United States
Environmental Protection
Agency

EPA/690/R-08/013F
Final
8-27-2008

Provisional Peer Reviewed Toxicity Values for

3,3'-Dimethylbenzidine
(CASRN 119-93-7)

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

Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
i.v.	intravenous
kg	kilogram
L	liter
LEL	lowest-effect level
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
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level
MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
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
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration

p-RfD	provisional oral reference dose
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	Provisional Peer Reviewed Toxicity Value
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR 3,3'-DIMETHYLBENZIDINE (CASRN 119-93-7)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).
2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.
3. Other (peer-reviewed) toxicity values, including:
   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a five-year basis and updated into the active database. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may 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), or OSRTI.

INTRODUCTION

No chronic RfD or RfC for 3,3'-dimethylbenzidine (*o*-tolidine) is available on IRIS (U.S. EPA, 2007), in the Health Effects Assessment Summary Table (HEAST) (U.S. EPA, 1997) or in the Drinking Water Standards and Health Advisories list (U.S. EPA, 2006). Although a cancer assessment for 3,3'-dimethylbenzidine is not available on IRIS (U.S. EPA, 2007) or the Drinking Water list (U.S. EPA, 2006), the HEAST (U.S. EPA, 1997) lists a cancer weight-of-evidence classification of B2 and an oral slope factor of 9.2 per (mg/kg-day) for 3,3'-dimethylbenzidine, based on increased incidence of mammary tumors in rats orally treated with the chemical for 30 days (Griswold et al., 1968). The source document for this cancer assessment was a Health and Environmental Effects Profile (HEEP) for 3,3'-Dimethylbenzidine (U.S. EPA, 1987). The HEEP and a subsequent Cancer Reportable Quantity document (U.S. EPA, 1988a) that derived an oral slope factor of 27.4 per (mg/kg-day) from the same data are the only relevant documents included on the Chemical Assessments and Related Activities (CARA) lists (U.S. EPA, 1991, 1994).

The International Agency for Research on Cancer (IARC, 1972a, 1987) has classified 3,3'-dimethylbenzidine in category Group 2B, possible human carcinogen, based on sufficient evidence in animals and no data in humans. The National Toxicology Program (NTP, 2007) Eleventh Report on Carcinogens lists 3,3'-dimethylbenzidine as reasonably anticipated to be a human carcinogen based on sufficient evidence in animals. NTP (1991a) has studied the carcinogenic potential of 3,3'-dimethylbenzidine as part of the Benzidine Dye Initiative (NTP, 1982), an intensive research program designed to evaluate the toxic and carcinogenic effects of benzidine congeners and related dyes.

The American Conference of Governmental Industrial Hygienists (ACGIH, 2001, 2006) categorized 3,3'-dimethylbenzidine in group A3, as a confirmed animal carcinogen with unknown relevance to humans, but did not set a Threshold Limit Value (TLV) due to an absence

of inhalation data. Based on its potential carcinogenicity, the National Institute for Occupational Safety and Health (NIOSH, 2007) established a REL of 0.02 mg/m^3 as a 60-minute ceiling for 3,3'-dimethylbenzidine. The Occupational Safety and Health Administration (OSHA, 2007) does not have a PEL for 3,3'-dimethylbenzidine, but jointly published with NIOSH (1981) a health hazard alert that concluded that this chemical may present a cancer risk to workers and recommended that worker exposure be reduced to the lowest feasible level. CalEPA (2001) has not derived a REL or cancer potency factor for 3,3'-dimethylbenzidine.

No Agency for Toxic Substances and Disease Registry (ATSDR, 2007) Toxicological Profile or World Health Organization (WHO, 2007) Environmental Health Criteria document are available for 3,3'-dimethylbenzidine. Literature searches were conducted from the 1960's through December, 2006 for studies relevant to the derivation of provisional toxicity values for 3,3'-dimethylbenzidine. Databases searched included: TOXLINE (Special, including NTIS subfile), MEDLINE (including PubMed cancer subset), BIOSIS, TSCATS/TSCATS2, CCRIS, DART/ETIC, GENETOX, HSDB, RTECS and Current Contents.

REVIEW OF PERTINENT DATA

Human Studies

No information was located regarding effects of 3,3'-dimethylbenzidine in humans, except as part of a mixture. Most occupational exposures to 3,3'-dimethylbenzidine involve mixed exposure with benzidine and/or other biphenyl amine compounds (ACGIH, 2001). Elevated risks for cancer of the urinary tract have been found in workers exposed to combinations of 3,3'-dimethylbenzidine and benzidine (ACGIH, 2001). However, because benzidine is a known human bladder carcinogen (IARC, 1972b, 1982, 1987), these data are not helpful in assessing the carcinogenic potential of 3,3'-dimethylbenzidine *per se* (ACGIH, 2001). Although 3,3'-dimethylbenzidine is structurally similar to (is a congener of) benzidine, there is no indication that it is metabolized to benzidine (IARC, 1972a, NTP, 1991a).

As indicated above, most studies involving occupational exposure to 3,3'-dimethylbenzidine included confounding exposure to benzidine. Workers in a study by Quellet-Hellstrom and Rench (1996) were not exposed to benzidine, although they were still exposed to other benzidine congeners at higher levels than to 3,3'-dimethylbenzidine. This study included a cohort of 704 workers (585 men and 119 women) employed between mid-1965 and 1989 in an arylamine production plant in Connecticut. The chemicals produced at the facility during this time period (in order of production volume) were 3,3'-dichlorobenzidine, *o*-dianisidine and 3,3'-dimethylbenzidine. Benzidine had been produced in the plant prior to mid-1965, but only workers hired after June 15, 1965 and never exposed to benzidine were included in the cohort. Cancer incidence data were collected by matching the cohort roster from the company medical department with the following sources: cancer cases registered at the Connecticut Tumor Registry (CTR), death certificates of deceased workers indicating cancer associated with death and cancer cases reported by the employees via mail survey confirmed by attending physician. There were 27 total cases of cancer identified, 23 among male workers (including 3 cases of non-melanoma skin cancer that were not considered cases for this study) and 4 among female

workers. Among male workers, there were statistically significant increases in standardized incidence ratio (SIR) for bladder cancer (SIR = 8.3; 95% CI: 3.3 to 17.1) based on 7 observed cases and testicular cancer (SIR=11.4; 95% CI: 1.4 to 41.1) based on 2 observed cases. The male workers diagnosed with testicular cancer had no reported exposure to arylamines and one had worked only 15 days, so these cancers were not considered to be exposure-related. The only elevated SIR among female workers was for breast cancer, but this was not statistically significant (3 cases; SIR=1.9; CI 95%: 0.4 to 5.6).

Workers were scored for cumulative exposure to arylamines and sorted into no-, low- and high-exposure groups (Quellet-Hellstrom and Rench, 1996). Workers were also stratified according to length of follow-up (<5 yrs or 5+ yrs). No bladder cancer was seen in workers with less than 5 years of follow-up. Among those with greater than 5 years of follow-up, the risk of bladder cancer increased with increasing exposure. The SIR increased from 0 in the no-exposure group (based on 0 cases) to 6.4 (95% CI: 0.8-23.1) in the low-exposure group based on 2 cases and 17.3 (95% CI: 5.6-40.5) in the high-exposure group based on 5 cases. The high-exposure group in which the bladder cancer cases were concentrated comprised chemical operators who worked with the arylamines over a long period of time and mechanics who came into close contact with the chemicals when repairing equipment (short, intense exposures). The authors noted that the average age of 52 years among those diagnosed with bladder cancer in this cohort was relatively young for this disease (compared with 68 years for Connecticut men overall). All of the bladder cancer cases were current or ex-smokers. Smoking is a known risk factor for bladder cancer and was considered by the authors to probably have contributed to the bladder cancer risk observed in this study. Due to the mixed chemical exposures and confounding effect of smoking, it is not clear to what extent 3,3'-dimethylbenzidine may have contributed to the observed bladder cancer risk in the exposed workers.

Animal Studies

Oral Exposure

NTP (1991a) performed short-term, subchronic and chronic oral studies in rats. In the short-term study, groups of 5 male and 5 female 48-day old F344/N rats were exposed to 0, 600, 1250, 2500, 5000 or 7500 ppm of 3,3'-dimethylbenzidine dihydrochloride in drinking water for 14 days. Based on reported water consumption and body weight data and the molecular weight of 3,3'-dimethylbenzidine dihydrochloride, doses of 3,3'-dimethylbenzidine can be estimated as 0, 40, 90, 111, 127 and 142 mg/kg-day in males and 0, 47, 75, 139, 150 and 189 mg/kg-day in females. Animals were observed twice daily. Feed and water consumption were recorded by cage (5 animals/cage) weekly and twice weekly, respectively. Body weight was recorded initially and weekly thereafter. All animals were necropsied and the following organ weights were measured: brain, heart, right kidney, liver, lung, right testicle and thymus. Complete histopathological examinations were performed on all control animals, males exposed to 5000 ppm and females exposed to 7500 ppm. Based on the findings in these groups, selected tissues were examined in the lower-dose groups.

All 5 males and 1/5 females in the 7500 ppm group died, as did 1/5 males at 5000 ppm (NTP, 1991a). All deaths occurred by day 13 of the study. A specific cause of death was not

reported, but deaths were probably related to marked decreases in water consumption and body weight in the higher-dose animals. At 2500 ppm and above, the animals actually lost weight during the study, leading to marked terminal body weight deficits of 37-61% compared to controls. At 1250 ppm, the animals gained weight, but more slowly than controls, leading to terminal body weights that were 11-14% lower than controls. There were corresponding dose-related decreases in water consumption compared to controls in both males and females. The deficit in water consumption was 25-30% at 600 ppm and increased to 83-88% at 7500 ppm. Feed consumption data were not reported. Clinical observations included thinness and kyphosis (hunched-back) in all treated groups, along with urine stains, skin cold to touch, rough hair coat, ataxia and reddish discharge at eyes and nares in the 7500 ppm groups. Gross necropsy revealed absence of body fat in the 5000 and 7500 ppm groups, small thymus gland in the 2500 ppm and 5000 ppm groups, and small seminal vesicles in the 7500 ppm males. Organ weight changes were observed in the 2500, 5000 and 7500 ppm groups, but were considered by the researchers to be secondary to the marked body weight changes in these groups. Histopathological examination showed liver lesions in males at $\geq$2500 ppm and females at $\geq$5000 ppm, including hepatocyte necrosis and brown pigmentation of the cells lining the hepatic sinusoids (incidence data not reported). Other effects at $\geq$2500 ppm were increased severity of nephropathy and bone marrow hypocellularity. Lymphocytic atrophy of the thymus, spleen and mandibular and mesenteric lymph nodes, necrosis and vacuolation of adrenal cortical cells, focal acinar cell hypertrophy of the pancreas and, in males; increased immature sperm forms in the testis and epididymis were also reported, although dose levels for these effects were not specified. The low dose of 600 ppm (40-47 mg/kg-day) is a LOAEL for clinical signs and a marked decrease in water consumption in rats exposed for 14 days.

In the NTP (1991a) subchronic study, groups of 10 male and 10 female 55-day old F344/N rats were exposed to 0, 300, 500, 1000, 2000 or 4000 ppm of 3,3'-dimethylbenzidine dihydrochloride in drinking water for 13 weeks. The average amount of 3,3'-dimethylbenzidine consumed per day can be estimated as 0, 16, 22, 44, 86 or 144 mg/kg-day in males and 0, 18, 27, 50, 100 or 266 mg/kg-day in females, calculated as described above for the 14-day study. Animals were observed twice daily. Feed and water consumption were recorded by cage (5 animals/cage) weekly and twice weekly, respectively. Body weight was recorded initially and weekly thereafter. At week 13, blood samples were collected from all surviving animals for hematology and clinical chemistry analyses that included liver enzymes and thyroid hormones. At necropsy, weights of the following organ weights were recorded: brain, heart, liver, lung, right kidney, right testis and thymus. Complete histopathological examinations were performed on all controls, the two high-dose groups (2000 and 4000 ppm), and all animals that died or were killed moribund. Based on the findings in these groups, selected organs were examined in the lower-dose groups.

All rats receiving 4000 ppm and 4/10 males and 3/10 females receiving 2000 ppm, died before study termination (NTP, 1991a). Deaths occurred on weeks 2 through 4 in the 4000 ppm group and weeks 6 through 13 in the 2000 ppm group. Deaths appear to have been related to marked decreases in water consumption and body weight in the high-dose groups. Final mean body weight of treated rats relative to controls was decreased 9-12% in males and females at 300 ppm, with the deficit increasing to 42-48% at 2000 ppm. Water consumption relative to controls was decreased 20% in males and 43% in females at 300 ppm, with the deficit increasing to 54-

64% at 2000 ppm (based on data for weeks 7 and 13). Feed consumption data were not reported. Clinical observations included red nasal exudate, thinness, stains on fur and urine stains in all treatment groups, appearing as early as week 1 or 2 in the 2000 and 4000 ppm groups. Hematology and clinical chemistry results are shown in Table 1. Hematological findings included significant dose-related decreases in hematocrit and red blood cell count in males at ≥1000 ppm and females at all dose levels. Hemoglobin was reduced only in females at 2000 ppm. Leukocytes were significantly increased in males and females at 1000 ppm. Clinical chemistry changes included significant increases in serum sorbitol dehydrogenase (SDH) in all male and female groups, alanine aminotransferase (ALT) in males at 1000 ppm and females at 2000 ppm, lactate dehydrogenase (LDH) in males at 1000 ppm and blood urea nitrogen (BUN) in males at 2000 ppm. There were significant decreases in serum thyroxin (T4) levels in all male and female treatment groups and significant decreases in serum triiodothyronine (T3) values in all treated females. Serum levels of thyroid stimulating hormone (TSH) were not different from controls.

Table 1. Significant Hematology and Clinical Chemistry Findings in Rats Treated with 3,3'-Dimethylbenzidine Dihydrochloride in Drinking Water for 13 Weeks (NTP, 1991a)

Males	**0 mg/kg-day**	**16 mg/kg-day**	**22 mg/kg-day**	**44 mg/kg-day**	**86 mg/kg-day**
Parameter					
Hematocrit (%)	45.2 ± 0.54[a]	48.2 ± 0.76	44.8 ± 0.74	40.3 ± 0.79[c]	40.4 ± 0.57[c]
Erythrocytes (10^6/μL)	8.78 ± 0.094	9.15 ± 0.132	8.59 ± 1.151	7.9 ± 0.146[c]	7.9 ± 0.105[c]
Leukocytes (10^3/μL)	5.4 ± 0.146	5.0 ± 0.264	5.4 ± 0.197	6.7 ± 0.363[c]	7.0 ± 0.732[b]
Lymphocytes (10^3/μL)	3.96 ± 0.256	3.82 ± 0.219	4.12 ± 0.200	5.02 ± 0.240[b]	5.57 ± 0.510[b]
Monocytes (10^3/μL)	0.13 ± 0.023	0.08 ± 0.022	0.12 ± 0.018	0.15 ± 0.030	0.30 ± 0.064[b]
BUN (mg/dL)	17.9 ± 0.62	18.5 ± 0.81	18.8 ± 0.80	20.4 ± 0.99	25.0 ± 3.08[b]
LDH (IU/L)	590 ± 49.47	762 ± 59.90[b]	663 ± 52.99	1018 ± 36.62[c]	623 ± 53.88
SDH (IU/L)	8.7 ± 0.616	13.8 ± 0.854[c]	26.5 ± 4.145[c]	32.7 ± 2.511[c]	14.3 ± 1.498[c]
ALT (mg/dL)	40 ± 2.46	33 ± 1.42	47 ± 5.99	54 ± 3.89[b]	43 ± 5.85
T4 (μg/dL)	4.73 ± 0.178	3.06 ± 0.158[c]	2.98 ± 0.181[c]	3.06 ± 0.136[c]	2.80 ± 0.163[c]
Females	**0 mg/kg-day**	**18 mg/kg-day**	**27 mg/kg-day**	**50 mg/kg-day**	**100 mg/kg-day**
Parameter					
Hematocrit (%)	48.3 ± 0.68	46.1 ± 0.69	44.6 ± 0.72[c]	41.4 ± 0.95[c]	37.6 ± 0.78[c]
Hemoglobin (g/dL)	16.3 ± 0.16	16.0 ± 0.13	15.8 ± 0.19	15.8 ± 0.21	15.4 ± 0.42[b]
Erythrocytes (10^6/μL)	8.86 ± 0.098	8.06 ± 0.262[c]	8.13 ± 0.131[c]	7.58 ± 0.175[c]	7.07 ± 0.173[c]
Leukocytes (10^3/μL)	4.3 ± 0.152	4.2 ± 0.292	4.9 ± 0.376	5.7 ± 0.283[c]	5.9 ± 0.658[b]
Lymphocytes (10^3/μL)	3.25 ± 0.116	3.64 ± 0.284	4.11 ± 0.334	4.50 ± 0.186[c]	4.86 ± 0.409[c]
SDH (IU/L)	5.5 ± 0.654	9.4 ± 0.581[c]	16.0 ± 2.708[c]	14.8 ± 1.504[c]	13.0 ± 1.612[c]
ALT (mg/dL)	30 ± 1.4	27 ± 1.28	30 ± 2.08	37 ± 2.74	51 ± 7.86[c]
T3 (ng/dL	102.42 ± 3.11	72.58 ± 4.48[c]	69.58 ± 5.26[c]	55.24 ± 3.50[c]	47.43 ± 5.60[c]
T4 (μg/dL)	2.50 ± 0.091	1.87 ± 0.092[c]	1.77 ± 0.131[c]	1.69 ± 0.087[c]	1.95 ± 0.198[c]

[a] mean ± standard error for groups of 10 animals.
[b] significantly different ($p \leq 0.05$) from control group by Dunn's or Shirley's test
[c] $p \leq 0.01$

Gross necropsy revealed paucity of body fat and reddening of the glandular mucosa of the stomach of treated rats (dose groups not specified) (NTP, 1991a). Histopathological examination showed lesions in the liver (hepatocyte necrosis and brown pigmentation within sinusoidal lining cells) and kidneys (nephropathy, karyomegaly of renal tubule epithelial cells); atrophy of the

thymus, spleen, mandibular and mesenteric lymph nodes and bone marrow; pancreatic acinar degeneration; and, in males, immature sperm forms in the testis and epididymis. The incidences and/or severity (severity scores provided for renal lesions only) of all of these lesions were dose-related (see Table 2). Although most of these effects were seen only in the 2000 and 4000 ppm dose groups, liver and kidney lesions were seen in the lower-dose groups as well. The most sensitive effect, pigmentation of liver sinusoid lining cells, was significantly increased in females of all dose groups. The low dose of 300 ppm (16 mg/kg-day in males and 18 mg/kg-day in females) in this 13-week rat study is a LOAEL for liver histopathology (pigmentation), serum chemistry changes (increased SDH, decreased T3 and T4), hematological effects (decreased hematocrit and RBC count), decreases in water intake and body weight, and clinical signs.

In the NTP (1991a) chronic study, 6-week old male and female F344/N rats were exposed to 3,3'-dimethylbenzidine dihydrochloride in drinking water at concentrations of 0, 30, 70 or 150 ppm (NTP, 1991a). Although originally intended to be a 2-year study with interim sacrifices at 9 and 14 months, the study was instead terminated at 14 months due to high mortality among the treated rats. The study included a total of 70 rats/sex in the control group, 45/sex at 30 ppm, 75/sex at 70 ppm and 70/sex at 150 ppm. Of these, 10/sex in the control and 150 ppm groups were used for the scheduled sacrifice at 9 months. Time weighted average (TWA) doses of 3,3'-dimethylbenzidine are estimated as 0, 1.3, 3.0 and 8.3 mg/kg-day in males and 0, 2.2, 5.1 and 9.6 mg/kg-day in females, in the control, low-, mid- and high-dose groups, respectively (dose estimates reported by NTP were adjusted for differences in molecular weight between 3,3'-dimethylbenzidine and 3,3'-dimethylbenzidine dihydrochloride). The animals were observed twice daily. Body weights were recorded initially, once weekly for 14 weeks, at week 17 and once per month thereafter. Feed consumption was measured one week/month, and water consumption was measured one week per month in 3-day and 4-day segments. Clinical observations were made at body weight determinations. Necropsy and complete histopathology examination were performed on all animals. Organ weights (liver, kidney, brain), hematology, clinical chemistry (including liver enzymes and thyroid hormones) and urinalysis were assessed only in rats sacrificed at 9 months.

Only rats from the control and high-dose (150 ppm) groups were examined in the scheduled sacrifice at 9 months (NTP, 1991a). In high-dose males and females sacrificed at 9 months, body weight was decreased 17-20% compared to controls. Both absolute and relative liver weights were significantly increased about 2-fold in the high-dose rats of both sexes. Smaller, but statistically significant, increases were seen in absolute and relative kidney weight in both sexes. Hematology results showed significant decreases in hematocrit, hemoglobin and red blood cell count in high-dose males and females (Table 3). Serum chemistry findings suggested effects on the liver (significant increases in SDH and ALT in high-dose males and females), kidney (significant increases in BUN and creatinine in high-dose males) and thyroid (significant decreases in T4 and increases in TSH in high-dose males and females). Urine volume was significantly and markedly decreased in high-dose groups of both sexes, with corresponding increases in urine osmolality, specific gravity, protein concentration and creatinine concentration.

Table 2. Histological Lesions in Rats in 13-Week Drinking Water Studies of 3,3'-Dimethylbenzidine Dihydrochloride (NTP, 1991a)

	Male							Female					
Dose (mg/kg-day)	**0**	**16**	**22**	**44**	**86**	**144**		**0**	**18**	**27**	**50**	**100**	**266**
LIVER													
hepatocyte necrosis	0/10	0/10	0/10	0/10	7/10[g]	3/10		0/10	1/10	6/10[g]	4/10[f]	7/10[g]	7/9[g]
pigment	0/10	1/10	0/10	0/10	10/10[g]	9/10[g]		0/10	10/10[g]	10/10[g]	10/10[g]	9/10[g]	8/9[g]
KIDNEY													
nephropathy	10/10 (1.1)[a]	_[b]	10/10 (1.0)	10/10 (1.6)	10/10 (2.6)	10/10 (2.6)		2/10 (1.0)	5/10 (1.0)	10/10[g] (1.0)	10/10[g] (1.0)	10/10[g] (2.2)	7/9[g] (2.1)
karyomegaly[c]								0/10	0/10	0/10	7/10[g]	9/10[g]	0/9
THYMUS													
atrophy	0/10	_	_	0/10	5/6[g]	9/9[g]		0/10	_	_	2/10	7/8[g]	5/5[g]
SPLEEN													
atrophy	0/10	_	_	0/10	5/10[g]	10/10[g]		0/10	_	_	0/10	4/10[f]	9/9[g]
MANDIBULAR LYMPH NODE													
atrophy	0/10	_	_	0/10	7/10[g]	10/10[g]		1/10	_	_	0/10	5/10	7/7[g]
MESENTERIC LYMPH NODE													
atrophy	0/10	_	_	0/10	1/10	2/9		0/10	_	_	0/10	4/10[f]	6/7[g]
BONE MARROW													
hypocellularity (atrophy)	0/10	_	_	0/10	8/10[g]	10/10[g]		0/10	_	_	0/10	10/10[g]	9/9[g]
PANCREAS													
degeneration[d]	0/10	_	_	0/10	4/10[f]	10/10[g]		0/10	_	_	_	2/10	8/9[g]
TESTES													
immature sperm	0/10	_	_	1/10	3/10	7/10[g]		NA[e]	NA	NA	NA	NA	NA

[a] values in parentheses are average severity grades for affected animals; 1=minimal, 2=slight, 3=moderate
[b] organ not examined in animals at this dose level
[c] terminology preferred by Pathology Working Group for the lesion diagnosed as megalocytosis by the laboratory pathologist
[d] terminology preferred by Pathology Working Group for the lesion diagnosed as acinar hypertrophy by the laboratory pathologist
[e] not applicable
[f] significantly different ($p \leq 0.05$) from the control group by Fisher exact test
[g] $p \leq 0.01$

Table 3. Significant Hematology, Clinical Chemistry and Urinalysis Findings in Rats Treated with 3,3'-Dimethylbenzidine Dihydrochloride in Drinking Water for 9 Months (NTP, 1991a)

	Males		Females	
Parameter	**0 mg/kg-day**	**8.3 mg/kg-day**	**0 mg/kg-day**	**9.6 mg/kg-day**
Hematology				
Hematocrit (%)	43.9 ± 0.71[a]	35.5 ± 1.06[d]	45.6 ± 0.72	34.5 ± 0.51[d]
Hemoglobin (g/dL)	16.6 ± 0.16	15.1 ± 0.34[d]	16.1 ± 0.12	14.7 ± 0.33[d]
Erythrocytes (10^6/µL)	8.61 ± 0.13	6.90 ± 0.22[d]	8.26 ± 0.11	6.10 ± 0.08[d]
Leukocytes (10^3/µL)	8.0 ± 0.21	10.0 ± 0.64[d]	4.4 ± 0.39	7.2 ± 0.72[d]
MCH (pg) [b]	19.3 ± 0.20	22.0 ± 0.40[d]	19.5 ± 0.27	24.2 ± 0.39[d]
MCHC (%)	37.6 ± 0.42	42.7 ± 0.73[d]	35.4 ± 0.55	42.8 ± 0.79[d]
MCV (μ^3)	51.0 ± 0.18	51.5 ± 0.32	55.1 ± 0.23	56.4 ± 0.19[d]
Serum Chemistry				
BUN (mg/dL)	18.7 ± 1.17	28.6 ± 5.33[c]	16.9 ± 0.43	14.9 ± 1.21[d]
Creatinine (mg/dL)	0.69 ± 0.05	1.00 ± 0.13[c]	0.64 ± 0.02	0.65 ± 0.02
Serum glucose (mg/dL)	147 ± 5.6	150 ± 5.8	120 ± 3.2	190 ± 18.1[d]
ALT (mg/dL)	73.3 ± 9.87	85.1 ± 4.78[c]	30.2 ± 3.70	98.6 ± 42.5[d]
SDH (IU/L)	14.8 ± 1.90	31.1 ± 3.86[d]	7.1 ± 1.43	71.4 ± 40.24[d]
T3 (ng/dL)	81.7 ± 8.45	114.0 ± 8.16[c]	104.8 ± 3.88	94.7 ± 4.67
T4 (µg/dL)	3.5 ± 0.26	2.2 ± 0.30[d]	3.44 ± 0.15	2.44 ± 0.20[d]
TSH (ng/mL)	337.6 ± 25.3	501.3 ± 54.5[c]	321.1 ± 23.4	486.2 ± 73.6[c]
Urinalysis				
Urine osmolality (mOSM/kg)	1350 ± 271	2730 ± 303[c]	1568 ± 246	2247 ± 164[c]
Osmolality ratio (urine/serum)	4.21 ± 0.86	8.44 ± 0.92[c]	4.96 ± 0.77	7.03 ± 0.53[c]
Urine creatinine (mg/dL)	163.7 ± 32.1	271.2 ± 26.0[c]	151.3 ± 21.3	245.4 ± 14.1[d]
Urine volume (mL/16 h)	7.3 ± 1.24	2.5 ± 0.53[d]	3.1 ± 0.46	0.9 ± 0.14[d]
Urine specific gravity	1.03 ± 0.00	1.06 ± 0.00[d]	1.04 ± 0.00	1.06 ± 0.00[d]
Urine protein (mg/dL)	51.00 ± 10.69	300.00 ± 0.00[d]	45.00 ± 12.58	300.00 ± 0.00[d]
Creatinine excretion rate (mg/16 h)	8.91 ± 0.33	5.57 ± 0.49[d]	3.71 ± 0.32	2.07 ± 0.32[d]

[a] mean ± standard error for groups of 10 animals.
[b] rank transformed data analyzed.
[c] significantly different ($p \leq 0.05$) from control group by Wilcoxon's test
[d] $p \leq 0.01$

Histopathological examination of high-dose rats at 9 months (NTP, 1991a) revealed nonneoplastic (hepatocellular hypertrophy, fatty change, cystic degeneration) and neoplastic (neoplastic nodules, hepatocellular carcinomas) effects in the liver, nonneoplastic effects in the spleen (atrophy in males and females) and kidney (increased incidence in females and severity in both sexes of nephropathy) and preneoplastic and neoplastic (malignant and benign) lesions in the lung, skin, oral cavity, preputial/clitoral gland, small intestine, large intestine and Zymbal's gland in both males and females (see Table 4). NTP commented that the short latency of neoplastic effects was unusual and shows the carcinogenic potency of 3,3'-dimethylbenzidine. Based on the non-neoplastic effects observed after 9 months of exposure, including histopathology in the liver, spleen and kidneys, and hematology, serum chemisty, and urinalysis changes, a subchronic LOAEL of 150 ppm (8.3-9.6 mg/kg-day) was identified for rats. A NOAEL was not identified because lower-dose groups were not evaluated at 9 months.

Table 4. Treatment-Related Lesions in Rats in the 9 Month Exposure Evaluation (NTP, 1991a)

	Male		Female	
Dose (mg/kg-day)	**0**	**8.3**	**0**	**9.6**
No. animals examined	10	10	10	10
LIVER				
hepatocellular carcinoma	0	2	0	0
neoplastic nodule[a]	0	5[e]	0	1
hepatocyte hypertrophy	0	10[f]	0	10[f]
basophilic focus	0	10[f]	0	0
fatty change[b]	1	10[f]	0	10[f]
cystic degeneration	0	7[f]	0	0
LUNG				
alveolar/bronchiolar carcinoma	0	1	0	0
alveolar/bronchiolar adenoma	0	0	0	1
alveolar epithelium hyperplasia	0	7[f]	0	1
SKIN				
basal cell carcinoma	0	1	0	0
sebaceous gland adenoma	0	1	0	0
squamous papilloma	0	0	0	1
ORAL CAVITY				
squamous cell carcinoma	0	0	0	1
PREPUTIAL/CLITORAL GLAND				
adenoma	0	1	0	2
carcinoma	0	2	0	3
SMALL INTESTINE				
mucinous adenocarcinoma	0	2	0	0
LARGE INTESTINE				
adenomatous polyp	0	3	0	0
ZYMBAL'S GLAND				
carcinoma	0	2	0	3
adenoma	0	1	0	2
squamous papilloma	0	3	0	1
squamous hyperplasia	0	3	0	1
focal hyperplasia	0	1	0	0
KIDNEY				
nephropathy[c]	10 (1.0)	10 (3.4)	3 (1.0)	10 (3.0)
SPLEEN				
lymphoid atrophy[d]	0	10[f]	0	7[f]

[a] term previously used for lesions currently classified as hepatocellular adenoma
[b] diagnosed as cytoplasmic vacuolization by the study pathologist
[c] values in parentheses are average severity grades; 1= minimal, 2= mild, 3= moderate, 4=marked
[d] diagnosed as lymphoid depletion by the study pathologist
[e] significantly different ($p \leq 0.05$) from the control group by Fisher exact test
[f] $p \leq 0.01$

In the main (14-month) part of the study, dose-related increases in mortality were seen in both sexes at all dose levels starting soon after the 9-month (36-week) mark (NTP, 1991a). All high-dose males were dead by week 55, at which time fewer than 25% of high-dose females remained alive. Survival to the end of the study at 14 months was significantly reduced in mid- and high-dose males and females and marginally reduced even in low-dose males (p=0.06) and females (p=0.05). The early deaths resulted from tumor formation. Body weight gain was reduced throughout the study in high-dose males and mid- and high-dose females. At study termination, body weights were reduced by about 30% in high-dose males and 20-25% in mid- and high-dose females, compared with controls. Water intake compared to controls was reduced 20-30% in high-dose males for the first 13 weeks of the study and in high-dose females for the first year of the study. In both groups, water consumption was higher than controls over the last few weeks of the study (increased 135% in males and 40% in females). Data on food intake were not reported.

Nonneoplastic lesions occurred in the liver in males and females from all treated groups (NTP, 1991a). As summarized in Table 5, significant increases were found for cystic degeneration and fatty change, as well as foci of cellular alteration (basophilic, eosinophilic and mixed-cell foci; possible precursors to neoplasms) and hematopoietic cell proliferation (presumably secondary to inflammation associated with neoplasms). The hepatotoxicity was considered by NTP to be of mild severity. The other noteworthy nonneoplastic effect was a dose-related increase in severity of nephropathy in males (incidence at or near 100% in all groups, minimal-to-mild severity in controls and low- and mid-dose males and moderate-to-marked in high-dose males) and in incidence and severity of nephropathy in females (incidence 78% in controls and close to 100% in all treated groups, severity minimal-to-mild in control and low-dose females and moderate in mid- and high-dose females). Based mainly on cystic degeneration and foci of cellular alteration in the liver, this study identified a LOAEL of 30 ppm (1.3 mg/kg-day in males and 2.2 mg/kg-day in females) for chronic toxicity in rats.

Table 5. Incidence of Nonneoplastic Liver Lesions in Rats in 14-month Exposure Evaluation (NTP, 1991a)

	Male				Female			
Dose (mg/kg-day)	**0**	**1.3**	**3.0**	**8.3**	**0**	**2.2**	**5.1**	**9.6**
cystic degeneration	0/60	24/45[a]	67/75[a]	51/60[a]	0/60	3/45	12/74[a]	11/60[a]
focal or multifocal necrosis	3/60	4/45	10/75	5/60	0/60	3/45	7/74[a]	2/60
fatty change	1/60	2/45	1/75	7/60[a]	0/60	0/45	4/74	2/60
basophilic focus	1/60	31/45[a]	54/75[a]	27/60[a]	0/60	13/45[a]	11/74[a]	3/60
eosinophilic focus	0/60	0/45	57/75[a]	53/60[a]	0/60	7/45[a]	57/74[a]	38/60[a]
mixed cell focus	0/60	37/45[a]	54/75[a]	30/60[a]	0/60	34/45[a]	49/74[a]	32/60[a]
hematopoietic cell proliferation	0/60	2/45	27/75[a]	15/60[a]	0/60	7/45[a]	19/74[a]	8/60[a]

[a] $p \leq 0.05$ by Fisher exact test, based on effective rates

Tissue masses were observed on the head, back and ventral posterior area of the rats after as few as 24 weeks (NTP, 1991a). These masses represented primarily Zymbal's gland tumors, epithelial skin tumors and preputial/clitoral gland tumors, respectively, all of which were found at significantly increased incidence in treated rats (see Table 6). Significant increases in tumor incidence also occurred in the liver, oral cavity, small and large intestine, mammary gland, lung, brain and mesothelium. While the tumor increases occurred primarily in the mid- and high-dose groups, the most sensitive tumors (basal cells in males, Zymbal's and clitoral gland tumors in females) were increased in the low-dose groups as well. NTP concluded there was clear evidence of carcinogenic activity of 3,3'-dimethylbenzidine in male and female F344/N rats in this study.

A previous cancer study in rats included a group of twenty 45-day-old Sprague-Dawley females that was administered 3,3'-dimethylbenzidine by gavage in sesame oil, in 10 doses of 50 mg/rat (total dose 500 mg/rat) at 3 day intervals over 30 days, followed by a 9-month observation period (Griswold et al., 1968). Using the reference body weight for female Sprague-Dawley rats of 0.204 kg (U.S. EPA, 1988b), it can be estimated that this dosing regimen provided an average dose of approximately 81 mg/kg-day of 3,3'-dimethylbenzidine over the 30 day dosing period. A negative control group (pooled from several experiments) comprised 140 young female Sprague-Dawley rats given the sesame oil vehicle alone. Endpoints evaluated included morbidity/mortality checks twice daily, body weight and inspection for abnormal tissue masses weekly, gross necropsy and histological examination of mammary tissue, intestinal tract, pituitary, liver, ovaries, adrenals and any gross lesions. Of the 20 rats exposed to 3,3'-dimethylbenzidine, 16 survived the 9 month observation period and were necropsied; 3 of these 16 rats (19%) had mammary lesions. The lesions identified by microscopic examination in these 3 rats were 4 carcinomas and 1 hyperplasia. In the control group, 132/140 rats survived to necropsy. Of these, 5 (4%) had mammary lesions (3 carcinomas, 1 fibroadenoma and 1 hyperplasia). The difference in the incidences of treated and control rats with mammary lesions is statistically significant (p=0.04; Fisher exact test conducted for this review). Identification of a NOAEL or LOAEL for systemic toxicity is precluded by inadequate reporting of non-neoplastic effects.

A cancer bioassay in mice was conducted by Schieferstein et al. (1989). There were 7 groups of 120 male and 120 female BALB/c mice that were exposed to 0, 5, 9, 18, 35, 70 or 140 ppm of 3,3'-dimethylbenzidine dihydrochloride in drinking water. Average doses of 3,3'-dimethylbenzidine (calculated from dose estimates for 3,3'-dimethylbenzidine dihydrochloride at specific time periods in the paper, and adjusted for differences in molecular weight between 3,3'-dimethylbenzidine and 3,3'-dimethylbenzidine dihydrochloride) were 0, 0.4, 0.8, 1.5, 2.8, 7.4 and 11 mg/kg-day in males and 0, 0.4, 0.7, 1.4, 2.6, 5.4 and 11 mg/kg-day in females. Sacrifices were scheduled for weeks 13, 26, 39, 52, 78 and 116 (24/sex/dose at each time, except 8/sex/dose at week 39 and 16/sex/dose at week 52). Endpoints evaluated included body weight and water consumption (averaged over three 4-week periods), survival and histopathological examination of 40 selected tissues. The probable cause of death or morbidity was determined for each dead or moribund animal. Body weight was similar to controls in males and females of all dose groups throughout the study. Water consumption was also similar to controls in all groups, except high-dose males, which had 10-20% lower water intake than controls throughout the study. There was no dose-related effect on mortality in male or female mice. The number of

Table 6. Treatment-Related Neoplastic Lesions in Rats in the 14-month Exposure Evaluation (NTP, 1991a)[a]

	Male				Female			
Dose (mg/kg-day)	**0**	**1.3**	**3.0**	**8.3**	**0**	**2.2**	**5.1**	**9.6**
SKIN								
keratoacanthoma	1/60	1/44	8/67[b]	5/27[b]	0/60[c]	0/45	0/75	1/60
sebaceous gland adenoma	0/60	0/44	7/72[b]	5/49[b]	0/60[c]	0/45	1/75	1/60
basal cell adenoma	0/60	10/44[b]	52/72[b]	29/45[b]	0/60	3/45	5/64[b]	5/41[b]
basal cell carcinoma	0/60	1/44	4/68	2/43	0/60	0/45	5/69[b]	4/46[b]
basal cell adenoma or carcinoma	0/60	11/44[b]	54/72[b]	30/45[b]	0/60	3/45	10/69[b]	9/46[b]
squamous cell papilloma	0/60	0/45	8/72[b]	15/55[b]	0/60	1/45	6/72[b]	5/55[b]
squamous cell carcinoma	0/60	2/45	10/74[b]	13/59[b]	0/60	2/45	4/64	7/41[b]
squamous cell papilloma or carcinoma	0/60	2/45	17/74[b]	27/59[b]	0/60	3/45	9/72[b]	12/55[b]
ZYMBAL'S GLAND								
adenoma	1/60	1/44	13/72[b]	16/54[b]	0/60	4/45[b]	11/72[b]	12/57[b]
carcinoma	0/60	2/45	21/74[b]	23/60[b]	0/60	2/45	22/74[b]	35/59[b]
adenoma or carcinoma	1/60	3/45	32/74[b]	36/60[b]	0/60	6/45[b]	32/74[b]	42/59[b]
PREPUTIAL GLAND (♂) or CLITORAL GLAND (♀)								
adenoma	2/60	4/44	4/72	8/49[b]	0/60	9/45[b]	32/73[b]	17/58[b]
carcinoma	0/60[c]	0/45	2/75	1/60	0/60	5/45[b]	11/72[b]	18/55[b]
adenoma or carcinoma	2/60	4/44	6/72	9/49[b]	0/60	14/45[b]	42/73[b]	32/58[b]
LIVER								
neoplastic nodule	0/60	0/44	29/72[b]	26/49[b]	0/60	0/45	7/58[b]	3/36[b]
hepatocellular carcinoma	0/60	0/45	12/72[b]	12/55[b]	0/60[c]	0/45	1/74	1/60
neoplastic nodule or hepatocellular carcinoma	0/60	0/45	35/72[b]	33/55[b]	0/60	0/45	7/58[b]	4/36[b]
ORAL CAVITY								
squamous cell papilloma	0/60[c]	0/45	3/75	2/60	0/60	3/45	7/73[b]	9/59[b]
squamous cell carcinoma	0/60[c]	0/45	1/75	3/60	0/60	1/45	2/64	4/41[b]
squamous cell papilloma or carcinoma	0/60	0/44	4/67	5/32[b]	0/60	3/45	9/73[b]	13/59[b]
SMALL INTESTINE								
adenomatous polyp	0/60[c]	0/45	1/75	1/60	0/60[c]	1/45	1/75	0/60
adenocarcinoma	0/60	0/45	3/74	8/59[b]	0/60	0/45	2/72	5/57[b]
adenomatous polyp or adenocarcinoma	0/60	0/45	4/74	8/59[b]	0/60	1/45	3/72	5/57[b]
LARGE INTESTINE								
adenomatous polyp	0/60	0/44	6/67[b]	9/38[b]	0/60	1/45	6/70[b]	4/46[b]
adenocarcinoma	0/60	0/45	0/67	7/36[b]	0/60[c]	0/45	1/75	1/60
adenomatous polyp or adenocarcinoma	0/60	0/45	6/67[b]	15/38[b]	0/60	1/45	7/70[b]	4/46[b]
MAMMARY GLAND	--	--	--	--				
Adenocarcinoma					0/60	1/45	3/71	6/51[b]
LUNG								
alveolar/bronchiolar adenoma	1/60	0/45	7/73	6/57[b]	1/60	1/45	3/63	3/41
alveolar/bronchiolar carcinoma	0/60[c]	0/45	1/75	0/60	0/60[c]	0/45	0/74	1/60
alveolar/bronchiolar adenoma or carcinoma	1/60	0/45	8/73[b]	6/57[b]	1/60	1/45	3/63	4/41
MESOTHELIUM (ALL ORGANS)					--	--	--	--
mesothelioma (benign/malignant)	0/60	0/45	3/67	4/38[b]				

[a] incidence based on effective rates (denominator is number of animals alive at first occurrence of tumor type in any dose group), except as noted
[b] $p \leq 0.05$ by Fisher exact test
[c] only overall rates reported by NTP and reproduced here

dead/moribund mice ranged from 17-25 in male groups and 15-21 in female groups (including controls). The primary cause of death was neoplasms in both males and females. In males, there was a statistically significant trend for increased death due to neoplasms with increased dose. Histopathological examination revealed that among male mice found dead or moribund there was a dose-related increase in the incidence of lung alveolar cell adenomas or adenocarcinomas (see Table 7). Pairwise comparisons showed a significant increase in the high-dose group and marginally significant increases in the 35- and 70-ppm groups ($0.05 < p < 0.1$). The incidence of lung tumors was not increased in male mice at scheduled sacrifices or in female mice. Although a number of skin neoplasms were found in treated mice, the low frequency and sporadic occurrence did not provide evidence of a treatment-related effect. The only reported information on nonneoplastic lesions was a slight increase in incidence of spleen erythropoiesis among dead or moribund females that was not clearly dose-related. Identification of a NOAEL or LOAEL for systemic toxicity is precluded by inadequate reporting of non-neoplastic effects.

A cancer bioassay in hamsters was conducted by Saffiotti et al. (1967). Groups of 30 male and 30 female Syrian golden hamsters were fed 0.1% 3,3'-dimethylbenzidine in the diet beginning at 10 weeks of age for the remainder of their life span. The researchers estimated that an average of 60 mg/week of 3,3'-dimethylbenzidine was consumed, corresponding to an approximate dose of 62 mg/kg-day using the average of the reference body weights for male and female hamsters (U.S. EPA, 1988b). A control group of unspecified size received only the powdered rat food diet for the duration of the study. Endpoints evaluated included food consumption measured weekly; animal observation and body weights recorded every two weeks; and survival. All animals were necropsied. The bladder, liver, kidney, adrenals and all tumors and gross lesions were examined for histopathology. No treatment-related effects were found.

As reported in a brief abstract, a similar study by the same researchers using 0.3% 3,3'-dimethylbenzidine in the diet (approximately 186 mg/kg-day), reported to be the highest tolerated level, was also negative for carcinogenicity in hamsters (Sellakumar et al., 1969). The only reported information on nonneoplastic lesions was a note that there was no bladder pathology in the study with 0.1% dietary 3,3'-dimethylbenzidine (Saffiotti et al., 1967). Identification of a NOAEL or LOAEL for systemic toxicity is precluded by inadequate reporting of non-neoplastic effects in both studies.

Given 200 mg of 3,3'-dimethylbenzidine by capsule daily for 8-9 months (total dose of 50 g/dog), 1/4 female mongrel dogs developed bladder cancer (papillary tumor and cystitis) 8 years later (Ferber, 1977). The average daily dose during the exposure period was estimated to be 20 mg/kg-day, assuming a reference dog body weight of 10.1 kg (U.S. EPA, 1988b). No tumors were observed in the other 3 dogs.

Inhalation Exposure

No information was located regarding effects of inhalation exposure to 3,3'-dimethylbenzidine in animals.

Table 7. Lung Alveolar Cell Adenomas or Adenocarcinomas Observed in BALB/c Mice Exposed to 3,3'-Dimethylbenzidine Dihydrochloride in Drinking Water (Schieferstein et al., 1989)

	Dose (mg/kg-day)						
Males	**0**	**0.4**	**0.8**	**1.5**	**2.8**	**7.4**	**11**
13 week sacrifice	0/24[a]	0/24	0/24	0/24	0/24	0/24	0/24
26 week sacrifice	0/23	1/24	1/24	0/23	0/23	0/24	0/24
39 week sacrifice	0/8	0/8	1/8	0/8	2/8	0/8	0/8
52 week sacrifice	1/15	3/16	1/14	5/14	2/15	4/16 (1)	2/16
78 week sacrifice	11/23	4/20	8/18	8/23 (2)	5/18	7/21	8/20
112 week sacrifice	3/10 (1)	5/10 (3)	0/4	6/10	3/8 (1)	4/7 (1)	4/7 (1)
Found dead or moribund	5/16 (2)	7/16 (2)	5/25 (2)	5/18 (2)	7/24 (6)	11/20 (5)	13/20 (10)[b]
Females	**0**	**0.4**	**0.7**	**1.4**	**2.6**	**5.4**	**11**
13 week sacrifice	0/24	0/24	0/24	1/24	0/24	0/24	0/24
26 week sacrifice	0/24	0/24	0/24	0/24	0/24	1/24	0/24
39 week sacrifice	0/8	0/8	1/8	0/8	0/8	1/8	0/8
52 week sacrifice	0/16	1/15	2/16	1/13	3/16	0/16	4/16 (1)
78 week sacrifice	4/21	1/23	8/20 (1)	5/21 (1)	4/20	2/21	5/18 (2)
112 week sacrifice	1/7	2/8	4/9	4/5 (2)	5/11 (3)	5/10	3/11 (1)
Found dead or moribund	7/19 (5)	4/17 (3)	3/19 (3)	4/20 (2)	5/17 (2)	4/15 (2)	4/18 (2)

[a] incidence reported for adenomas or adenocarcinomas combined; number of mice with adenocarcinomas shown in ( )
[b] $p \leq 0.05$

Other Studies

Carcinogenicity Studies (Parenteral Exposure)

As summarized by U.S. EPA (1987) and NTP (1991a), early cancer studies found that 3,3'-dimethylbenzidine was carcinogenic in rats when administered by chronic weekly subcutaneous injections or subcutaneous implantation (Pliss, 1963; Pliss and Zabezhinsky, 1970; Spitz et al., 1950). These studies were generally limited by small numbers of animals, lack of concurrent controls and use of toxic doses (NTP, 1991a). Tumors were induced in various body tissues distant from the site of administration, including Zymbal gland, mammary gland, preputial gland, forestomach, skin, liver, lungs, external auditory canal and/or hematopoietic system. Increased incidences of mammary and lung tumors also occurred in the offspring of mice injected subcutaneously during gestation (Golub et al., 1974).

Toxicity and Carcinogenicity of Related Compounds

There is *in vivo* evidence in humans, rats and dogs indicating that some 3,3'-dimethylbenzidine-based azo dyes are metabolized to 3,3'-dimethylbenzidine (ACGIH, 2001; Boeniger, 1978; NIOSH, 1981; NTP, 1991a). Rats treated with one of these dyes, C.I. Acid Red 114, in the drinking water for up to two years developed a similar array of tumors as found for 3,3'-dimethylbenzidine (NTP, 1991b). Trypan blue and Evans blue, two 3,3'-dimethylbenzidine-based dyes, were carcinogenic in rats when injected subcutaneously or intraperitoneally (IARC 1975a,b, 1987). 3,3'-Dimethylbenzidine-based dyes (trypan blue, Evans blue, benzopurpurin 4B) had no effect on testicular development in male offspring of pregnant mice treated with 1000 mg/kg-day doses by gavage on days 8-12 of gestation (Gray and Ostby, 1993). The offspring were examined 44-50 days after birth (post-puberty) and/or 86-87 days after birth (young adult).

Genotoxicity

The genotoxicity of 3,3'-dimethylbenzidine has been evaluated in a variety of test systems. Based on results of studies summarized by U.S. EPA (1987), NTP (1991a), and Chung et al. (2006), as well as more recent studies (Claxton et al., 2001; Oda, 2004), 3,3'-dimethylbenzidine is genotoxic in both bacteria and eukaryotes. Extensive testing in *Salmonella typhimurium* has shown that 3,3'-dimethylbenzidine produces reverse mutations in frameshift-sensitive strains (e.g., TA98 and TA1538) with metabolic activation; response was weak or negative in the absence of metabolic activation or in tester strains designed to detect base-pair substitutions (e.g., TA100 and TA1535; Reid et al., 1984) or that are sensitive to reactive oxygen species (e.g., TA102; Makena and Chung, 2007). Also in bacteria, 3,3'-dimethylbenzidine produced positive results in a growth differential test using repair-deficient and proficient strains of *Escherichia coli*. In eukaryotes, 3,3'-dimethylbenzidine produced positive results with or without metabolic activation in tests for forward mutation in mouse L5178Y lymphoma cells, DNA repair in isolated rat and hamster hepatocytes, unscheduled DNA synthesis in HeLa cells and sister chromatid exchange and chromosomal aberrations in cultured Chinese hamster ovary cells. 3,3'-Dimethylbenzidine also produced positive results in a cell transformation assay using Fischer rat embryo cells. *In vivo* assays in mice for micronucleus formation in bone marrow and inhibition of DNA synthesis in the testis also were positive. *In vivo* studies with *Drosophila* found that 3,3'-dimethylbenzidine induced sex-linked recessive lethal mutations when administered by feeding or injection, but did not induce reciprocal translocations.

Using the NIH 3T3 DNA transfection assay, Reynolds et al. (1990) analyzed tumors (both benign and malignant) from several tissues of control and treated rats from the NTP carcinogenicity bioassays for 3,3'-dimethylbenzadine dihydrochloride (NTP, 1991a) for the presence of activated *ras* oncogene (specifically H-*ras* or N-*ras*). While spontaneous tumors in control animals had a very low frequency of oncogene activation (1/38), 81% (13/16) of the tumors from 3,3'-dimethylbenzidine-treated animals[1] contained activated oncogenes. Southern blot analysis was used to identify the activated oncogenes as primarily H-*ras* oncogenes (12/13

[1] The researchers do not distinguish between tumors from animals treated with 3,3'-dimethylbenzadine dihydrochloride and those treated with C.I. Acid Red 114. They appear to have considered animals from both groups together as 3,3'-dimethylbenzidine exposed.

tumors) in the tumors from animals treated with 3,3'-dimethylbenzidine. Mutations at codons 12, 13 and 61 were identified in the altered H-*ras* oncogenes from these tumors.

Oda (2004) showed, using the SOS/umu assay system, that the human acetyltransferase encoded by the gene NAT1 was primarily responsible for activating 3,3'-dimethylbenzidine to a genotoxic intermediate. Strains of *S. typhimurium* overexpressing NAT1 (strain NM6001) and NAT2 (strain NM6002) were tested for induction of umuC gene expression, expressed as an increase in β-galactosidase activity in culture. The 3,3'-dimethylbenzidine treatment did not cause gene induction in the parent strain (NM6000), which lacked acetyltransferase activity; in contrast, dose-dependent induction was observed in the NM6001 strain. Gene induction in the NM6002 strain was much lower, indicating that the NAT1 enzyme was more important than NAT2 in the production of DNA damage.

Role of Metabolism in Carcinogenicity

Metabolism is believed to play an important role in the carcinogenic action of benzidines, many of which are known to be metabolized to DNA-reactive metabolites (Morgan et al. 1994). However, little information is available on the metabolism of 3,3'-dimethylbenzidine. Dieteren (1966) analyzed the urine of workers engaged in the manufacture of 3,3'-dimethylbenzidine (o-tolidine) and reported detection of diacetylated and hydroxylated metabolites, as well as the parent compound. None of the compounds detected in urine were quantified. Mongrel dogs given intraperitoneal doses of 70 mg/kg 3,3'-dimethylbenzidine excreted about 40% of the dose in the urine, with about 4% excreted as parent compound; the balance was reported to consist of "the conjugated form" without further identification (Sciarini and Meigs, 1961). Rats treated with 3,3'-dimethylbenzidine excreted N-acetyl 3,3'-dimethylbenzidine, N,N'-diacetyl-dimethylbenzidine and parent compound in the urine (Tanaka et al. 1982, as cited in NTP, 1991a).

Metabolism of 3,3'-dimethylbenzidine may be similar to that of the related compound benzidine. ATSDR (2001) and Morgan et al. (1994) reviewed the available data on metabolism of benzidine, and ATSDR (2001) provides a thorough discussion of the role of metabolism in the carcinogenicity of benzidine. Although the metabolism of benzidine is quite complex, the three major reactions are N-acetylation, N-oxidation and N-glucuronidation. Each of these pathways leads to reactive metabolites that interact with DNA (ATSDR, 2001; Morgan et al., 1994). A number of known metabolites of benzidine are mutagenic, more so than the parent compound; mono- and diacetylated metabolites have been shown to be about 10 times as mutagenic as benzidine, while N-hydroxy-N,N'-diacetylbenzidine glucuronide is about 100 times as mutagenic (after release of the glucuronide moiety) (Morgan et al., 1994). N-acetylated benzidine DNA adducts have been observed in rodents and in humans (ATSDR, 2001).

Species differences in the metabolism of benzidine have been observed both *in vivo* and *in vitro* (ATSDR, 2001). For example, studies using liver slices showed that dog liver does not acetylate benzidine, in contrast to both rat and human liver. Rat liver produced more N,N'-diacetylbenzidine, while human liver produced more N-acetylbenzidine. Both *in vivo* and *in vitro* data indicate that dogs produce glucuronidated metabolites of benzidine (ATSDR, 2001). Species differences in metabolic pathways are believed to contribute to tumor site specificity that

differs between species (e.g., benzidine is associated with liver tumors in rats but with bladder tumors in dogs and humans) (ATSDR, 2001). It is not known whether these differences in metabolism and tumor site specificity also extend to 3,3'-dimethylbenzidine, as data on its metabolism is sparse and there are no data with which to clearly identify human tissues susceptible to 3,3'-dimethylbenzidine carcinogenesis.

FEASIBILITY OF DERIVING PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR 3,3'-DIMETHYLBENZIDINE

No data were located regarding nonneoplastic health effects of 3,3'-dimethylbenzidine in humans. Pertinent data on nonneoplastic effects in animals are available only from the NTP (1991a) subchronic and chronic studies of 3,3'-dimethylbenzidine dihydrochloride in rats.

Subchronic RfD

In the subchronic NTP (1991a) study, rats were exposed to 0, 300, 500, 1000, 2000 or 4000 ppm in drinking water for 13 weeks. The low dose of 300 ppm (16 mg/kg-day in males and 18 mg/kg-day in females) was a LOAEL for effects on the liver indicated by histopathology (brown pigmentation in sinusoidal lining cells) and serum chemistry changes (increased SDH), thyroid (indicated by decreases in serum T3 and T4) and blood (decreases in red blood cell count and hematocrit). General toxicity at this dose was also indicated by decreases in water intake (20-43%) and body weight (9-12% lower than controls) and the presence of clinical signs of toxicity (thinness, urine stains, red nasal exudate), although the extent of occurrence of clinical signs at this dose was not reported. At higher doses, there were also effects on the kidneys (nephropathy), spleen (atrophy), bone marrow (atrophy), thymus (atrophy), lymph nodes (atrophy), pancreas (degeneration) and testis (immature sperm). Early deaths occurred at 2000 ppm (86-100 mg/kg-day) and above.

The chronic NTP (1991a) study included interim sacrifices in rats exposed to 0 or 150 ppm in drinking water (8.3 mg/kg-day in males and 9.6 mg/kg-day in females) for 9 months, which represents a subchronic duration. Effects were similar to those observed in the 13-week study: hepatotoxicity indicated by histopathology (hepatocellular hypertrophy, fatty change, cystic degeneration) and serum chemistry (increased SDH and ALT), nephrotoxicity indicated by histopathology (nephropathy) and serum chemistry (increased BUN and creatinine), hypothyroid indicated by changes in serum hormone levels (decreased T4 and increased TSH), anemia (decreased red blood cell count, hemoglobin and hematocrit), splenic atrophy and reduced body weight (17-20% lower than controls). In addition, neoplastic effects were found in both males and females, including malignant and benign tumors in the liver, lung, skin, oral cavity, preputial/clitoral gland, intestines and Zymbal's gland.

The NTP (1991a) studies of 3,3'-dimethylbenzidine do not provide a suitable basis for deriving a subchronic p-RfD, because sufficiently low doses were not employed. The low dose in the 13-week study (16-18 mg/kg-day) was a LOAEL that produced a variety of toxic effects, including overt generalized effects on body weight and clinical signs of toxicity. The 9-month sacrifice in the chronic study found many of the same toxic effects as the 13-week study,

including a large reduction in body weight, at half the dose of the 13-week study (8.3-9.6 mg/kg-day). These data suggest that a threshold for nonneoplastic effects of 3,3'-dimethylbenzidine could occur at far lower doses than were tested for subchronic exposure. Therefore, it would not be appropriate to base a risk assessment value for non-carcinogenic end points on these data.

Chronic RfD

In the chronic NTP (1991a) study, rats were exposed to 0, 30, 70 or 150 ppm in drinking water for 14 months. This study was terminated at 14 months due to high mortality in all treated groups associated with tumor formation. Significant increases in tumor incidence were found for skin, Zymbal's gland, preputial/clitoral gland, liver, oral cavity, small and large intestine, mammary gland, lung and mesothelium, primarily in the 70 and 150 ppm groups, but also in the 30 ppm males (basal cell adenoma) and females (Zymbal's gland adenoma, clitoral gland adenoma and carcinoma). Nonneoplastic effects unrelated to tumor formation occurred in the liver (cystic degeneration) and kidney (nephropathy). The low dose of 30 ppm (1.3 mg/kg-day in males and 2.2 mg/kg-day in females) was a LOAEL for both effects.

The 1.3-2.2 mg/kg-day chronic LOAEL did not clearly approach a threshold for nonneoplastic effects due to a high incidence of liver cystic degeneration in males (53% compared to 0% in controls) at the lowest tested dose in the study. Additionally, tumors and related mortality occurred at this dose. Therefore, because the available chronic data do not identify a NOAEL and suggest that a threshold for nonneoplastic effects could occur at far lower doses than were tested, it is not appropriate to derive a chronic p-RfD for 3,3'-dimethylbenzidine.

FEASIBILITY OF DERIVING PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR 3,3'-DIMETHYLBENZIDINE

Derivation of RfC values for 3,3'-dimethylbenzidine is precluded by a lack of inhalation toxicity data.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR 3,3'-DIMETHYLBENZIDINE

Weight-of-Evidence Descriptor

Information on the carcinogenicity of 3,3'-dimethylbenzidine in humans mainly consists of studies of occupational exposure that included confounding exposure to benzidine. Risks for urinary tract cancer were increased in workers with mixed exposure to 3,3'-dimethylbenzidine and benzidine (ACGIH, 2001), but because benzidine is a known human bladder carcinogen (IARC, 1972b, 1982, 1987), the data are insufficient for determining whether 3,3'-dimethylbenzidine alone is carcinogenic. In arylamine production workers exposed to 3,3'-dimethylbenzidine and other benzidine congeners, but not to benzidine, Quellet-Hellstrom and Rench (1996) found a significant exposure-related increase in bladder cancer. No conclusions

regarding the carcinogenicity of 3,3'-dimethylbenzidine alone can be drawn from this study due to the mixed benzidine congener exposure and because all bladder cancer cases were current or ex-smokers (smoking is a known risk factor for bladder cancer that probably contributed to the observed cancer risk).

There is no indication that 3,3'-dimethylbenzidine is metabolized to benzidine (IARC, 1972a; NTP, 1991a), but concern for the carcinogenicity of 3,3'-dimethylbenzidine in humans is raised by its structural similarity to benzidine, as well as human and animal data indicating that some carcinogenic 3,3'-dimethylbenzidine-based azo dyes are metabolized to 3,3'-dimethylbenzidine (ACGIH, 2001; Boeniger, 1978; IARC, 1975a,b; NIOSH, 1981; NTP, 1991a, 1991b).

There is sufficient evidence of 3,3'-dimethylbenzidine carcinogenicity in experimental animals based on results of oral bioassays in rats and mice (Griswold et al., 1968; NTP, 1991a; Schieferstein et al., 1989). In the NTP (1991a) study, rats that were exposed to 3,3'-dimethylbenzidine dihydrochloride in drinking water showed development of malignant and benign tumors in the liver, lung, skin, oral cavity, preputial/clitoral gland, intestines and Zymbal's gland after only 9 months, with no tumors of any type in controls at 9 months. This was a planned 24-month study that was terminated at 14 months due to high tumor-related mortality. At 14 months, significant increases in tumor incidence were found for skin, Zymbal's gland, preputial/clitoral gland, liver, oral cavity, small and large intestine, mammary gland, lung, and mesothelium. NTP (1991a) concluded that this bioassay presented clear evidence of carcinogenic activity in male and female F344/N rats. Rats (Sprague-Dawley) that were exposed to 3,3'-dimethylbenzidine by gavage on 3 days/week for 30 days and subsequently observed for 9 months, had a significantly increased incidence of mammary tumors (Griswold et al., 1968). A two-year drinking water study of 3,3'-dimethylbenzidine dihydrochloride in mice found an increased incidence of lung alveolar cell adenomas or adenocarcinomas in males found dead or moribund during the study, although not in males killed at scheduled times or in females (Schieferstein et al., 1989). Lifetime dietary studies of 3,3'-dimethylbenzidine in hamsters reported no evidence of carcinogenicity, but were limited by small group sizes, use of single dose levels, examination of only a few tissues and insufficient reporting (Saffiotti et al., 1967; Sellakumar et al., 1969). Supporting evidence for the carcinogencity of 3,3'-dimethylbenzidine in animals is provided by limited subcutaneous studies in rats and mice that found systemic tumor induction in a wide variety of target tissues (Golub et al., 1974; Pliss, 1963; Pliss and Zabezhinsky, 1970; Spitz et al., 1950) and oral studies of a 3,3'-dimethylbenzidine-derived dye that found similar results (NTP, 1991b).

The weight of the evidence is adequate to demonstrate carcinogenic potential to humans. 3,3'-Dimethylbenzidine has tested positive for carcinogenicity by the oral route in animal studies in more than one species, sex, strain and site, and these findings are supported by results of subcutaneous injection studies of 3,3'-dimethylbenzidine and oral studies of a 3,3'-dimethylbenzidine-derived dye. Additionally, 3,3'-dimethylbenzidine is structurally similar to (is a congener of) benzidine, a known human carcinogen. In accordance with the U.S. EPA (2005a) cancer guidelines, the weight-of-evidence indicates that 3,3'-dimethylbenzidine is *likely to be carcinogenic to humans*.

Mode of Action Discussion

The U.S. EPA (2005a) Guidelines for Carcinogen Risk Assessment defines mode of action as a sequence of key events and processes starting with the 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 include mutagenic, mitogenic, anti-apoptotic (inhibition of programmed cell death), cytotoxic with reparative cell proliferation and immunologic suppression.

There are no data on the carcinogenicity of 3,3'-dimethylbenzidine in humans. Available evidence in laboratory animals indicates that oral exposure to 3,3'-dimethylbenzidine leads to tumors in a wide variety of tissues including skin, Zymbal's gland, preputial/clitoral gland, liver, oral cavity, small and large intestine, mammary gland, lung and mesothelium. A weight of evidence evaluation supports a determination that 3,3'-dimethylbenzidine is carcinogenic by a mutagenic mode of action. Determination of the mode of action of carcinogens is addressed in Section 5 of the 2005 Cancer Supplementary Guidance (U.S. EPA, 2005b) as follows: "Determinations of chemicals that are operating by a mutagenic mode of action entails evaluation of test results for genotoxic endpoints, metabolic profiles, physiochemical properties, and structure-activity relationships." Evaluation of each of these elements is discussed below in the mode of action discussion.

Mutagenic Mode of Action

Key Events — The proposed mode of action for 3,3'-dimethylbenzidine carcinogenicity consists of the following key events: 1) metabolism to DNA-reactive metabolites, 2) binding to DNA, 3) mutation of *ras* and possibly other oncogenes, and 4) proliferation of initiated cells. Most of the support for this proposed mode of action is derived from the study published by Reynolds et al. (1990), coupled with data from *in vitro* genotoxicity testing and from studies of the related compounds, 3,3'-dimethoxybenzidine and benzidine. Reynolds et al. (1990) showed codon-specific mutations, primarily in the H- *ras* oncogene, in a wide variety of rat tumors induced by 3,3'-dimethylbenzidine[2]. In addition, a large majority of both benign and malignant tumors (81%) from rats treated with 3,3'-dimethylbenzidine contained activated H- *ras* or N- *ras* oncogenes, while only 1 of 38 spontaneous tumors from control rats contained activated oncogenes. The much higher incidence of activated *ras* oncogenes and mutational specificity at codons 12, 13 and 61 provide support for a genotoxic mode of action for the benign and malignant tumors observed in rats treated with 3,3'-dimethylbenzidine.

Additional support for a mutagenic mode of action is provided by *in vitro* genotoxicity tests, as described in more detail above in the Genotoxicity section. 3,3'-Dimethylbenzidine has consistently given positive results in genotoxicity testing in bacteria and eukaryotes. In bacteria, metabolic activation is required to obtain positive mutagenicity results, but in mammalian cells,

[2] Tumors were from rats treated with 3,3'-dimethylbenzidine dihydrochloride or C.I. Acid Red 114, a dye derived from 3,3'-dimethylbenzidine. Available data indicate that benzidine dyes are cleaved via azo reduction by intestinal flora to release the parent benzidine molecule (in this case, 3,3'-dimethylbenzidine), which is then taken up by the gastrointestinal tract. Thus, exposure to the dye is believed to result in risks comparable to those associated with exposure to the parent benzidine compound (Morgan et al., 1994).

3,3'-dimethylbenzidine produced positive results with or without metabolic activation in tests for gene mutation, DNA repair, unscheduled DNA synthesis, sister chromatid exchange and chromosomal aberrations (U.S. EPA, 1987; NTP, 1991a). *In vivo* assays for micronucleus formation and inhibition of DNA synthesis in mice and mutations in *Drosophila* were also positive (U.S. EPA, 1987; NTP, 1991a).

There is also evidence for a mutagenic mode of action for the structurally similar compound benzidine. A number of known metabolites of benzidine are mutagenic, more so than the parent compound: mono- and diacetylated metabolites have been shown to be about 10 times as mutagenic as benzidine, while N-hydroxy-N,N'-diacetylbenzidine glucuronide is about 100 times as mutagenic (after release of the glucuronide moiety) (Morgan et al., 1994). DNA adducts with N-acetylated benzidine derivatives have been observed in both rodents and in humans (ATSDR, 2001).

Strength, Consistency, Specificity of Association — Data from Reynolds et al. (1990) show codon-specific mutations in the *ras* oncogene in a large majority of the tumors from animals treated with 3,3'-dimethylbenzidine or its derivative dye C.I. Acid Red 114 in NTP (1991a, 199b) cancer bioassays. The high incidence of these mutations (in 12/13 tumors with activated *ras* oncogene) and the high incidence of *ras* gene activation (13/16 tumors) in tumors from treated animals, in contrast with the very low incidence of oncogene activation in spontaneous tumors from control animals (1/38) provides support for the role of mutation in the *ras* oncogene as a precursor to tumor formation in rats treated with 3,3'-dimethylbenzidine. A similar finding of *ras* oncogene activation (21/34 tumors) and mutations in these three codons (19/21 tumors with activated *ras* oncogene) was observed in tumors from rats treated with 3,3'-dimethoxybenzidine or its derivative dye C.I. Direct Blue 15 (Reynolds et al., 1990), providing additional support for the importance of *ras* gene activation via mutation in the tumorigenicity of these compounds.

Additional evidence for the association between mutagenesis and tumor formation results from the observation that 3,3'-dimethylbenzidine exposure caused tumors in a wide variety of rat tissues, including skin, Zymbal's gland, preputial/clitoral gland, liver, oral cavity, small and large intestine, mammary gland, lung, and mesothelium, many of which are sites at which tumors are infrequently found in F344 rats (NTP, 1991a) and also caused tumors in exposed mice (Schieferstein et al., 1989). Induction of tumors at multiple sites and in different species is characteristic of carcinogens acting via mutagenesis (U.S. EPA, 2005a). In addition, the short latency-to-tumor formation (a high incidence was observed in rats after only 9 months, while no tumors were observed in controls at this time; NTP, 1991a) is suggestive of a mutagenic effect.

Dose-Response Concordance — No data with which to evaluate the dose-response concordance between mutagenesis and tumor formation after 3,3'-dimethylbenzidine exposure are available. Reynolds et al. (1990) did not report the dose distribution of mutations or activated oncogenes in the tumors evaluated. Furthermore, the high incidence of tumors in all dose groups of the NTP (1991a) rat bioassay may have obscured a dose-response relationship for mutation and/or oncogene activation.

Temporal Relationships — In rats exposed to 3,3'-dimethylbenzidine, tumors were observed in a significant fraction of the exposed animals after only 9 months of exposure (no tumors were observed in controls at this time), and the bioassay was terminated at 14 months due to high tumor-related mortality (NTP, 1991a). There are no data from any studies on the incidence or types of mutations induced prior to tumor formation in tissues subsequently developing tumors; thus, the temporal relationship between mutagenesis and the development of tumors cannot be assessed.

Biological Plausibility and Coherence — The biological plausibility of a mutagenic mode of action for 3,3'-dimethylbenzidine is supported by evidence of mutations leading to *ras* oncogene activation in tumors from rats treated with 3,3'-dimethylbenzidine (Reynolds et al., 1990). This study provides the critical link between *in vitro* evidence for mutagenicity and tumor formation in a specific species. Similar findings with the related compound 3,3'-dimethoxybenzidine and the lack of oncogene activation in spontaneous tumors from untreated rats (Reynolds et al., 1990) augment the database supporting this particular mode of action for benzidine congeners. Evidence for a variety of mutagenic metabolites observed both *in vitro* and *in vivo* after exposure to the structurally similar carcinogen benzidine supports the plausibility of this mode of action. In addition, in a study of known carcinogenic aryl amines that included benzidine, the extent and persistence of measured DNA adducts in Beagles given oral doses of a series of these compounds correlated with their potency to form bladder tumors (Beland et al., 1983), providing further support for the relationship between mutagenesis and tumor formation for aryl amines in general.

Early-Life Susceptibility — According to the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (Supplemental Guidance) those exposed to carcinogens with a mutagenic mode of action are assumed to have increased early-life susceptibility. Data on 3,3'-dimethylbenzidine are not sufficient to develop separate risk estimates for childhood exposure. There are no data comparing the tumorigenicity of 3,3'-dimethylbenzidine after exposure during early life with tumorigenicity after exposure during adulthood. Exposure to 3,3'-dimethylbenzidine was commenced at about 6 weeks of age and continued through adulthood in the chronic bioassays in rats (NTP, 1991a; Griswold et al., 1968); the age at which exposure was commenced in the one bioassay in mice was not clearly reported, but was apparently after 4 weeks of age (Scheiferstein et al., 1989). Limited information from the structurally-related compound benzidine provides some toxicokinetic plausibility for age-dependent differences in susceptibility to benzidine carcinogenesis. Human expression of N-acetyl transferase 2 (NAT2) and glucuronosyl transferase (UGT), two enzymes involved in benzidine metabolism, varies developmentally, with adult activity being reached at 1-3 years of age (NAT2) and 6-18 months (UGT). However, the importance of these two enzymes in the metabolic pathways for 3,3'-dimethylbenzidine and in the formation of critical DNA-reactive metabolites after exposure to this congener is not known. Oda (2004) found that the NAT1 enzyme was more important than NAT2 in the production of DNA damage from 3,3'-dimethylbenzidine in the SOS/umu assay system.

Conclusions — A weight of evidence evaluation supports a mutagenic mode of action for 3,3'-dimethylbenzidine tumorigenicity. *In vitro* studies provide evidence that 3,3'-dimethylbenzidine is capable of eliciting genotoxic effects in both bacteria and eukaryotic cells.

More importantly, analysis of tumors from rats exposed to 3,3-dimethylbenzidine showed codon-specific mutations in *ras* oncogenes in a large majority of tumors, while only one spontaneous tumor from a control rat contained an activated *ras* oncogene (Reynolds et al., 1990). Finally, the finding of tumors at multiple sites and in multiple species, as well as the brief latency to tumor formation, provide additional support for a mutagenic mode of action for 3,3'-dimethylbenzidine. Because a mutagenic mode of action for carcinogenicity is proposed for 3,3'-dimethylbenzidine, a linear approach would be appropriate to extrapolate from the point of departure in the derivation of the oral slope factor (U.S. EPA, 2005a). There are no data with which to develop separate estimates of risk from childhood exposure to 3,3'-dimethylbenzidine.

An important uncertainty in the quantitative cancer assessment for 3,3'-dimethylbenzidine stems from the limited information on its metabolism. Although metabolic activation may be a critical step in the cancer mode of action for 3,3'-dimethylbenzidine, the important DNA-reactive metabolites have not been established. Information on the closely related compound benzidine suggests species variations in metabolism and DNA-reactive metabolites that lead to species differences in tumor site (ATSDR, 2001) and possibly to species differences in potency. Because there are few data on metabolites of 3,3'-dimethylbenzidine in different species, and insufficient data on human tumors associated with 3,3'-dimethylbenzidine exposure, it is not known whether these species variations also pertain to 3,3'-dimethylbenzidine.

Quantitative Estimates of Carcinogenic Risk

Oral Exposure

In the NTP (1991a) chronic study, 6-week old male and female F344/N rats were exposed to 3,3'-dimethylbenzidine dihydrochloride in drinking water at concentrations of 0, 30, 70 or 150 ppm, which are equivalent to estimated time-weighted average doses of 0, 1.3, 3.0, and 8.3 mg/kg-day in males and 0, 2.2, 5.1, and 9.6 mg/kg-day in females, in the control, low-, mid-, and high-dose groups respectively. Cancer dose-response modeling for 3,3'-dimethylbenzidine was performed using the most prominent tumors: basal cell tumors (adenoma or carcinoma) in the skin of male rats, squamous cell tumors (papilloma or carcinoma) in the skin of male rats, liver tumors (neoplastic nodules or hepatocellular carcinoma) in male rats, Zymbal's gland tumors (adenoma or carcinoma) in male and female rats, and clitoral gland tumors (adenoma or carcinoma) in female rats. Incidences used for dose-response modeling were based on the number of animals alive at first occurrence of the tumor being modeled in any dose group, as reported by NTP (1991a).

In addition, dose-response modeling was performed for all significantly increased skin tumors combined in male and female rats and for all significantly increased tumors (across sites) combined in male and female rats. Combined skin tumors were modeled because significant increases were found for six different tumor types in the skin (keratoacanthoma, sebaceous gland adenoma, basal cell adenoma, basal cell carcinoma, squamous cell papilloma and squamous cell carcinoma). Although distinct from each other in terms of the specific cell type/location affected and the degree of development of the neoplasm, the fact that all of these tumor types were increased suggests a nonspecific effect on the skin. Therefore, it appeared reasonable to estimate overall skin cancer risk by modeling the combined skin tumor incidence data.

Combined incidence of all significantly increased tumors across sites was modeled for a similar reason. 3,3'-Dimethylbenzidine produced significant increases in tumor incidence in many tissues, including skin, Zymbal's gland, preputial/clitoral gland, liver, oral cavity, small intestine, large intestine, mammary gland, lung and mesothelium. The lack of tissue specificity of the neoplastic response, which is characteristic of a mutagenic mode of action, means that the overall risk of tumor formation is spread throughout the body. Because all of these tumors contribute to the overall cancer risk, dose-response assessment based on any one type will underestimate risk of developing cancer. Therefore, it is reasonable to estimate overall cancer risk for this chemical based on combined tumor incidence. Combining incidence data for significantly increased tumors across sites is an appropriate way to estimate risk for carcinogens that produce tumors at multiple sites (U.S. EPA, 2005a).

The combined incidence data (effective rates) were extracted from the individual animal data reported by NTP (1991a). Although this method of estimating overall tumor risk can sometimes underestimate risk by inflating the control tumor incidence, that was not an issue for 3,3'-dimethylbenzidine, at least for females, as combined tumor incidence in female controls was 0/60 for skin tumors and 1/60 for all significantly increased tumors. All tumor incidence data used for dose-response modeling are shown in Table 8.

In accordance with the U.S. EPA (2005a) cancer guidelines, the $BMDL_{10}$ (lower bound on dose estimated to produce a 10% increase in tumor incidence over background) was estimated using the U.S. EPA (2000) benchmark dose methodology. The incidence data were analyzed using the multistage model available in the BMDS program (version 1.4.1) developed by U.S. EPA. The polydegree was chosen as the lowest degree polynomial providing an adequate fit to the data, as indicated by the chi-square goodness-of-fit test producing a *p*-value greater than or equal to 0.1. The high-dose group was dropped where necessary to achieve an adequate fit. Risk was calculated as extra risk. Confidence bounds were calculated by the BMDS software using a maximum likelihood profile method.

Modeling results are shown in Table 8. An adequate fit was achieved for all tumor types except combined skin tumors in males, in most cases after dropping the high-dose group. In both males and females, the lowest $BMDL_{10}$ values were for combined tumors, with the value in females being approximately half that in the males.

Human equivalent doses ($BMDL_{10\,HED}$) were calculated for each animal $BMDL_{10}$ using U.S. EPA's cross-species scaling factor of body weight raised to the 3/4 power (U.S. EPA, 2005a). Using this scaling factor, the straight dose (mg) in humans is obtained by multiplying the straight animal dose (mg) by the ratio of human:animal body weight raised to the 3/4 power. For doses expressed per unit body weight (mg/kg or mg/kg-day), the relationship is reciprocal and the human dose (mg/kg) is obtained by multiplying the animal dose (mg/kg) by the ratio of animal:human body weight raised to the 1/4 power. The $BMDL_{10\,HED}$ represents the chronic daily dose (mg/kg-d) expected to result in 10% extra risk for tumor development extrapolated from the animal bioassay data. The $BMDL_{10\,HED}$ values are shown in Table 8.

Table 8. BMD Modeling of Cancer Incidence (Effective Rates) in Rats Treated with 3,3'-Dimethylbenzidine in Drinking Water for 14 Months (NTP, 1991a)

Tumor Type	Sex	Dose (mg/kg-day)				Poly-degree	χ^2 *p*-value	Benchmark Dose (mg/kg-day)		
		0	1.3	3	8.3			BMD_{10}	$BMDL_{10}$	$BMDL_{10\ HED}$[a]
Skin										
Basal cell adenoma or carcinoma	male	0/60	11/44	54/72	30/45	2[b]	1.00	0.74	0.36	0.098
Squamous cell papilloma or carcinoma	male	0/60	2/45	17/74	27/59	1	0.65	1.42	1.12	0.30
Combined skin tumors	male	1/60	13/44	61/74	41/59	NA	NA	NA	NA	NA
Liver										
Neoplastic nodule or hepatocellular carcinoma	male	0/60	0/45	35/72	33/55	3[b]	0.29	1.66	1.44	0.39
Zymbal's gland										
Adenoma or carcinoma	male	1/60	3/45	32/74	36/60	2[b]	0.35	1.35	0.94	0.26
Combined sites[c]										
Combined tumors	male	5/60	19/45	73/74	58/60	2[b]	0.14	0.53	0.35	0.095
		0	**2.2**	**5.1**	**9.6**					
Skin										
Combined skin tumors	female	0/60	6/45	19/72	21/54	1	0.94	1.87	1.48	0.35
Zymbal's gland										
Adenoma or carcinoma	female	0/60	6/45	32/74	42/59	1	0.45	0.93	0.78	0.18
Clitoral gland										
Adenoma or carcinoma	female	0/60	14/45	42/73	32/58	1[b]	1.00	0.63	0.50	0.12
Combined sites[d]										
Combined tumors	female	1/60	25/45	70/74	58/59	1	0.22	0.23	0.19	0.045

[a] human cancer equivalent dose of the $BMDL_{10}$ calculated as: animal $BMDL_{10}$ x $(W_{animal} / W_{human})^{1/4}$ where W_{human} = 70 kg (human reference body weight) and W_{animal} = 0.384 kg for male rats and 0.218 kg for female rats (time weighted average body weights for the low-dose group in the study)

[b] high dose group dropped

[c] includes all sites with statistically increased tumor incidences (skin, zymbal's gland, preputial gland, liver, oral cavity, small intestine, large intestine, lung and mesothelium)

[d] includes all sites with statistically increased tumor incidences (skin, zymbal's gland, clitoral gland, liver, oral cavity, small intestine, large intestine, and mammary gland), as well as the lung (marginal increase in females, but presumed treatment-related due to statistical increase in males)

An oral slope factor calculated from adult exposure is derived from the $BMDL_{10\ HED}$, the 95% lower bound on the human equivalent exposure associated with a 10% extra cancer risk, by dividing the risk (as a fraction; in this case 0.1) by the $BMDL_{10\ HED}$, and represents an upper bound risk estimate for continuous lifetime exposure without consideration of increased early-life susceptibility due to the 3,3'-dimethylbenzidine mutagenic mode of action (see below). Since a linear, mutagenic mode of action has been implicated for 3,3'-dimethylbenzidine-induced tumors, a linear extrapolation to low doses was performed. In order to linearly extrapolate cancer risks from the $BMDL_{10\ HED}$ to the origin, a cancer oral slope factor was calculated as the ratio $0.1/BMDL_{10\ HED}$. Taking the $BMDL_{10\ HED}$ of 0.045 mg/kg-day for combined tumors in female rats as the point of departure, a provisional *unadjusted* oral slope factor of 2.2 $(mg/kg\text{-}day)^{-1}$ is calculated as follows:

$$\begin{aligned} p\text{-}OSF_{(unadjusted)} &= 0.1 / BMDL_{10\ HED} \\ &= 0.1 / 0.045\ mg/kg\text{-}day \\ &= 2.2\ (mg/kg\text{-}day)^{-1} \end{aligned}$$

An adjustment was used for shorter-than-lifetime observation period (U.S. EPA, 1980). The NTP (1991a) bioassay was terminated after only 14 months (compared to the reference rat life span of 24 months), due to early mortality associated with tumor formation. In the NTP (1991a) study, a short duration of observation was imposed by the development of tumors however, it was not clear that a sufficient period of time had elapsed to fully evaluate the carcinogenicity of 3,3'-dimethylbenzidine in the low-dose treated rats. Specifically, as illustrated in Table 8, the combined tumor incidence in male and female rats of the mid- and high-dose treatment groups approached almost 100%, whereas there was an approximate 50% tumor incidence in the low-dose treatment group. Due to the truncated experimental protocol in the NTP (1991a) study it is not known how an increased duration (i.e. the full 2-year lifetime exposure) may have influenced the tumor incidence in the low-dose treated rats. Therefore, an adjustment factor of $(L/Le)^3$ was applied to the *unadjusted* p-OSF, where L = the lifetime of the animal and Le = the duration of experimental dosing. Using this adjustment, a provisional **oral slope factor of 11 $(mg/kg\text{-}day)^{-1}$** is derived as follows:

$$\begin{aligned} p\text{-}OSF &= p\text{-}OSF_{(unadjusted)} \times (L/Le)^3 \\ &= 2.2\ (mg/kg\text{-}day)^{-1} \times (24\ months/14\ months)^3 \\ &= 11\ (mg/kg\text{-}day)^{-1} \end{aligned}$$

The oral slope factor for 3,3'-dimethylbenzidine should not be used with exposures exceeding the point of departure ($BMDL_{10\ HED}$ = 0.045 mg/kg-day), because above this level the fitted dose-response model better characterizes what is known about the carcinogenicity of 3,3'-dimethylbenzidine. For exposures exceeding the point of departure, the uncertainty in risk associated with the OSF may be significantly increased.

The human equivalent dose was also calculated for the central estimate associated with the selected point of departure, the BMD_{10} for combined tumors in female rats (0.23 mg/kg-day). A $BMD_{10\ HED}$ of 0.054 mg/kg-day was calculated. The *unadjusted* slope of the linear extrapolation from the central estimate (0.054 mg/kg-day) is 1.8 $(mg/kg\text{-}day)^{-1}$ and the adjusted slope is 9 $(mg/kg\text{-}day)^{-1}$.

A weight of evidence evaluation has concluded that 3,3'-dimethylbenzidine is carcinogenic by a mutagenic mode of action. According to the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b) those exposed to carcinogens with a mutagenic mode of action are assumed to have increased early-life susceptibility. Data on 3,3'-dimethylbenzidine are not sufficient to develop separate risk estimates for childhood exposure. The oral slope factor of 11 $(mg/kg\text{-}day)^{-1}$ calculated from data from adult exposure does not reflect presumed early-life susceptibility for this chemical, 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 are given in Section 6 of the Supplemental Guidance (U.S. EPA, 2005b) which establishes ADAFs for three specific age groups. The current ADAFs and their age groupings are 10 for <2 years, 3 for 2 to <16 years, and 1 for 16 years and above. The 10-fold and 3-fold adjustments in slope factor are to be combined with age-specific exposure estimates when estimating cancer risks from early life (<16 years of age) exposure to 3,3'-dimethylbenzidine. These ADAFs and their age groups were derived from the 2005 Supplemental Guidance (U.S. EPA, 2005b), and they may be revised over time. The most current information on the application of ADAFs for cancer risk assessment can be found at www.epa.gov/cancerguidelines/. In estimating risk, EPA recommends using age-specific values for both exposure and cancer potency; for 3,3'-dimethylbenzidine, age-specific values for cancer potency are calculated using the appropriate ADAFs. A cancer risk is derived for each age group and these are summed across age groups to obtain the total risk for the exposure period of interest.

Inhalation Exposure

There are no human or animal carcinogenicity data from which to derive an inhalation unit risk for 3,3'-dimethylbenzidine.

REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). 2001. Documentation of the threshold limit values for chemical substances. 7th Edition. Cincinnati, OH.

ACGIH (American Conference of Governmental Industrial Hygienists). 2006. Threshold limit values for chemical substances and physical agents and biological exposure indices. Cincinnati, OH.

ATSDR (Agency for Toxic Substances and Disease Registry). 2001. Toxicological Profile for Benzidine. U.S. Department of Health and Human Services, Public Health Service. PB/2001/109102. Online. http://www.atsdr.cdc.gov/toxpro2.html

ATSDR (Agency for Toxic Substances and Disease Registry). 2007. Toxicological Profile Information Sheet. U.S. Department of Health and Human Services, Public Health Service. Online. http://www.atsdr.cdc.gov/toxpro2.html

Beland, F.A., D.T. Beranek, K.L. Dooley et al. 1983. Arylamine-DNA adducts in vitro and in vivo: Their role in bacterial mutagenesis and urinary bladder carcinogenesis. Environ Health Perspect. 49:125-134.

Boeniger, M. 1978. The carcinogenicity and metabolism of azo dyes, especially those derived from benzidine. NIOSH Report. DHEW, Public Health Service, Center for Disease Control. (Unpublished) (Cited in NIOSH, 1981).

CalEPA (California Environmental Protection Agency). 2007. Benzyl Chloride. Air Toxic Hot Spots Program Risk Assessment Guidelines. Office of Environmental Health Hazard Assessment. Online. http://www.oehha.ca.gov/air/hot%5Fspots/.

Chung, K-T, S-C Chen, and L.D. Claxton. 2006. Review of the Salmonella typhimurium mutagenicity of benzidine, benzidine analogues, and benzidine-based dyes. Mutat. Res. 612: 58–76.

Claxton, L.D., T.J. Hughes and K.T. Chung. 2001. Using base-specific *Salmonella* tester strains to characterize the types of mutation induced by benzidine and benzidine congeners after reductive metabolism. Food Chem. Toxicol. 39(2):1253-1261.

Dieteren, H.M.L. 1966. The biotransformation of o-tolidine: A qualitative investigation. Arch. Environ. Health. 12:30-32.

Ferber, K.H. 1977. Carcinogenicity of orthotolidine in the urinary bladder of the dog. Unpublished report submitted to NIOSH by Ferber KH, Allied Chemical Division, Buffalo Dye Plant, Specialty Chemicals Division, Buffalo. p. 11 (Cited in U.S. EPA, 1987).

Golub, N.I., T.S. Kolesnichenko and L.M. Shabad. 1974. Oncogenic action of some nitrogen compounds on the progeny of experimental mice. Bull. Exp. Biol. Med. (USSR). 78:1402-1404. (Cited in U.S. EPA, 1987).

Gray, L.E., Jr. and J.S. Ostby. 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. Fund. Appl. Toxicol. 20(2):177-183.

Griswold, D.P., Jr., A.E. Casey, E.K. Weisburger et al. 1968. The carcinogenicity of multiple intragastric doses of aromatic and heterocyclic nitro or amino derivatives in young female Sprague-Dawley rats. Cancer Res. 28:924-933.

IARC (International Agency for Research on Cancer). 1972a. 3,3'-Dimethylbenzidine (o-Tolidine). IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. Lyon, France. 1:87-91.

IARC (International Agency for Research on Cancer). 1972b. Benzidine. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. Lyon, France. 1:80-86.

IARC (International Agency for Research on Cancer). 1975a. Evans Blue. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. Lyon, France. 8:151-156.

IARC (International Agency for Research on Cancer). 1975b. Trypan Blue. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. Lyon, France. 8:267-278.

IARC (International Agency for Research on Cancer). 1982. Benzidine and its sulphate, hydrochloride and dihydrochloride. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Lyon, France. 29:149-183.

IARC (International Agency for Research on Cancer). 1987. Overall evaluation of carcinogenicity: An updating of IARC Monographs Volumes 1 to 42. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Lyon, France. Supplement 7:62, 123-125.

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

Morgan D.L., J.K Dunnick, T. Goehl et al. 1994. Summary of the National Toxicology Program benzidine dye initiative. Environ. Health Perspect. 102(Suppl2):63-78.

NIOSH (National Institute for Occupational Safety and Health). 1981. Health Hazard Alert: Benzidine-, o- Tolidine-, and o- Dianisidine-Based Dyes. DHEW 81-106. Washington, DC.

NIOSH (National Institute for Occupational Safety and Health). 2007. NIOSH Pocket Guide to Chemical Hazards. Online. http://www.cdc.gov/niosh/npg/.

NTP (National Toxicology Program). 1982. Department of Health, Education and Welfare. Proposed NTP initiative on benzidine and benzidine congener based azo dyes. Memorandum. 1980. Produced 2/1/82. U.S. EPA/OPTS Document No. 40-8329024. Fiche# OTS0507295.

NTP (National Toxicology Program). 1991a. NTP Technical Report on Toxicology and Carcinogenesis Studies of 3,3'-dimethylbenzidine dihydrochloride (CAS No. 612-82-8) in F344/N Rats (Drinking Water Studies). National Toxicology Program, Research Triangle Park, N.C. Toxicity Report No. 390.

NTP (National Toxicology Program). 1991b. NTP Technical Report on Toxicology and Carcinogenesis Studies of C.I. Acid Red 114 (CAS No. 6459-94-5) in F344/N Rats (Drinking Water Studies). National Toxicology Program, Research Triangle Park, N.C. Toxicity Report No. 405.

NTP (National Toxicology Program). 2007. 11th Report on Carcinogens. Online. http://ntp.niehs.nih.gov/index.cfm?objectid=32BA9724-F1F6-975E-7FCE50709CB4C932.

Oda, Y. 2004. Analysis of the involvement of human N-acetyltransferase 1 in the genotoxic activation of bladder carcinogenic arylamines using a SOS/umu assay system. Mutat. Res. 554(1-2):399-406.

OSHA (Occupational Safety and Health Administration). 2007. OSHA Standard 1910.1000 Table Z-1. Part Z, Toxic and Hazardous Substances. Online. http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=9992.

Pliss, G.B. 1963. On some regular relationships between carcinogenicity of aminodiphenyl derivatives and the structure of substance. Acta Un. Int. Cancer. 19:499-501. (Cited in U.S. EPA, 1987 and NTP, 1991a).

Pliss, G.B. and M.A. Zabezhinsky. 1970. Properties of orthotolidine (3,3'-dimethylbenzidine). J. Natl. Cancer Inst. 55:181-182. (Cited in U.S. EPA, 1987 and NTP, 1991a).

Quellot-Hellstrom, R. and J.D. Rench. 1996. Bladder cancer incidence in arylamine workers. J. Occup. Environ. Med. 38(12):1239-1247.

Reid, T.M., C.Y. Wang, C.M. King, and K.C. Morton. 1984. Mutagenicity of some benzidine congeners and their N-acetylated and N,N'-diacetylated derivatives in different strains of Salmonella typhimurium. Environ. Mutagen. 6:145-151.

Reynolds, S.H., R.M. Patterson, J.H. Mennear et al. 1990. *ras* Gene activation in rat tumors induced by benzidine congeners and derived dyes. Cancer Res. 50:266-272.

Saffiotti, U., F. Cefis, R. Montesano et al. 1967. Induction of bladder cancer in hamsters fed aromatic amines. In: Bladder Cancer, A Symposium (Duchmann, W.B. and Tampe, Ed.) Aesculapius Publishing Co., Birmingham, AL. p. 129-135.

Schieferstein, G.J., Y. Shinora, R.R. Allen et al. 1989. Carcinogenicity study of 3,3'-dimethylbenzidine dihydrochloride in BALB/c mice. Food Chem. Toxicol. 27(12):801-806.

Sciarini L J. and J. W. Meigs. 1961. Biotransformation of the benzidines III. Studies on diorthotolidine, dianisidine, and dichlorobenzidine: 3,3'-disubstituted congeners of benzidine (4,4;-diaminobiphenyl). Arch. Environ. Health. 2:108-112.

Sellakumar, A.R., R. Montesano and U. Saffiotti. 1969. Aromatic amines carcinogenicity in hamsters. Proc. Am. Assoc. Cancer Res. 10:78. (Cited in U.S. EPA, 1987).

Spitz, S., W.H. Maguigan and K. Dobriner. 1950. The carcinogenic action of benzidine. Cancer. 3:789-804. (Cited in U.S. EPA, 1987 and NTP, 1991a).

Tanaka K., T. Mii, S. Marui et al. 1982. Some aspects of metabolism and mutagenicity of o-tolidine and an o-tolidine-based azo dye. Indust. Health. 20:227-235. (As cited in NTP, 1991a).

U.S. EPA. 1980. Federal Register Notice. Vol. 45, no. 231:79352.

U.S. EPA. 1987. Health and Environmental Effects and Profiles for 3,3-Dimethylbenzidine (HEEP). Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response, Washington, DC.

U.S. EPA. 1988a. Evaluation of the Potential Carcinogenicity of 3,3'-Dimethylbenzidine (119-93-7). Prepared by the Carcinogen Assessment Group, Office of Health and Environmental Assessment, Washington, DC for the Office of Solid Waste and Emergency Response, Washington, DC. EPA/600/8-91/118.

U.S. EPA. 1988b. Recommendations for and Documentation of Biological Values for Use in Risk Assessment. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response, Washington, DC. EPA/600/6-87/008. NTIS PB 88-179874.

U.S. EPA. 1991. Chemical Assessments and Related Activities (CARA). Office of Health and Environmental Assessment, Washington, DC. April.

U.S. EPA. 1994. Chemical Assessments and Related Activities (CARA). Office of Health and Environmental Assessment, Washington, DC. December.

U.S. EPA. 1997. Health Effects Assessment Summary Tables. FY-1997 Update. 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. July. EPA/540/R-97/036. NTIS PB97-921199.

U.S. EPA. 2000. Benchmark Dose Technical Guidance Document. Risk Assessment Forum, Washington, DC. External Review Draft. EPA/630/R-00/001.

U.S. EPA. 2004. 2004 Edition of the Drinking Water Standards and Health Advisories. Office of Water, Washington, DC. EPA 822-R-02-038. Washington, DC. Online. http://www.epa.gov/waterscience/drinking/standards/dwstandards.pdf.

U.S. EPA. 2005a. Guidelines for Cancer Risk Assessment. Risk Assessment Forum, Washington, DC. EPA/630/P-03/001F. Online. http://www.epa.gov/raf.

U.S. EPA. 2005b. Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens. Risk Assessment Forum, Washington, DC. EPA/630/R-03/003F. Online. http://www.epa.gov/raf.

U.S. EPA. 2007. Integrated Risk Information System (IRIS). Online. Office of Research and Development, National Center for Environmental Assessment, Washington, DC. http://www.epa.gov/iris/.

WHO (World Health Organization). 2007. Online catalogs for the Environmental Health Criteria Series. Online. http://www.inchem.org/pages/ehc.html.