3,3'-Dichlorobenzidine; CASRN 91-94-1

Human health assessment information on a chemical substance is included in the IRIS database only after a comprehensive review of toxicity data, as outlined in the IRIS assessment development process. Sections I (Health Hazard Assessments for Noncarcinogenic Effects) and II (Carcinogenicity Assessment for Lifetime Exposure) present the conclusions that were reached during the assessment development process. Supporting information and explanations of the methods used to derive the values given in IRIS are provided in the guidance documents located on the IRIS website.

STATUS OF DATA FOR 3,3'-Dichlorobenzidine

File First On-Line 08/01/1990

Category (section)	Assessment Available?	Last Revised
Oral RfD (I.A.)	not evaluated	
Inhalation RfC (I.B.)	message	11/01/1991*
Carcinogenicity Assessment (II.)	yes	08/01/1990*

*A comprehensive review of toxicological studies was completed (July 14, 2006) - please see sections I.B. and II.D.2. for more information.

I. Chronic Health Hazard Assessments for Noncarcinogenic Effects

I.A. Reference Dose for Chronic Oral Exposure (RfD)

Substance Name — 3,3'-Dichlorobenzidine
CASRN — 91-94-1

Not available at this time.

I.B. Reference Concentration for Chronic Inhalation Exposure (RfC)

Substance Name — 3,3'-Dichlorobenzidine
CASRN — 91-94-1

The health effects data for 3,3'-dichlorobenzidine were reviewed by the U.S. EPA RfD/RfC Work Group and determined to be inadequate for derivation of an inhalation RfC. For additional information on the health effects of this chemical, interested parties are referred to the EPA documentation listed below.

U.S. EPA. 1988. Health and Environmental Effects Document for 3,3'- Dichlorobenzidine. Prepared by the Office of Health and Environmental Assessment, Environmental Criterial and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response, Washington, DC.

Agency Work Group Review — 09/12/1991

Screening-Level Literature Review Findings — A screening-level review conducted by an EPA contractor of the more recent toxicology literature pertinent to the RfC for 3,3'-Dichlorobenzidine conducted in November 2001 did not identify any critical new studies. IRIS users who know of important new studies may provide that information to the IRIS Hotline at hotline.iris@epa.gov or (202)566-1676.

EPA Contacts:

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202)566-1676 (phone), (202)566-1749 (FAX) or hotline.iris@epa.gov (internet address).

II. Carcinogenicity Assessment for Lifetime Exposure

Sustance Name -- 3,3'-Dichlorobenzidine
CASRN — 91-94-1
Last revised -- 08/01/1990

Section II provides information on three aspects of the carcinogenic assessment for the substance in question; the weight-of-evidence judgment of the likelihood that the substance is a human carcinogen, and quantitative estimates of risk from oral exposure and from inhalation exposure. The quantitative risk estimates are presented in three ways. The slope factor is the result of

application of a low-dose extrapolation procedure and is presented as the risk per (mg/kg)/day. The unit risk is the quantitative estimate in terms of either risk per ug/L drinking water or risk per ug/cu.m air breathed. The third form in which risk is presented is a drinking water or air concentration providing cancer risks of 1 in 10,000, 1 in 100,000 or 1 in 1,000,000. The rationale and methods used to develop the carcinogenicity information in IRIS are described in The Risk Assessment Guidelines of 1986 (EPA/600/8-87/045) and in the IRIS Background Document. IRIS summaries developed since the publication of EPA's more recent Proposed Guidelines for Carcinogen Risk Assessment also utilize those Guidelines where indicated (Federal Register 61(79):17960-18011, April 23, 1996). Users are referred to Section I of this IRIS file for information on long-term toxic effects other than carcinogenicity.

II.A. Evidence for Human Carcinogenicity

II.A.1. Weight-of-Evidence Characterization

Classification — B2; probable human carcinogen

Basis — Based on statistically significantly increased tumor incidences in rats, mice and dogs. Additional support is provided by positive evidence of genotoxicity and structural relationship to the known human bladder carcinogen benzidine.

II.A.2. Human Carcinogenicity Data

Inadequate. Although benzidine has been classified as a human bladder carcinogen on the basis of human studies, three epidemiologic studies (Gerarde and Gerarde, 1974; Gadian, 1975; MacIntyre, 1975) on 3,3'-dichlorobenzidine have found no evidence for an association with human bladder cancer. These studies have been criticized by IARC (1982), however, because of their small cohort size, limited statistical power, relatively brief exposure periods, and incomplete follow-up.

II.A.3. Animal Carcinogenicity Data

Sufficient. In a study by Pliss (1959), 15 female and 35 male Rappolovskii white rats received 10 to 20 mg 3,3'-dichlorobenzidine of unknown purity per day in feed 6 days/week for 12 months; the total intake was calculated to be 4.5 g/rat. Animals were observed for their lifetimes. Of 29 rats surviving at the time of the appearance of the first tumor, 22 animals had tumors at a variety of sites: zymbal gland (7 animals), skin (3), mammary gland (7), ileum (2), bladder (3), hemopoetic (3), connective tissue (2), salivary gland (2), liver (1), and thyroid (1). The authors did not report the distribution of tumors between males and females or the distribution between

malignant and benign tumors. Ten of the 22 rats had multiple tumors. No tumors were reported in 130 control animals.

Stula et al. (1975) fed 3,3'-dichlorobenzidine to 50 male and 50 female ChR-CD rats at 1000 ppm (50 mg/kg/day) in the diet. The compound was administered for the duration of the study, which had been intended to last 2 years. The average length of time on test was 349 days (range of 143 to 488) for females and 353 (range of 118 to 486) for males. The reason for the early mortality was not stated. Male and female control animals (range of 118 to 486) (50/group) were fed the standard diet and observed for up to 2 years. An interim sacrifice of 6 rats/group was conducted at 12 months and was not included in the final tumor analysis. In males, statistically significant increases in tumor incidences were observed at three sites: granulocytic leukemia (9/44 treated vs. 2/44 control), mammary adenocarcinoma (7/44 vs. 0/44) and zymbal gland carcinoma (8/44 vs. 0/44). In female rats, mammary adenocarcinomas were the only tumors showing a significant increase in incidence (26/44 vs. 3/44).

In another study by Stula et al. (1978), 6 female beagle dogs were given 100 mg 3,3'-dichlorobenzidine by capsule 3 times/week for 6 weeks and then 100 mg 5 times/week for 7 years. The total duration of the study was 7.1 years. Two animals died during the course of the study. No tumors were found in the animal that died after 3.5 years while the animal that died after 6.6 years had both an undifferentiated liver carcinoma and a papillary transitional cell carcinoma of the bladder. Of the 4 animals remaining at the end of the study, 3 had liver carcinoma and all 4 had bladder papillary transitional cell carcinoma. Six untreated control animals, observed for 8 to 9 years, had no liver or bladder neoplasms.

Osanai (1976) fed 3,3'-dichlorobenzidine (0.1% in the diet) to 26 male ICR/JCL mice. Thirty-nine control mice were fed the standard diet. Treated and control animals were sacrificed at 6 and 12 months. Statistically significant increased incidences of hepatomas were observed as a result of treatment at both 6 months (8/8 treated vs 0/5 control) and 12 months (18/18 treated vs 2/21 control). The remaining 13 control animals were sacrificed at 18 months; 5 animals in this group had hepatomas.

Saffiotti et al. (1967) fed technical grade 3,3-dichlorobenzidine (40% dihydrochloride, 60% free base) to Syrian golden hamsters (30/sex) at a concentration of 0.1% for their lifespans. The control group consisted of 20 hamsters/sex. This treatment did not cause significant carcinogenic effects or bladder pathology. In an extension of this study, Sellakumar et al. (1969) fed similar groups of hamsters a diet containing 0.3% 3,3-dichlorobenzidine, which was determined to be the maximum tolerated dose. At this level, four transitional cell bladder carcinomas were observed (sex of animals not specified). Some liver-cell and cholangiomatous tumors were also seen, although the incidences were not reported.

Golub et al. (1975) have presented evidence suggesting that 3,3'- dichlorobenzidine acts as a transplacental carcinogen in mice following subcutaneous injection and the studies of Pliss (1959, 1963) indicate that the compound can cause tumors at multiple sites when administered by the subcutaneous route.

II.A.4. Supporting Data for Carcinogenicity

Numerous studies have shown that 3,3'-dichlorobenzidine is mutagenic in Salmonella typhimurium both in the presence and absence of metabolic activation by S-9 liver preparations. The compound has also been shown to cause increased sister chromatid exchange in a human B-lymphoblastoid cell line (Shiraishi, 1986) and increased unscheduled DNA synthesis in HeLa cells (Martin et al., 1978).

Dichlorobenzidine is structurally similar to benzidine, which is a known bladder carcinogen in humans.

II.B. Quantitative Estimate of Carcinogenic Risk from Oral Exposure

II.B.1. Summary of Risk Estimates

Oral Slope Factor — 4.5E-1 per (mg/kg)/day

Drinking Water Unit Risk — 1.3E-5 per (ug/L)

Extrapolation Method — Linearized multistage procedure, extra risk

Drinking Water Concentrations at Specified Risk Levels:

Risk Level	Concentration
E-4 (1 in 10, 000)	8E+0 ug/L
E-5 (1 in 100,000)	8E-1 ug/L
E-6 (1 in 1,000,000)	8E-2 ug/L

II.B.2. Dose-Response Data (Carcinogenicity, Oral Exposure)

Tumor Type — mammary adenocarcinoma
Test animals — rat/ChR-CD, female
Route — diet
Reference — Stula et al., 1975

Dose			Tumor Incidence
Administered (ppm)	**Animal Transformed (mg/kg/day)**	**Human Equivalent (mg/kg/day)**	
0	0	0	3/44
1000	50	8.5	26/44

II.B.3. Additional Comments (Carcinogenicity, Oral Exposure)

The slope factor incorporates an increase by a factor of $(730/488)^3$, the ratio of the lifetime of the rat to the survival period for the rat, because of the greatly reduced survival in the exposed group. The animal transformed dose was determined by assuming a 5% food consumption value for rats. Human equivalent doses were derived by multiplying the animal transformed dose by $(\text{wt. animal/wt. human})^3$, assuming body weight of the rat is 0.35 kg and body weight of an adult human is 70 kg.

The unit risk should not be used if the water concentration exceeds 8E+2 ug/L, since above this concentration the slope factor may differ from that stated.

II.B.4. Discussion of Confidence (Carcinogenicity, Oral Exposure)

With only one exposed group, response cannot reflect any curvature in the underlying dose-response relationship. Thus, the model could be far above or below true risk. A slope factor of 1.7 per (mg/kg)/day was derived from the data of Stula et al. (1978) using the incidence of hepatic carcinomas (4/5 treated vs. 0/6 control) in female beagles.

II.C. Quantitative Estimate of Carcinogenic Risk from Inhalation Exposure

None

II.D. EPA Documentation, Review, and Contacts (Carcinogenicity Assessment)

II.D.1. EPA Documentation

Source Document — U.S. EPA, 1980, 1988

The 1988 Health and Environmental Effects Document for 3,3'-Dichlorobenzidine and the 1980 Ambient Water Quality Criteria Document for Dichlorobenzidine have received OHEA review.

II.D.2. EPA Review (Carcinogenicity Assessment)

Agency Work Group Review — 07/23/1986, 05/25/1988, 11/30/1988

Verification Date — 11/30/1988

A comprehensive review of toxicological studies published through July 2006 was conducted. No new health effects data were identified that would be directly useful in the revision of the existing carcinogenicity assessment for 3,3'-Dichlorobenzidine and a change in the assessment is not warranted at this time. For more information, IRIS users may contact the IRIS Hotline at hotline.iris@epa.gov or (202)566-1676.

II.D.3. EPA Contacts (Carcinogenicity Assessment)

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202)566-1676 (phone), (202)566-1749 (FAX) or hotline.iris@epa.gov (internet address).

VI. Bibliography

Substance Name — 3,3'-Dichlorobenzidine
CASRN — 91-91-1

VI.A. Oral RfD References

None

VI.B. Inhalation RfC References

U.S. EPA. 1988. Health and Environmental Effects Document for 3,3'- Dichlorobenzidine. Prepared by the Office of Health and Environmental Assessment, Environmental Criterial and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emrgency Response, Washington, DC.

VI.C. Carcinogenicity Assessment References

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Shiraishi, Y. 1986. Hypersensitive character of Bloom syndrome B-lymphoblastoid cell lines usable for sensitive carcinogen detection. Mutat. Res. 175: 179-187.

Stula, E.F., H. Sherman, J.A. Zapp and J.W. Clayton. 1975. Experimental neoplasia in rats from oral administration of 3,3'-dichlorobenzidine, 4,4'- methylene-bis(2- chloroanaline), and 4,4'-methylene-bis(2-methylanaline). Toxicol. Appl. Pharmacol. 31: 159-176.

Stula, E.F., J.R. Barnes, H. Sherman, C.F. Reinhardt and J.A. Zapp. 1978. Liver and urinary bladder tumors in dogs from 3,3'-dichlorobenzidine. J. Environ. Pathol. Toxicol. 1(4): 475-490.

U.S. EPA. 1980. Ambient Water Quality Criteria Document for Dichlorobenzidine. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Water Regulations and Standards, Washington, DC. NTIS PB 81- 11717/AS.

U.S. EPA. 1988. Health and Environmental Effects Document for 3,3'- Dichlorobenzidine. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response, Washington, DC.

VII. Revision History

Substance Name — 3,3'-Dichlorobenzidine
CASRN — 91-94-1

Date	Section	Description
08/01/1990	II.	Carcinogen assessment on-line
11/01/1991	I.B.	Inhalation RfC message on-line
12/03/2002	I.B., II.D.2.	Screening-Level Literature Review Findings message has been added.
09/28/2006	I.B., II.D.2.	Screening-Level Literature Review Findings message has been removed and replaced by comprehensive literature review conclusions.

VIII. Synonyms

Substance Name — 3,3'-Dichlorobenzidine
CASRN — 91-94-1
Last Revised — 08/01/1990

- 91-94-1
- BENZIDINE, 3,3'-DICHLORO-
- (1,1'-BIPHENYL)-4,4'-DIAMINE, 3,3'-DICHLORO-
- C.I. 23060
- CURITHANE C 126
- 4,4'-DIAMINO-3,3'-DICHLOROBIPHENYL
- 3,3'-DICHLORBENZIDIN [CZECH]
- 3,3'-DICHLOROBENZIDINA [SPANISH]
- 3,3'-DICHLOROBENZIDIN [CZECH]
- DICHLOROBENZIDINE
- 3,3'-DICHLOROBENZIDINE
- DICHLOROBENZIDINE BASE
- O,O'-DICHLOROBENZIDINE

- 3,3'-DICHLORO-(1,1'-BIPHENYL)-4,4'-DIAMINE
- 3,3'-DICHLORO-4,4'-BIPHENYLDIAMINE
- 3,3'-DICHLOROBIPHENYL-4,4'-DIAMINE
- 3,3'-DICHLORO-4,4'-DIAMINOBIPHENYL
- HSDB 1632
- NSC 154073
- RCRA WASTE NUMBER U073