United States
Environmental Protection
Agency

EPA/690/R-13/008F
Final
6-12-2013

Provisional Peer-Reviewed Toxicity Values for

3,3′-Dimethoxybenzidine
(CASRN 119-90-4)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

AUTHORS, CONTRIBUTORS, AND REVIEWERS

CHEMICAL MANAGER

Jason C. Lambert, PhD, DABT
National Center for Environmental Assessment, Cincinnati, OH

DRAFT DOCUMENT PREPARED BY

ICF International
9300 Lee Highway
Fairfax, VA 22031

PRIMARY INTERNAL REVIEWERS

Anuradha Mudipalli, MSc, PhD
National Center for Environmental Assessment, Research Triangle Park, NC

Dan D. Petersen, PhD, DABT
National Center for Environmental Assessment, Cincinnati, OH

This document was externally peer reviewed under contract to
Eastern Research Group, Inc.
110 Hartwell Avenue
Lexington, MA 02421-3136

Questions regarding the contents of this document may be directed to the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300).

TABLE OF CONTENTS

COMMONLY USED ABBREVIATIONS

BMC	benchmark concentration
BMCL	benchmark concentration lower bound 95% confidence interval
BMD	benchmark dose
BMDL	benchmark dose lower bound 95% confidence interval
HEC	human equivalent concentration
HED	human equivalent dose
IUR	inhalation unit risk
LOAEL	lowest-observed-adverse-effect level
$LOAEL_{ADJ}$	LOAEL adjusted to continuous exposure duration
$LOAEL_{HEC}$	LOAEL adjusted for dosimetric differences across species to a human
NOAEL	no-observed-adverse-effect level
$NOAEL_{ADJ}$	NOAEL adjusted to continuous exposure duration
$NOAEL_{HEC}$	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
POD	point of departure
p-OSF	provisional oral slope factor
p-RfC	provisional reference concentration (inhalation)
p-RfD	provisional reference dose (oral)
RfC	reference concentration (inhalation)
RfD	reference dose (oral)
UF	uncertainty factor
UF_A	animal-to-human uncertainty factor
UF_C	composite uncertainty factor
UF_D	incomplete-to-complete database uncertainty factor
UF_H	interhuman uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
WOE	weight of evidence

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR 3,3′-DIMETHOXYBENZIDINE (CASRN 119-90-4)

BACKGROUND

A Provisional Peer-Reviewed Toxicity Value (PPRTV) is defined as a toxicity value derived for use in the Superfund Program. PPRTVs are derived after a review of the relevant scientific literature using established Agency guidance on human health toxicity value derivations. All PPRTV assessments receive internal review by a standing panel of National Center for Environment Assessment (NCEA) scientists and an independent external peer review by three scientific experts.

The purpose of this document is to provide support for the hazard and dose-response assessment pertaining to chronic and subchronic exposures to substances of concern, to present the major conclusions reached in the hazard identification and derivation of the PPRTVs, and to characterize the overall confidence in these conclusions and toxicity values. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of this substance.

The PPRTV review process provides needed toxicity values in a quick turnaround timeframe while maintaining scientific quality. PPRTV assessments are updated approximately on a 5-year cycle for new data or methodologies that might impact the toxicity values or characterization of potential for adverse human health effects and are revised as appropriate. It is important to utilize the PPRTV database (http://hhpprtv.ornl.gov) to obtain the current information available. When a final Integrated Risk Information System (IRIS) assessment is made publicly available on the Internet (www.epa.gov/iris), the respective PPRTVs are removed from the database.

DISCLAIMERS

The PPRTV document provides toxicity values and information about the adverse effects of the chemical and the evidence on which the value is based, including the strengths and limitations of the data. All users are advised to review the information provided in this document to ensure that the PPRTV used is appropriate for the types of exposures and circumstances at the site in question and the risk management decision that would be supported by the risk assessment.

Other U.S. Environmental Protection Agency (EPA) programs or external parties who may choose to use PPRTVs are advised that Superfund resources will not generally be used to respond to challenges, if any, of PPRTVs used in a context outside of the Superfund program.

QUESTIONS REGARDING PPRTVS

Questions regarding the contents and appropriate use of this PPRTV assessment should be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300).

INTRODUCTION

3,3′-Dimethoxybenzidine is an intermediate in the production of bisazobiphenyl dyes used for coloring textiles, paper, plastic, rubber, and leather and in the production of *o*-dianisidine diisocyanate for use in isocyanate-based adhesives and polyurethane elastomers (NTP, 1990). The empirical formula for 3,3′-dimethoxybenzidine is $C_{14}H_{16}N_2O_2$ (see Figure 1). A table of physicochemical properties is provided below (see Table 1). In this document, "statistically significant" denotes a *p*-value <0.05.

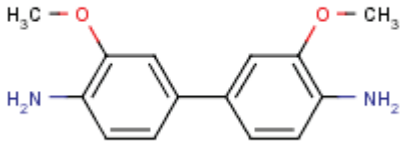


Figure 1. 3,3′-Dimethoxybenzidine Structure

Table 1. Physicochemical Properties of 3,3′-Dimethoxybenzidine[a]

Property (unit)	Value
Boiling point (ºC)	356
Melting point (ºC)	137.5
Density (g/cm^3)	Not available
Vapor pressure (Pa at 20 ºC)	Negligible
pH (unitless)	Not available
Solubility in water (g/100 mL at 18.5 ºC)	0.006
Relative vapor density (air = 1)	Not available
Molecular weight (g/mol)	244.3
Flash point (°C)	206
Octanol/water partition coefficient (unitless)	1.81

[a]Values from http://www.cdc.gov/niosh/ipcsneng/neng1582.html except for boiling point which was retrieved from http://chem.sis.nlm.nih.gov/chemidplus/.

The EPA's Integrated Risk Information System (IRIS) (U.S. EPA, 2011) does not list a chronic oral reference dose (RfD), a chronic inhalation reference concentration (RfC), or a cancer assessment for 3,3′-dimethoxybenzidine. Subchronic or chronic RfDs or RfCs for 3,3′-dimethoxybenzidine are not listed in the HEAST (U.S. EPA, 2010) or the Drinking Water Standards and Health Advisories list (U.S. EPA, 2006). HEAST (U.S. EPA, 2010) reports a

cancer weight-of evidence (WOE) classification of Group B2 (*Probable Human Carcinogen*), an oral slope factor (OSF) of 1.4×10^{-2} (mg/kg-day)$^{-1}$, and an oral unit risk factor of 4.0×10^{-7} (μg/L)$^{-1}$ based on increased incidence of forestomach papillomas in hamsters (Sellakumar et al., 1969). The 1994 CARA list (U.S. EPA, 1994) includes a Health and Environmental Effects Profile (HEEP) for 3,3′-dimethoxybenzidine reporting a human carcinogen potency factor (q1*) of 1.41×10^{-2} (mg/kg-day)$^{-1}$ for oral exposure but does not include any noncancer toxicity values. No occupational exposure limits for 3,3′-dimethoxybenzidine have been derived by the American Conference of Governmental Industrial Hygienists (ACGIH, 2009), the National Institute of Occupational Safety and Health (NIOSH, 2010), or the Occupational Safety and Health Administration (OSHA, 2006). The International Agency for Research on Cancer (IARC, 2000) has reviewed the carcinogenic potential of 3,3′-dimethoxybenzidine and placed it in Group 2B, "*Possibly carcinogenic to humans*." The toxicity of 3,3′-dimethoxybenzidine has not been reviewed by the Agency for Toxic Substances and Disease Registry (ATSDR, 2010) or the World Health Organization (WHO, 2010). 3,3′-Dimethoxybenzidine is classified as "*Reasonably Anticipated to be a Human Carcinogen*" based on sufficient data from animal studies in the *12th Report on Carcinogens* (NTP, 2011). No noncancer toxicity values for exposure to 3,3′-dimethoxybenzidine have been derived by the California Environmental Protection Agency (CalEPA, 2008, 2009). CalEPA (2009) has prepared a quantitative estimate of carcinogenic potential for 3,3′-dimethoxybenzidine and reports a No Significant Risk Level (NSRL) of 0.15 μg/day.

Literature searches were conducted from 1900 through August 2011 for studies relevant to the derivation of provisional toxicity values for 3,3′-dimethoxybenzidine, CAS No. 119-90-4. Searches were conducted using EPA's Health and Environmental Research Online (HERO) database of scientific literature. HERO searches the following databases: AGRICOLA; American Chemical Society; BioOne; Cochrane Library; DOE: Energy Information Administration, Information Bridge, and Energy Citations Database; EBSCO: Academic Search Complete; GeoRef Preview; GPO: Government Printing Office; Informaworld; IngentaConnect; J-STAGE: Japan Science & Technology; JSTOR: Mathematics & Statistics and Life Sciences; NSCEP/NEPIS (EPA publications available through the National Service Center for Environmental Publications [NSCEP] and National Environmental Publications Internet Site [NEPIS] database); PubMed: MEDLINE and CANCERLIT databases; SAGE; Science Direct; Scirus; Scitopia; SpringerLink; TOXNET (Toxicology Data Network): ANEUPL, CCRIS, ChemIDplus, CIS, CRISP, DART, EMIC, EPIDEM, ETICBACK, FEDRIP, GENE-TOX, HAPAB, HEEP, HMTC, HSDB, IRIS, ITER, LactMed, Multi-Database Search, NIOSH, NTIS, PESTAB, PPBIB, RISKLINE, TRI; and TSCATS; Virtual Health Library; Web of Science (searches Current Content database among others); World Health Organization; and Worldwide Science. The following databases outside of HERO were searched for toxicity values: ACGIH, ATSDR, CalEPA, EPA IRIS, EPA HEAST, EPA HEEP, EPA OW, EPA TSCATS/TSCATS2, NIOSH, NTP, OSHA, and RTECS.

REVIEW OF POTENTIALLY RELEVANT DATA (CANCER AND NONCANCER)

Table 2 provides information for all of the potentially relevant studies relating to 3,3′-dimethoxybenzidine toxicity. Entries for the principal studies are bolded.

Table 2. Summary of Potentially Relevant Data for 3,3′-Dimethoxybenzidine (CASRN 119-90-4)

Category	Number of Male/Female, Strain, Species, Study Type, and Duration	Dosimetry[b]	Critical Effects	NOAEL[b,c]	LOAEL[b,c]	Reference (Comments)	Notes[a]
Human							
1. Oral (mg/kg-day)[b]							
None							
2. Inhalation (mg/m^3)[b]							
Subchronic	None						
Chronic	None						
Developmental	None						
Reproductive	None						
Carcinogenic	438 (sex not reported), occupational, duration not reported	Not reported	A total of 88 cases of uroepithelial cancer consisting of 67 in bladder; 5 in upper urinary tract; 16 in bladder and upper urinary tract	None	Not reported	Hamasaki et al. (1996); (abstract) (subjects were exposed to a mixture of compounds that included 3,3′-dimethoxybenzidine)	
	400/0, occupational, duration not reported	Not reported	A total of 6 workers with bladder cancer	None	Not reported	Frumin et al. (1990) (subjects were exposed to a mixture of compounds that included 3,3′-dimethoxybenzidine)	
	585/119, occupational, 8624 person-years	Not reported	Bladder cancer	None	Not reported	Ouellet-Hellstrom and Rench (1996); (subjects were exposed to a mixture of compounds that included 3,3′-dimethoxybenzidine)	

Table 2. Summary of Potentially Relevant Data for 3,3′-Dimethoxybenzidine (CASRN 119-90-4)

Category	Number of Male/Female, Strain, Species, Study Type, and Duration	Dosimetry[b]	Critical Effects	NOAEL[b,c]	LOAEL[b,c]	Reference (Comments)	Notes[a]
Animal							
			1. Oral (mg/kg-day)[b]				
Subchronic (Screening Value)	**10/sex, F344N, rat, drinking water, ad libitum, 91 days**	**0, 13, 22, 39, 70, 120 (male); 0, 24, 49, 60, 103, 187 (female)**	**Increased relative kidney and liver weights in males and females; decreased thymus weights in males**	**None**	**13**	**NTP (1990); Morgan et al. (1989)**	**PS (noncancer)**
Chronic/ Carcinogenicity	3/3 per dose, 14/15 (10 mg/day dose), F344, rats, oral by gavage, 52 weeks	0, 0.2, 0.6, 1.9, 5.6, 18.8, 56.4 (male) 0, 0.3, 0.9, 3.1, 9.4, 31.2, 93.6 (female)	Decreased survival time and body weight; tumors in lower intestinal tract, skin, ear, and forestomach (incidence not statistically significant compared to control)	None	None	Hadidian (1968) (animals were followed for 6-months after exposure concluded)	
	42, sex and strain unreported, rat, orally by gavage, 14 months	0, 33 (first 3 weeks of study), 16 (over subsequent 13 months of study)	Decreased survival time	None	None	Pliss (1963, 1965), as cited by NTP (1990) (animals initially received 30 mg gavage doses 3x/week for the first 3 weeks of study but due to poor survival was reduced to 15 mg for an additional 13 months)	

Table 2. Summary of Potentially Relevant Data for 3,3′-Dimethoxybenzidine (CASRN 119-90-4)

Category	Number of Male/Female, Strain, Species, Study Type, and Duration	Dosimetry[b]	Critical Effects	NOAEL[b,c]	LOAEL[b,c]	Reference (Comments)	Notes[a]
Chronic/ Carcinogenicity	**45−75/45−75 per dose, F344N, rat, drinking water, ad libitum, 21 months**	**0, 6, 12, 21 (male); 0, 7, 14, 23 (female)**	Increased liver lesions; hematopoietic cell proliferation in the spleen; thrombi in the atrium; histiocytic cellular infiltration in the lung; **Increased tumors in multiple organs including: Zymbal gland, preputial gland, clitoral gland, skin basal cells, skin squamous cells, small intestines, large intestines, oral cavity, liver, mammary gland; increased mortality due to tumors**	None	None	**NTP (1990); Morgan et al. (1990)** (high mortality rate at all doses tested)	**PS** (cancer)
	10 rats/sex from control and high dose group, F344N, rat, drinking water, ad libitum, 9 months	0, 21 (male), 23 (female)	Increased kidney, liver weight; decreased hemoglobin, erythrocytes, hematocrit, mean corpuscular hemoglobin	None	21	NTP (1990); Morgan et al. (1990) (the 9-month time point was a scheduled interim sacrifice in the 21-month study; low- and mid-dose animals not examined at interim sacrifice)	
	120/120 per dose, BALBc, mouse, drinking water, ad libitum, 112 weeks	0, 6, 12, 23, 46, 91, 182 (male); 0, 6, 13, 26, 52, 102, 204 (female)	Decreased body weight gain; no carcinogenic effects	91	182	Schieferstein et al. (1990)	
	30/30, Syrian golden, hamster, feed, ad libitum, lifetime	0, 57 (male); 0, 54 (female)	No carcinogenic effects	None	None	Saffiotti et al. (1967)	

Table 2. Summary of Potentially Relevant Data for 3,3′-Dimethoxybenzidine (CASRN 119-90-4)

Category	Number of Male/Female, Strain, Species, Study Type, and Duration	Dosimetry[b]	Critical Effects	NOAEL[b,c]	LOAEL[b,c]	Reference (Comments)	Notes[a]
Chronic/ Carcinogenicity	Number, sex, and strain not reported, hamster, study type and duration not reported	171, 571 (male); 161, 536 (female)	Forestomach papillomas	None	None	Sellakumar et al. (1969)	
2. Inhalation (mg/m^3)[b]							
Subchronic	None						
Chronic	None						
Developmental	None						
Reproductive	None						
Carcinogenic	None						

[a]Notes: IRIS = Utilized by IRIS, date of last update; PS = Principal study; NPR = Not peer reviewed.
[b]Dosimetry: Animal doses presented. For all discontinuous exposures, NOAEL and LOAEL values are converted to a continuous (daily) exposure.
[c]Not reported by the study authors; determined from available data for this document.

HUMAN STUDIES

Oral and Inhalation Exposures

No information is available regarding oral exposure of humans to 3,3′-dimethoxybenzidine. No studies investigating the effects of subchronic inhalation exposure to 3,3′-dimethoxybenzidine in humans have been identified. Chronic inhalation exposure to 3,3′-dimethoxybenzidine in humans has been evaluated in occupational studies involving the production or usage of benzidine and benzidine congeners (Frumin et al., 1990; Hamasaki et al., 1996; Ouellet-Hellstrom and Rench, 1996). No human studies involving exposure to 3,3′-dimethoxybenzidine alone were identified.

Hamasaki et al. (1996) evaluated a cohort of 438 workers (sex not reported) employed in a plant producing and using aromatic amines including benzidine sulfate, beta-naphthylamine, alpha-naphthylamine, and 3,3′-dimethoxybenzidine. The results presented here are as reported in the abstract because the original publication was only available in Japanese. Among the 438 workers, a total of 88 cases of uroepithelial cancer occurred from 1949 to 1995, resulting in an incidence rate of 20.1%. The average exposure time of individuals with cancer was 7.40 years. The average latency period was 26.79 years, and the average age of onset was 52.59 years. The duration of exposure of all workers evaluated was not provided. Of the 88 cases, 67 reported tumor sites in the bladder only and another 16 reported tumor sites in the bladder and upper urinary tract. A total of 28 of the workers with cancer died of uroepithelial cancer (31.8%). The authors reported survival rates of 87.9%, 74.0%, 65.9%, and 56.3% for 5, 10, 15, and 20 years, respectively.

Frumin et al. (1990) investigated the occurrence of bladder cancer in textile dyeing and printing workers. A total of 400 male workers were evaluated over a 4-year period using urine cytology, during which time, 2 workers were diagnosed with bladder cancer. The authors presented case reports of these two workers along with three other workers that self-reported bladder cancers and one worker that was not diagnosed with bladder cancer until 2 years after the screening process. All of the workers evaluated mixed dyes and pigments and applied them to cloth. As a result, these workers were exposed to a large number of dyes including 3,3′-dimethoxybenzidine, 3,3′-dimethylbenzidine, and benzidine. The duration of exposure of all workers evaluated was not provided. The average latency period was 23.3 years and ranged from 16 to 32 years. A total of six workers were diagnosed with bladder cancer. These 6 workers had a mean age of 56.5 years at time of cancer detection; according to the study authors, this age is 9−14 years less than the mean age at detection of nonoccupational bladder cancer in men. The study authors concluded that occupational exposure to benzidine dyes and dyes made from benzidine congeners cause an increased risk of bladder cancer. However, the authors noted that their screening method and low number of cases did not allow for a statistical analysis of their results.

Ouellet-Hellstrom and Rench (1996) evaluated a cohort of 704 workers (585 men, 119 women) employed in a plant that produced 3,3′-dichlorobenzidine, 3,3′-dimethylbenzidine, and 3,3′-dimethoxybenzidine. Plant records were used to identify workers employed at the plant between June 15, 1965, and December 31, 1989. Workers who may have been exposed to benzidine were excluded from the study. Plant records were cross-referenced with the company's medical records, death certificates, and the Connecticut Tumor Registry to identify cancer cases. In addition, a survey was sent out by mail to all members of the cohort for which a

current address was available. Additional mailings and telephone calls were used as follow-up measures for nonrespondents. A total of 8624 person-years of observation were collected from the cohort. A total of 24 malignant cancer cases were identified, including cancer of the buccal cavity, bladder cancer, kidney cancer, brain cancer, breast cancer, and testicular cancer. Only bladder cancer and testicular cancer were statistically significant. Of the three workers diagnosed with testicular cancer, two were never exposed to the dyes, and the third was only exposed for 15 days. An eight-fold increase in the risk of bladder cancer was observed in individuals exposed to the dyes. All of the workers diagnosed with bladder cancer were current or ex-smokers. The authors concluded that an association exists between exposure to the dyes and bladder cancer, and that while smoking is known to be related to bladder cancer, it would not by itself explain such a large increase in bladder cancer incidence. Because workers were likely exposed to multiple dyes, this study was not able to determine cancer risks from one specific dye.

ANIMAL STUDIES

Oral Exposure

The effects of oral exposure of animals to 3,3′-dimethoxybenzidine have been evaluated in short-term (NTP, 1990), subchronic- (Morgan et al., 1989; NTP, 1990) and chronic-duration (Hadidian et al., 1968; NTP, 1990; Schieferstein et al., 1990; Pliss, 1963, 1965; Saffiotti et al., 1967; Sellakumar et al., 1969) studies.

Short-term Study

NTP (1990) sponsored a 14-day drinking water study with 3,3′-dimethoxybenzidine dihydrochloride (purity 98%) in F344N rats. Groups of five male and five female rats were exposed to 0, 200, 350, 750, 1500, or 4500 ppm for two consecutive weeks. Based on body-weight data and water consumption data reported in the study, daily doses of 3,3′-dimethoxybenzidine dihydrochloride are estimated as 0, 18, 29, 57, 101, and 127 mg/kg-day in males and 0, 19, 32, 61, 141, and 214 mg/kg-day in females, respectively. Water and feed were provided ad libitum. Animals were observed for mortality and clinical signs twice daily. The study authors recorded body weights before treatment and on Treatment Days 7 (males) or 4 (females) and also on Treatment Day 14. The rats were necropsied, and relative organ weights for the brain, lungs, heart, liver, kidney, right testis, and thymus were recorded. The study authors performed comprehensive histopathology on several tissues (including gross lesions, tissue masses, associated lymph nodes, and 33 organs) on all rats in the 4500 ppm group. In addition, the spleen, bone marrow (sternum), and thymus were examined histologically in male rats in the 1500 ppm group, and bone marrow (sternum) was examined histologically in 1500 ppm female rats. This study was peer reviewed and performed in accordance with Good Laboratory Practice (GLP) regulations.

All rats lived until the end of the study (NTP, 1990). Organ weight results are presented in Table B.1. Final mean body weights of animals dosed with 4500 ppm were decreased when compared to initial body weights. Water consumption was decreased in a dose-dependent manner. Relative liver weights were increased at 200 ppm and at doses of 750 ppm and greater in males and 1500 ppm and greater in females. No effects on relative liver weights were observed in males treated with 350 ppm. Relative kidney weights were increased at doses of 350 ppm and greater in males and 1500 ppm and greater in females. The study authors noted that no microscopic changes were observed in these organs. However, detailed results of the histopathological examinations were not provided. Increases in relative brain, lung, heart, and

right testis weights were measured in males treated with 4500 ppm. Relative brain and thymus weights were increased in females treated with 4500 ppm. Lymphoid depletion was observed in the spleen of males and females and in the thymus of males at 4500 ppm. Animals dosed with 4500 ppm that lost weight also had bone marrow hypocellularity. Based on increased relative kidney weights in males, a LOAEL of 350 ppm (average daily dose of 29 mg/kg-day) and a NOAEL of 200 ppm (average daily dose of 18 mg/kg-day) are established for 2-week oral exposure to 3,3′-dimethoxybenzidine in rats.

Subchronic-duration Studies

The study by NTP (1990) is selected as the principal study for deriving the screening subchronic p-RfD. NTP (1990) reported a 13-week oral study in which groups of 10 male and 10 female Fischer 344N rats were administered 0, 170, 330, 630, 1250, or 2500 ppm 3,3′-dimethoxybenzidine dihydrochloride (purity 98%) in drinking water. Results of this study were also reported by Morgan et al. (1989). Respective corresponding daily doses were estimated as 0, 13, 22, 39, 70, and 120 mg/kg-day for males and 0, 24, 49, 60, 103, and 187 mg/kg-day for females, respectively (Morgan et al., 1989). Animals were obtained from Frederick Cancer Research Facility at 4 weeks of age and acclimated to laboratory conditions for at least 2 weeks prior to study initiation. Food was provided ad libitum, and fresh water was supplied twice weekly. Animals were observed daily for mortality and clinical signs of toxicity. Body weights and food consumption were measured once per week. Water consumption was measured twice per week. At study termination, blood samples were collected from the retro-orbital sinus of all animals for hematology. At the end of the treatment period, all surviving animals were sacrificed and necropsied. Selected organs were weighed, and complete histopathological examinations were performed. This study was peer reviewed and performed in accordance with GLP guidelines.

All animals survived until the end of the study (Morgan et al., 1989; NTP, 1990). No signs of clinical toxicity were reported. Water consumption decreased in a dose-dependent manner (see Table B.2). At 1250 ppm, water consumption was decreased by approximately 33% in males and 56% in females after 13 weeks of exposure when compared to controls. At 2500 ppm, a 43% decrease in water consumption was observed in males and a 60% decrease in females when compared to controls. Decreased body weights were noted in males (see Table B.3) at 1250 (10%) and 2500 ppm (19%) and in females (see Table B.4) at 2500 ppm (8%). The study authors reported significant treatment-related increases in relative organ weights for the liver and kidney in males of all exposure groups (see Table B.3) and the liver at ≥630 ppm and the kidney at ≥330 ppm in females (see Table B.4). Significantly decreased relative thymus weights were seen in males at all doses; however, this effect was not observed in females at any dose (data not shown). Statistically significant changes were reported for leukocyte, lymphocyte, and neutrophil counts in males and neutrophil and erythrocyte counts and hematocrit values in females. However, the study authors concluded that none of these changes were reliable based on nonoptimal experimental sampling procedure (e.g., mechanical stress of harvested cells). Decreased creatinine was seen in all males and females treated with 3,3′-dimethoxybenzidine dihydrochloride (see Table B.5). The study authors concluded that these changes could be due to loss of muscle mass or the result of assay interference from bilirubin or hemoglobin. Mean serum triiodothyronine (T3) was decreased in females at ≥330 ppm; no significant effects were seen in males. Decreases in mean serum thyroxine (T4) were seen in all treated males and in females at ≥330 ppm (see Table B.5). The authors noted

that because thyrotropin (TSH) remained unchanged, these changes are not a direct effect on the thyroid gland and are most likely due to competition for the carrier protein for these hormones. No other treatment-related effects in clinical chemistry or hematology parameters were reported.

Chronic nephropathy and foci of regenerative tubular epithelium were reported in females at 2500 ppm after 90 days of treatment. Chronic nephropathy was seen in all control and treated male rats and high-dose (2500 ppm) female rats (Morgan et al., 1989). However, increased severity of the lesions was seen in 2500 ppm males (1.8) compared to control (1.0) and 1250 ppm males (1.0). Increased pigment (lipofuscin) in the cytoplasm of thyroid follicular cells was observed in all males and females at 1250 (severity 1.6 and 1.5, respectively) and 2500 ppm (severity = 1.5 and 3.0, respectively) with no effects seen in the thyroid of any control animals (Morgan et al., 1989). For this study, a LOAEL of 170 ppm (13 mg/kg-day) is determined based on increased relative organ-weight changes in the liver and kidney, and decreased relative thymus weight in male rats; a NOAEL is not established.

Chronic-duration Studies

Hadidian et al. (1968) reported the effects of 3,3′-dimethoxybenzidine administered orally by gavage to male and female F344 rats. Three rats per sex per dose were administered 0, 0.1, 0.3, 1, 3, or 30 mg/day 3,3′-dimethoxybenzidine 5 days per week for 52 weeks. A total of 14 males and 15 females were administered 10 mg/day 3,3′-dimethoxybenzidine by the same route and procedure. The study authors noted that 10 mg/day was one-third the maximum tolerated dose of 3,3′-dimethoxybenzidine and felt that using a larger number of animals at this dose would best reveal the carcinogenic effects of the compound. Based on reference average body weights for this strain of rat (U.S. EPA, 1988), the estimated duration-adjusted (5/7 days per week exposure) daily doses are 0, 0.2, 0.6, 1.9, 5.6, 18.8, and 56.4 mg/kg-day for males and 0, 0.3, 0.9, 3.1, 9.4, 31.2, and 93.6 mg/kg-day for females, respectively. The test material was dissolved in a vehicle consisting of NaCl, sodium carboxymethylcellulose, polysorbate 80, and benzyl alcohol. The purity of the 3,3′-dimethoxybenzidine used was not reported. Following administration of the test substance for 52 weeks, the animals were observed for an additional 6 months. Animals were examined for signs of clinical toxicity five times per week during the treatment. Body weight was measured every other week. Following the 6-month observation period, all animals were sacrificed. Organ weights for the liver, spleen, kidneys, adrenal glands, and the pituitary were obtained. Gross necropsies were performed on the liver, spleen, kidneys, adrenal glands, pituitary, lungs, esophagus, stomach, intestines, bladder, gonads, thyroids, and mammary glands. Tissues appearing abnormal during gross examination were examined for histopathology.

Average survival time decreased in male and female rats of all treatment groups >0.3 mg/day (see Table B.6) (Hadidian et al., 1968). However, statistical analysis was not reported and could not be conducted because the average survival time of control animals was not reported. Body weights also decreased in a dose-dependent fashion in female rats (see Table B.6). No effects on liver weight were seen. Organ weights for other tissues were not reported. The study authors noted that organ weights for the spleen, adrenal glands, and the pituitary were rarely affected by treatment. No dose-related trends in nonneoplastic lesions were seen. For neoplastic lesions, the study authors concluded that treatment with 3,3′-dimethoxybenzidine resulted in intestinal tract adenocarcinomas, skin carcinomas, ear duct carcinomas, and a tumor of the forestomach (see Table B.7). However, statistical analysis of

these results indicates that none of the tumors are statistically increased compared to controls. The small number of animals evaluated precludes the determination of a NOAEL or LOAEL.

Pliss (1963, 1965) administered 3,3′-dimethoxybenzidine (purity unknown) via gavage to 42 rats (sex, strain unknown). Pliss (1963) is a review of benzidine and its derivatives and only briefly mentions tumors found following exposure to 3,3′-dimethoxybenzidine. Pliss (1965) is written in a foreign language. Therefore, the summary of these studies is based on information provided by NTP (1990). Animals were administered 30 mg 3,3′-dimethoxybenzidine via gavage 3 times per week. Due to poor survival, the dose was reduced to 15 mg after 3 weeks and administered for 13 months. No further information was available for study protocol, and study results were limited to survival and positive tumor results seen at terminal sacrifice. Based on reference body weight of rats of unknown sex and strain (U.S. EPA, 1988), estimated daily doses are 0, 33 (first 3 weeks of study), and 16 mg/kg-day (over subsequent 13 months). Of the 42 rats treated with 3,3′-dimethoxybenzidine, only 18 survived until the end of the study. No data regarding time of mortality were reported. The survival rate of the control animals was not provided. Of the 18 animals that survived until the end of the study, Zymbal gland tumors were reported in 2 animals (sex unknown), and 1 animal had an ovarian tumor. The study authors noted that none of the 50 control animals developed tumors at these sites. Due to the high rate of mortality seen in treated animals and poor study design/reporting, no LOAEL or NOAEL can be determined from this study.

NTP (1990) evaluated the effects of 3,3′-dimethoxybenzidine in a 21-month chronic study in rats. Results of this study are also reported by Morgan et al. (1990). Fischer 344N rats were obtained from Simonsen Laboratories at 4 weeks of age and acclimated to laboratory conditions for 14−21 days. Groups of 45−75 male and female Fischer 344N rats were exposed to 0, 80, 170, or 330 ppm 3,3′-dimethoxybenzidine dihydrochloride (purity 98%) in drinking water. Corresponding estimated daily doses were 0, 6, 12, and 21 mg/kg-day for males and 0, 7, 14, and 23 mg/kg-day for females, respectively (Morgan et al., 1990). Animals were observed twice daily for signs of clinical toxicity and weighed once a week for the first 15 weeks and once a month afterwards. A total of 10 animals per sex from the control and 330 ppm groups only were selected for interim sacrifice following 9 months of exposure. Hematology, serum chemistry, and urine analyses were performed during the 9-month interim sacrifice only. Gross necropsy and histologic examinations were performed on all animals. Organ weights were obtained during necropsy. This study was peer reviewed and conducted according to GLP guidelines.

At 9 months, significant treatment-related increases in relative kidney and liver weights were observed in both male and female rats of the 330 ppm groups (NTP, 1990) (see Table B.8). In males, decreased hemoglobin, erythrocyte counts, hematocrit, and mean corpuscular hemoglobin were observed and were indicative of mild anemia. No evidence of renal damage was seen from the urinalysis. The study authors also reported basophilic and/or eosinophilic foci of altered cells in the liver (8/10 males and 5/10 females). However, results for control rats were not reported. Carcinomas of the preputial gland (1/10 rats) and Zymbal gland (2/10 rats) were observed in males. In one female rat, a carcinoma of the clitoral gland was observed (NTP, 1990). None of these lesions were observed in the control animals during this interim sacrifice. Based on increased relative organ weights in the liver and kidney, and hematology effects seen in males, a LOAEL of 330 ppm (21 mg/kg-day) is determined for 9 months of exposure in rats.

Because animals from the mid- and low-dose groups were not evaluated during the 9-month interim sacrifice, a NOAEL cannot be established.

Dose-dependent decreases in body weight and water consumption were observed in both male and female rats after 21 months of exposure (NTP, 1990) (see Table B.9). Clinical signs reported by the study authors included increased incidence of tissue masses on the head, over the dorsum, and in the genital area of dosed groups. Significant increased mortality due to tumors was reported in both males and females at all doses (see Table B.10), resulting in the shortening of the study from 24 to 21 months. The study authors noted that the decreased survival was mostly due to the formation of neoplasms of the skin, Zymbal gland, preputial gland (in males), and clitoral and mammary glands (in females). Tumors first appeared following 32 weeks of exposure in the Zymbal gland and clitoral gland (in females). Treatment-related increases in nonneoplastic lesions were observed in the lung, liver, heart, and spleen (see Table B.11). Neoplastic lesions were reported in multiple tissues including the Zymbal gland, preputial gland, clitoral gland, skin basal cells, skin squamous cells, small intestines, large intestines, oral cavity, liver, and mammary gland (see Table B.12). NTP (1990) concluded that there was clear evidence of carcinogenic activity of 3,3′-dimethoxybenzidine in both male and female rats. Due to the increased rate of mortality seen in all dose groups treated with 3,3′-dimethoxybenzidine, determination of a NOAEL or a LOAEL is not feasible.

Schieferstein et al. (1990) conducted a 2-year chronic toxicity and carcinogenicity study in mice. BALBc mice (up to 24/sex/dose group) were given 0, 20, 40, 80, 160, 315, or 630 ppm 3,3′-dimethoxybenzidine dihydrochloride (≥99.5% pure) in their drinking water for 112 weeks. Based on recommended water consumption and reference body weight values (U.S. EPA, 1988), corresponding daily doses are estimated here at 0, 6, 12, 23, 46, 91, and 182 mg/kg-day in males and 0, 6, 13, 26, 52, 102, and 204 mg/kg-day in females. Methods of measuring water consumption, food consumption, or body weight were not reported in the study. Animals were sacrificed and necropsied on Weeks 13, 26, 39, 52, 78, and 112. Mice that died during study were also necropsied. Complete histopathological examinations were recorded for all animals.

No treatment-related changes in mortality were observed at any dose (Schieferstein et al., 1990). Histopathological analysis also revealed no treatment-related effects. Decreased water consumption was reported in high-dose male and female mice (data not provided), and the study authors reported that this may have been due to an unpleasant taste and not necessarily reflective of 3,3′-dimethoxybenzidine toxicity. No data on organ weights were provided in the study. Decreases in weight gain were noted in high-dose males (10.7%) and females (13.3%). The study authors noted that the decrease in weight gain may be related to the decreased water consumption and may not be reflective of 3,3′-dimethoxybenzidine toxicity. However, the authors also noted that a 10% or greater decrease in body-weight gain can alter normal lifespan by mechanisms not related to tumor induction. Therefore, a LOAEL of 630 ppm (182 mg/kg-day) and a NOAEL of 315 ppm (91 mg/kg-day) are identified based on decreased body-weight gain in male mice.

Saffiotti et al. (1967) investigated the effects of aromatic amines on bladder cancer in hamsters. Groups of 30 male and 30 female Syrian golden hamsters were administered 0 or 0.1% (w/w) 3,3′-dimethoxybenzidine (purity unspecified) in the diet, ad libitum, from 8 weeks of age through the remainder of the lifespan. No further information on testing duration was given; however, the average lifespan of a hamster is 2.5 years (U.S. EPA, 1988). Based on the

estimated average chemical intake of 60 mg/week provided by the study authors and average reference body weights for mature Syrian golden hamsters (U.S. EPA, 1988), daily doses are estimated at 0 and 57 mg/kg-day for males, and 0 and 54 mg/kg-day for females. Animals were weighed and evaluated for clinical toxicity every 2 weeks during the study. It is unknown if clinical chemistry parameters were evaluated by the study authors as this is not discussed in the study. At the end of the study, histopathological examinations were conducted on all bladders and most kidneys, livers, and adrenal glands. In addition, any other organs where gross lesions were observed were also examined microscopically. Because this study is presented as a book chapter, it is not clear whether or not it is peer reviewed. GLP compliance is also unknown.

Survival rates are not reported (Saffiotti et al., 1967). Data concerning body weights, clinical toxicity, clinical chemistry, and organ weights are not provided. The study authors reported that no treatment-related tumors were seen in any organs evaluated except for the bladder where a small transitional cell carcinoma was seen in a single male that died during Week 144 of treatment. Histopathological data for groups or individuals were not provided. Because this study provides limited data and appears to focus mainly on induction of bladder cancer, determination of a LOAEL or NOAEL is not feasible.

Sellakumar et al. (1969) evaluated the effects of 3,3′-dimethoxybenzidine in hamsters. No information on strain, sex, husbandry, test duration, or compound purity is given. The authors of this document noted that the study groups were similar to those reported by Saffiotti et al. (1967). However, no further explanation of study groups is given. Animals were treated with 0.3% or 1% (w/w) 3,3′-dimethoxybenzidine in the diet. Assuming the protocol for this study is similar to the previous study on 3,3′-dimethoxybenzidine conducted by Saffiotti et al. (1967), daily doses are estimated as 171 and 571 mg/kg-day for males, and 161 and 536 mg/kg-day for females (based on estimated weekly chemical intakes of 180 mg at 0.3% and 600 mg at 1.0%, and reference body weights for mature Syrian golden hamsters [U.S. EPA, 1988]). Discussion on examination protocol is limited to findings in the bladder, liver, bile duct, and forestomach. This study is presented as an abstract for a conference proceeding, and no further study details were found. Therefore, it is unknown if the information is peer reviewed. GLP compliance is also unknown.

Survival data were not reported (Sellakumar et al., 1969). The study authors reported the induction of 4 transitional cell bladder carcinomas, liver cell and cholangiomatous tumors (number not reported), and diffuse chronic intrahepatic obstructing cholangitis (63%) in the 0.3% group. Results for controls were not provided. Therefore, evaluation of these effects for significance is not feasible. At 1.0%, 3,3′-dimethoxybenzidine had no effect on the formation of bladder or liver tumors but caused a 37% increase in forestomach papillomas, compared to 2% in controls. No information was provided to determine if these effects were seen in males, females, or both. Due to the limited amount of information provided for this study, it is not feasible to determine a NOAEL or LOAEL.

Developmental and Reproduction Studies

Gray and Ostby (1993) published a developmental study using two dimethoxybenzidine-based dyes, Chicago Sky Blue (CSB) and Azoic Diazo Component 48 (ADC) (purities unreported). CSB is a tetrasodium salt of a naphthalene, dimethoxybenzidine and disulphonate conjugate, whereas ADC's chemical structure is virtually identical to a dimethoxybenzidine. Female CD-1 mice (number not reported) were administered 0 or

1000 mg/kg-day CSB or ADC orally by gavage in a vehicle of 0.2 mL water on Gestation Days (GDs) 8−12. Dams were weighed both before and after exposure on GD 7 and GD 13, respectively. Litters were randomly reduced to seven pups each. Female pups were discarded, and male pups were weaned on Day 30 and necropsied on Days 46−47 and 187−190. Body, right testis, cauda epididymis, and seminal vesicle weights were reported. Cauda epididymal sperm counts and testicular sperm head counts were measured. Histopathological examinations were conducted on the testes. Treatment with ADC resulted in a 4.0 to 1.2 g ($p < 0.05$) decrease in maternal-weight gain during the treatment period. Treatment with CSB yielded no change in maternal parameters. No significant treatment-related effects on the development of male mice were observed after treatment with either CSB or ADC. Based on the decrease in maternal-weight gain, a maternal LOAEL of 1000 mg/kg-day is established; no maternal NOAEL is determined. No developmental LOAEL is established. The developmental NOAEL for this study is 1000 mg/kg-day.

Inhalation Exposure

Subchronic-duration Studies

No studies could be located regarding the effects of subchronic inhalation exposure of animals to 3,3′-dimethoxybenzidine.

Chronic-duration Studies

No studies could be located regarding the effects of chronic inhalation exposure of animals to 3,3′-dimethoxybenzidine.

Developmental and Reproduction Studies

No studies could be located regarding the effects of inhaled 3,3′-dimethoxybenzidine on reproduction or fetal development.

OTHER DATA (SHORT-TERM TESTS, OTHER EXAMINATIONS)

Little information on the toxicokinetics of 3,3′-dimethoxybenzidine is available. 3,3′-Dimethoxybenzidine has been detected in the urine of workers following occupational exposure (IARC, 1974). Rodgers et al. (1983) reported that 3,3′-dimethoxybenzidine was rapidly metabolized by rats after intravenous administration; specifically, 30 minutes after an intravenous injection of ^{14}C-3,3′-dimethoxybenzidine, <2% of the bolus dose was recovered as parent compound from the exposed animal. Three days after oral exposure, approximately 85% of the administered ^{14}C-3,3′-dimethoxybenzidine dose was excreted in the feces or urine with greater than 90% of the excreted radiolabel in the form of metabolites (Rodgers et al., 1983). Although a full metabolic profile has not been established for 3,3′-dimethoxybenzidine, GC/MS analyses indicated a multitude of different phase II conjugates including *N*-acetylated, *O*-demethylated, hydroxylated, and glucuronidated species (Rodgers et al., 1983).

Table 3 summarizes the studies examining genotoxicity (e.g., clastogenicity, mutagenicity) of 3,3′-dimethoxybenzidine. Anderson and Styles (1978), Chung et al. (2000), Haworth et al. (1983), Krishna et al. (1986), Martin and Kennelly (1981), Probst et al. (1981), and Messerly et al. (1987) indicate the mutagenicity of 3,3′-dimethoxybenzidine in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 when metabolically activated. Makena and Chung (2007) also found dimethoxybenzidine to be mutagenic in *Salmonella* strain TA102 with metabolic activation. De France et al. (1986), Gregory et al.

(1981), and Prival et al. (1984) investigated the mutagenic effects of certain dimethoxybenzidine-based dyes. De France et al. (1986) found no significant results while Gregory et al. (1981) and Prival et al. (1984) concluded that these dyes are mutagenic when chemically-reduced to release benzidine. *E. coli* strains W3110 and P3478 were exposed to 3,3′-dimethoxybenzidine by Fluck et al. (1976), but the study yielded inconclusive results.

Martelli et al. (2000) conducted a study examining the effects of 3,3′-dimethoxybenzidine on rat and human hepatocytes, as well as human urinary bladder cells in vitro and rat urinary bladder cells in vivo. This study revealed dose-dependent DNA fragmentation and increased frequencies of micronucleated cells in both rat and human hepatocytes, as well as increased DNA damage to urinary bladder cells in vitro and in vivo. Galloway et al. (1987, 1985) provided evidence of sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells with and without metabolic activation after exposure to 3,3′-dimethoxybenzidine dihydrochloride. In a study involving *Drosophila melanogaster* conducted by Yoon et al. (1985), no sex-linked mutagenic effects were observed after exposure to 3,3′-dimethoxybenzidine. In an NIH 3T3 transfection assay, Reynolds et al. (1990) analyzed several benign and malignant tumors from control and treated rats from the carcinogenic study conducted by NTP (1990). Reynolds et al. (1990) reported a high percentage of dimethoxybenzidine-induced tumors containing activated H-ras and N-ras oncogenes, compared to low percentages of spontaneously occurring tumors in control rats, indicating that the increased tumor incidence of treated rats was directly related to the mutagenicity of the chemical.

Table 3. Other Studies

Test	Materials & Methods	Results	Conclusions	References
Genotoxicity	*Salmonella* strains TA98, TA100, TA1535 and TA1538 were exposed to 4, 20, 100, 500, or 2500 µg/plate 3,3′-dimethoxybenzidine in an Ames assay. Cultures were evaluated for mutagenic activity with metabolic activation by Aroclor 1254-induced rat liver S9.	Authors reported positive results for all strains.	These results indicate that 3,3′-dimethoxybenzidine is mutagenic in *Salmonella* strains TA98, TA100, TA1535, and TA1538 with metabolic activation.	Anderson and Styles (1978)
Genotoxicity	*Salmonella typhimurium* strains TA98 and TA100 were exposed to 3, 10, 30, 100, 300, or 1000 µg/plate 3,3′-dimethoxybenzidine in an Ames assay. Resulting cultures were examined for mutagenicity with and without Aroclor 1254-induced rat liver S9.	Authors reported positive results in both strains with metabolic activation.	These results suggest mutagenicity of 3,3′-dimethoxybenzidine in strains TA98 and TA100 with metabolic activation.	Chung et al. (2000)
Genotoxicity	*Salmonella* strains TA98, TA100, TA 1535, and TA1537 were exposed to 0−1000 µg/plate (concentrations vary over 3 laboratory test locations) 3,3′-dimethoxybenzidine in an Ames assay as part of an evaluation of 250 chemicals. Cultures were evaluated for mutagenic activity without metabolic activation, and with Aroclor 1254-induced rat and hamster liver S9 activation.	Authors reported positive results at all three testing facilities but did not report findings regarding specific strains, dose levels, state of metabolic activation, nor did they present statistical analysis.	These results suggest mutagenicity of 3,3′-dimethoxybenzidine to various strains of *Salmonella typhimurium*.	Haworth et al. (1983)
Genotoxicity	*Salmonella* strains SV50 and TA98 were exposed to 0.03, 0.10, 0.30, or 1 mg/plate 3,3′-dimethoxybenzidine in an Ames assay and a complete azo dye protocol experiment. Cultures were evaluated for mutagenicity without metabolic activation, and with activation by hamster liver S9 and Aroclor 1254-induced rat liver S9 fraction.	Authors reported a positive result in strain TA98 with rat liver S9 fraction and negative results with and without metabolic activation in SV50 for the Ames assay. Statistically significant positive results were reported in strain SV50 for the azo dye protocol experiment, but the number of revertants/plate did not double.	These results suggest mutagenicity of 3,3′-dimethoxybenzidine in strain TA98 with metabolic activation.	Krishna et al. (1986)

Table 3. Other Studies

Test	Materials & Methods	Results	Conclusions	References
Genotoxicity	*Salmonella* strains TA98 and TA1538 were exposed to 20, 100, 500, or 2500 µg/plate 3,3′-dimethoxybenzidine in an Ames assay examining mutagenicity of azo dyes. Cultures were evaluated for mutagenicity with sodium phenobarbitone-induced rat liver S9.	Authors reported statistically significant positive results in both strains with metabolic activation by rat liver S9.	These results suggest mutagenicity of 3,3′-dimethoxybenzidine in strains TA98 and TA1538 with metabolic activation.	Martin and Kennelly (1981)
Genotoxicity	*Salmonella* strain TA98 was exposed to 1.0 µmol/plate while strain TA100 was exposed to 0.5 µmol/plate 3,3′-dimethoxybenzidine. Cultures were evaluated for mutagenicity with and without Aroclor 1254-induced male Sprague-Dawley rat liver S9 fraction.	Authors reported negative results in both strains without metabolic activation, and positive results in both strains with activation.	These results indicate that 3,3′-dimethoxybenzidine is mutagenic with exogenous metabolic activation in strains TA98 and TA100.	Messerly et al. (1987)
Genotoxicity	*Salmonella* strains TA98 and TA100 were exposed to dihydrochloride salt of 3,3′-dimethoxybenzidine in an Ames assay examining the mutagenic effects of benzidine-based dyes.	Results were positive in both strains when dyes were first reduced with sodium dithionate.	3,3′-dimethoxybenzidine dihydrochloride is mutagenic in strains TA98 and TA100 when reduced to release benzidine.	Gregory et al. (1981), as cited by NTP (1990)
Genotoxicity	*Salmonella* strain TA98 was exposed to 0−1000 nmol/plate (exact concentrations unreported) 3,3′-dimethoxybenzidine and an unreported number of hydrazone dyes in which it is incorporated. Cultures were evaluated for mutagenic activity with metabolic activation by hamster liver S9.	Authors reported no significant mutagenic effects of 3,3′-dimethoxybenzidine or its corresponding hydrazone dyes.	Dyes of the hydrazone class containing 3,3′-dimethoxybenzidine are not considered mutagenic because of their resistance to enzymatic reduction.	De France et al. (1986)

Table 3. Other Studies

Test	Materials & Methods	Results	Conclusions	References
Genotoxicity	*Salmonella* strain TA98 was exposed to 0, 0.1, 0.3, or 1.0 µmol/plate 3,3′-dimethoxybenzidine dihydrochloride and 3 monoazo dyes incorporating it in an Ames assay modified to include preincubation with flavin mononucleotide (FMN). Cultures were evaluated for mutagenicity with hamster liver S9 fraction.	Authors reported positive results for 2 of the 3 *o*-dianisidine dyes containing 3,3′-dimethoxybenzidine in the presence of FMN.	Any soluble compound that can be reduced to release free 3,3′-dimethoxybenzidine considered mutagenic under the conditions of this assay.	Prival et al. (1984)
Genotoxicity	*Salmonella* strain TA102 was exposed to 5, 10, 50, or 100 µg/plate 3,3′-dimethoxybenzidine in a preincubation Ames assay and evaluated for mutagenicity with and without activation by Aroclor 1254-induced rat liver S9 fraction.	Authors reported strongly positive results with rat liver S9, and negative results without rat liver S9.	These results suggest mutagenicity of 3,3′-dimethoxybenzidine in *Salmonella* strain TA102 with metabolic activation.	Makena and Chung (2007)
Genotoxicity	*Salmonella* strains C3076, D3052, G46, TA98, TA100, TA1535, TA1537, and TA1538, and *E. coli* strains WP1 and WP2 were exposed to unreported concentrations of 3,3′-dimethoxybenzidine in an Ames assay. Cultures were evaluated for mutagenic activity with and without Aroclor 1254-induced liver S9 fraction. Authors also conducted an autoradiographic assay for unscheduled DNA synthesis (UDS).	For the Ames assay, authors reported positive results in strains TA100, TA98, and TA1538 with metabolic activation, and negative results in these strains without activation and in all other strains. Authors also reported positive results for the hepatocyte UDS test.	These results suggest mutagenicity of 3,3′-dimethoxybenzidine in strains TA100, TA98, and TA1538 with metabolic activation, which can also be detected by a test for UDS.	Probst et al. (1981)
Genotoxicity	*E. coli* strains W3110 and P3478 were exposed to 500 µg/plate 3,3′-dimethoxybenzidine using a rapid screening technique. Cultures were evaluated for mutagenic effects without metabolic activation.	Test results were negative for both strains.	Insolubility of the test substance could have prevented it from diffusing through the agar and reaching the indicator organism, resulting in inconclusive results.	Fluck et al. (1976)

Table 3. Other Studies

Test	Materials & Methods	Results	Conclusions	References
Genotoxicity	Male Sprague-Dawley rat hepatocytes were exposed to 0, 56, 100, or 180 µmol/plat 3,3′-dimethoxybenzidine for 20 hours in a DNA damage/alkaline elution assay.	Authors reported a dose-dependent increase in frequency of DNA single-strand breaks and/or alkali-labile sites (DNA elution rate).	Exposure of rat hepatocytes to 3,3′-dimethoxybenzidine causes DNA fragmentation in rat hepatocytes in a dose-dependent manner.	Martelli et al. (2000)
Genotoxicity	Human hepatocytes taken from 2 donors (1 male, 1 female) were exposed to 0, 56 (male hepatocytes only), 100, or 180 µmol/plate 3,3′-dimethoxybenzidine for 20 hours in a DNA damage assay.	Authors reported a dose-dependent increase in DNA elution rate in primary cultures.	Exposure of human hepatocytes to 3,3′-dimethoxybenzidine causes DNA fragmentation in a dose-dependent manner.	Martelli et al. (2000)
Genotoxicity	Human urinary bladder cells taken from 5 donors (4 male, 1 female) were exposed to 0, 100 (3 of 5 donors), or 180 µmol/plate 3,3′-dimethoxybenzidine for 20 hours and evaluated in a Comet assay.	Authors reported increased nuclear DNA damage in all bladder cells exposed to 3,3′-dimethoxybenzidine.	3,3′-dimethoxybenzidine causes increased damage to nuclear DNA in human bladder cells.	Martelli et al. (2000)
Genotoxicity	Male Sprague-Dawley rat hepatocytes were exposed to 56, 100, or 180 µmol/plate 3,3′-dimethoxybenzidine for 48 hours in a micronucleus assay.	Authors reported increased frequencies of micronucleated cells at the highest dose level in 1 of 3 experiments. Pooled data showed a dose-dependent increase in micronucleated cell frequency with significance reported at 100 and 180 µmol.	Exposure of rat hepatocytes to 3,3′-dimethoxybenzidine may cause increased frequencies of micronucleated cells.	Martelli et al. (2000)
Genotoxicity	Male Sprague-Dawley rats were administered 960 mg/kg of 3,3′-dimethoxybenzidine in a single treatment by gastric intubation. Distilled water (0.01 mg/g body weight) was used as the vehicle with 0.5% carboxymethylcellulose as a suspending agent. Rats were sacrificed 4 hours after exposure. Liver and urinary bladder cells were removed and evaluated for DNA damage.	Authors reported no clinical signs of toxicity in any of the rats. DNA damage was observed in urinary bladder cells collected from all animals in the form of migration of the DNA from the urinary bladder mucosa. Authors reported no DNA damage in liver cells.	Exposure of rats to 3,3′-dimethoxybenzidine results in increased DNA damage in the urinary bladder mucosa but not in the liver.	Martelli et al. (2000)

Table 3. Other Studies

Test	Materials & Methods	Results	Conclusions	References
Genotoxicity	Chinese hamster ovary cells were exposed to various unreported doses of 3,3′-dimethoxybenzidine dihydrochloride and evaluated for sister chromatid exchanges, and chromosomal aberrations.	In 1985, authors reported positive evidence of sister chromatid exchanges with and without metabolic activation, and negative results for chromosomal aberrations with and without activation. A reanalysis of the chromosomal aberration data in 1987 revealed a weakly positive result without metabolic activation, and a positive result with activation.	These results indicate induction of chromosomal aberrations and sister chromatid exchanges by 3,3′-dimethoxybenzidine in Chinese hamster ovary cells both with and without metabolic activation.	Galloway et al. (1985), Galloway et al. (1987)
Genotoxicity	Adult male *Drosophila melanogaster* were exposed to 3,3′-dimethoxybenzidine by feeding (100 ppm) or injection (200 ppm) and evaluated for the induction of sex-linked recessive lethal.	Negative for sex-linked mutations induced by injection or feeding.	Results indicate that 3,3′-dimethoxybenzidine does not cause sex-linked mutations in adult male *Drosophila melanogaster.*	Yoon et al. (1985)
Genotoxicity	Benign and malignant tumors were obtained from control and treated rats in the NTP (1990) study and evaluated for the presence of activated oncogenes in a NIH 3T3 DNA transfection assay.	Tumors of rats treated with 3,3′-dimethoxybenzidine contained a higher percentage of activated H-ras and N-ras oncogenes than the single spontaneous tumor from a control rat.	Results suggest that 3,3′-dimethoxybenzidine and other benzidine derived compounds cause point mutations in the ras gene family in rats.	Reynolds et al. (1990)

DERIVATION OF PROVISIONAL VALUES

Table 4 presents a summary of noncancer reference values. Table 5 presents a summary of cancer values. The cancer toxicity value was converted to human equivalent dose (HED) units, and the conversion process is presented in the section on derivation of provisional cancer potency values. IRIS data are indicated in the table if available.

Table 4. Summary of Reference Values for 3,3′-Dimethoxybenzidine (CASRN 119-90-4)

Toxicity Type (Units)	Species/ Sex	Critical Effect	*p*-Reference Value	POD Method	POD	UF_c	Principal Study
Subchronic p-RfD** (mg/kg-day)	Rat/M	Increased relative liver weight	1×10^{-3}	LOAEL	13	10,000	NTP (1990)
Chronic p-RfD (mg/kg-day)	None						
Subchronic p-RfC (mg/m^3)	None						
Chronic p-RfC (mg/m^3)	None						

**Oral Screening value provided in Appendix A.

Table 5. Summary of Cancer Values for 3,3′-Dimethoxybenzidine (CASRN 119-90-4)

Toxicity Type	Species/ Sex	Tumor Type	Cancer Value	Principal Study
p-OSF	Rat/M	Combined tumor types	$1.6\ (mg/kg\text{-}day)^{-1}$	NTP (1990)
p-IUR	None	None	None	None

FEASIBILITY OF DERIVING PROVISIONAL SUBCHRONIC AND CHRONIC ORAL REFERENCE DOSES

It is inappropriate to derive a provisional subchronic or chronic p-RfD for 3,3′-dimethoxybenzidine. No quantitative human studies examining the effects of subchronic or chronic oral exposure to 3,3′-dimethoxybenzidine alone have been identified. The available human studies involve occupational exposure to a mixture of compounds including 3,3′-dimethoxybenzidine. In animals, useful dose-response data for nonneoplastic effects following subchronic or chronic exposure are limited to the NTP (1990) study of 3,3′-dimethoxybenzidine dihydrochloride in rats following 13 weeks or 21 months of exposure, respectively. After 13-weeks of 3,3′-dimethoxybenzidine exposure, male and female rats exhibited significant changes in relative organ weights and hematological/serum chemistry parameters, as well as chronic nephropathy and accumulation of pigment in follicular cells of the thyroid. However, as a function of dose, changes in relative organ weight were the most

sensitive effects observed. Compared to control, significantly increased relative liver weight and decreased relative thyroid weight were observed in male rats at the lowest 3,3′-dimethoxybenzidine exposure dose. A subchronic p-RfD cannot be confidently derived here due to the high level of uncertainty associated with the lack of reliable study data; however a "screening level" value for subchronic oral exposure is provided in Appendix A.

As previously discussed, the NTP (1990) chronic study was terminated at 21 months because of significantly decreased survival at all doses tested, primarily due to extensive neoplastic formation. Treatment-related increases in tumors were seen in the liver, small intestines, large intestines, Zymbal gland, preputial gland, oral cavity, and skin. For nonneoplastic lesions, dose-dependent increases were seen in the liver, spleen, heart, and lungs. Because of the exceedingly high rates of mortality and neoplastic effects at all doses, a NOAEL for nonneoplastic effects is not identified suggesting that a threshold for nonneoplastic effects may occur at a dose much lower than those tested. As such, a chronic p-RfD cannot be derived. In addition, a "screening level" value for chronic oral exposure cannot be supported.

FEASIBILITY OF DERIVING PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION REFERENCE CONCENTRATIONS

Due to a complete lack of exposure-response data for the inhalation route in any species, it is inappropriate to derive a subchronic or chronic p-RfC for 3,3′-dimethoxybenzidine. Derivation of "screening values" is also precluded.

CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR

Table 6 identifies the cancer WOE descriptor for 3,3′-dimethoxybenzidine.

Table 6. Cancer WOE Descriptor for 3,3′-Dimethoxybenzidine			
Possible WOE Descriptor	**Designation**	**Route of Entry (Oral, Inhalation, or Both)**	**Comments**
"Carcinogenic to Humans"	Not selected	N/A	No human cancer studies involving exposure to 3,3′-dimethoxybenzidine alone are available.
"Likely to Be Carcinogenic to Humans"	**Selected**	**Oral administration by drinking water**	**Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the available evidence of carcinogenicity in rats orally exposed to 3,3′-dimethoxybenzidine supports the *"Likely to Be Carcinogenic to Humans"* descriptor. There are limited available human data; what human data are available comes from studies or reports of human exposure to mixtures that included 3,3′-dimethoxybenzidine. NTP (1990) reported treatment-related increases in a number of tumor types located in multiple tissues including the Zymbal gland, preputial gland, clitoral gland, skin basal cells, skin squamous cells, small intestines, large intestines, oral cavity, liver, and mammary gland in rats exposed to 3,3′-dimethoxybenzidine orally by drinking water for 21 months (see Table B.12). Tumors were also reported at the 9-month interim sacrifice. The observation of tumors in multiple animal tissues following a short latency period, and significant evidence of mutagenicity and clastogenicity in several experimental cell systems, including human, is suggestive of a mutagenic carcinogen. In addition, 3,3′-dimethoxybenzidine has been classified as "*Reasonably Accepted to be a Human Carcinogen*" by the 1*2th Report on Carcinogens* (NTP, 2011). Studies evaluating the carcinogenic potential of inhaled 3.3′-dimethoxybenzidine in animals were not identified. Occupational studies indicate increased incidences of bladder cancer in workers exposed to 3,3′-dimethoxybenzidine (Frumin et al., 1990; Hamasaki et al., 1996; Ouellet-Hellstrom and Rench, 1996). However, these workers were employed in textile dyeing and printing facilities and were exposed to other compounds as well. No studies involving exposure to 3,3′-dimethoxybenzidine alone were identified.**
"Suggestive Evidence of Carcinogenic Potential"	Not selected	N/A	The evidence from human and animal data is more than suggestive of carcinogenicity, which raises a concern for carcinogenic effects but is judged sufficient for a stronger conclusion.
"Inadequate Information to Assess Carcinogenic Potential"	Not selected	N/A	Available adequate information exists to assess carcinogenic potential.
"Not Likely to Be Carcinogenic to Humans"	Not selected	N/A	No strong evidence of noncarcinogenicity in humans or animals is available.

MODE-OF-ACTION (MOA) DISCUSSION

The *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) define mode of action (MOA) as "a sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation". Examples of possible modes of carcinogenic action for any given chemical include "mutagenicity, mitogenensis, inhibition of cell death, cytotoxicity with reparative cell proliferation, and immunologic suppression".

Human studies involving exposure to 3,3′-dimethoxybenzidine alone are not available. However, occupational studies involving the exposure of 3,3′-dimethoxybenzidine simultaneously with benzidine or benzidine congeners indicate that 3,3′-dimethoxybenzidine may cause tumors in the bladder. In animals, the available evidence suggests that tumors observed following oral exposure to 3,3′-dimethoxybenzidine arise from genetic mechanisms (e.g., oncogene activation; see "Key Events" section below). In rats, tumors have been reported in a number of tissues including the Zymbal gland, preputial gland, clitoral gland, skin, small intestines, large intestines, oral cavity, liver, and mammary gland. Based on the weight of the evidence, it is determined that 3,3′-dimethoxybenzidine is carcinogenic by a mutagenic MOA.

Mutagenic Mode of Action (MOA)

Key Events

For 3,3′-dimethoxybenzidine, the proposed MOA involves the occurrence of a number of key events. These include (1) metabolic activation of parent compound to reactive intermediates that bind covalently to DNA, (2) genetic alteration of oncogenes including Ras, and (3) tumor formation following proliferation of initiated cells. Reynolds et al. (1990) provided data to support this MOA. The authors evaluated a wide range of neoplasms formed in the rat following exposure to 3,3′-dimethoxybenzidine and found codon-specific mutations in the H-ras and N-ras oncogenes with the large majority found in H-ras. Additionally, an evaluation of malignant and benign tumors from rats treated with 3,3′-dimethoxybenzidine showed that 62% contained activated H-ras or N-ras genes compared with detection of the activated oncogenes in 1/38 spontaneous tumors from control rats (Reynolds et al., 1990). The increased incidence of activated Ras oncogenes coupled with mutational specificity at codons 13 and 61 of H-ras suggest that the increased incidence of both benign and malignant tumors observed in rats exposed to 3,3′-dimethoxybenzidine is related to its mutagenic effects.

Support for a mutagenic MOA is also provided by in vitro tests as described in Table 3. In a number of *Salmonella* strains, 3,3′-dimethoxybenzidine was shown to cause mutagenicity following metabolic activation. Positive results for clastogenicity/mutagenicity (sister chromatid exchanges, micronucleated cells, DNA damage, and chromosomal aberrations) were seen in mammalian cells both with and without metabolic activation. In vivo data in rats indicated an increase in DNA damage to urinary bladder cells.

Evidence also exists for a mutagenic MOA for the structurally similar compound benzidine and a number of its metabolites (Morgan et al., 1994). Morgan et al. (1994) also indicated that a number of metabolites of benzidine are more mutagenic than the parent compound. Mono- and diacetylated metabolites were indicated to be about 10 times as

mutagenic as benzidine while *N*-hydroxy-*N*,*N*′-diacetylbenzidine glucoronide was about 100 times as mutagenic as benzidine, following incubation with β-glucoronidase to release the hydroxylated diacetylamine.

Strength, Consistency, Specificity of Association

Reynolds et al. (1990) evaluated a large number of tumor types from animals treated with 3,3′-dimethoxybenzidine (NTP, 1990) or its derivative dye, C.I. Direct Blue 15 (NTP, 1992), and found that the majority contained codon-specific mutations in the ras oncogene. The role of the ras oncogene in the carcinogenic effects of 3,3′-dimethoxybenzidine is supported by the high incidence of the codon-specific mutations (19/21 tumors with an activated Ras oncogene) and the high incidence of ras gene activation (21/34) in tumors from treated animals as compared to the low incidence of oncogene activation in spontaneous tumors obtained from control animals (1/38). Reynolds et al. (1990) reported similar findings in tumors obtained from rats treated with 3,3′-dimethylbenzidine. In tumors from treated animals, Ras gene activation was seen in 13/16 tumors and codon-specific mutations in 12/13 tumors with activated Ras oncogene.

Dose-Response Concordance

Data to evaluate the dose-response concordance between mutagenesis and tumor formation following exposure to 3,3′-dimethoxybenzidine are unavailable. No data indicating the dose distribution of mutations or activated oncogenes in the tumors evaluated by Reynolds et al. (1990) were provided.

Temporal Relationships

For 3,3′-dimethoxybenzidine, the temporal relationship between mutagenesis and tumor formation cannot be assessed at this time. Reynolds et al. (1990) evaluated tumors collected from rats exposed to 3,3′-dimethoxybenzidine for types of mutations. However, data on the incidence or types of mutations formed prior to the generation of neoplasms in these tissues are not available.

Biological Plausibility and Coherence

Reynolds et al. (1990) provides data supporting the biological plausibility of a mutagenic MOA for 3,3′-dimethoxybenzidine. In tumors from rats treated with 3,3′-dimethoxybenzidine, point mutations were detected at codons 12, 13, and 61 and shown to lead to activation of Ras oncogenes. Similar findings were seen in the structurally similar compound 3,3′-dimethylbenzidine (Reynolds et al., 1990). Combined with a lack of oncogene activation in tumors taken from control animals, and the large body of in vitro evidence indicating clastogenicity/mutagenicity, these data suggest a mutagenic MOA for 3,3′-dimethoxybenzidine.

An evaluation of the results from chronic studies in rats exposed to 3,3′-dimethoxybenzidine provides additional evidence of an association between mutagenesis and tumor formation (NTP, 1990). After only 9 months of exposure, carcinomas of the preputial, clitoral, and Zymbal glands were seen in treated rats but not in controls, although the statistical significance of these neoplasias is not clear. Following 21 months of exposure to 3,3′-dimethoxybenzidine, a number of rare tumors were reported in rats including those found in the intestinal tract, Zymbal gland, skin, and oral cavity (NTP, 1990). These data are supportive of a mutagenic MOA, because most mutagenic compounds are associated with multiple, unusual tumor sites and a short latency period for tumorigenesis (NTP, 1990).

Early-Life Susceptibility

An increased early-life susceptibility is assumed in individuals exposed to carcinogens with a mutagenic MOA (U.S. EPA, 2005b). For 3,3′-dimethoxybenzidine, sufficient data are not available to develop separate risk estimates for childhood exposure because no information evaluating tumor formation during early life after exposure to 3,3′-dimethoxybenzidine has been reported.

Conclusions

The weight of evidence (WOE) for 3,3′-dimethoxybenzidine tumorigenicity supports a mutagenic MOA. Data from a battery of in vitro studies in both bacteria and eukaryotic cells show that exposure to 3,3′-dimethoxybenzidine causes clastogenic/mutagenic effects (see Table 3). Formation of codon-specific mutations in Ras oncogenes in tumors taken from rats exposed to 3,3′-dimethoxybenzidine was also seen while only one tumor from control rats contained an activated Ras oncogene (Reynolds et al., 1990). Lastly, the reporting of rare tumors at multiple sites, along with the short latency period before tumor formation provide further support for a mutagenic MOA for 3,3′-dimethoxybenzidine (NTP, 1990). Because a mutagenic MOA for the carcinogenic effects of 3,3′-dimethoxybenzidine is proposed, a linear approach is used to extrapolate from the POD to determine the p-OSF (U.S. EPA, 2005b). No data are available to develop estimates of risk from early-life exposure to 3,3′-dimethoxybenzidine.

DERIVATION OF PROVISIONAL CANCER POTENCY VALUES

Derivation of Provisional Oral Slope Factor (p-OSF)

The 21-month study by NTP (1990) is selected as the principal study. The cancer endpoint is the incidence of combined primary tumor types in male rats; combined tumor data in female rats were also evaluated. This study is generally well conducted, and the data from this study support a quantitative cancer dose-response assessment. This study is a peer-reviewed technical report from the NTP, has been performed according to GLP principles, and otherwise meets the standards of study design and performance with numbers of animals, examination of potential toxicity endpoints, and presentation of information. Details are provided in the "Review of Potentially Relevant Data" section. The NTP (1990) chronic study represents the only study in the database with useful data for deriving the p-OSF. As previously discussed in the "Review of Potentially Relevant Data," other chronic studies for 3,3′-dimethoxybenzidine suffer from a number of deficiencies including small sample size, poor reporting of data, and low rates of survival.

NTP (1990) reported treatment-related tumor types in a number of tissues in both male and female rats after exposure to 3,3′-dimethoxybenzidine in drinking water for 21 months. Tissues with observed tumors include the Zymbal gland, preputial gland, clitoral gland, skin basal cells, skin squamous cells, small intestines, large intestines, oral cavity, liver, mesothelium, and mammary gland. Cancer-dose-response modeling was performed for all of these tumor types; it should be noted that some tumor types were not included in the dose-response modeling analyses due to an irregular dose response (e.g., incidence decreases with increasing dose such as mammary fibroadenomas in female rats). Incidence data used for dose-response modeling were based on effective rates (the number of animals alive during the first occurrence of the tumor being modeled) as reported by NTP (1990). The effective rates for combined tumor types were extracted from the individual animal data provided by NTP (1990).

The significant increases in tumor incidence seen in multiple tissues are characteristic of a mutagenic MOA and indicate that the overall risk of tumor formation is spread throughout the body. Because all of these tumors contribute to the overall cancer risk, an underestimation of risk of cancer development would result from a dose-response assessment based on any one type. Therefore, the overall cancer risk for 3,3′-dimethoxybenzidine based on combined tumor incidence was evaluated in both male and female rats. For carcinogens that produce tumors at multiple sites, combining incidence is an appropriate way to estimate cancer risk (U.S. EPA, 2005a).

The following dosimetric adjustments were made for oral drinking water treatment in adjusting doses for cancer analysis (p-OSF). The low-dose conversion is shown below for convenience:

$\mathbf{(DOSE_{HED})_{NTP,\ 1990}}$	=	$\mathbf{(Dose)_{NTP,\ 1990} \times}$ **body-weight adjustment**
Body-weight adjustment	=	$(BW_A \div BW_H)^{1/4}$
where,		
BW_H	=	70 kg (human reference body) (U.S. EPA, 1997)
BW_A	=	0.363 kg (average body weight for male F344 rats) (Morgan et al., 1989)
Body-weight adjustment	=	$(0.363 \div 70)^{1/4} = 0.26835$
$(DOSE_{HED})_{NTP,\ 1990}$	=	$(Dose)_n \times 0.26835$
	=	6 mg/kg-day × 0.26835
$\mathbf{(DOSE_{HED})_{NTP,\ 1990}}$	=	**1.61 mg/kg-day**

Table 7 presents BMD input data for incidence of combined primary tumors in male rats exposed to 3,3′-dimethoxybenzidine by drinking water for 21 months (see Table B.12 for incidences of individual tumor types). Combined tumor incidence in female rats was also evaluated by BMD analysis; however, the male rat data provided a slightly lower BMD_{10HED} and $BMDL_{10HED}$.

Table 7. BMD Input for Combined Primary Tumors in Male F344/N Rats Exposed to 3,3′-Dimethoxybenzidine Dihydrochloride for 21 Months[a]

$(Dose)_n$ (mg/kg-day)	$(DOSE_{HED})_n$ (mg/kg-day)	Number of Rats	Response (Combined Tumor Incidence)
0	0	59	22[b]
6	1.61	45	41
12	3.22	75	70
21	5.64	60	60

[a]NTP (1990).
[b]16/59 control male rats had preputial adenoma or carcinoma; the incidence of other tumor types did not exceed 2/59.

Table 8 presents the BMD modeling results. Adequate model fit is obtained for incidence of combined primary tumors using the multistage-cancer model, and the BMD modeling results yields a BMD_{10HED} of 0.122 mg/kg-day and a $BMDL_{10HED}$ of 0.095 mg/kg-day. The OSF calculated from adult exposure is derived from the $BMDL_{10HED,}$ the 95% lower bound on the human equivalent exposure associated with a 10% extra cancer risk (represented by the 0.1 BMR in the calculation of a p-OSF below). It is representative of an upper bound risk estimate for continuous lifetime exposure. As discussed in the MOA section, 3,3′-dimethoxybenzidine is a mutagenic carcinogen. However, because no data on early-life susceptibility are available, the $BMDL_{10HED}$ is representative of an upper bound risk estimate for continuous lifetime exposure without consideration of increased susceptibility during childhood. Because a linear, mutagenic MOA has been determined for neoplasms caused by 3,3′-dimethoxybenzidine, a linear extrapolation to low dose was calculated as the ratio $0.1/BMDL_{10HED}$, as shown below.

Table 8. Model Predictions for Combined Malignant Tumors in the Male Rat Exposed to 3,3′-Dimethoxybenzidine Dihydrochloride in Drinking Water for 21 Months[a]

Model	Goodness of Fit *p*-Value[b]	AIC[c] for Fitted Model	BMD_{10HED}[c] (mg/kg-day)	$BMDL_{10HED}$[c] (mg/kg-day)	Conclusions
Multistage Cancer	0.18	149.289	0.122	0.095	Selected as lowest BMDL for POD

[a]NTP (1990).
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]AIC = Akaike Information Criteria; BMD = benchmark dose; BMDL lower confidence limit (95%) on the benchmark dose.

$$\textbf{p-OSF}_{\textbf{(unadjusted)}} = 0.1 \div BMDL_{10HED}$$
$$= 0.1 \div 0.095 \text{ mg/kg-day}$$
$$= \textbf{1.1 (mg/kg-day)}^{-1}$$

An adjustment was performed for a shorter-than-lifetime observation period (U.S. EPA, 1980). The NTP (1990) rat bioassay was terminated after 21 months (compared to the experimental rat life span of 24 months), due to increased mortality from the formation of tumors. Therefore, it is unclear if a sufficient period of time had elapsed to fully evaluate the carcinogenicity of 3,3′-dimethoxybenzidine at the lowest dose. Because of the truncated experimental protocol seen in the NTP (1990) study, it is unknown how a full 2-year exposure to 3,3′-dimethoxybenzidine may have influenced the tumor incidence in low-dose rats. As a result, an adjustment factor of $(L/Le)^3$ is applied to the unadjusted p-OSF, where L = the lifetime of the animal (in this case, the experimental lifetime) and Le = the duration of experimental dosing. Using this adjustment, **a p-OSF of 1.6 (mg/kg-day)$^{-1}$** is derived as follows:

$$\begin{aligned} \textbf{p-OSF} &= \text{p-OSF}_{(\text{unadjusted})} \times (\text{L} \div \text{Le})^3 \\ &= 1.1\ (\text{mg/kg-day})^{-1} \times (24\ \text{months} \div 21\ \text{months})^3 \\ &= \textbf{1.6 (mg/kg-day)}^{\mathbf{-1}} \end{aligned}$$

The p-OSF for 3,3′-dimethoxybenzidine should not be used with exposures exceeding the point of departure ($BMDL_{10\ HED}$ = 0.095 mg/kg-day), because above this level the fitted dose-response model better characterizes what is known about the carcinogenicity of 3,3′-dimethoxybenzidine.

The human equivalent dose was also calculated for the central estimate (BMD_{10}) associated with the selected point of departure (combined tumors in male rats); A $BMD_{10\ HED}$ of 0.122 mg/kg-day was calculated. The *unadjusted* slope of the linear extrapolation from the central estimate (0.122 mg/kg-day) is 0.8 $(\text{mg/kg-day})^{-1}$ and the adjusted slope is 1.2 $(\text{mg/kg-day})^{-1}$.

Based on a WOE evaluation, 3,3′-dimethoxybenzidine is carcinogenic by a mutagenic MOA. Carcinogens with a mutagenic MOA are assumed to be associated with an increased early-life susceptibility (U.S. EPA, 2005b). However, no sufficient data are available to develop separate risk estimates for childhood exposure to 3,3′-dimethoxybenzidine. Therefore, the p-OSF of 1.6 $(\text{mg/kg-day})^{-1}$ calculated from adult exposure data is not reflective of the presumed early-life susceptibility for this compound, and age-dependent adjustment factors (ADAFs) should be applied to this parameter when assessing cancer risks. Example evaluations of cancer risks based on age at exposure and ADAFs for three specific age groups have been established as indicated in Section 6 of the *Supplemental guidance for assessing susceptibility from early-life exposure to carcinogens* (U.S. EPA, 2005b). Currently, the ADAFs and their age groups are 10 for <2 years, 3 for 2 to <16 years, and 1 for ≥16. When estimating cancer risk from early life (<16 years of age) exposure to 3,3′-dimethoxybenzidine, the 10-fold and 3-fold adjustments in slope factor should be combined with age-specific exposure estimates. These ADAFs and their age groups may be revised over time, and the most current guidance on assessing susceptibility from childhood exposure to carcinogens can be found at www.epa.gov/cancerguidelines/. When estimating risk for exposure to 3,3′-dimethoxybenzidine, it is recommended that age-specific values for both exposure and cancer potency be used and that age-specific values for cancer potency are determined using the appropriate ADAFs. For each age group, a cancer risk is derived with these values summed across age groups to obtain the total risk for the exposure period of interest.

Derivation of Provisional Inhalation Unit Risk (p-IUR)

No human or animal studies examining the carcinogenicity of 3,3′-dimethoxybenzidine following inhalation exposure have been identified. Therefore, derivation of an IUR is precluded.

APPENDIX A. PROVISIONAL SCREENING VALUES

DERIVATION OF A SCREENING SUBCHRONIC p-RFD VALUE

For reasons noted in the main PPRTV document, it is inappropriate to derive provisional toxicity values for 3,3′-dimethoxybenzidine. However, information is available for this chemical which, although insufficient to support derivation of a provisional toxicity value, under current guidelines, may be of limited use to risk assessors. In such cases, the Superfund Health Risk Technical Support Center summarizes available information in an Appendix and develops a "screening value." Appendices receive the same level of internal and external scientific peer review as the PPRTV documents to ensure their appropriateness within the limitations detailed in the document. Users of screening toxicity values in an appendix to a PPRTV assessment should understand that there is considerably more uncertainty associated with the derivation of an appendix screening toxicity value than for a value presented in the body of the assessment. Questions or concerns about the appropriate use of screening values should be directed to the Superfund Health Risk Technical Support Center.

The 13-week rat study by NTP (1990) is selected as the principal study for the derivation of a screening subchronic p-RfD. This study is a range-finding study in a peer-reviewed report conducted for the NTP, National Institutes of Health and has been performed according to GLP principles, and otherwise meets the standards of study design and performance with regards to the numbers of animals and the examination of potential toxicity. This study and its results were also published as a peer-reviewed article (Morgan et al., 1989)**.** Details are provided in the "Review of Potentially Relevant Data" section and are summarized in Table A.1 below. After 13 weeks of 3,3′-dimethoxybenzidine exposure, male and female rats experienced significant changes in relative organ weights and hematological/serum chemistry parameters, as well as chronic nephropathy and accumulation of pigment in follicular cells of the thyroid. However, the original study authors ascribed the observed hematological/serum chemistry changes in part to mechanical hemolysis during sample processing. Furthermore, while the study authors noted reduced T3 and T4 levels in male and female rats, the changes were not significant compared to controls, and thyrotropin (TSH, which is responsive to T3 and T4 levels) concentrations in these animals were not different from controls. Therefore, relative organ-weight changes, pigmentation of thyroid follicular cells, and chronic nephropathy were considered in the selection of a critical effect for subchronic exposure. While nephropathy and thyroid follicular cell pigmentation occurred primarily at higher 3,3′-dimethoxybenzidine exposure doses, significant organ-weight changes occurred at doses lower than all other effects considered (see Table A.1). Relative liver and kidney weights were statistically significantly increased as a function of increasing dose in both male and female rats; males appeared to be more sensitive than females to the liver-weight changes, while the kidney-weight changes were comparable in males and females. Thymus weights were statistically significantly reduced in male rats at all doses tested; however, compared to controls, thymus weights in females were unchanged even at the highest 3,3′-dimethoxybenzidine dose. As such, decreased relative thymus weights were not further considered. In male rats, the magnitude of change in relative liver weight at the lowest exposure dose was greater than in the kidney, and greater than that observed in liver or kidney of female rats at the lowest dose (see Tables B.3 and B.4). Therefore, increased relative liver weight in male rats is chosen as the critical effect for derivation of a subchronic oral screening value.

Table A.1. Summary of Relevant Subchronic Oral Systemic Toxicity Studies for 3,3′-Dimethoxybenzidine

References	#/Sex (M/F), Species	Exposure (mg/kg-day)	Frequency/ Duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Endpoint
NTP (1990)	10/10, rat	Male: 0, 13, 22, 39, 70, or 120 Female: 0, 24, 49, 60, 103, or 187	Ad libitum in drinking water for 13 weeks	Male: 22 Female: 24	Male: 39 Female: 49	Increased relative kidney weight
NTP (1990)	10/10, rat	Male: 0, 13, 22, 39, 70, or 120 Female: 0, 24, 49, 60, 103, or 187	Ad libitum in drinking water for 13 weeks	Male: none Female: 49	Male: 13 Female: 60	Increased relative liver weight
NTP (1990)	10/10, rat	Male: 0, 13, 22, 39, 70, or 120 Female: 0, 24, 49, 60, 103, or 187	Ad libitum in drinking water for 13 weeks	Male: none Female: 187	Male: 13 Female: none	Decreased relative thymus weight in males
NTP (1990); Morgan et al. (1989)	10/10, rat	Male: 0, 70, or 120 Female: 0, 103, or 187	Ad libitum in drinking water for 13 weeks	Male: none Female: none	Male: 70 Female: 103	Thyroid pigment in follicular cells
NTP (1990); Morgan et al. (1989)	10/10, rat	Male: 0, 70, or 120 Female: 0, 103, or 187	Ad libitum in drinking water for 13 weeks	Female: none	Female: 103	Kidney multifocal chronic nephropathy

BMD modeling was conducted with the EPA's BMD software (BMDS version 1.4.1). For continuous data such as the male rat relative liver weight (see Table B.3.), the data were modeled with all the continuous models available within the software. An adequate fit was judged based on the goodness-of-fit-*p*-value, scaled residue at the range of benchmark response (BMR), and visual inspection of the model fit. Among all the models attempted, none provided adequate fit to the liver-weight data; therefore, the LOAEL of 13 mg/kg-day is used as the POD for derivation of a subchronic oral screening value as follows:

$$\textbf{Screening Subchronic p-RfD} = \text{LOAEL} \div UF_C$$
$$= 13 \text{ mg/kg-day} \div 10{,}000$$
$$= \mathbf{1 \times 10^{-3} \text{ mg/kg-day}}$$

Table A.2 summarizes the uncertainty factors for the subchronic oral screening value for 3,3′-dimethoxybenzidine.

Table A.2. Uncertainty Factors for Screening Subchronic p-RfD for 3,3′-Dimethoxybenzidine (NTP, 1990)

UF	Value	Justification
UF_A	10	A UF_A of 10 is applied for interspecies extrapolation to account for potential toxicokinetic and toxicodynamic differences between rats and humans. There are no data to determine whether humans are more or less sensitive than rats to liver effects of 3,3′-dimethoxybenzidine.
UF_D	10	A UF_D of 10 is applied because there are no acceptable two-generation reproduction studies or developmental studies.
UF_H	10	A UF_H of 10 is applied for intraspecies differences to account for potentially susceptible individuals in the absence of information on the variability of response in humans.
UF_L	10	A UF_L of 10 is applied because the POD was developed using a LOAEL.
UF_S	1	A UF_S of 1 is applied because a subchronic study (NTP, 1990) was utilized as the principal study.
UF_C	10,000	

APPENDIX B. DATA TABLES

Table B.1. Selected Organ Weight to Body-Weight Ratios in F344N Rats Exposed to Oral 3,3′-Dimethoxybenzidine for 14 Days[a]

Parameter	Exposure Group (Daily Average Dose, mg/kg-day)					
Male rat	**0 ppm**	**200 ppm (18)**	**350 ppm (29)**	**750 ppm (57)**	**1500 ppm (101)**	**4500 ppm (127)**
Final body weight (g)	235 ± 1.2	241 ± 6.2 (+3)	235 ± 4.0 (0)	232 ± 7.2 (−1)	225 ± 9.9 (−4)	141 ± 4.2** (−40)
Relative organ weights (mg/g)						
Brain	7.3 ± 0.11	7.6 ± 0.22 (+4)	7.6 ± 0.08 (+4)	7.5 ± 0.12 (+3)	7.8 ± 0.27 (+7)	11.9 ± 0.43** (+63)
Lungs	4.0 ± 0.09	4.3 ± 0.16 (+8)	4.2 ± 0.10 (+5)	4.2 ± 0.09 (+5)	4.1 ± 0.09 (+3)	5.5 ± 0.29** (+38)
Heart	2.8 ± 0.08	3.1 ± 0.23 (+11)	2.9± 0.08 (+4)	3.0 ± 0.15 (+7)	3.0 ± 0.03 (+7)	3.3 ± 0.07** (+18)
Liver	43.4 ± 0.74	46.7 ± 0.41* (+8)	45.0 ± 0.70 (+4)	48.2 ± 0.45** (+11)	51.5 ± 0.41** (+19)	47.8 ± 3.60** (+10)
Kidney	3.5 ± 0.08	3.9 ± 0.27 (+11)	3.9 ± 0.15* (+11)	3.8 ± 0.10* (+09)	4.0 ± 0.09** (+14)	5.1 ± 0.25** (+46)
Right testis	5.3 ± 0.15	5.4 ± 0.24 (+02)	5.3 ± 0.08 (0)	5.6 ± 0.14 (+6)	5.6 ± 0.13 (+6)	7.7 ± 0.26** (+45)
Female rat	**0 ppm**	**200 ppm (19)**	**350 ppm (32)**	**750 ppm (61)**	**1500 ppm (141)**	**4500 ppm (214)**
Final body weight (g)	163 ± 4.2	163 ± 4.1 (0)	160 ± 1.9 (−2)	156 ± 2.9 (−4)	157 ± 4.2 (−4)	135 ± 3.3** (−17)
Relative organ weights (mg/g)						
Brain	10.2 ± 0.34	10.4 ± 0.26 (+2)	11.0 ± 0.40 (+8)	10.6 ± 0.21 (+4)	10.4 ± 0.26 (+2)	11.9 ± 0.49* (+17)
Liver	37.0 ± 0.95	39.2 ± 0.96 (+6)	37.9 ± 16 (+2)	39.3 ± 0.46 (+6)	41 ± 0.57** (+11)	45.6 ± 1.50** (+23)
Kidney	3.7 ± 0.15	3.7 ± 0.23 (0)	3.7 ± 0.08 (0)	3.9 ± 0.08 (+5)	4.1 ± 0.13* (+11)	4.6 ± 0.23** (+24)
Thymus	2.2 ± 0.10	2.3 ± 0.10 (+5)	2.4 ± 0.24 (+9)	2.1 ± 0.08 (−5)	2.0 ± 0.07 (−9)	1.7 ± 0.10** (−23)

[a]NTP (1990).
Notes: Means ± SE (% change relative to controls) ; $n = 5$ for each group; p values are vs. the controls by Dunn's or Shirley's test.
*$p \leq 0.05$; **$p \leq 0.01$.

Table B.2 Water Consumption of Male and Female F344N Rats Exposed to Oral 3,3′-Dimethoxybenzidine for 13 Weeks[a]

Parameter	Exposure Group (Daily Average Dose, mg/kg-day)					
Male rat	**0 ppm**	**170 ppm (13)**	**330 ppm (22)**	**630 ppm (39)**	**1250 ppm (70)**	**2500 ppm (120)**
Water consumption (ml/animal/day)						
Week 7[b]	21	21 (0)	17 (−19)	16 (−24)	13 (−38)	12 (−43)
Week 13[b]	21	22 (+5)	20 (−5)	17 (−19)	14 (−33)	12 (−43)
Female rat	**0 ppm**	**170 ppm (24)**	**330 ppm (49)**	**630 ppm (60)**	**1250 ppm (103)**	**2500 ppm (187)**
Water consumption (ml/animal/day)						
Week 7[b]	27	23 (−15)	29 (+7)	16 (−41)	13 (−52)	10 (−63)
Week 13[b]	25	21 (−16)	29 (+16)	14 (−44)	11 (−56)	10 (−60)

[a]NTP (1990).
[b]Value (% change relative to controls).

Table B.3. Selected Organ Weight to Body-Weight Ratios in the F344N Male Rat Exposed to Oral 3,3′-Dimethoxybenzidine for 13 Weeks[a]

	Exposure Group (Daily Average Dose, mg/kg-day)					
Parameter	**0 ppm**	**170 ppm (13)**	**330 ppm (22)**	**630 ppm (39)**	**1250 ppm (70)**	**2500 ppm (120)**
Sample size	10	10	10	10	10	10
Final body weight (g)	326 ± 6.18	319 ± 5.58 (−2)	325 ± 4.54 (0)	318 ± 5.69 (−2)	295 ± 5.51** (−10)	265 ± 5.45** (−19)
Relative organ weights (mg/g)						
Liver	25.1 ± 0.20	27.7 ± 0.19** (+10)	27.9 ± 0.21** (+11)	29.3 ± 0.30** (+17)	31.3 ± 0.35** (+25)	32.8 ± 0.58** (+31)
Right kidney	3.0 ± 0.04	3.1 ± 0.04* (+3)	3.2 ± 0.04** (+7)	3.4 ± 0.04** (+13)	3.5 ± 0.06** (+17)	4.0 ± 0.06** (+33)
Thymus	1 ± 0.03	0.9 ± 0.02* (−18)	0.9 ± 0.06** (−18)	0.9 ± 0.04** (−18)	0.8 ± 0.06** (−27)	0.8 ± 0.01** (−27)

[a]NTP (1990).
Notes: Means ± SE (% change relative to controls); *p* values are vs. the controls by Dunn's or Shirley's test.
*$p \leq 0.05$; **$p \leq 0.01$.

Table B.4. Selected Organ Weight to Body-Weight Ratios in F344N Female Rats Exposed to Oral 3,3′-Dimethoxybenzidine for 13 Weeks[a]

Parameter	Exposure Group (Daily Average Dose, mg/kg-day)					
	0 ppm	170 ppm (24)	330 ppm (49)	630 ppm (60)	1250 ppm (103)	2500 ppm (187)
Sample size	10	10	10	10	10	10
Final body weight (g)	179 ± 2.20	176 ± 2.22 (−2)	178 ± 1.65 (−1)	175 ± 1.46 (−2)	174 ± 3.44 (−3)	164 ± 2.63* (−8)
Relative organ weights (mg/g)						
Liver	25.9 ± 0.40	26.2 ± 0.36 (+1)	27.0 ± 0.39 (+4)	28.4 ± 0.97** (+10)	28.3 ± 0.24** (+9)	30.2 ± 0.46** (+17)
Right kidney	3.2 ± 0.05	3.3 ± 0.05 (+3)	3.5 ± 0.05** (+9)	3.9 ± 0.06** (+22)	4.0 ± 0.09** (+25)	4.2 ± 0.05** (+31)

[a]NTP (1990).
Notes: Means ± SE (% change relative to controls); *p* values are vs. the controls by Dunn's or Shirley's test.
*$p \leq 0.05$; **$p \leq 0.01$.

Table B.5. Selected Serum Chemistry of Male and Female F344N Rats Exposed to Oral 3,3′-Dimethoxybenzidine for 13 Weeks[a,b]

Parameter	Exposure Group (Daily Average Dose, mg/kg-day)					
Male rat	**0 ppm**	**170 ppm (13)**	**330 ppm (22)**	**630 ppm (39)**	**1250 ppm (70)**	**2500 ppm (120)**
Serum creatinine (mg/dl)	0.67 ± 0.015	0.58 ± 0.013** (−13)	0.57 ± 0.015** (−15)	0.50 ± 0.030** (−25)	0.61 ± 0.028** (−19)	0.56 ± 0.034** (−16)
Triiodothyronine (T_3) (ng/dl)	67.0 ± 2.68	67.0 ± 4.41 (0)	69.1 ± 3.31 (+3)	65.9 ± 2.46 (−2)	65.5 ± 1.85 (−2)	58.6 ± 3.13 (−13)
Thyroxine (T_4) (µg/dl)	4.0 ± 0.14	3.4 ± 0.22* (−15)	3.6 ± 0.16* (−10)	2.9 ± 0.14** (−27)	3.4 ± 0.16** (−15)	2.8 ± 0.19** (−30)
Thyrotropin (TSH) (ng/ml)	609 ± 55.3[c]	527 ± 39.2[d] (−13)	639 ± 74.4[e] (+5)	592 ± 27.0 (−3)	668 ± 74.0[d] (+10)	476 ± 52.3[e] (−22)
Female rat	**0 ppm**	**170 ppm (24)**	**330 ppm (49)**	**630 ppm (60)**	**1250 ppm (103)**	**2500 ppm (187)**
Serum creatinine (mg/dl)	0.71 ± 0.031	0.62 ± 0.025* (−13)	0.61 ± 0.038* (−14)	0.54 ± 0.029** (−24)	0.62 ± 0.025* (−13)	0.57 ± 0.021** (−20)
Triiodothyronine (T_3) (ng/dl)	98.4 ± 2.16	97.7 ± 4.54 (−1)	79.4 ± 3.63** (−19)	68.3 ± 2.87** (−31)	63.3 ± 2.01** (−36)	57.2 ± 2.49** (−42)
Thyroxine (T_4) (µg/dl)	3.9 ± 0.17	3.4 ± 0.17 (−13)	3.2 ± 0.23* (−18)	2.4 ± 0.05** (−38)	2.0 ± 0.17** (−49)	2.0 ± 0.14** (−49)
Thyrotropin (TSH) (ng/ml)	461 ± 21.7[c]	697 ± 62.9[f] (+51)	730 ± 79.2[e] (+58)	606 ± 47.8[g] (+31)	962 ± 246.1[e] (+109)	605 ± 138.8[d] (+31)

[a]NTP (1990).
[b]Mean ± SE for groups of 10 animals, unless otherwise specified (% change relative to controls).
[c]Five animals were examined.
[d]Nine animals were examined.
[e]Eight animals were examined.
[f]Six animals were examined.
[g]Seven animals were examined.
*$p < 0.05$.
**$p < 0.01$.

Table B.6 Mean Survival Time and Mean Final Body Weights of Male and Female F344 Rats Following Oral Exposure to 3,3′-Dimethoxybenzidine for 52 Weeks[a,b]

Parameter	Exposure Group (Daily Average Dose mg/kg-day)					
Male rat	**0.1 mg/day (0.2)**	**0.3 mg/day (0.6)**	**1.0 mg/day (1.9)**	**3.0 mg/day (5.6)**	**10 mg/day (18.8)[c]**	**30 mg/day (56.4)**
Survival time (days)[d]	568	568 (0)	503 (−11)	519 (−9)	506 (−11)	394 (−31)
Mean body weight[d] (g)	418	425 (+2)	359 (−14)	357 (−15)	393 (−6)	343 (−18)
Female rat	**0.1 mg/day (0.3)**	**0.3 mg/day (0.9)**	**1.0 mg/day (3.1)**	**3.0 mg/day (9.4)**	**10 mg/day (31.2)[e]**	**30 mg/day (93.6)**
Survival time[d]	548	548 (0)	517 (−6)	383 (−30)	447 (−18)	462 (−16)
Mean body weight (g)[d]	293	305 (+4)	249 (−15)	247 (−16)	232 (−21)	223 (−24)

[a]Hadidian et al. (1968).
[b]*n* = 3 except where otherwise specified.
[c]*n* = 14.
[d]Value (% change relative to lowest exposure dose group).
[e]*n* = 15.

Table B.7. Incidence of Neoplasms in Male and Female F344 Rats Following Oral Exposure to 3,3′-Dimethoxybenzidine for 52 Weeks[a,b]

Parameter		Exposure Group (Daily Average Dose mg/kg-day)						
Male Rat		**0 mg/day[c]**	**0.1 mg/day (0.2)**	**0.3 mg/day (0.6)**	**1.0 mg/day (1.9)**	**3.0 mg/day (5.6)**	**10 mg/day (18.8)[d]**	**30 mg/day (56.4)**
Ear:	Squamous cell carcinoma	2	0	0	1	1	3	0
Skin:	Basal cell carcinoma	0	0	0	0	0	2	0
	Squamous cell carcinoma	0	0	0	0	0	1	1
Stomach:	Papilloma	0	0	0	0	0	1	0
Pituitary:	Adenoma	2	0	0	0	0	1	0
Intestine:	Adenocarcinoma	0	0	0	0	0	2	0
Colon:	Adenocarcinoma	0	0	0	0	0	0	1
Testes:	Interstitial cell	123	2	2	2	2	2	0
Multiple organs:	Metastasis	0	0	0	1	0	1	0
Female Rat		**0 mg/day[c]**	**0.1 mg/day (0.3)**	**0.3 mg/day (0.9)**	**1.0 mg/day (3.1)**	**3.0 mg/day (9.4)**	**10 mg/day (31.2)[e]**	**30 mg/day (93.6)**
Ear:	Squamous cell carcinoma	0	0	0	0	0	2	1
Skin:	Basal cell carcinoma	0	0	0	0	0	0	1
	Squamous cell carcinoma	0	0	0	0	0	2	0
Uterus:	Carcinoma	0	0	0	0	0	1	0
	Endometrial carcinoma	0	0	0	0	0	1	0
Mammary gland:	Adenocarcinoma	0	0	0	0	1	2	0
	Fibroadenoma	10	0	0	0	0	1	1
Bladder:	Papilloma	1	0	0	0	1	0	1
Multiple organs:	Metastasis	0	0	0	0	0	1	0
	Lipoma	0	0	1	0	0	0	0

[a]Hadidian et al. (1968).
[b]Number of rats observed with lesion; $n = 3$ except where otherwise specified.
[c]$n = 330$ (240 vehicle controls and 90 untreated controls).
[d]$n = 14$.
[e]$n = 15$.

Table B.8. Selected Organ Weight to Body-Weight Ratios in F344N Rats Exposed to Oral 3,3′-Dimethoxybenzidine for 9 Months[a]

Parameter	Exposure Group (Daily Average Dose, mg/kg-day)	
Male rat	**0 ppm**	**330 ppm (21)**
Sample size	10	10
Final body weight (g)	390 ± 7.7	373 ± 8.4 (−4)
Relative organ weights (mg/g)		
Liver	25.5 ± 0.40	28.7 ± 0.67* (+13)
Kidney	6.1 ± 0.11	7.0 ± 0.12* (+15)
Female rat	**0 ppm**	**330 ppm (23)**
Sample size	10	10
Final body weight (g)	232 ± 3.9	223 ± 3.3 (−4)
Relative organ weights (mg/g)		
Liver	26.9 ± 0.47	29.7 ± 0.69* (+10)
Kidney	6.2 ± 0.16	7.3 ± 0.15* (+18)

[a]NTP (1990).
Notes: Means ± SE (% change relative to controls); *p* values are vs. the controls by Dunn's or Shirley's test.
*$p \leq 0.05$.

Table B.9. Water Consumption, Mean Body Weight, and Survival of Male and Female F344N Rats Exposed to 3,3′-Dimethoxybenzidine in Drinking Water for 21 Months[a]

Parameter	Exposure Group (Daily Average Dose, mg/kg-day)			
Male rat	**0 ppm**	**80 ppm (6)**	**170 ppm (12)**	**330 ppm (21)**
Water consumption[b]	26.6 ± 3.3	25.0 ± 5.1 (−6)	25.8 ± 6.2 (−3)	22.0 ± 4.9 (−17)
Mean body weight[c]	373	366 (−2)	359 (−4)	354 (−5)
Survival[d]	44/60 (73%)	8/45 (18%)	0/75	0/60
Female rat	**0 ppm**	**80 ppm (7)**	**170 ppm (14)**	**330 ppm (23)**
Water consumption[b]	20.1 ± 2.9	19.9 ± 4.6 (−1)	19.4 ± 3.1 (−3)	15.7 ± 4.1 (−22)
Mean body weight[c]	251	244 (−3)	234 (−7)	231 (−8)
Survival[d]	45/60 (75%)	15/45 (33%)	6/75 (8%)	0/60

[a]NTP (1990).
[b]Grams of water consumed per rat per day (% change relative to controls).
[c]Estimated over the duration of the study (% change relative to controls).
[d]Animals surviving until study termination (% alive at termination of study at 21 months).

Table B.10. Survival Rates in F344N Rats Exposed to 3,3′-Dimethoxybenzidine in Drinking Water for 21 Months[a]

Parameter	Exposure Group (Daily Average Dose, mg/kg-day)			
Male rat	**0 ppm**	**80 ppm (6)**	**170 ppm (12)**	**330 ppm (21)**
Sample size	60	45	75	60
Natural deaths[b]	9	9 (20)	25 (33)	14 (23)
Moribund	7	28 (62)	50 (67)	46 (77)
Animals surviving to end of study	44	8 (18)	0 (0)	0 (0)
Survival *p*-values[c]	<0.001	<0.001	<0.001	<0.001
Female rat	**0 ppm**	**80 ppm (7)**	**170 ppm (14)**	**330 ppm (23)**
Sample size	60	45	75	60
Natural deaths	5	3 (7)	9 (12)	9 (15)
Moribund	10	27 (60)	60 (80)	51 (85)
Animals surviving until end of study	45	15 (33)	6 (8)	0 (0)
Survival *p*-values[c]	<0.001	<0.001	<0.001	<0.001

[a]NTP (1990).
[b]Number (% of exposure group).
[c]Life table pairwise comparisons.

Table B.11. Selected Nonneoplastic Lesions in F344N Rats Exposed to 3,3′-Dimethoxybenzidine in Drinking Water for 21 Months[a]

Parameter		Exposure Group (Daily Average Dose, mg/kg-day)			
Male rat		**0 ppm**	**80 ppm (6)**	**170 ppm (12)**	**330 ppm (21)**
Number examined		60	45	74	60
Liver:	Cystic degeneration[b]	13 (22)	23** (51)	34** (46)	28** (47)
	Centrilobular degeneration[b]	0 (0)	4* (9)	9** (12)	10** (17)
	Eosinophilic focus[b]	6 (10)	15** (33)	35** (47)	38** (63)
	Hematopoietic cell proliferation[b]	2 (3)	15** (33)	39** (53)	41** (68)
	Necrosis[b]	4 (7)	15** (33)	18** (24)	17** (28)
	Regeneration[b]	5 (8)	7 (16)	22** (30)	18** (30)
	Cytoplasmic vacuolization[b]	2 (3)	2 (4)	7 (9)	10** (17)
Spleen:	Hematopoietic cell proliferation[b]	3 (5)	13[c]* (31)	43[d]* (57)	38* (63)
Heart:	Atrium thrombi[b]	3 (5)	15[e]* (34)	27* (36)	23* (38)
Lung:	Histiocytic cellular infiltration[b]	0 (0)	3[e] (7)	10* (14)	6* (10)
Female rat		**0 ppm**	**80 ppm (7)**	**170 ppm (14)**	**330 ppm (23)**
Number examined		60	44	75	60
Liver:	Cystic degeneration[b]	1 (2)	2 (5)	1 (1)	5 (8)
	Centrilobular degeneration[b]	1 (2)	3 (7)	8* (11)	5 (8)
	Eosinophilic focus[b]	5 (8)	7 (16)	20** (27)	28** (47)
	Hematopoietic cell proliferation[b]	1 (2)	18** (41)	43** (57)	41** (68)
	Necrosis[b]	1 (2)	3 (7)	13** (17)	18** (30)
	Regeneration[b]	6 (10)	3 (7)	5 (7)	4 (7)
	Cytoplasmic vacuolization[b]	3 (5)	1 (2)	4 (5)	3 (5)
Spleen:	Hematopoietic cell proliferation[b]	3 (5)	22* (50)	50* (67)	47* (78)
Heart:	Atrium thrombi[b]	0 (0)	1[f] (2)	0 (0)	1 (2)
Lung:	Histiocytic cellular infiltration[b]	0 (0)	3[f] (7)	4 (5)	18* (30)

[a]NTP (1990).
[b]Number (% of exposure group examined).
[c]42 animals were examined.
[d]75 animals were examined.
[e]44 animals were examined.
[f]45 animals were examined.
Notes: *$p < 0.05$ vs. controls; **$p < 0.01$ vs. controls.

Table B.12. Selected Analysis of Primary Tumors in Male and Female F344N Rats Exposed to 3,3′-Dimethoxybenzidine Dihydrochloride in Drinking Water for 21 Months[a,b]

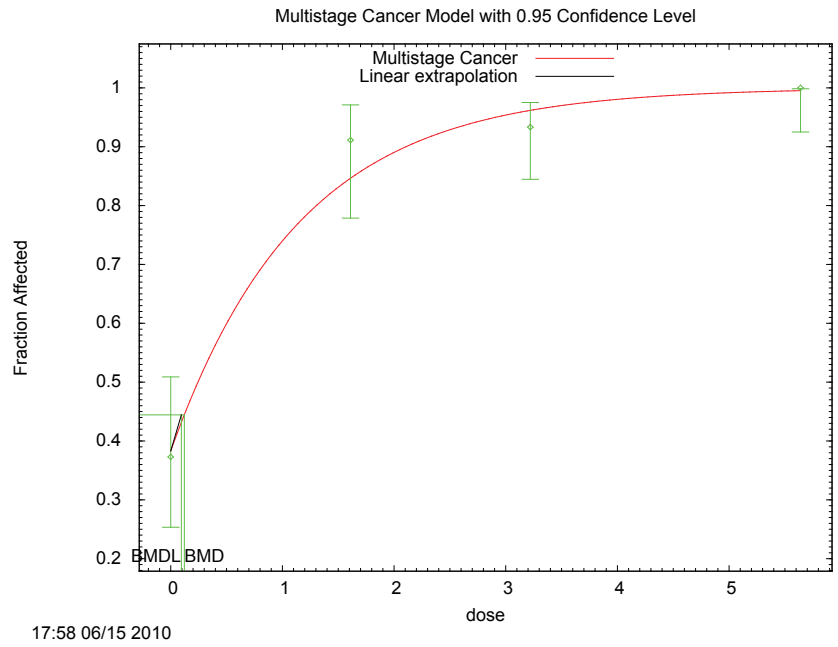
Parameter		Exposure Group (Daily Average Dose mg/kg-day)			
Male Rat		**0 ppm**	**80 ppm (6)**	**170 ppm (12)**	**330 ppm (21)**
Liver:	Neoplastic nodule	0/58	3/39	7/54**	6/35**
	Neoplastic nodule or hepatocellular carcinoma	1/58	4/39	7/54*	8/35**
Large intestine:	Adenomatous polyp	0/59	1/44	4/73	5/57*
	Adenocarcinoma	0/59	0/42	4/67	3/50
	Adenomatous polyp or adenocarcinoma	0/59	1/44	8/73**	8/57**
Small intestine:	Adenocarcinoma	0/59	4/44*	7/75*	5/60*
Zymbal gland:	Adenoma	0/58	4/44*	11/71**	9/53**
	Carcinoma	0/58	7/45**	14/75**	21/60**
	Adenoma or carcinoma	0/58	10/45**	25/75**	30/60**
Preputial gland:	Carcinoma	2/59	6/42	15/73**	19/59**
	Adenoma or carcinoma	16/59	12/42	33/73*	29/59*
Oral cavity:	Squamous cell papilloma	1/59	7/44*	10/73*	9/57**
	Squamous cell papilloma or carcinoma	1/59	8/44**	10/73*	11/57**
Skin:	Basal cell adenoma	1/59	31/42**	47/67**	35/50**
	Basal cell carcinoma	1/59	4/44	18/71**	17/54**
	Basal cell adenoma or carcinoma	2/59	32/44**	54/71**	40/54**
	Squamous cell papilloma	0/58	5/42*	7/62**	5/41*
	Squamous cell carcinoma	0/59	9/42**	24/65**	21/48**
	Squamous cell papilloma or carcinoma	0/59	13/42**	28/65**	22/48**
All Tissues:	Mesothelioma	2/59	1/44	7/72*	6/56**
Combined Sites[c]	Combined tumors	22/59	41/45**	70/75**	60/60**
Female Rat		**0 ppm**	**80 ppm (7)**	**170 ppm (14)**	**330 ppm (23)**
Liver:	Neoplastic nodule or hepatocellular carcinoma	0/59	1/44	0/47	3/38
Large intestine:	Adenomatous polyp or adenocarcinoma	0/59	1/44	1/48	3/35*
Zymbal gland:	Adenoma	0/59	3/44	4/48*	3/35*
	Carcinoma	1/60	10/45**	17/74**	13/59**
	Adenoma or carcinoma	1/60	12/45**	21/74**	16/59**
Skin:	Basal cell adenoma	0/59	3/44	3/48	2/35
	Basal cell adenoma or carcinoma	0/59	4/44*	3/48	2/35
Clitoral gland:	Adenoma	5/58	15/44**	13/73	16/55**
	Carcinoma	2/58	17/44**	41/74**	30/55**
	Adenoma or Carcinoma	7/58	27/44**	48/74**	41/55**
Mammary gland:	Adenocarcinoma	1/60	2/45	14/73**	20/57**

Table B.12. Selected Analysis of Primary Tumors in Male and Female F344N Rats Exposed to 3,3′-Dimethoxybenzidine Dihydrochloride in Drinking Water for 21 Months[a,b]

Parameter		Exposure Group (Daily Average Dose mg/kg-day)			
Uterus:	Adenoma	0/56	3/34	1/19	2/8*
	Adenoma or Carcinoma	0/59	4/44*	2/48	2/35
Combined Sites[d]	Combined tumors	5/60	38/45**	65/74**	53/59**

[a]NTP (1990).
[b]Number of tumor-bearing animals/effective number of animals, i.e., number of animals living during first occurrence of tumors in any dose group.
[c]Includes sites with statistically increased tumor incidences (liver, small intestines, large intestine, Zymbal gland, preputial gland, oral cavity, and skin).
[d]Includes sites with statistically increased tumor incidences (liver, large intestine, Zymbal gland, skin, clitoral gland, mammary gland, and uterus).
Notes: *$p < 0.05$ vs. controls; **$p < 0.01$ vs. controls.

APPENDIX C. BMD MODELING OUTPUTS FOR 3,3′-DIMETHOXYBENZIDINE



Combined Significant Tumors in Males (NTP, 1990).

Output for selected model: Multistage Cancer

NTP, 1990: Combined Significant Tumors in Males

```
=============================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\1\NTP_1990_SigTumor_M_MultiCanc_1.(d)
        Gnuplot Plotting File:  C:\1\NTP_1990_SigTumor_M_MultiCanc_1.plt
                                                Tue Jun 15 17:58:58 2010
=============================================================================

[add notes here]
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the probability function is:

  P[response] = background + (1-background)*[1-EXP(
            -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

  The parameter betas are restricted to be positive


  Dependent variable = DichPerc
  Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
```

Total number of parameters in model = 4
Total number of specified parameters = 0
Degree of polynomial = 3

Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0
Beta(1) = 0
Beta(2) = 0
Beta(3) = 5.80808e+017

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Beta(2) -Beta(3)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Background	Beta(1)
Background	1	-0.44
Beta(1)	-0.44	1

Parameter Estimates

		95.0% Wald Confidence Interval		
Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
Background	0.38265	*	*	*
Beta(1)	0.864045	*	*	*
Beta(2)	0	*	*	*
Beta(3)	0	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-70.8357	4			
Fitted model	-72.6447	2	3.618	2	0.1638
Reduced model	-117.058	1	92.4447	3	<.0001

AIC: 149.289

Goodness of Fit
Scaled

Dose	Est._Prob.	Expected	Observed	Size	Residual
0.0000	0.3826	22.576	22.000	59	-0.154
1.6100	0.8464	38.088	41.000	45	1.204
3.2200	0.9618	72.134	70.000	75	-1.285
5.6400	0.9953	59.717	60.000	60	0.534

Chi^2 = 3.41 d.f. = 2 P-value = 0.1818

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.121939

BMDL = 0.095041

BMDU = 0.218288

Taken together, (0.095041, 0.218288) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 1.05218

APPENDIX D. REFERENCES

ACGIH (American Conference of Industrial Hygienists). (2009) 2009 TLVs and BEIs: Threshold limit values for chemical substances and physical agents and biological exposure indices. Cincinnati, OH: ACGIH. 594528

Anderson, D; Styles, JA. (1978) An evaluation of 6 short-term tests for detecting organic chemical carcinogens: Appendix II- The bacterial mutation test. *Br J Cancer* 37(6):924−930. 594532

ATSDR (Agency for Toxic Substances and Disease Registry). (2010) Toxicological profile information sheet. U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA. Available online at http://www.atsdr.cdc.gov/toxprofiles/index.asp. Accessed on 2/25/2010. 684152

CalEPA (California Environmental Protection Agency). (2008) All OEHHA acute, 8-hour and chronic reference exposure levels (chRELs) as on December 18, 2008. Office of Environmental Health Hazard Assessment, Sacramento, CA. Available online at http://www.oehha.ca.gov/air/allrels.html. Accessed on 2/25/2010. 595416

CalEPA (California Environmental Protection Agency). (2009) OEHHA toxicity criteria database. Office of Environmental Health Hazard Assessment, Sacramento, CA. Available online at http://www.oehha.ca.gov/risk/\ChemicalDB/index.asp. Accessed on 2/25/2010. 595417

Chung, KT; Chen, SC; Wong, TY; et al. (2000) Mutagenicity studies of benzidine and its analogs: Structure-activity relationships. *Toxicol Sci* 56(2):351−356. 225330

De France, BF; Carter, MH; Josephy, PD. (1986) Comparative metabolism and mutagenicity of azo and hydrazone dyes in the Ames test. *Food Chem Toxicol* 24(2):165−169. 625777

Fluck, ER; Poirier, LA; Ruelius, HW. (1976) Evaluation of a DNA polymerase-deficient mutant of E. coli for the rapid detection of carcinogens. *Chem Biol Interact* 15(3):219−231. 074993

Frumin, E; Velez, H; Bingham, E; et al. (1990) Occupational bladder cancer in textile dyeing and printing workers: Six cases and their significance for screening programs. *J Occup Med* 32:887−890. 225334

Galloway, SM; Armstrong, MJ; Reuben, C; et al. (1987) Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. *Environ Mol Mutagen*, 10:1−175. 007768

Galloway, SM; Bloom, AD; Resnick, M; et al. (1985) Development of a standard protocol for in vitro cytogenetic testing with Chinese hamster ovary cells: comparison of results for 22 compounds in two laboratories. *Environ Mutagen* 7:1−51. 625785

Gray, LE Jr; Ostby, JS. (1993) The effects of prenatal administration of azo dyes on testicular development in the mouse: A structure activity profile of dyes derived from benzidine, dimethylbenzidine, or dimethoxybenzidine. *Toxicol Sci* 20(2):177−183. 068031

Gregory, AR; Elliott, J; Kluge, P. (1981) Ames testing of Direct Black 38 parallels carcinogenicity testing. *J Appl Toxicol* 1(6):308−313. 625786

Hadidian, Z; Fredrickson, TN; Weisburger, EK; et al. (1968) Tests for chemical carcinogens Report on the activity of derivatives of aromatic amines, nitrosamines, quinolines, nitroalkanes, amides, epoxides, aziridines, and purine antimetabolites. *J Natl Cancer Inst* 41(4):985−1036. 062691

Hamasaki, T; Aramaki, K; Hida, T; et al. (1996) [Clinical study of occupational uroepithelial cancer]. *J UOEH* 18:247−259. 229151

Haworth, S; Lawlor, T; Mortelmans, K; et al. (1983) Salmonella mutagenicity test results for 250 chemicals. *Environ Mutagen* 5(Suppl 1):3−142. 028947

IARC (International Agency for Research on Cancer). (1974) Some aromatic amines, hydrazine and related substances, N-nitroso compounds and miscellaneous alkylating agents. IARC monographs on the evaluation of carcinogenic risks to humans, vol 4. WHO. Available online at http://monographs.iarc.fr/ENG/Monographs/vol4/volume4.pdf. 231090

IARC (International Agency for Research on Cancer). (2000) Some industrial chemicals. IARC monographs on the evaluation of carcinogenic risks to humans, volume 77. Geneva: WHO. Available online at http://monographs.iarc.fr/ENG/Monographs/vol77/index.php. 201837

Krishna, G; Xu, J; Nath, J. (1986) Comparative mutagenicity studies of azo dyes and their reduction products in Salmonella typhimurium. *J Toxicol Environ Health* 18:111−119. 625787

Makena, P; Chung, KT. (2007) Evidence that 4-aminobiphenyl, benzidine, and benzidine congeners produce genotoxicity through reactive oxygen species. *Environ Mol Mutagen* 48(5):404−413. 625788

Martelli, A; Robbiano, L; Carrozzino, R; et al. (2000) DNA damage induced by 3,3' dimethoxybenzidine in liver and urinary bladder cells of rats and humans. *Toxicol Sci* 53:71−76. 233260

Martin, CN; Kennelly, JC. (1981) Rat liver microsomal azoreductase activity on four azo dyes derived from benzidine, 3,3′-dimethylbenzidine or 3,3′-dimethoxybenzidine. *Carcinogenesis* 2(4):307−312. 625791

Messerly, EA; Fekete, JE; Wade, DR; et al. (1987) Structure-mutagenicity relationships of benzidine analogues. *Environ Mol Mutagen* 10(3):263−274. 062707

Morgan, DL; Jameson, CW; Mennear, JH; et al. (1989) Thirteen-week toxicity studies of 3,3′-dimethoxybenzidine and CI direct blue 15 in the fisher 344 rat. *Toxicology* 59(3):297−309. 094786

Morgan, DL; Bucher, JR; Huff, JE; et al. (1990) Multi-organ carcinogenicity of 3,3′-dimethoxybenzidine dihydrochloride given in drinking water to F344/N rats. *Int J Toxicol* 9:79−91. 061197

Morgan, DL; Dunnick, JK; Goehl, T; et al. (1994) Summary of the National Toxicology Program benzidine dye initiative. *Environ Health Perspect* 102(Suppl 2):63−78. 069233

NIOSH (National Institute for Occupational Safety and Health). (2010) NIOSH pocket guide to chemical hazards. Index of chemical abstracts service registry numbers (CAS No.). Center for Disease Control and Prevention, U.S. Department of Health, Education and Welfare, Atlanta, GA. Available online at http://www.cdc.gov/niosh/npg/npgdcas.html. 625692

NTP (National Toxicology Program). (1990) Toxicology and carcinogenesis studies of 3,3′-dimethoxybenzidine dihydrochloride (CAS No. 20325-40-0) in F344/N rats (drinking water studies). U.S. Department of Health and Human Services, Public Health Service, Research Triangle Park, NC; TR 372. Available online at http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr372.pdf.

NTP (National Toxicology Program). (1992) Toxicology and carcinogenesis studies of C.I. direct blue 15 (CAS No. 2429-74-5) in F344 Rats (drinking water studies). National Toxicology Program. Research Triangle Park, N.C. Available online at http://ntp.niehs.nih.gov/?objectid=070952A0-F5B1-A1F1-DB952886A955ACCA.

NTP (National Toxicology Program). (2011) 12th Report on carcinogens. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC. Available online at http://ntp.niehs.nih.gov/?objectid=03C9AF75-E1BF-FF40-DBA9EC0928DF8B15.

OSHA (Occupational Safety and Health Administration). (2006) Table Z-1 limits for air contaminants: occupational safety and health standards, subpart Z, toxic and hazardous substances. U.S. Department of Labor, Washington, DC; OSHA Standard 1910.1000. Available online at http://63.234.227.130/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=9992. Accessed on 2/22/2010. 670067

Ouellet-Hellstrom, R; Rench, JD. (1996) Bladder cancer incidence in arylamine workers. J *Occup Environ Med* 38(12):1239−1247. 376069

Pliss, GB. (1963) On some regular relationships between carcinogenicity of aminodiphenyl derivatives and the structure of substance. *Acta Unio Internat Cont Canc* 19:499−501. 376071

Pliss, GB. (1965) [On the carcinogenic properties of ortho-toluidine and dianisidine]. *Gigiena Truda i Professional'nye Zabolevaniya* 9(7):18−22. 377970

Prival, MJ; Bell, SJ; Mitchell, VD; et al. (1984) Mutagenicity of benzidine and benzidine-congener dyes and selected monoazo dyes in a modified Salmonella assay. *Mutat Res* 136:33−47. 625794

Probst, GS; McMahon, RE; Hill, LE; et al. (1981) Chemically-induced unscheduled DNA synthesis in primary rat hepatocyte cultures: a comparison with bacterial mutagenicity using 218 compounds. *Environ Mutagen* 3:11−32. 018887

Reynolds, SH; Patterson, RM; Mennear, JH; et al. (1990) ras Gene activation in rat tumors induced by benzidine congeners and derived dyes. *Cancer Res* 50(2):266−272. 062314

Rodgers, RM; Garvie-Gould, C; Scott, KF; et al. (1983) Metabolism, distribution, and excretion of the carcinogenic aromatic amine, 3,3′-dimethoxybenzidine in the rat: formation of mutagenic urinary and biliary metabolites. *Drug Metab Dispos* 11(4):293−300. 062715

Saffiotti, U; Cefis, F; Montesano, R; et al. (1967) Induction of bladder cancer in hamsters fed aromatic amines. In: WB Deichman; KR Lampe; ed. Bladder cancer: A symposium. Birmingham, AL: Aesculapius Publishing Company, pp. 129−135. 062317

Schieferstein, GJ; Sheldon, WG; Allen, RR; et al. (1990) Oncogenic evaluation of 3,3′-dimethoxybenzidine dihydrochloride in BALB/c mice. *Int J Toxicol* 9:71−77. 094675

Sellakumar, AR; Montesano, R; Saffiotti, U. (1969) Aromatic amines carcinogenicity in hamsters: Abstract #309. Presented at American Association for Cancer Research, San Francisco, CA. 376236

U.S. EPA (Environmental Protection Agency). (1980) Appendix C−Guidelines and methodology used in the preparation of health effect assessment chapters of the consent decree water criteria documents. *Federal Register* 45:79347−79357.

U.S. EPA (Environmental Protection Agency). (1988) Recommendations for and documentation of biological values for use in risk assessment. Environmental Criteria and Assessment Office, Cincinnati, OH; EPA/600/6-87/008. Available online at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=34855#Download. 064560

U.S. EPA (Environmental Protection Agency). (1994) Chemical assessments and related activities (CARA). Office of Health and Environmental Assessment, Washington, DC; EPA/600/R-94/904. Available online at http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=60001G8L.txt. Accessed on 2/25/2010. 596444

U.S. EPA (Environmental Protection Agency). (1997) Exposure factors handbook (final report) 1997. U.S. Environmental Protection Agency, Washington, DC; EPA/600/P-95/002Fa-c. Available online at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=12464. 594981

U.S. EPA (Environmental Protection Agency). (2005a) Guidelines for carcinogen risk assessment. Risk Assessment Forum, Washington, DC; EPA/630/P-03/001F. Federal Register 70(66):17765−17817. Available online at http://www.epa.gov/raf/publications/pdfs/CANCER_GUIDELINES_FINAL_3-25-05.PDF. Accessed on 6/12/2010. 086237

U.S. EPA (Environmental Protection Agency). (2005b) Supplemental guidance for assessing susceptibility from early-life exposure to carcinogens. Risk Assessment Forum, Washington, DC; EPA/630/R-03/003F. Available online at http://www.epa.gov/ttn/atw/childrens_supplement_final.pdf. Accessed on 6/12/2010. 088823

U.S. EPA (Environmental Protection Agency). (2006) 2006 Edition of the drinking water standards and health advisories. Office of Water, Washington, DC; EPA/822/R-06/013. Washington, DC. Available online at http://www.epa.gov/waterscience/drinking/standards/dwstandards.pdf. Accessed on 2/25/2010. 091193

U.S. EPA (Environmental Protection Agency). (2010) Health effects assessment summary tables (HEAST). Prepared by the Office of Research and Development, National Center for Environmental Assessment, Cincinnati OH for the Office of Emergency and Remedial Response, Washington, DC. Available online at http://epa-heast.ornl.gov/. Accessed on 2/25/2010. 595422

U.S. EPA (Environmental Protection Agency). (2010) Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at http://www.epa.gov/iris/. Accessed on 2/25/2010. 595423

WHO (World Health Organization). (2010) Online catalogs for the Environmental Health Criteria series. Available online at http://www.who.int/topics/environmental_health/en/. Accessed on 2/25/2010. 595424

Yoon, JS; Mason, JM; Valencia, R; et al. (1985) Chemical mutagenesis testing in Drosophila. IV. Results of 45 coded compounds tested for the National Toxicology Program. *Environ Mol Mutagen* 7(3):349−367. 194373