handled benzidine or other carcinogens. Also, the characteristic latency period for induction of bladder cancer by chemicals is quite long, exceeding 16 years for benzidine (Haley, 1975), and may not have elapsed for many workers. Finally, most of these studies have focused solely upon bladder cancer as the disease of interest. As discussed below, this approach may be misleading and fallacious in view of the pattern of DCB carcinogenesis in animals and the nature of cancer observed in DCB process workers.

Gadian (1975) examined the health records of 59 workers at a dyestuff plant in Great Britain who were exposed from 1953 through 1973 to DCB only and compared them to those working with both benzidine and DCB, and to unexposed populations. This time was justified as the average latency period for chemically-induced bladder cancer in humans (ca. 18 years). It was calculated that the DCB process worker was actually exposed to DCB for a maximum of 10 hours per work week. Men whose total DCB exposure was less than 245 hours (six months' full-time work) were excluded from the study, leaving 35 segregated DCB workers. These 35 workers, representing a total of 68,505 hours of DCB exposure, had no urinary tract tumors, no other tumors, and two deaths from other causes (coronary thrombosis, cerebral hemorrhage). In contrast, among 14 mixed benzidine and DCB workers with 16,200 hours exposure (approximately 60 percent worked with benzidine, 40 percent worked with DCB), three men developed tumors of the bladder, and one man developed carcinoma of the bronchus. One death from coronary thrombosis occurred. Since the use of benzidine ceased in 1964, the mixed group had a longer time to develop tumors than the DCB-segregated group. Therefore, the DCB-alone hours worked during the same period (1953-1964) as the mixed group was 31,945 hours. These results, while admitting that the population studied was small, were taken as evidence that DCB can be safely used if the provisions of the Carcinogenic Substances Regulations are observed.

MacIntyre (1975) also surveyed the health history of a DCB-utilizing plant in Great Britain. It was noted that the vast majority (209 out of 217) of production and service workers had

received first exposure to DCB less than 20 years before the time of the report, indicating that the latent period for tumor formation might not have elapsed. Only 3 of the 217 exposed workers were deceased. The causes of death were amytrophic lateral sclerosis (age 55 years, 15 years of DCB exposure, 39 years since first exposed), carcinoma of the lung (age 61 years, one year of DCB exposure, 12 years since first exposed), and pneumonia (age 70 years, 10 years of DCB exposure, 43 years since first exposed). Three other employees who had not been exposed to DCB died of bronchial carcinoma. All employees exposed to DCB since 1965 have received cytological testing twice yearly, with all tests proving negative. A 1974 meeting of occupational physicians is also cited, stating that in Europe approximately 1,000 persons have been exposed to DCB with a zero incidence of bladder cancer.

Gerarde and Gerarde (1974) reported the results of an epidemiological study of workers exposed to DCB in manufacture and utilization in a plant in the United States. A survey of the number of DCB-exposed workers who developed neoplasms and the type of neoplasm was presented. These included lung cancer (2 workers), leukemia-bone marrow (1), lipoma (6), rectum-papilloma (3), sigmoid colon carcinoma (2), prostate carcinoma (1), breast muscle myoblastoma (1), and skin basal cell epithelioma (1). A total of 17 workers of the total of 207 workers surveyed had developed neoplasms.

The etiology of bladder cancer was discussed and the data treated using several epidemiological and statistical approaches. Accordingly, if DCB were as potent as benzidine as a bladder carcinogen and the latent period long enough, a total of 22 cases of

bladder cancer out of 163 DCB production workers would have been observed, whereas none were seen. The possible induction by DCB of tumors at sites other than the bladder was not considered.

Summary

Based upon existing data, there is little doubt that DCB is carcinogenic in several animal species including rats, mice, hamsters, and dogs. According to current methodology, the experimental evidence serves as an indication that a potential carcinogenic risk is posed to man. DCB induces tumors in a variety of tissues in animals, with mammary, hematopoietic, and skin (Zymbal's gland) tissue being the most affected. Many of the tumors have been characterized as malignant.

CRITERION FORMULATION

Existing Guidelines and Standards

The American Conference of Governmental Industrial Hygienists (ACGIH, 1977) has recommended that no exposure to DCB by any route should be permitted, because of a demonstrated high carcinogenic response in animals. Strict regulations have recently been promulgated by the Occupational Safety and Health Administration to minimize or eliminate occupational exposure to DCB (29 CFR 1910). To date, no standards have been placed on permissable levels of DCB in the environment or in food.

Current Levels of Exposure and Special Groups at Risk

It is estimated that between 250 and 2,500 workers receive exposure to DCB in the U.S., compared to 62 for benzidine (Fishbein, 1977). Given the stringent precautions which must be taken in the manufacture and use of DCB, the level of exposure may be minimal at present, although no data is available. However, past exposure of individuals working without benefit of protective measures must present a cause for concern. In addition, the general population may receive exposure to DCB through contaminated drinking water or food (fish), although there is no significant evidence for this at the present.

Additional groups that may be at risk include workers in the printing or graphic arts professions handling the DCB-based azo pigments. DCB may be present as an impurity in the pigments, and there is very limited evidence to suggest that DCB may be metabolically liberated from the azo pigment. More information is needed on the levels of exposure to and metabolism of these pigments.

Basis and Derivation of Criterion

The safe dose of DCB in water was calculated from the carcinogenicity assays, using a linearized multistage model described in the Human Health Methodology Appendices to the October 1980 Federal Register notice which announced the availability of this document. The calculation assumes a risk of 1 in 100,000 of developing cancer as a result of daily consumption of 2 liters of water and 6.5 g DCB-contaminated fish or shellfish having a bioconcentration factor of 312. Although several carcinogenicity studies are available for use in calculating a criterion for DCB in drinking water, only the work of Stula and coworkers (1975, 1978) was considered, since the studies by Pliss (1959, 1963) lack appropriate control data. More specifically, the data on induction of hepatic carcinomas in female beagle dogs (Stula, et al. 1978) were chosen as a base for the calculation. Based on these data, a DCB criterion of 0.103 µg/l is judged to be adequate to protect the population consuming the water. This dose is low from an occupational viewpoint and should justify efforts to eliminate exposure of workers to DCB.

Under the Consent Decree in NRDC v. 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." DCB is suspected of being a human carcinogen. Because there is no recognized safe concentration for a human carcinogen, the recommended concentration of DCB 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 DCB 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, the U.S. 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 (daily intake)	Risk Levels and Corresponding Criteria(1)		
	10^{-7}	10^{-6}	10^{-5}
2 l of drinking water and consumption of 6.5 g of fish and shellfish (2)	0.001 µg/l	0.010 µg/l	0.103 µg/l
Consumption of fish and shellfish only.	0.002 µg/l	0.02 µg/l	0.204 µg/l

(1) Calculated by applying a linearized multistage model as mentioned above. Appropriate bioassay data used in the calculation are presented in the Appendix. Since the extrapolation model is linear at low doses, the additional lifetime risk is directly proportional to the water concentration. Therefore, water concentrations 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) Fifty percent of DCB exposure results from the consumption of aquatic organisms which exhibit an average bioconcentration potential of 312-fold. The remaining 50 percent of DCB exposure results from drinking water.

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

Although total exposure information for DCB 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. Care must be taken to remember that the proposed criterion is derived from animal experiments using pure DCB. In the environment, DCB undergoes degradation to other possibly toxic compounds such as benzidine. The possible additional risk posed by these breakdown products should be considered in the overall assessment of DCB.

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APPENDIX

Summary and Conclusions Regarding the Carcinogenicity of 3,3'-Dichlorobenzidine (DCB)*

3,3'-Dichlorobenzidine (DCB) is used as an intermediate in the synthesis of dyes and pigments. It is structurally related to carcinogenic aromatic amines, which have been used in the dye and pigment industries.

Five epidemiological studies of employees handling DCB in chemical plants in the United States, Great Britain, and Japan have provided no evidence of DCB-induced cancers. However, investigative problems associated with these studies, such as too short a follow-up time and small sample size, make them unreliable as the sole basis for making conclusions about human cancer risks from DCB.

DCB has induced carcinomas in three species of experimental animals receiving oral doses of the chemical. Dogs (female) developed papillary transitional cell carcinomas of the urinary bladder and hepatocellular carcinomas. Hamsters developed transitional cell bladder carcinomas, liver cell, and cholangiomatous tumors. Rats developed mammary adenocarcinomas (male and female), granulocytic leukemia (males), and Zymbal's gland carcinomas (males).

Two studies of the mutagenicity of DCB showed that it was mutagenic in two Salmonella typhimurium tester strains (TA1538, TA98) in the presence and absence of an S-9 liver enzyme system. DCB also transformed cultured rat embryo cells, and the transformed cells were tumorigenic when transplanted into newborn rats.

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

The carcinogenic, mutagenic, and transforming activities of DCB in laboratory organisms and its chemical similarity to benzidine, a human bladder carcinogen, are strong evidence that it is likely to be a human carcinogen.

The water quality criterion for DCB is based on the induction of hepatic carcinomas in female beagle dogs, given an oral dose of 100 mg 3,3'-dichlorobenzidine, three times per week for six weeks, then five times per week continuously for up to 7.1 years (Stula, et al. 1978). The concentration of DCB in water, calculated to keep the lifetime cancer risk below 10^{-5}, is 0.103 µg/l.

Summary of Pertinent Data

The water quality criterion for DCB is based on the induction of hepatic carcinomas in female beagle dogs, given an oral dose of 100 mg DCB, three times per week for six weeks, then five times per week continuously for periods up to 7.1 years (Stula, et al. 1978). The criterion was calculated from the following parameters:

Dose (mg/kg/day)	Incidence (No. responding/No. tested)
0	0/6
7.36	4/5

le = 2,593 days
Le = 2,593 days
L = 3,159 days

w = 11.391 kg
R = 312 l/kg

With these parameters the carcinogenic potency factor for humans, q_1^*, is 1.692 $(mg/kg/day)^{-1}$. The resulting water concentration for DCB, calculated to keep the individual lifetime cancer risk below 10^{-5}, is 0.103 µg/l.

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