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Provisional Peer-Reviewed Toxicity Values for

Diphenylamine
(CASRN 122-39-4)

Superfund Health Risk Technical Support Center
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COMMONLY USED ABBREVIATIONS

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

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR DIPHENYLAMINE (CASRN 122-39-4)

BACKGROUND

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

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

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

DISCLAIMERS

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

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

QUESTIONS REGARDING PPRTVS

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

INTRODUCTION

Diphenylamine is produced in the United States in fairly large amounts and is primarily used in dye manufacturing and as a nitrocellulose explosive and celluloid stabilizer (Hazardous Substances Data Bank [HSDB], 2010). In the analytical chemistry field, it is used in the detection of nitrates, chlorates, and other oxidizing substances. It is also used as a rubber antioxidant and accelerator, solid rocket propellant, fungicide and herbicide, storage preservative for apples, topical application in antiscrewworm mixtures in the pharmaceutical industry, and as a stabilizer for formaldehyde copolymers, epoxy resins, polyvinyl chloride, and polyoxyethylene. A foliar application of 1% diphenylamine in dust formation reportedly has decreased injury caused by ozone to various leaves of plants, including apple, bean, muskmelon, and petunia (HSDB, 2010). HSDB (2010) reports that diphenylamine is used as a potential model to research human bilateral disorder (an autosomally inherited kidney disease) by using the chemical to study polycystic kidneys in animals. The empirical formula for diphenylamine is $C_{12}H_{11}N$ (see Figure 1). A table of the physicochemical properties is provided below (see Table 1).

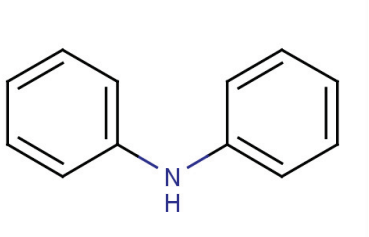

Figure 1. Diphenylamine Structure

Table 1. Physicochemical Properties Table (Diphenylamine)[a]

Property (Unit)	Value
Boiling point (ºC)	302
Melting point (ºC)	52.9[b]
Density (g/cm^3)	1.16
Vapor pressure (Pa at 25ºC)	0.089
pH (unitless)	NA
Solubility in water (mg/L at 20ºC)	53
Vapor density (air = 1)	5.82
Molecular weight (g/mol)	169.23

[a]Values from HSDB unless otherwise specified; http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB
[b]Values from ChemID Plus Advanced;
http://chem.sis.nlm.nih.gov/chemidplus/ProxyServlet?objectHandle=DBMaint&actionHandle=default&nextPage=jsp/chemidheavy/ResultScreen.jsp&ROW_NUM=0&TXTSUPERLISTID=0000122394
NA = not available

A chronic oral Reference Dose (RfD) of 2.5×10^{-2} mg/kg-day for diphenylamine is included in the IRIS database (U.S. EPA, 2010a). This value is based on decreased body-weight gain and increased liver and kidney weights in dogs in a 2-year feeding study (Thomas et al., 1967a). However, no Reference Concentration (RfC) or cancer assessment is available on IRIS (U.S. EPA, 2010a). Diphenylamine is not included on the Drinking Water Standards and Health Advisories List (U.S. EPA, 2009). No RfD or RfC values have been reported in the HEAST (U.S. EPA, 2010b). The CARA list (U.S. EPA, 1994a) has a Health and Environmental Effects Profile (HEEP) for diphenylamine (U.S. EPA, 1985). The toxicity of diphenylamine has not been reviewed by ATSDR (2008) or the World Health Organization (WHO, 2010). CalEPA (2008) has not derived toxicity values for exposure to diphenylamine. The American Conference of Governmental Industrial Hygienists (ACGIH) has set an 8-hour time-weighted average (TWA) of 10 mg/m^3 for occupational exposures to diphenylamine (ACGIH, 2010) based on industrial poisoning symptoms such as bladder and skin problems, abnormal heartbeat, and high blood pressure. This value is also the recommended exposure limit (REL) set by the National Institute of Occupational Safety and Health (NIOSH, 2005) and the personal exposure limit (PEL) set by the Occupational Safety and Health Administration (OSHA, 2010).

The HEAST (U.S. EPA, 2010b) does not report any cancer values for diphenylamine. The International Agency for Research on Cancer (IARC, 2010) has not reviewed the carcinogenic potential of diphenylamine. Diphenylamine is not included in the *11th Report on Carcinogens* (NTP, 2005). CalEPA (2008) has not prepared a quantitative estimate of carcinogenic potential for diphenylamine. ACGIH has classified diphenylamine as a Group A4 carcinogen—*Not Classifiable as a Human Carcinogen* (ACGIH, 2010).

Literature searches were conducted on sources published from 1900 through April 2011 for studies relevant to the derivation of provisional toxicity values for diphenylamine, CAS Number 122-39-4. Searches were conducted using EPA's Health and Environmental Research Online (HERO) database of scientific literature. HERO searches the following databases: AGRICOLA; American Chemical Society; BioOne; Cochrane Library; DOE: Energy Information Administration, Information Bridge, and Energy Citations Database; EBSCO: Academic Search Complete; GeoRef Preview; GPO: Government Printing Office; Informaworld; IngentaConnect; J-STAGE: Japan Science & Technology; JSTOR: Mathematics & Statistics and Life Sciences; NSCEP/NEPIS (EPA publications available through the National Service Center for Environmental Publications [NSCEP] and National Environmental Publications Internet Site [NEPIS] database); PubMed: MEDLINE and CANCERLIT databases; SAGE; Science Direct; Scirus; Scitopia; SpringerLink; TOXNET (Toxicology Data Network): ANEUPL, CCRIS, ChemIDplus, CIS, CRISP, DART, EMIC, EPIDEM, ETICBACK, FEDRIP, GENE-TOX, HAPAB, HEEP, HMTC, HSDB, IRIS, ITER, LactMed, Multi-Database Search, NIOSH, NTIS, PESTAB, PPBIB, RISKLINE, TRI, and TSCATS; Virtual Health Library; Web of Science (searches Current Content database among others); World Health Organization; and Worldwide Science. The following databases outside of HERO were searched for risk assessment values: ACGIH, ATSDR, CalEPA, EPA IRIS, EPA HEAST, EPA HEEP, EPA OW, EPA TSCATS/TSCATS2, NIOSH, NTP, OSHA, and RTECS.

A number of unpublished studies with diphenylamine were located in a formal government risk assessment document (European Communities, 2008), where they are reported only as summaries, although full references are given. Given that these are acknowledged in the

assessment as full reports and not, for example, as abstracts from a scientific conference, all listed therein are considered as relevant and valid for use in this assessment. It is also acknowledged here that these report summaries are evaluated in this assessment as to their accuracy and internal consistency.

REVIEW OF POTENTIALLY RELEVANT DATA (CANCER AND NONCANCER)

Table 2 provides an overview of the relevant database for diphenylamine and includes all potentially relevant repeated short-term-, subchronic-, and chronic-duration studies. No-observed-adverse-effect levels (NOAELs), lowest-observed-adverse-effect levels (LOAELs), and benchmark dose lower limits/benchmark concentration lower limits (BMDLs/BMCLs) are provided in human equivalent doses/human equivalent concentrations (HEDs/HECs) for comparison except that oral noncancer values are not converted to HEDs and are identified in parentheses as (Adjusted). Developmental studies are not adjusted for continuous exposure. Principal studies are identified. Entries for the principal studies are bolded. **The phrase, "statistical significance" used throughout the document, indicates a *p*-value of <0.05.**

Table 2. Summary of Potentially Relevant Data for Diphenylamine (CASRN 122-39-4)

Notes[a]	Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[b]	Critical Effects	NOAEL[b]	BMDL/ BMCL[b]	LOAEL[b]	Reference (Comments)
Human								
				1. Oral (mg/kg-day)[b]				
	Subchronic	None						
	Chronic	None						
	Developmental	None						
	Reproductive	None						
	Carcinogenic	None						
				2. Inhalation (mg/m^3)[b]				
	Subchronic	None						
	Chronic/ Carcinogenic	220 male cases and 440 male controls, occupational case-control study at rubber and tire factories	Exposure concentration not measured	Statistically Significantly elevated ($p < 0.05$) odds ratios for bladder cancer compared to expected rates for milling (OR = 1.91) and calendar operation (OR = 2.21) jobs	Not Determinable	Not run	Not Determinable	Checkoway et al. (1981) Effects from many chemicals used in the industry; effects specific to diphenylamine could not be separated.
	Developmental	None						
	Reproductive	None						
	Carcinogenic	33,815 males, retrospective cohort study of cancer mortality in the British rubber industry	Exposure concentration not measured	Significant ($p < 0.001$) excess of cancer deaths; lung cancer and stomach cancers most common cancer types	Not Determinable	Not run	Not Determinable	Parkes et al. (1982) Effects from many chemicals used in the industry; effects specific to diphenylamine could not be separated.

Table 2. Summary of Potentially Relevant Data for Diphenylamine (CASRN 122-39-4)

Notes[a]	Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[b]	Critical Effects	NOAEL[b]	BMDL/ BMCL[b]	LOAEL[b]	Reference (Comments)
Animal								
				1. Oral (mg/kg-day)[b]				
	Short-term	Female (number not specified), Sprague-Dawley rat, diet, 3–6 weeks	0 or 2451[c]	Gross cysts in the corticomedullary region of the kidney and other morphological changes	Not Determinable	Not run	2451[d]	Eknoyan et al. (1976)
NPR	Short-term	6/6, Fischer rat, gavage, 28 days	0, 111, 333, or 1000[e]	Decreased body-weight gain; increased liver, spleen, and kidney weights; anemia; histopathological changes in forestomach, renal tubules, and bone marrow	111[f]	Not run	1000[f]	Yoshida et al. (1989)
NPR	Subchronic	4/4, beagle dog, gelatin capsule, 90 days	0, 10, 25, or 50[e]	Treatment-related changes not observed in either sex	50[f]	Not run	Not Determinable[f]	Krohmer (1992a)
NPR	Subchronic	10/10, rat (strain not reported), diet, 90 days	Males: 0, 9.8, 25, 78, 236, or 791[c] Females: 0, 12, 32, 96, 303, or 978[c]	Increased mean relative liver, spleen, kidney, and body weight	Males: 78[d] Females: 12[d]	Not run	Males: 236[d] Females: 32[d]	Dow Chemical Company (1958); LOAEL identified by causing 10% increase in relative liver weight in female rats considered to be biologically significant.
NPR	Subchronic	10/10, Sprague-Dawley rat, diet, 90 days	Males: 0, 9.6, 96, 550, or 1200[e] Females: 0, 12, 110, 650, or 1300[e]	Increased absolute and relative liver weights; decreased hematocrit; and histopathological changes in kidneys, spleen, and liver	Female:12[f] Male: 96[f]	Not run	Female: 110[f] Male: 550[f]	Krohmer (1992b); LOAEL identified by causing statistically significant increase in relative and absolute liver weight

Table 2. Summary of Potentially Relevant Data for Diphenylamine (CASRN 122-39-4)

Notes[a]	Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[b]	Critical Effects	NOAEL[b]	BMDL/ BMCL[b]	LOAEL[b]	Reference (Comments)
PS/NPR	**Subchronic**	**15/15, CD-1 mouse, diet, 90 days**	**Males: 0, 1.7, 94, 444, or 926[c] Females: 0, 2.1, 107, 555, or 1101[c]**	**Splenic effects (i.e., hemosiderosis and congestion), increased relative and absolute liver weight, increased absolute kidney weight (males only) and relative kidney weight (females only); decreased ovary weight in female mice**	**Female: 2.1[d] Male: 94[d]**	**11.51 for increased incidence of spleen hemosid-erosis in male mice.**	**Female: 107[d] Male: 444[d]**	**Botta (1992); NOAEL and LOAEL identified for increased incidence of splenic effects (i.e., hemosiderosis and congestion).**
IRIS, 1993	Chronic	2/2, purebred beagle dog, 2 years	2.5, 25, 250	Decreased body-weight gain; increased liver and kidney weights	2.5	Not run	25	Thomas et al. (1967a); NOAEL and LOAEL formerly identified in the IRIS review of Diphenylamine.
NPR	Chronic	4/4, beagle dog, gelatin capsule, 52 weeks	0, 10, 25, or 100[e]	Changes in clinical chemistry (bilirubin, BUN) and hematological (platelet) parameters	10[f]	Not run	25[f]	Botta (1994a)
	Chronic	2/2, beagle dog, diet, 2 years	Males: 2.1, 21, or 208[c] Females: 1.9, 19, or 185[c]	Severely inhibited growth; reduced hemoglobin levels; increased liver weight; possibly increased kidney and spleen weights; some hemosiderosis of the spleen, kidney, and bone marrow	Not determinable	Not run	Not determinable	DeEds (1963a); Because raw data were not provided in the study report and the use of a control group was not specified, a LOAEL and a NOAEL cannot be determined from this study.
	Chronic	20/20, albino rat (strain not specified), diet, 734 days	Males: 0, 0.72, 7.2, 72, 362, or 723[c] Females: 0.82, 8.2, 82, 410, or 820[c]	Depressed growth; reduced feed consumption (>10%); moderate anemia; increased incidence of cystic dilated renal tubules and chronic interstitial nephritis	Not determinable	Not run	Not determinable	DeEds (1963b); Study authors reported that kidney lesions were not related to diphenylamine exposure.

Table 2. Summary of Potentially Relevant Data for Diphenylamine (CASRN 122-39-4)

Notes[a]	Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[b]	Critical Effects	NOAEL[b]	BMDL/ BMCL[b]	LOAEL[b]	Reference (Comments)
NPR	Chronic	Male (number not specified), Sprague-Dawley rat, diet, 76 weeks	500[e]	Focal proliferation of distal tubular and collecting duct cells at Week 5; cystic dilatations with focal necrosis in collecting ducts at Week 10	Not determined[f]	Not run	Not determined[f]	Evan et al. (1978); Study authors reported that study was not designed for toxicological purposes.
	Chronic	0/6, albino rat (strain not specified), 226 days	0, 21, 82, 410, 820, or 1230[c]	Dose-related decrease in final body weight; pigmentation in liver, spleen, adrenals, heart, and kidneys; kidney cysts	82[d]	Not run	410[d] for microscopic changes in the kidney.	Thomas et al., 1957
NPR	Chronic/ Carcinogenicity	10/10, Sprague-Dawley rat, diet, 2 years	Males: 0, 8.1, 29, 150, or 300[e] Females: 0, 7.5, 25, 140, or 290[e]	Changes in hematological parameters (hematocrit, hemoglobin, erythrocytes); histopathological changes in the spleen, liver, and kidneys; no treatment-related increase in tumors was observed	7.5[f]	Not run	25[f]	Botta (1994b)
	Chronic/ Carcinogenicity	20/20, albino Slonaker-Addis rat, diet, 2 years	Males: 0, 0.72, 7.2, 72, 362, or 723[c] Females: 0, 0.82, 8.2, 82, 410, or 820[c] HED males: 0, 0.21, 2.1, 21, 104, or 207 HED females: 0, 0.21, 2.1, 21, 107, or 213	Decreased body weight and increased incidence and severity of chronic nephritis	Males: 72[d] Females: 82[d]	Not run	Males: 361.5[d] Females: 410[d]	Thomas et al. (1967b); according to study authors, tumor incidence was due to senility of the animals and not related to treatment of the compound.
	Chronic/ Carcinogenicity	0/20, Sprague-Dawley rat, gavage, single dose, observed for up to 6 months	1.7[c]; HED: 0.44	No results specific to diphenylamine exposure presented	1.7	Not run	None	Griswold et al. (1966)
NPR	Chronic/ Carcinogenicity	60/60, CD-1 mouse, diet, 78 weeks	Males: 0, 73, 370, or 760[e] Females: 0, 91, 460, or 940[e]	Decreased body-weight gain; decreased survival; hematological changes (MCV, MCH, hematocrit, erythrocytes), gross and microscopic pathological alterations (spleen, liver, kidneys, urinary bladder); tumors comparable between groups	N/A	Not run	N/A	Botta (1994c)

Table 2. Summary of Potentially Relevant Data for Diphenylamine (CASRN 122-39-4)

Notes[a]	Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[b]	Critical Effects	NOAEL[b]	BMDL/ BMCL[b]	LOAEL[b]	Reference (Comments)
NPR	Chronic/ Carcinogenicity	150/150, CD-1 albino mouse, diet, 92 weeks	Males: 0, 8.7, 17, or 43 Females: 0, 9.3, 18, or 45 HED males: 0, 1.3, 2.6, or 6.6 HED females: 0, 7.1, 14, or 35	No effects noted (tumor or nontumor), including in timing or incidence of tumors	N/A	Not run	N/A	Ford et al. (1972); Only abstract was available for this study.
NPR	Chronic/ Carcinogenicity	125 (sex not specified), NRMI outbred albino mouse, gavage, dosed 78 days over the 18-month study duration	300[e] HED: 6.2	Tumor incidence reported to be unrelated to diphenylamine exposure and comparable to controls	N/A	Not run	N/A	Holmberg et al. (1983)
	Developmental	0/16, New Zealand white rabbit, gavage, GDs 7–19	0, 33, 100, or 300	No maternal or developmental effects	Maternal and developmental: 300[d]	Not run	Not Determinable	Edwards et al. (1983b)
NPR	Developmental	0/25, Sprague-Dawley rat, gavage, GDs 6–15	0, 10, 50, or 100[e]	Maternal: increased spleen weights, enlarged spleens, and blackish-purple colored spleen Developmental: no effects	Maternal: 50[f] Developmental: $\geq$ 100[f]	Not run	Maternal: 100[f] Developmental: Not Determinable[f]	Rodwell (1992)
NPR	Reproductive	28/28, Sprague-Dawley rat, diet, two generations	Males: 0, 40, 115, or 399[e] Females: 0, 46, 131, or 448[e]	Parental: gross pathology changes in spleen and microscopic changes in kidneys, liver and spleen Developmental: decreased F2 body weight Reproductive: decreased litter size in F1 and F2 generations	Parental (males and females, respectively): <40[f] and <46[f] Developmental: 46[f] Reproductive: 131[f]	Not run	Parental (Males:115[f] Females: 131[f] respectively): Developmental: 131[f] Reproductive: 448[f]	Rodwell (1993)
	Reproductive	3/12, Slonaker-Addis rat, diet, two generations	Males: 0, 90, 226, or 451[c] Females: 0, 101, 252, or 503[c]	Dose-dependent decrease in litter size in F1 and F2 generations; in second mating, number of pups per litter at birth significantly lower compared to controls at all 3 dose levels; no firm conclusions possible	Not Determinable	Not run	Not Determinable	Thomas et al. (1967c) Study authors concluded that results were not conclusive.

Table 2. Summary of Potentially Relevant Data for Diphenylamine (CASRN 122-39-4)

Notes[a]	Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[b]	Critical Effects	NOAEL[b]	BMDL/ BMCL[b]	LOAEL[b]	Reference (Comments)
2. Inhalation (mg/m^3)[b]								
	Subchronic	None						
	Chronic	None						
	Developmental	None						
	Reproductive	None						
	Carcinogenic	None						

[a]Notes: IRIS = utilized by IRIS, date of last update; PS = principal study, NPR = not peer reviewed, N/A= not applicable.
[b]Dosimetry: NOAEL, BMDL/BMCL, and LOAEL values are converted to adjusted daily dose, human equivalent dose (HED in mg/kg-day) or human equivalent concentration (HEC in mg/m^3) units. All exposure values of long-term exposure (4 weeks and longer) are converted from a discontinuous to a continuous (weekly) exposure. Values for inhalation (cancer and noncancer) and oral (cancer only) are further converted to an HEC/HED. Values from animal developmental studies are not adjusted to a continuous exposure.
HED = $NOAEL_{adj} \times (\text{body weight animal} \div \text{body weight human})^{0.25}$.
[c]Adjusted Daily Dose (ADD) = administered dose × food consumption per day × (1 ÷ body weight) × (days dosed ÷ total days).
[d]NOAEL/LOAEL determined from study report.
[e]Dose conversion presented by European Communities (2008).
[f]NOAEL/LOAEL reported by European Communities (2008).

HUMAN STUDIES

Oral Exposures

The effects of oral exposure to diphenylamine on humans have not been evaluated in subchronic- or chronic-duration, developmental, reproductive, or carcinogenicity studies.

Inhalation Exposures

The effects of chronic-duration and carcinogenic exposure to various amines, including diphenylamine, on humans were explored in two occupational studies (Checkoway et al., 1981; Parkes et al., 1982). While a specific exposure pathway was not identified by Checkoway et al. (1981) or Parkes et al. (1982), it may be inferred that because the exposure was in an occupational setting, the primary route of exposure was most likely via inhalation pathway along with dermal exposure. Effects of inhalation exposure to diphenylamine on humans have not been evaluated in subchronic-duration, developmental, or reproductive studies.

Chronic-duration Studies

In a case-control study, Checkoway et al. (1981) investigated the association between certain jobs and work areas in rubber and tire factories and bladder cancer. All subjects were hourly workers from five rubber factories in Akron, Ohio. Bladder cancer cases (232 total; 220 men and 12 women) were identified from a hospital record review or from death certificates obtained for mortality studies. A control:case ratio of 2:1 was employed; controls from the hourly paid workers were matched to cases based on company, sex, race, and year of birth (± 2 years). Investigators gathered work histories and grouped workers into 21 job title groups based upon similarity of materials and machinery used. Exposure to specific chemicals was not measured. The mean exposure duration among subjects diagnosed with bladder cancer was approximately 25 years compared to the mean exposure duration of 24 years in the corresponding control group. In order to estimate the risk of bladder cancer by job type, study authors calculated odds ratios (ORs) and performed chi-squared (χ^2) tests of association using the methods of Mantzel and Haenzel. Student's *t*-tests for paired samples were used to compare years and ages of initial hire, and χ^2 tests for linear trend were used to examine dose-response relationships.

Investigators limited analysis to males due to the small number of female bladder cancer cases (220 males compared to 12 females). Among males from the 113 cases identified using death certificates, 107 cases with reliable histological classification were established from hospital listings. Transitional cell carcinoma was observed in 88 (82%) of the 107 cases; other notable observations included squamous cell carcinoma (4/107), adenocarcinoma (1/107), allantoid papilloma (7/107), and undifferential carcinoma not otherwise specified (7/107). When odds ratios were calculated by the 21 different job types, only those job types suspected as having the highest exposure—milling (OR = 1.91) and calendar operation (OR = 2.21)—had statistically significantly elevated ($p < 0.05$) odds ratios compared to expected rates. The work group for final inspection of tires also had a weak association with bladder cancer risk (OR = 1.49). An apparent increase in relative risk was observed in the milling and calendar operation jobs with increased exposure duration, although tests for linear trend were not significant. However, a similar association was not observed in the final inspection of the tires job category.

When one company was analyzed separately, it appeared that the bladder cancer risk for the three job titles presented above was localized to this particular plant. The study authors stated that milling and calendar operations involve handling and heating uncured stock, which could lead to exposure from volatilized rubber chemicals. Diphenylamine is a common rubber chemical that may produce aromatic amines, but there are other carcinogenic chemicals produced during the process as well. The study authors concluded that association between certain job types and bladder cancer must be viewed with caution because the effects of the complex chemical mixture used in the rubber industry could not be separated. Furthermore, the study authors stated that their analysis was limited because confounding factors such as effects of smoking, coffee consumption, and other lifestyle factors were not considered. Because of the limitations of this study, a NOAEL or LOAEL cannot be identified.

In a retrospective cohort study, Parkes et al. (1982) investigated the association between death from cancer and working in the British rubber industry. The study included 33,815 men employed in 13 tire or rubber manufacturing plants in Scotland and England, divided into 3 cohorts (I, II, and III) based upon the date they began work in the industry. Subjects were first employed between 1946 and 1960 and were followed until 1975. Investigators required that all workers had worked continuously for a minimum of 1 year in the industry and also required that the workers had survived for a minimum of 9 years after the first year of employment in order to allow for a long latency period for cancer. Factory records and a subsequent screening of the Office of Population Censuses and Surveys in England were used to ascertain current or former workers' status at the end of the study period. Details were collected from death certificates, with deaths coded according to the International Classification of Diseases (ICD). Jobs were placed in 1 of 10 categories based on similar characteristics. The expected numbers of deaths were calculated using national age-specific mortality rates for England and Scotland. A *p*-value was calculated for the difference between observed and expected deaths. Standardized mortality ratios (SMRs) were calculated when appropriate.

The follow-up rate was high (98.5%); 4882 men were identified as dead, and 28,410 were traced as alive at the end of the study. Investigators determined a significant ($p < 0.001$) overall excess of cancer deaths with a total of 1359 recorded deaths compared to 1221 expected deaths. However, Cohorts I (men entering the industry between 1946 and 1950) and III (men entering the industry between 1956 and 1960) were the only cohorts showing excess deaths. The largest excess was seen in the general rubber goods sector of Cohort I (SMR = 144). Furthermore, specific occupations in the industry, including component building, preparation, assembly, vulcanizing, curing, molding press, autoclave, pan, inspection, painting, trimming, site workers, internal transport, and general truck drivers, were primarily responsible for the statistically significant ($p < 0.05$ to $p < 0.001$) increase in deaths. The most common causes of death were lung cancer (observed: 638, expected: 517; $p < 0.01$) and stomach cancer (observed: 183; expected: 141.8; $p < 0.01$). For lung cancer, almost the entire excess came from Cohort I, partially due to the small numbers in Cohorts II and III. For stomach cancer, most of the excess also came from Cohort I. Although the study authors noted that the smaller number of deaths precludes any meaningful examination by occupation, statistically significant excesses occurred in the following job categories: compounding, weighing, mixing, reforming, and washing ($p < 0.01$); component building, preparation, and assembly ($p < 0.05$); finished goods, packaging, and dispatch ($p < 0.01$); and site workers, internal transport, and general truck drivers ($p < 0.001$). Bladder cancer was only slightly associated with work in the tire sector of Cohort I

(nonsignificant), mainly in jobs involving heating of the rubber product. The study authors noted that it is likely that only men entering the industry before 1950 were exposed to known bladder carcinogens that were later withdrawn from production. There were also statistically significant ($p < 0.05$ to $p < 0.001$) excesses in esophageal cancer in all three cohorts, although these cancers were less common than lung and stomach cancers.

This study did not collect information on smoking and other lifestyle choices and, thus, did not include these confounding factors in the overall analysis. In addition, the investigators did not calculate social class-specific mortality, which would have helped control for potential bias introduced by class. This study is also limited by the inability to separate the effects of exposure to a number of different chemicals in the industry. Because of the limitations of this study, a NOAEL or LOAEL cannot be identified.

ANIMAL STUDIES

As described above, a number of unpublished studies with diphenylamine were located in a formal government risk assessment document (European Communities, 2008), where they are reported as thorough descriptive summaries along with the full reference to the complete report. It is clear that these summaries have been prepared from existing full reports and not abstracts only as may exist, for example, from a scientific conference. Given that these summaries are acknowledged and utilized in the European assessment, all those listed are considered as relevant and valid for use in this assessment. The adjusted doses listed in these summaries were provided in the former risk document (European Communities, 2008) and were not calculated.

Oral Exposures

The effects of oral exposure to diphenylamine on animals have been evaluated in 2 short-term studies (Eknoyan et al., 1976; Yoshida et al., 1989), 4 subchronic-duration studies (Krohmer, 1992a,b; Dow Chemical Company, 1958; Botta, 1992), 12 chronic-duration and/or carcinogenicity studies (Botta, 1994a,b,c; DeEds, 1963a,b; Evan et al., 1978; Ford et al., 1972; Griswold et al., 1966; Holmberg et al., 1983; Thomas et al., 1967a,b; Thomas et al., 1957), 2 developmental toxicity studies (Edwards et al., 1983b; Rodwell, 1992), and 2 reproductive toxicity studies (Rodwell, 1993; Thomas et al., 1967c).

Short-term Studies

In a peer-reviewed study to evaluate renal function and histopathology as a consequence from exposure to diphenylamine, Eknoyan et al. (1976) administered 2.5% diphenylamine in the diet for 3–6 weeks to female Sprague-Dawley rats (number not specified) that weighed between 70 and 90 g. The estimated average daily converted dose for diphenylamine is 2451 mg/kg-day with control animals receiving diet (Wayne Rat Chow, Allied Mills, Inc., Chicago, IL) only. It is unclear whether this study complied with Good Laboratory Practice (GLP).

The study authors found gross cysts in the corticomedullary region of approximately 10% of all kidneys and morphological alterations in the kidneys of all experimental animals. The most common change was cystic dilatation of the collecting ducts, although the study authors occasionally noted proteinaceous casts in the dilated tubules. Some animals had focal dilatation of the cortical collective ducts and distal tubules. The duration of the diphenylamine treatment affected the severity and extent of these morphological changes. There was a significant but temporary drop (*p*-value not reported) in maximal urine osmolality 1 week after the beginning of

treatment and at 2 weeks when this measure was decreased to 1401 ± 96 milliosmoles (mOsm) compared to 3224 ± 144 mOsm before treatment. The osmolality remained steady for the rest of the treatment period. Micropuncture studies revealed focal regions of dilated tubules in treated rats, although gross cysts were not apparent on the kidney surface.

The study authors concluded that, based on these results, the nephron (specifically the terminal portion of the collecting duct) is the site most likely affected by diphenylamine exposure to at least temporarily decrease the ability to concentrate urine in treated rats. Based on the results presented above, a LOAEL of 2.5% (2451 mg/kg-day) is identified in this study for significant reduction in maximal urine concentrating ability and for the appearance of gross cysts in the corticomedullary region of the kidney. A NOAEL cannot be identified in this study. This study is limited by the use of a small number of animals per investigated outcome and the use of a single dose of diphenylamine in feed.

In a 28-day unpublished study, Yoshida et al. (1989) examined the oral toxicity of diphenylamine in Fischer rats. The full study is not available for review. A summary of the study is available on page 78 of the European Union Risk Assessment Report on diphenylamine (Yoshida et al. 1989 as discussed in European Communities, 2008).

> *In a guideline conform oral 28 day study Fischer rats of both sex received 111, 333 and 1000 mg/kg bw/d diphenylamine by gavage. Thirty-six animals were divided into 6 groups of equal numbers, 4 groups being used for treatment and the remainder for investigation of recovery. Inhibition of body weight gain, increase of liver, spleen and kidney weights and anemia were observed in the high dose group in both sexes. The same group demonstrated mucosal hyperplasia in the forestomach, dilatation, degeneration or necrosis of renal tubules in the corticomedullary junction and hyperplasia in the bone marrow histopathologically. Slight increase[s] of spleen, liver and kidney weights as well as slight degeneration of renal tubules were evident in several animals of the 333 mg/kg bw/d dose group. Repair of histopathologic lesions and anemia occurred after 14 days. As there were no toxic effects in the low dose group a NOAEL of 111 mg/kg bw/d was derived under these experimental conditions (Yoshida et al. 1989).*

No other information or statistical analysis is available. A LOAEL of 1000 mg/kg-day was identified in the European Union Risk Assessment Report on diphenylamine (Yoshida et al. 1989 as discussed in European Communities, 2008; Table 4.1.2.6).

Subchronic-duration Studies

Krohmer (1992a) conducted a 90-day unpublished study examining the oral toxicity of diphenylamine in purebred beagle dogs (four/sex/group; Krohmer 1992a as discussed in European Communities, 2008). The full study is not available for review. A study summary is available on page 81 of the European Union Risk Assessment Report on diphenylamine (European Communities, 2008).

> *Groups of four pure-bred beagle dogs of each sex received technical-grade diphenylamine (purity, > 99%) in gelatin capsules at doses of 0, 10, 25 or 50 mg/kg bw per day for 90 days. They were observed for deaths, clinical signs, body weight, food consumption, ophthalmological, haematological, clinical chemical and urinary*

parameters, organ weights, and gross and histopathological appearance. There were no deaths, and no treatment-related changes were seen in any of the above parameters. Statistically significant increases were seen, however, in some clinical chemical parameters including albumin content, the albumin:globulin ratio in males, and bilirubin content in females at the high dose. These effects may have been incidental. The NOAEL was 50 mg/kg bw/day, the highest dose tested.

An unpublished study by Dow Chemical Company (1958) examined the effects of dietary subchronic-duration exposure to diphenylamine on rats. Ten rats (strain not reported) per sex per group were administered doses of 0-, 0.01%- (100-ppm), 0.03%- (300-ppm), 0.1%- (1000-ppm), 0.3%- (3000-ppm), or 1%- (10,000-ppm) diphenylamine (purity not reported) neat in the diet for 90 days. Rats were 45 days old at the time of the first dose. Information about adherence to GLP guidelines is not provided in the study report. Rats were housed two per cage, and food (Purina lab chow pellets) and water were available ad libitum. Appropriate body weight data and food consumption data for dose conversion were not provided in the study. Therefore, average values provided for the available rat strains by EPA (1994b) for body weight (0.235 kg for males and 0.1728 kg for females) and food consumption (0.0212 kg for males and 0.0174 kg for females) are used in the dosimetric calculation. Adjusted daily doses were 0, 9.8, 25, 78, 236, or 791 mg/kg-day for males and 0, 12, 32, 96, 303, or 978 mg/kg-day for females, respectively. Rats were weighed twice weekly during the first 28 days of the study and once per week thereafter. Food consumption was recorded for the first month of the study. Study authors observed animals for clinical signs of toxicity, and dead or moribund animals were autopsied. Hematological values were obtained at necropsy from five females at the 0, 303 and 978 mg/kg-day dose levels. The study authors recorded lung, heart, liver, kidneys, spleen, and testes weights at study termination. These organs, as well as the pancreas and adrenals, were also examined histologically. Use of statistical methods was not reported, and it appears that this report was not peer reviewed.

The Dow Chemical Company (1958) reported no evidence of critical effects in males in the 96 and 303 mg/kg-day dose groups. It is unclear if this study was performed according to GLP standards. The study authors noted the appearance of weakness of the hind legs in both sexes at the 1.0%-dose level after 50 days of treatment. Additionally, growth retardation was noted in both sexes with the effect being more pronounced in male rats. The study authors also reported an elevated level of mortality in male rats (further details not provided), but they attributed this finding mainly to upper respiratory infections. Average hemoglobin values of female rats decreased slightly in a dose-related manner (see Table B.1). Table B.2 provides data for body weights at necropsy and relative organ weights for male and female rats. Average relative liver weight was statistically significantly ($p < 0.01$) increased in females at all dose levels and in males at doses ≥78 mg/kg-day of diphenylamine. Average relative spleen weight was statistically significantly increased ($p < 0.01$) in females at doses ≥32 mg/kg-day of diphenylamine and in males at the highest dose level. Average relative kidney weights were increased in both males and females treated with 1.0% diphenylamine (791 mg/kg-day for males and 978 mg/kg-day for females). The study authors reported a brown coloration of tissues in females at the three highest dose levels and in males at the two highest dose levels. The study authors reported central lobular necrosis in the livers, increased interstitial nephritis in the kidneys, and marked congestion in the spleens of both sexes at the highest dose level. Incidence data for these changes were not available in the study report. Based on these results, LOAELs of

0.03% (32 mg/kg-day) in female rats and 0.3% (236 mg/kg-day) in male rats are identified in this study because of a 10%-increase in relative liver weight, considered to be biologically significant. NOAELs of 0.01% (12 mg/kg-day) and 0.1% (78 mg/kg-day) are identified from this study in female and male rats, respectively.

Krohmer (1992b) conducted a 90-day unpublished study examining the oral toxicity of diphenylamine in Sprague-Dawley rats. The full study is not available for review. A study summary is available on pages 79–80 of the European Union Risk Assessment Report on diphenylamine (European Communities, 2008).

> *Groups of 10 male and 10 female Sprague Dawley rats received technical-grade diphenylamine in the diet at 0, 150, 1500, 7500, or 15000 ppm for 90 days, equal to doses of 0, 9.6, 96, 550, and 1200 mg/kg bw per day in males and 0, 12, 110, 650, and 1300 mg/kg bw per day in females. The frequency of darkening of the urine increased with dose, starting with one female at 1500 ppm and 100% of rats at 15000 ppm. Haematological measures indicated decreased erythrocyte counts and haemoglobin values, which were statistically significantly different from those of controls in animals at 7500 and 15,000 ppm at termination. The haematocrit values were statistically significantly lower than those of controls in females at the three highest doses. Small, statistically significant increases in alkaline phosphatase activity, albumin content, and albumin:globulin ratio in males and glucose and albumin content and albumin:globulin ratio in females were observed at 7500 and 15000 ppm. The cholesterol concentration increased with dose in females and was statistically significantly different from that of controls at the three higher doses. In males, the absolute and relative weights of the liver and spleen increased with dose and were statistically significantly raised at 7500 and 15000 ppm; the relative weights of the kidney and gonad also increased with dose and were also statistically significant at the two higher doses. In females, the absolute and relative weights of the liver increased with dose, and the change in relative weights was statistically significant at doses > 1500 ppm. The kidneys were dark in animals of each sex at 7500 and 15000 ppm, and about 60% of the females at the high dose had dark and/or enlarged livers. The spleens of both males and females at the two higher doses were congested. Histopathological examination revealed an increased incidence of haematopoiesis and pigment in the liver, haematopoiesis, haemosiderosis, and congestion in the spleen, and pigmented kidneys in animals of each sex at 7500 and 15000 ppm. The spleens of all females at 1500 ppm also showed an increase from minimal to slight haematopoiesis and haemosiderosis. The NOAEL was 150 ppm, equal to 12 mg/kg bw per day, on the basis of increased clinical signs of toxicity, clinical chemical changes, organ weights, and gross and histopathological appearance.*

A LOAEL of 110 mg/kg-day and NOAEL of 12 in females was identified in the European Union Risk Assessment Report on diphenylamine (Krohmer 1992b as discussed in European Communities, 2008; Table 4.1.2.6).

The subchronic-duration study by Botta (1992) is selected as the principal study for the derivation of a subchronic p-RfD. Botta (1992) conducted a 90-day unpublished, nonpeer-reviewed study examining the effects of technical-grade diphenylamine on Swiss-derived CD-1 mice. Fifteen mice per sex per dose received 0-, 10-, 52-, 260-, or

5200-ppm diphenylamine (purity not given) in feed. Adjusted daily doses provided in the principal study (Botta, 1992) are 0, 1.7, 94, 444, or 926 mg/kg-day for males and 0, 2.1, 107, 555, or 1101 mg/kg-day for females. This study adhered to GLP guidelines. All animals were observed for clinical signs daily, including mortality/moribundity checks twice daily. Body weight and food consumption were recorded weekly, and ophthalmology examinations were conducted prior to treatment initiation and during the final week of the study. Food consumption was recorded throughout the study period. A complete postmortem examination was performed on all mice that died or were sacrificed in a moribund state. After 90 days of dietary exposure to diphenylamine, remaining animals were fasted overnight, final body weight was recorded, and mice were euthanized in a CO_2 chamber. Animals were bled for clinical chemistry analyses. Selected organs (liver, kidneys, heart, spleen, pituitary, brain/brainstem, testes/epididymides, ovaries, and adrenals) were weighed, and the terminal organ and body weights were used to calculate relative organ-/body-weight ratios. Tissues were collected and prepared for histopathological examination. Statistical analysis (Dunnett's Test) was performed on body weight, food consumption, hematology, and organ-weight data.

Six unscheduled deaths occurred during the study (Botta, 1992); there were three female deaths in the control group and three male deaths in the high-dose group. It was not explained by the study authors if these deaths were treatment related. Slight treatment-related effects were observed at 525 ppm (94 mg/kg-day for males and 110 mg/kg-day for females) with the appearance of brownish-yellow pigment and extra-medullary hematopoiesis in the liver. At the third highest dose group (94 mg/kg-day) in females and the fourth highest dose group (560 mg/kg-day) in males, there were statistically significant increased incidences of congestion and hemosiderosis in the spleen. As the dosage was increased, not only were the incidence and severity of the lesions in the spleen and liver increased, but pigment in kidneys, increased cellularity in the bone marrow, and cystitis (in the 5250-ppm group [926 mg/kg-day for males and 1101 mg/kg-day for females]) were also present. Treatment-related increases in relative organ weights were seen in the liver and spleen in the highest dose groups (2625 [444 mg/kg-day for males and 555mg/kg-day for females] and 5250 ppm [926 mg/kg-day for males and 1101 mg/kg-day for females]). There were also increases in absolute liver weight observed in males which were statistically significant at the second highest dose (444 mg/kg-day) tested and were both biologically (≥10% increase) and statistically significant at the highest dose (926 mg/kg-day) group. Absolute kidney weight was biologically significantly increased at the highest dose (926 mg/kg-day) in males coupled with increased relative kidney weight in female mice in the highest dose (1101 mg/kg-day) group, which was biologically significant. Relative ovary weight was statistically significantly decreased at the middle (107 mg/kg-day) and high (1101 mg/kg-day) dose group in female mice. Table 2 presents data for the effect on diphenylamine exposure on organ and body weight. Hematology parameters showing treatment-related effects included statistically significant decreases in red blood cell (RBC) count and hematocrit (data not shown). Also, statistically significant increases in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) in the two highest dose groups were observed (data not shown). The highest dose group also showed a marked increase in reticulocytes (data not shown). In the 525-ppm group (94 mg/kg-day for males and 110 mg/kg-day for females), there was a statistically significant increase in MCHC (data not shown). Based on these results, LOAELs of 107 mg/kg-day in female mice and 444 mg/kg-day in male rats are identified in this study by causing statistically significant increased incidences of congestion and hemosiderosis in the spleen. NOAELs of

2.1 mg/kg-day and 94 mg/kg-day are identified from this study in female and male rats, respectively. The data for these effects (i.e., congestion and hemosiderosis in the spleen) are further evaluated with the BMDS modeling program for determination of a POD for the screening subchronic p-RfD in Appendices A and C.

Chronic-duration Studies

IRIS (U.S. EPA, 2010a) has provided an RfD. However, subsequent to the IRIS file, additional studies that may be relevant have been discovered. These studies are summarized in this PPRTV assessment. These additional studies do not provide a lower or more credible POD than the study formerly used by IRIS (i.e., Thomas et al., 1967a). In the Thomas et al 1967a study, groups of sixteen beagle dogs (two per sex per group) were treated orally with diphenylamine in concentrations of 0.01% (2.5 mg/kg bw/d), 0.1% (25 mg/kg bw/d), and 1.0% (250 mg/kg bw/d) in the diet for 2 years. Mid- and high-dose animals developed marked growth retardation after 1 year. A dose-dependent anemia was seen in the same treatment groups, being pronounced in the high-dose group and moderate in the mid-dose group. After 2 years, the erythrocytes of the dogs on the 1.0%-diet showed a moderate decreased resistance to hypotonicity. Liver function as a result of sulfobromophthalein testing at Days 618 to 627 indicated a moderate degree of liver damage in the 1.0%-group. These animals also showed an increased organ weight of the liver with perilobular fatty changes and increased lipid content, a mild haemosiderosis of the spleen, kidneys, and bone marrow, and a slight increase in kidney weight (Thomas et al., 1967a). A LOAEL of 25 mg/kg-day was identified by IRIS for decreased body-weight gain and increased liver and kidney weights. A NOAEL was identified to be 2.5 mg/kg-day.

Botta (1994a) conducted a 52-week unpublished study examining the oral chronic toxicity of diphenylamine in beagle dogs (four/sex/group; Botta 1994a as discussed in European Communities, 2008). The full study is not available for review. The study summary available on pages 90–91 of the European Union Risk Assessment Report on diphenylamine (European Communities, 2008) is presented below.

> *Four beagles of each sex received diphenylamine (purity > 99%) by gelatin capsule at a dose of 0, 10, 25, or 100 mg/kg bw per day for 52 weeks and were observed for clinical signs, body weight, food consumption, ophthalmological, haematological, clinical chemical and urinary end-points, organ weights, and gross and histopathological appearance. No treatment-related clinical signs were seen at termination. One dog at the intermediate dose and two at the high dose had greenish hair. There were no deaths or treatment-related effects on body weight, food consumption, or ophthalmological parameters. Haematological examination revealed decreased mean erythrocyte counts (by 11% in comparison with controls), haemoglobin (9.3%), and haematocrit (8.7%) in males at the high dose; smaller decreases in these parameters were found in females. The platelet count increased with dose in males at the 13-, 26-, 39-, and 52-week evaluation periods, becoming statistically significant at the intermediate and high doses. There was a dose-related increase in mean total bilirubin concentration, which was statistically significant for animals at the intermediate and high doses throughout the study, in animals at the low dose at week 26, and in females only at week 39. The mean cholesterol concentration appeared to increase with dose at all evaluation periods but was statistically significantly increased only in males at the high dose at week 13 (by*

> *68%) and in females at the high dose at week 39 (by 37%). The blood urea nitrogen concentration was decreased in females at the intermediate (by 16%) and high doses (by 20%) at week 52. The mean absolute and relative weights of the liver and thyroid appeared to increase with dose in males, but only the mean absolute liver weight of males at the high dose was statistically significantly increased. The mean absolute and relative weights of the thyroid decreased with dose in females but did not reach statistical significance at any dose. There were no treatment-related gross or histopathological changes. The NOAEL for toxicity was 10 mg/kg bw per day on the basis of small clinical chemical changes.*

A LOAEL of 25 mg/kg-day based on "small clinical changes" was identified in the European Union Risk Assessment Report on diphenylamine (Botta 1994a as discussed in European Communities, 2008; Table 4.1.2.6).

In a published summary of chronic-duration toxicity studies on diphenylamine, DeEds (1963a,b) administered diphenylamine in feed to dogs and rats over a 2-year period. It is unclear whether either of these studies was GLP compliant. Raw data were not provided for either of these studies, and details regarding experimental methods were limited.

In the dog study (DeEds, 1963a), groups of two beagles per sex were fed diets of 0.01%- (100-ppm), 0.1%- (1000-ppm), or 1%- (10,000-ppm) diphenylamine (purity = 99.9%) over 2 years. Adjusted daily doses calculated using default data for body weight (U.S. EPA, 1994b) and food consumption (U.S. EPA, 1988) are 2.1, 21, or 208 mg/kg-day for males and 1.9, 19, or 185 mg/kg-day for females. Treated animals were examined for changes in growth, changes in hematological parameters, and changes in liver and kidney function. At study termination, animals were sacrificed and received a histopathological examination. Use of a control group was not specified in the study report.

Mortality data were not presented in the study report. Growth in dogs treated with 1%-diphenylamine was severely inhibited up to Day 400. (An outbreak of distemper after 400 days made further interpretation of growth difficult.) Between Days 724 and 731, dogs treated with 1%-diphenylamine experienced reduced hemoglobin levels and RBC counts, as well as crenated red blood cells that were fragile. Liver function tests revealed that retention of sulphobromophthalein may have been increased in dogs exposed to 1%-diphenylamine. Histopathological examination revealed no parasites, tumors, or other indications of disease. Liver weight (no data regarding absolute or relative weight) was increased at 1%-diphenylamine due to marked fatty change measured in extractable lipids and intracellular bilirubin present in moderate amounts. Changes in RBC fragility in the 1%-dose group were reflected by some hemosiderosis of the spleen, kidney, and bone marrow. The small numbers of animals made organ-weight data difficult to interpret, but the study authors noted that there might have been increased kidney and spleen weights in dogs treated with 1%-diphenylamine in diet. Because raw data were not provided in the study report and the use of a control group was not specified, firm conclusions regarding the toxic effect of diphenylamine exposure in dogs cannot be made from the study results. Hence, a LOAEL and a NOAEL cannot be determined from this study.

In the rat study (DeEds, 1963b), groups of 20 albino weanling rats per sex were given feed containing 0-, 0.001%- (10-ppm), 0.01%- (100-ppm), 0.1%- (1000-ppm), 0.5%-

(5000-ppm), or 1%- (10,000-ppm) diphenylamine for 734 days. Adjusted daily doses calculated using default data for body weight (U.S. EPA, 1994b) and food consumption (U.S. EPA, 1988) are 0, 0.72, 7.2, 72, 362, or 723 mg/kg-day for males and 0.82, 8.2, 82, 410, or 820 mg/kg-day for females. Treated animals were examined for growth and food consumption, survival, and changes in hematological parameters. At study termination, animals were sacrificed and received a histopathological examination.

Survival was not reduced in any group. Results indicated that growth inhibition did not occur at or below 0.1%, but at higher levels, growth was depressed. Because data tables were not provided, percent change in growth between treated groups and the control group cannot be determined. Food consumption was reduced by more than 10% in animals fed 1%-diphenylamine. At 1%-diphenylamine, animals experienced moderate anemia, indicated by reduced hemoglobin and RBC levels and an increase in circulating normoblasts (RBC precursors). Histopathological examination revealed that treated animals had cystic dilated renal tubules and chronic interstitial nephritis; however, these lesions were not different from controls at 0.01% and lower. The lesions were much more severe in the 0.1%- and 0.5%-groups compared to controls. The study authors stated that, based on a detailed record of pathological changes (data not presented) in various groups, the lesions were not related to diphenylamine exposure. Because data tables were not provided in the study report, firm conclusions regarding the toxic effect of diphenylamine exposure in rats cannot be made from the study results. Hence, a LOAEL and a NOAEL cannot be determined from this study.

Evan et al. (1978) conducted a 76-week study examining the oral toxicity of diphenylamine in male Sprague-Dawley rats (number not specified). The full study is not available for review. A summary of the study available on page 79 of the European Union Risk Assessment Report on diphenylamine (European Communities, 2008) is presented below.

> *In a study male Sprague Dawley rats were given 1.0% (500 mg/kg bw/d) diphenylamine in food up to 76 weeks. After 2 to 6 weeks the animals developed polyurea with diluted urine. The first histological change was noted after 5 weeks, and was described as focal proliferation of distal tubular and collecting duct cells. Focal areas of medullary tubuli appeared thickened because several cells were layered upon each other. By week 10 collecting ducts showed cystic dilatations with focal necrosis and increasing cast material in the duct lumina. As the study was not designed for toxicologic purposes, a NOAEL was not derived.*

Thomas et al. (1957) published a peer-reviewed study examining the effects of diphenylamine exposure to rats for 226 days (approximately 32 weeks). The study authors did not report whether this study was conducted according to GLP guidelines. Six albino female rats (strain not specified) per sex per group were administered 0-, 0.025 (250-ppm), 0.1%- (1000-ppm), 0.5%- (5000-ppm), 1.0%- (10,000-ppm), or 1.5%- (15,000-ppm) diphenylamine (purity not reported) in feed for 226 days. Adjusted daily doses calculated using default data for body weight (U.S. EPA, 1994b) and food consumption (U.S. EPA, 1988) are 0, 21, 82, 410, 820 for male rats, or 1230 mg/kg-day for female rats. No details regarding husbandry of rats were provided. Body weights were recorded at study termination, and food consumption was recorded (further details not provided). Animals were sacrificed at study termination and

necropsied. Microscopic examinations were performed on liver, kidney, spleen, adrenal, and heart tissue. The study authors did not specify any statistical tests used to analyze the data.

Thomas et al. (1957) reported no mortalities with the exception of one rat at the 0.10%-dose level that was removed from the study on Day 156 due to a severe respiratory infection. The study authors noted a dose-related decrease in final average body weight and stated that food consumption did not vary by more than 7.5% in all treated groups from control values (see Table B.3). The study authors observed a drop in the growth curve of rats in the 1.50%-dose group during the first few days of the study due to temporary lack of food intake. Enlargement of the kidneys was observed in rats in the 1.50%-diphenylamine dose group (30% larger than controls). Microscopic examination of sections of liver, kidney, spleen, adrenals, and heart revealed pigment deposition possibly due to blood destruction, along with multiple cystic structures in the kidneys. Microscopic examination also revealed brown pigmentation of Kupffer cells in the liver and in the tubular epithelial cells of the kidneys, particularly in the epithelial cells of the proximal convoluted tubules. This pigmentation was noted in rats administered ≥0.50%-diphenylamine, and the amount of pigmentation appeared to be correlated with the amount of diphenylamine consumed. The study authors also recorded the presence of cysts in the kidneys of rats at the highest dose level. A LOAEL of 0.50% (410 mg/kg-day) is identified in this study for microscopic changes in the kidney. A NOAEL of 0.10% (82 mg/kg-day) is also identified.

In a chronic-duration toxicity/carcinogenicity study, Botta (1994b) administered diphenylamine to Sprague-Dawley rats (10 animals per sex per dose group) in the diet for 2 years at concentrations of 0, 200, 750, 3750, or 7500 ppm in males and 0, 150, 500, 2500, or 5000 ppm in females. These concentrations are equivalent to average daily doses of 0, 8.1, 29, 150, or 300 mg/kg-day in males and 0, 7.5, 25, 140, or 290 mg/kg-day in females, as calculated by European Communities (2008). It is unclear whether this study is GLP compliant, although it appears that is has been peer reviewed. The full study is not available for review. The study review as provided in the European Union Risk Assessment Report (European Communities, 2008; pages 91–92) is summarized below.

> *Sprague-Dawley rats received diphenylamine (>99.0%) at dose levels of 0, 200, 750, 3750 or 7500 ppm in males (equal to 8.1, 28.8, 146.7, or 302.1 mg/kg/day) and 0, 150, 500, 2500, or 5000 ppm in females (equal to 7.5, 24.9, 137.8, or 286.1 mg/kg/day) in the diet for 2 years. A one year interim sacrifice of 10 animals per sex per dose group was used. There was no treatment related mortality noted, however, the study was terminated early due to increased mortality in the control and low dose animals. No effects were noted in ophthalmic examinations. The only treatment related clinical observation was a greenish tint to the hair coat in the urogenital or ventral cervical area which was assumed to be due to an...oxidative product of the interaction of the test article or a metabolite with urine or feces in the high mid and high dose groups. Systemic toxicity was noted at the high mid and high dose groups in both sexes as decreased mean body weights and body weight gains (statically significant). Food consumption was increased in the same dose groups; however, due to food spillage, when food consumption values exceeded two standard deviations from the mean, they were not included in calculation of the group mean food consumption. Treatment related effects were noted in*

hematology involving red cell elements mainly in the high mid and high dose groups. Increases in albumin levels, decreases in globulin levels and increased albumin/globulin ratios were noted but, the biological relevance to these changes is unknown since there was no related pathology. Some slight transient effects were also noted in alkaline phosphatase and total bilirubin also in serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT). Urinalysis did not reveal any specific treatment related effects except a slight increase in ketones in the high dose due to incomplete or partial interference of the test article causing a false positive reading. There was an increase in spleen weights in both sexes in the high mid and high dose groups at the interim sacrifice and terminal sacrifice. Gross necropsy observations revealed a roughened surface to the kidneys in the high dose groups. Treatment related non-neoplastic observations were splenic congestion, increased hemosiderosis and hematopoiesis in the spleen, pigment deposits in the kidneys, and increased hematopoiesis in the liver in the high mid and high dose groups. No treatment related increase in any tumor type or site was seen in either sex at any dose level. Methemoglobin was not measured in this study. For chronic toxicity the NOEL was 28.8 mg/kg/day in males and 24.9 mg/kg/day in females and the LOEL was 146.7 mg/kg/day in males and 137.8 mg/kg/day in females based on reduced mean body weight and body weight gains, changes in hematological parameters, splenic and kidney lesions and increased clinical signs of toxicity. There was no evidence of carcinogenicity.

A LOAEL of 25 mg/kg-day based on haematological and histological effects and NOAEL of 7.5 in female rats were identified in the European Union Risk Assessment Report on diphenylamine (Botta 1994b as discussed in European Communities, 2008; Table 4.1.2.6).

Thomas et al. (1967b) published a peer-reviewed 2-year study examining the chronic toxicity and carcinogenicity of diphenylamine administered in the diet to weanling albino Slonaker-Addis rats. Twenty rats per sex per group were administered 0-, 0.001%- (10-ppm), 0.01%- (100-ppm), 0.1%- (1000-ppm), 0.5%- (5000-ppm), or 1.0%- (10,000-ppm) diphenylamine (purity 99.9%) in feed with water provided ad libitum. Adjusted daily doses calculated using default data for body weight (U.S. EPA, 1994b) and food consumption (U.S. EPA, 1988) are 0, 0.72, 7.2, 72, 362, or 723 mg/kg-day for males and 0, 0.82, 8.2, 82, 410, or 820 mg/kg-day for females. Rats were housed five per cage. The study authors did not report whether this study adhered to GLP guidelines. Rats were weighed once per week during the first 5 months of the study and approximately once per month thereafter. The study authors recorded food consumption in 6- to 8-day intervals. All survivors at study termination were necropsied beginning on Day 734. Hematology data were collected only from select rats in the 1.0%-dose group. The Duncan method was used to test statistical difference in data between the treated and control groups.

Thomas et al. (1967b) generally reported 2–4 mortalities by Day 240 in males and females due to respiratory infection (see Table B.4). The study authors reported depressed average weights of females receiving ≥ 0.1%, which appears to be considerably less than 10% from control rats, and males receiving ≥ 0.5%, which appears to be somewhat greater than 10% (only graphical data provided). Average food consumption during the first 240 days was

significantly decreased for both sexes in the two highest dose groups. Hemoglobin and RBC counts of albino male and female Slonaker-Addis rats at the 1.00% dose level were decreased from controls but not significantly (see Table B.5). The study authors reported that diphenylamine-related lesions were limited to the urinary tract. Lesions included chronic nephritis, tubular cysts, and epithelial hyperplasia/metaplasia (see Table B.6) and were distinguishable from those of control rats at the ≥0.5%-diphenylamine dose levels. Irregularly dilated kidneys were occasionally filled with proteinaceous fluid or with iron-positive pigment resulting from blood-derived masses. Morphological changes were not observed in the glomeruli and were rarely observed in the proximal convoluted tubules. Mild epithelial hyperplasia or squamous metaplasia of the epithelial linings of the renal pelvis and bladder were observed, along with an accumulation of masses of pigment and protein in these organs. Interstitial lymphocyte accumulation and scarring were also noted, along with renal cystic changes. Frequent accumulation of neutrophils in the pigment-filled cysts was also reported. Severe inflammation was not observed in the absence of marked cystic changes. Based on the increased incidence of rats having a severity score for chronic nephritis "two plus" as well as the increase (significance not indicated) of the mean severity score over the controls (in Table 6 of the Thomas et al. 1967b study), the 0.5%-dose group appears to be a clear effect level. In contrast, the "break-point" for cystic change was not clear, but it was reported by the study authors to occur at the 0.1%-dose group.

Tumor incidence was reported by the study authors to be due to senility of the test rats and not due to exposure of diphenylamine. Adenomatous hyperplasia of the adrenal medulla, three of which were malignant, was reported to be the most commonly occurring tumor (see Table B.7). In addition, cystic dilation of the peripancreatic lymph node and occurrence of eosinophilic granuloma of the gastric submucosa were also frequently observed. The study authors also reported senile pancreatic and testicular atrophy as well as chronic respiratory disease.

Although urinary tract lesions were observed in treated animals, the incidences were comparable to those noted in the concurrent control group (see Table B.6). Lesions included cystic dilation of renal tubules and interstitial inflammation (see Table B.6) and were distinguishable from those of control rats at the ≥0.5%-diphenylamine dose levels. Based on these results, a LOAEL of 0.5% is identified for decreased body weight and increased incidence and severity of chronic nephritis in both female (410 mg/kg-day) and male (362 mg/kg-day) rats in this study. It should also be noted, however, that the decreases in weight were accompanied by significant decreases in food consumption. A NOAEL of 0.1% (82 mg/kg-day for females and 72 mg/kg-day for males) is also identified.

Griswold et al. (1966) published a peer-reviewed study on the mortality and carcinogenicity effects of a single maximum tolerated oral dose to each of 50 different chemicals in 20 female Sprague-Dawley rats. Evaluation was at 6 months after the single dose. Diphenylamine was administered intragastrically in sesame oil at 300 mg/rat. The adjusted daily doses calculated using default data for body weight (U.S. EPA, 1994b) and food consumption (U.S. EPA, 1988) is 1.7 mg/kg-day. A separate group of animals (n = 89) served as a control group, receiving sesame oil alone. Dimethylbenzanthracene, known to cause mammary gland tumors in this species under these time constraints (6 months), was used as the positive control. While purity of the chemicals tested in this study was not reported, the study authors stated that

chemicals were checked for purity, and when necessary, purified using appropriate techniques. Rats were housed five per cage and supplied food and water ad libitum. It is unclear whether this study adhered to GLP guidelines.

Two animals of the 20 administered diphenylamine died by 30 days postadministration with no others dying between 30 days and termination of the experiment at 6 months. Mortality was also observed among the controls, with 5/89 dying before the 6-month sacrifice. Histological descriptions of tissues that were considered to be neoplastic or suggestive of preneoplasia were listed in Table 2 of this study (including hyperplasia or metaplasia of the mammary glands, tubo-ovarian disease, lung lesions, as well as any other miscellaneous diseased tissues found during the course of the examinations). Tissues in which disease entities were not directly suggestive were not included in this study report table. As no mention of diphenylamine is made in this table, it is presumed that no such effects were seen with this compound.

In a chronic-duration toxicity/carcinogenicity study (Botta, 1994c), CD-1 mice (60 sex/group) were administered diphenylamine (>99%) in the diet at levels of 0, 525, 2625, or 5250 ppm (males: 73, 370, and 760 mg/kg/day; females: 90, 460, or 940 mg/kg/day) for 78 weeks. There was a significant treatment-related increase in overall mortality in the 2625- and 5250-ppm groups. The increased mortality was due to cystitis in males and amyloidosis in females. A greenish staining of the hair was the most frequently observed clinical sign, with some of the 525-ppm group and essentially all of the 2625- and 5250-ppm groups affected by the end of the study. Mean body-weight gain was significantly decreased in the 5250-ppm group males at the majority of the time points in the study. Haematological examination revealed decreased mean erythrocyte counts (by 11% in comparison with controls), haemoglobin (9.3%), and haematocrit (8.7%) in males at the high dose; smaller decreases in these parameters were found in females. Changes in the hematology parameters indicate that the chemical produced a regenerative anemia in the 2625- and 5250-ppm group males and females. On gross examination at the interim and terminal necropsies, the liver and spleen of the 2625- and 5250-ppm group animals were dark and enlarged. The absolute and relative weights of the liver and spleen were also increased in these animals. On histopathology at the interim and terminal necropsies, the 525-ppm group and above had increased incidence of hemosiderosis and congestion in the spleen; also, the 2625- and 5250-ppm groups had increased incidences and/or severity of hematopoiesis in the spleen and liver, and pigment in the reticuloendothelial cells of the liver. Pigment was also observed in the convoluted epithelial cells of the kidney of these groups at terminal necropsy. The incidence of pyelonephritis in the 5250-ppm group males was marginally increased. There were increased incidences of cystitis and dilatation of the urinary bladder and balanoposthitis in the penis and preputial area of the 2625- and 5250-ppm groups at both of the necropsies. For the 5250-ppm group females, the incidence of amyloidosis was increased in the thyroid, adrenals, kidneys (also in the 2625-ppm group), stomach, small intestines, ovaries, and uterus. For chronic toxicity, the LOAEL is identified to be 525 ppm (73 mg/kg/day for males and 91 mg/kg/day for females) based on histopathological lesions in the spleen. Because 525 ppm was the lowest dose tested, a NOAEL cannot be identified from this study. There was no evidence of carcinogenicity.

Ford et al. (1972; available as abstract only) conducted a 92-week study examining the chronic oral toxicity and carcinogenicity of diphenylamine in CD-1 albino mice. The full study is not available for review. A summary of the abstract available on page 94 of the European

Union Risk Assessment Report on diphenylamine (European Communities, 2008) is presented below. Adjusted daily doses calculated using default data for body weight (U.S. EPA, 1994b) and food consumption (U.S. EPA, 1988) are 0, 8.7, 17, and 43 mg/kg-day for male mice, or 9.3, 18, and 45 mg/kg-day for female mice.

> *Charles River CD-1 Albino mice of both sexes (150 per group) were fed diets containing 0, 0.005, 0.01, and 0.025% diphenylamine over a time period of 92 weeks. No effects of diphenylamine exposure were noted on the nature and incidence of histopathological changes in particular the times of appearance and incidence of tumors.*

Holmberg et al. (1983) conducted an 18-month study examining the chronic oral toxicity and carcinogenicity of diphenylamine in outbred albino mice. The full study is not available for review. A summary of the abstract available on page 90 of the European Union Risk Assessment Report on diphenylamine (European Communities, 2008) is presented below.

> *NMRI outbred Albino mice, 8 weeks old, were administered with 300 mg/kg bw/d diphenylamine in soy bean oil by gavage once a week during 18 months (78 times). A positive control group receiving dimethynitrosamine (DMNA) (15 mg/kg bw/d) was equally investigated. The diphenylamine treatment group consisted of 125 mice and the control group of 30 animals. Sacrifices were at 25 and 52 weeks. Total observation time lasted 126 weeks. There were no changes in the frequency of tumors as compared to the vehicle treated controls. In the diphenylamine group 22.9% of the animals developed tumors. The most common tumor type*[s] *were lymphomas (8.3%) and alveolar adenomas (16.5). The tumor incidence in the vehicle control group were 22.2% with 11.1% lymphomas and 11.1% alveolar adenomas. As a non neoplastic alteration an increased frequency of lymphohistiocytic nephritis occurred in diphenylamine treated animals. The results on tumor morphology and incidence did not reveal a diphenylamine related change of tumors compared to vehicle treated controls.*

A LOAEL or NOAEL was not identified for this study in the European Union Risk Assessment Report on diphenylamine (Holmberg et al. 1983 as discussed in European Communities, 2008).

Developmental Studies

Based on a range-finding preliminary study (Edwards et al., 1983a), study authors administered 0, 33, 100, or 300 mg/kg-day of diphenylamine (99.9% pure) via gastric intubation in 1%-methyl cellulose to groups of 16 pregnant female rabbits per dose from gestation days (GDs) 7–19 (Edwards et al., 1983b). Females had been mated with males and injected with luteinizing hormone to promote ovulation; the day of mating was considered GD 0. Dose volumes were adjusted according to body weights. Environmental conditions and feeding methods were the same as described in the preliminary study. All animals were observed daily for signs of toxicity and were weighed on GDs 1, 7, 9, 11, 15, 20, 24, and 29. Food intake was measured during periods between weighing. After sacrifice on GD 29, dams were dissected and examined pathologically, with special attention paid to ovaries and uteri. The investigators counted the number of corpora lutea, the number and distribution of live fetuses, and the number and distribution of embryonic/fetal deaths. Embryonic/fetal deaths were classified as early with only placenta seen at termination, late with both embryonic and placental remnants seen at termination, and abortion with only implantation scars seen at termination. Fetuses were

weighed and examined for abnormalities in the brain and skeleton. Those showing visible abnormalities were examined more thoroughly for histopathological changes. The study authors calculated group means for litter size and various litter parameters (as percentages). Kruskal-Wallis and Jonckheere tests were used to analyze nonparametric data.

All treated animals, but particularly those dosed with 100 or 300 mg/kg-day, experienced green discoloration of the urine. Data on animals that died or were removed from the study for welfare reasons (number not specified) were not considered. Mean food consumption was reduced at 300 mg/kg-day (statistical significance not reported). Animals dosed with 300 mg/kg-day experienced lower weight gain compared to controls. Mean body weight gain was slightly reduced at 33 mg/kg-day, but animals treated with 100 mg/kg-day gained more weight compared to controls (see Table B.8). The study authors stated that weight gain and weight loss were not treatment related. There were no statistically significant ($p < 0.05$) or dose-related differences in litter size or pre- and postimplantation losses among groups. Fetal weight was slightly lower than controls at all doses, but the differences were not statistically significant (see Table B.9). The four fetal malformations (two in the controls, one at 33 mg/kg-day, and one at 100 mg-kg/day) were reportedly unrelated to treatment with diphenylamine. Skeletal variations were not significantly different among groups (see Table B.9). Based on these results, a maternal and developmental NOAEL of 300 mg/kg-day is identified based on this study. A LOAEL cannot be identified in this study due to lack of maternal and developmental effects at the administered doses.

Rodwell (1992) conducted an unpublished teratology study examining the developmental effects of diphenylamine on Sprague-Dawley rats. The full study is not available for review. The study as summarized in the European Union Risk Assessment Report on diphenylamine (European Communities, 2008; page 98) is presented below.

> *In an unpublished teratology study by Rodwell, 1992, pregnant female Sprague-Dawley rats (25/group) received diphenylamine (99.9%) in corn oil by oral gavage at dose levels of 0, 10, 50, or 100 mg/kg bw/d from gestation day six through gestation day 15 inclusive; dams were sacrificed on gestation day 20. None of the rats died during the study. Maternal toxicity was evidenced by increased splenic weights, enlarged spleens and blackish-purple colored spleen in the dams at 100 mg/kg bw/d. The maternal toxicity NOAEL was 50 mg/kg bw/d and the LOAEL was 100 mg/kg bw/d. No developmental toxicity was seen at any dose level. The developmental toxicity NOAEL was equal to or greater than 100 mg/kg bw/d, the highest tested dose (cited from EPA RED report, 1998 and JMPR report 1998; the original study was not available).*

Reproductive Studies

In an unpublished two-generation reproductive study, Rodwell (1993) administered diphenylamine (99.8% purity) to Sprague-Dawley rats (28/sex/group) in the diet for 90 days at concentrations of 0, 500, 1500, or 5000 ppm. These concentrations are equivalent to average daily doses of 0, 40, 115, or 399 mg/kg-day in F0 males and 0, 46, 131, or 448 mg/kg-day in F0 females, as calculated in the European Union Risk Assessment Report on diphenylamine (Rodwell 1993 as discussed in European Communities, 2008). It is unclear whether this study is GLP compliant, although it appears that is has been peer reviewed. The full study is not

available for review. The study as summarized in the European Union Risk Assessment Report on diphenylamine (European Communities, 2008; page 96) is presented below.

> *Compound- related systemic toxicity was observed in a dose related manner among both sexes and generations at all dose levels. In general, females were more affected than males and F1 animals were more affected than F0 animals. Clinical signs (bluish colored fluid in the cage and bluish colored staining of the coat in both sexes, and swelling of mammary gland(s) or palpable lateral-ventral masses, primarily in females) were evident at 5000 ppm. Body weight was decreased at 1500 and 5000 ppm. At 5000 ppm, there was a 6–9% decrease in body weight values, as compared to control, for F0 males, 5–8% for F0 females, 22–28% for F1 males, and 11–23% for F1 females. At 1500 ppm, there was a 5–8% decrease in body weight values from controls for F0 females, 7–9% for F1 males, and 5% for F1 females. Food consumption (g/animal/day) was also decreased at 1500 and 5000 ppm. Kidney, spleen, and liver appeared to be the target organs as evidenced by weight differences from control at 5000 ppm in males and at 1500 and 5000 ppm in females and gross and microscopic findings at all dose levels in both sexes. Gross findings included enlarged and blackish-purple spleens. Microscopic findings included brown pigment in the proximal convoluted tubules of the kidney, hepatocytic hypertrophy, brown pigments in the Kupffer cells of the liver, congestion and hemosiderosis of the spleen. The systemic toxicity NOAEL was less than 500 ppm (40 mg/kg-bw/d in males and 46 mg/kg-bw/d in females). The LOAEL was less than or equal to 500 ppm based on gross pathological findings in the spleen (enlarged, discolored), and on microscopic findings in the kidney (brown pigment in the proximal convoluted tubule), liver (hepatic hypertrophy and brown pigment in the Kupffer cells), and spleen (congestion and hemosiderosis).*
>
> *Developmental toxicity was observed at 1500 and 5000 ppm, as evidenced by significantly decreased body weight for F1 pups at 5000 ppm throughout lactation (11–25% less than control), for F2 pups at 5000 ppm from LD 4 through 12 LD 21 (10%–29% less than control), and for F pups at 1500 ppm on LD 14 (10%) and LD 21 (12%). The developmental toxicity NOAEL was 500 ppm (46 mg/kg-bw/d for maternal animals) and the LOAEL was 1500 ppm (131 mg/kg bw/d for maternal animals) based on decreased F2 pup body weight in late lactation. Reproductive toxicity was noted as smaller litter sizes at birth (significant for the F2 litters) in both generations at 5000 ppm. The reproductive toxicity NOAEL was 1500 ppm (131 mg/kg-bw/d for maternal animals) and the LOAEL was 5000 ppm (448 mg/k- bw/d for maternal animals), based upon decreased litter size in both generations (cited from EPA RED report, 1998 and JMPR report, 1998; original publication not available).*

Thomas et al. (1967c) also published a peer-reviewed study examining the reproductive effects of diphenylamine on weanling Slonaker-Addis rats. Twelve, 5-week-old female and three male rats per group were administered concentrations of 0-, 0.1%- (1000-ppm), 0.25%- (2500-ppm), or 0.5%- (5000-ppm) diphenylamine (purity 99.9%) in feed for approximately 65 days and were mated at 100 days of age. Adjusted daily doses calculated using default data for body weight (U.S. EPA, 1994b) and food consumption (U.S. EPA, 1988) are 0, 90, 226, or 451 mg/kg-day for males and 0, 101, 252, or 503 mg/kg-day for females. (Average daily doses displayed in Table B.10 represent an average of the doses between males and females.) The

study authors did not report whether this study followed GLP guidelines. Rats (F0) were housed four females and one male per cage during mating, and males were rotated once per week to a cage of four different females for 3 weeks. When the first litter of offspring was weaned, a second round of mating was performed in this same manner. First generation (F1) offspring were also mated in the same manner described above to produce a second generation (F2).

The study authors reported a general trend of decreasing litter size with increasing dose in the first and second mating of the F0 generation (see Table B.10). The number of pups per litter at birth in the first F0 mating was significantly ($p < 0.05$) lower in the 0.5%-dose group compared to the control, while in the second mating, the number of pups per litter at birth was significantly ($p < 0.05$ or $p < 0.01$) lower compared to the concurrent control group for all three dose groups (see Table B.10). The number of pups per litter at birth and weaning in F1 animals exposed to 0.1%-diphenylamine was significantly ($p < 0.01$) lower than the control group; however, a similar effect was not observed in F1 animals exposed to 0.25%- and 0.5%-diphenylamine (see Table B.10). The study authors reported that average weights of the second-generation weanling pups were comparable to the control group for both males and females exposed to 0.1%- and 0.25%-diphenylamine, while weanling weights were significantly ($p < 0.01$) lower in the 0.5%-dose group compared to the concurrent controls. The study authors concluded that because weanling weight loss was not observed at the 0.1%- and 0.25%-dose levels, the weight loss observed in the 0.5%-dose group could not be firmly attributed to diphenylamine exposure. Because the study results are not conclusive, a LOAEL or NOAEL cannot be identified for this study.

Inhalation Exposures

The effects of inhalation exposure to diphenylamine on animals have not been evaluated in subchronic- or chronic-duration, developmental, reproductive, or carcinogenic studies.

OTHER STUDIES (SHORT-TERM TESTS, OTHER EXAMINATIONS)

A few studies pertaining to the mutagenicity, genotoxicity, clastogenecity, cytotoxicity, acute toxicity via oral and intraperitoneal (ip) exposure, and metabolism/toxicokinetics are available for diphenylamine (see Table 3). Mutagenicity studies (Mobil Oil Corporation, 1994a; Litton Bionetics, 1994; Braun et al., 1977; Probst et al., 1981a; Zeiger et al., 1988; Babish et al., 1983; Dolara et al., 1993) on diphenylamine conducted using a traditional or modified Ames assay in *Salmonella typhimurium* strains TA97, TA98, TA100, TA1000, TA1535, TA1537, TA1538, G46, C3076, and D3052 were all negative in the presence and absence of S-9 activation. Similarly, mutagenicity studies (Probst et al., 1981a) in *E. coli* strains WP2 and WP2uvrA were also negative. The murine lymphoma assay, unscheduled DNA synthesis in rat hepatocytes, DNA single strand breaks (SSBs) in rat hepatocytes and V79 cells, and sister chromatid exchanges (SCEs) in human lymphocytes were all negative as well when tested using various doses/concentrations of diphenylamine, either alone (Mobil Oil Corporation, 1994b; Probst et al., 1981b; Görsdorf et al., 1988) or in a mixture (Dolara et al., 1993). Cytogenicity studies examining increases in micronucleated cells following diphenylamine exposure were mostly negative (Mobil Oil Corporation, 1994c; Dolara et al., 1994). A study by Dolara et al. (1993) reported that slight cytogenicity was noted following treatment with diphenylamine based on nonsignificant increases in polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) ratios. One cytotoxicity test by Masubuchi et al. (2000) stated that diphenylamine was cytotoxic in vitro at concentrations ranging from 10 to 500 μM in cultured

male Wistar rat hepatocytes. Genotoxicity results were equivocal when determined in various test systems. Ardito et al. (1996) reported that diphenylamine was weakly genotoxic in human lymphocytes, while Lodovici et al. (1997) reported that diphenylamine produced free radicals capable of inducing genetic damage to the cell in livers of Wistar rats dosed with 0.14-mg/kg-day diphenylamine for 10 days. Contrary to these results, von der Hude et al. (1988) reported that diphenylamine-related genotoxicity was not noted in an SOS chromotest conducted using *E. coli* PQ37.

Acute oral toxicity studies (Lenz and Carlton, 1990a,b,c) indicated that acute exposures to diphenylamine via gavage were more toxic to hamsters when compared to rats and gerbils, with toxicity primarily noted in the kidneys. A single study (Lenz and Carlton, 1990d) in hamsters following ip administration with diphenylamine also suggested that kidneys were the target organ.

A metabolism and toxicokinetic study by Alexander et al. (1965) reported that major metabolites in rats, rabbits, and humans were 4-hydroxydiphenylamine and 4,4'-dihydroxydiphenylamine. Toxicokinetic studies in rats indicated that [^{14}C] diphenylamine was rapidly metabolized and excreted in the urine and bile. Metabolism studies in female NMRI mice suggested that *N*-hydroxyl-derivate of diphenylamine may be a potential metabolite of diphenylamine (Appel et al., 1987). Gutenmann and Lisk (1975a,b) conducted metabolism studies in a Holstein cow and fresh rumen and concluded that conjugates of diphenylamine were not observed in the hydrolyzed urine from the cow, or in the rumen. However, the study authors stated that microsomal hydroxylation of diphenylamine is possible in bovine systems, but these were not detected due to the limited analytical techniques employed in their test system.

Crocker et al. (1972) conducted a mechanistic study focused on the kidneys of fetal rats and their dams that were administered diphenylamine from several different commercial sources. The study authors also chromatographically isolated and tested the effects of major diphenylamine contaminants in pregnant rats and in their offspring. Commercial preparations of diphenylamine were administered in the feed of a group of pregnant Sprague-Dawley rats from GD 14 to term at high doses (1.5% or 2.5%, or about 2143 or 3571 mg/kg-day) to ensure that effects were seen. A total of three contaminants chromatographically isolated from aged preparations of diphenylamine, as well as purified diphenylamine itself, were dissolved in 70% ethanol and fed by gastric tube at doses estimated at about 0.05 mg/day to another group of pregnant rats for 7 days prior to delivery. Control groups of pregnant rats received either no diphenylamine in their food or 70% ethanol. Live offspring were removed from their mothers shortly after birth, and histology was performed upon the kidneys of both parent and offspring. Results indicated that cystic lesions were confined to the proximal nephron in newborn rats and were in contrast with the localization in the collecting ducts after long-term feeding of diphenylamine to adult rats. Variations observed in the incidence and severity of the cystic changes appeared to depend upon the commercial source of the diphenylamine. Renal lesions were also produced in newborn offspring of pregnant rats fed microgram quantities of one of the components isolated from diphenylamine, whereas none were seen after feeding of chromatographically pure diphenylamine.

In a GLP-compliant preliminary range-finding study, Edwards et al. (1983a) administered 0, 200, 400, or 600 mg/kg-day of diphenylamine (99.9% pure) in 1% methyl-cellulose via

gavage to groups of six pregnant female New Zealand white rabbits per day for 13 days. Rabbits were obtained from Cheshire Rabbit Farms, Ranch Rabbits, and Buxted Rabbit Co., Ltd. (England) and were between 12 and 20 weeks of age upon arrival. Animals were housed individually in metal cages in a room kept at 18 ± 3°C with a relative humidity of 55 ± 5%. Artificial lighting supplemented natural light between the hours of 7:00 am and 9:00 pm. Tap water and food (SDS Rabbit Diet) were provided ad libitum. Contaminant analysis indicated that no nonnutrient substance in feed/water or nutrient levels in the feed would interfere with the test system. Animals were observed daily for clinical signs of toxicity and other changes. All animals were weighed prior to treatment and every day during treatment. One animal was sacrificed early after persistent weight loss. All treated animals exhibited green discoloration of the urine. Food consumption was slightly reduced, and body weight was lowered in all dose groups, although recovery was seen in the 400-mg/kg-day group by the end of the study. Autopsy revealed no treatment-related macroscopic changes. The study authors did not report whether statistical tests were performed on the preliminary study data. It is unclear whether this study was peer reviewed.

Table 3. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Mutagenicity	Ames *Salmonella* (strain: TA1535) assay without metabolic activation; diphenylamine in DMSO at doses of 3, 5, 10, 15, 20, 30, 50, or 75 µg/µl per bacterial plate incubated for 48 hr at 37°C	Negative; reversion frequencies not substantially different from solvent or spontaneous controls	Diphenylamine was not mutagenic in TA1535 in the absence of metabolic activation	Mobil Oil Corporation (1994a)
Mutagenicity	Murine lymphoma mutagenesis assay with S-9 activation (at doses ranging from 0.58–0.007-µl/mL diphenylamine); S-9 fraction obtained from male S-D rats induced with Aroclor 1254; without S-9 activation (at doses ranging from 0.075–0.0056 µl/mL); counted induced forward mutations from the heterozygote (TK+/-) to the homozygote (TK-/-) in a matrix cytotoxic to TK +/-; cells exposed for 180 minutes and given 48 hours to express mutation before cloning	Toxicity not associated with dose; no significant increase in mutagenic frequency in those with acceptable (≥10%) total growth, with or without activation	Diphenylamine was not mutagenic in the murine lymphoma assay	Mobil Oil Corporation (1994b)
Mutagenicity	Ames *Salmonella* (strains TA1535, TA1537, TA1538, TA98, and TA100) assay with and without S-9 activation; S-9 fraction obtained from male S-D rat liver induced with Aroclor 1254; diphenylamine in DMSO at doses of 0.1, 1, 10, 100, or 500 µg per bacterial plate; incubated for 48 hours at 37°C	Diphenylamine toxic to all strains at 500 µg; mutagenicity results negative with and without activation	Diphenylamine was not mutagenic in TA1535, TA1537, TA1538, TA98, or TA100, both with and without metabolic activation.	Litton Bionetics (1994)
Mutagenicity	Host-mediated assay with *Salmonella typhimurium* strain TA1950; male NMRI mice injected via ip with 2 mL of bacterial culture; diphenylamine dissolved in NaCl administered simultaneously to mice via gavage at doses ranging from 1450–2900-µmoles/kg diphenylamine; replicate experiments were performed	No mutagenic activity observed in mice injected with TA1950 and treated with diphenylamine	Diphenylamine was not mutagenic to mice injected with *Salmonella typhimurium* strain TA1950	Braun et al. (1977)

Table 3. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Mutagenicity	Ames *Salmonella* assay (TA1538) with and without S-9 activation treated with 100-µg diphenylamine; incubation time unreported	No mutagenic activity observed following treatment with diphenylamine in the presence and absence of S-9 activation	Diphenylamine was not mutagenic to TA1538	Ferretti et al. (1977)
Mutagenicity	Modified Ames assay using *Salmonella* strains G46, TA1535, TA1000, C3076, TA1537, D3052, TA1538, and TA98, and *E. coli* strains WP2 and WP2 uvrA$^-$ with and without S-9 metabolic activation; S-9 fraction obtained from Aroclor 1254-treated rats; diphenylamine concentration range not specified; chemical incorporated into 4-gradient plates to give a 10-fold concentration range per plate	No mutagenic activity observed in any of the *Salmonella* or the *E. coli* strains, both in the presence and absence of S-9 activation	Diphenylamine was not mutagenic	Probst et al. (1981a)
Mutagenicity	Unscheduled DNA synthesis assay using Fischer-344 rat hepatocytes exposed to 100-nmoles/mL diphenylamine for a 5-hour incubation period	No unscheduled DNA synthesis observed in treated rat hepatocytes	Diphenylamine was not mutagenic	Probst et al. (1981b)
Mutagenicity	Ames assay using *Salmonella* strains TA97, TA98, TA100, and TA1535 exposed to concentrations of 0-, 1-, 3-, 10-, 33-, 66-, 100-, 166-, or 333-µg/plate diphenylamine and incubated for 2 days; strains first tested without metabolic activation; subsequently tested with S-9 activation if results from the first assay were positive	No mutagenic activity observed in any of the *Salmonella* strains tested	Diphenylamine was not mutagenic	Zeiger et al. (1988)
Mutagenicity	Alkaline elution assay used to detect DNA single strand breaks in V79 cells and rat hepatocytes; doses not specified	Positive results observed in rat hepatocytes only	No firm conclusions presented; results are presented in an abstract; complete article is not available	Görsdorf et al. (1988; abstract)

Table 3. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Mutagenicity	Ames assay using *Salmonella* strain TA100 exposed to concentrations of 15-, 21-, 29.3-, 41-, 57.3-, or 80-µg/plate diphenylamine with and without S-9 activation following an incubation for 18 and 48 hours; modified liquid suspension assay used to test for mutagenicity of the aqueous extract from sterilized nipples	No dose-related increase in revertants with or without S-9 activation; tests of mutagenicity in the aqueous extract positive (significant increases in reversions with and without activation in a number of different samples)	Diphenylamine was not mutagenic in TA100 when tested via the standard Ames assay; positive mutagenicity with the aqueous extract may be related to dimethyl- and diethylnitrosamines (as opposed to diphenylamine), which were readily detected in the extract	Babish et al. (1983)
Mutagenicity/Clastogenicity	Mixture of 15 pesticides, including 50-ppb diphenylamine, tested using the standard plate test (Ames) with and without metabolic activation (S-9) at concentrations up to 500 µg/plate in *Salmonella* strains TA1537, TA1538, TA98, TA100, TA1535, and TA102 Human lymphocytes tested for sister chromatid exchanges (SCEs) after incubation with 0.1–20 µg/mL of the mixture for 72 hours at 37°C (cells stained with acridine orange and observed with fluorescent microscope) Micronucleus assay using Wistar rat bone marrow (rats had been administered 1, 10, or 100 µg/kg of the mixture) in which polychromatic erythrocytes (PCEs) and normochromatic erythrocytes were (NCEs) counted with a fluorescent microscope	Concentrations of up to 500 µg/plate of pesticide mixture not mutagenic in Ames assay Small but statistically significant increase in SCEs compared to controls from dose of 1 µg/mL of the mixture Nonsignificant increase in the ratio between PCEs and NCEs in rat bone marrow (indicating slight toxicity)	The study authors concluded that the mixture did not have "appreciable genotoxic activity" in these test systems; they noted that nonmutagenic pesticides might have interfered with the DNA interaction of a genotoxic pesticide, such as diphenylamine, inhibiting its mutagenic potential	Dolara et al. (1993)

Table 3. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Clastogenicity	Micronucleus bone marrow assay; rats (strain not specified); 10 M/10F treated dermally for 90 days, 5 days/week, with 0-, 500-, or 2000-mg/kg-day diphenylamine; PCEs and NCEs scored for micronuclei; cytotoxicity also recorded	Ratio means of PCEs and NCEs not significantly different between treated and untreated groups; cytotoxicity not a factor in micronucleus induction evaluation; percentage of micronucleated PCEs and NCEs not different between treated animals and negative controls	Diphenylamine did not significantly increase micronucleated cells	Mobil Oil Corporation (1994c)
Clastogenicity	Human lymphocytes incubated with 1–20 µg/mL of a mixture of 15 pesticides, including 14.4%-diphenylamine (incubation period not specified); followed "standard" methods with Giemsa staining and classified for cytogenic abnormalities (e.g., altered chromosome numbers, gaps, deletions)	No significant changes in chromosome number; dose-dependent increase in the frequency of nonsynchronous centromeric separations that disappeared after benomyl was removed from the mixture	The mixture was not genotoxic in the absence of benomyl	Dolara et al. (1994)
Cytotoxicity	Freshly obtained male Wistar rat hepatocytes incubated with or without oligomycin and 0-, 10-, 25-, 50-, 100-, 250-, or 500-µM diphenylamine dissolved methanol (incubation time unreported); fructose added after 90 minutes of treatment with diphenylamine; samples of suspension removed after 60 minutes to analyze cellular ATP content	Pseudoenergetic mitochondrial swelling and uncoupling of mitochondrial oxidative phosphorylation, depleting cellular ATP	Diphenylamine was a cytotoxic agent under the experimental conditions tested in this study	Masubuchi et al. (2000)
Genotoxicity	Human lymphocytes obtained from whole blood, exposed to diphenylamine at concentrations of 0.6, 6, or 60 µg/mL and incubated for up to 48 hours with and without metabolic activation (rat liver induced with phenobarbital and benzoflavone, or S-9 fraction); frequency of SCEs analyzed	Significant ($p < 0.05$) increase in SCEs in cells treated with 6 µg/mL compared to controls without activation; similar increase not seen with or without activation with S-9 after 4 hours of incubation	Diphenylamine was reported to be a weak, direct-acting genotoxic agent.	Ardito et al. (1996)

Table 3. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Genotoxicity	6 male Wistar rats administered 0.14-mg/kg-day diphenylamine dissolved in corn oil for 10 days; animals sacrificed and livers examined for elevated levels of 8-OH-2-deoxyguanosine (8-OH-2DG)	Significantly ($p < 0.05$) increased levels of 8-OH-2DG in rat liver DNA relative to controls	In vivo, diphenylamine produced free radicals capable of inducing genetic damage to cells	Lodovici et al. (1997)
Genotoxicity	SOS chromotest using *E. coli* PQ37 with and without S-9 metabolic activation; S-9 fraction obtained from Aroclor 1254-idnuced Wistar rats and Syrian hamsters; culture incubated for 2 hours	No genotoxicity observed following exposure to diphenylamine	Diphenylamine was not genotoxic	von der Hude et al. (1988)
Acute Oral Toxicity	10 male Syrian hamsters per dose group administered 400-, 600-, or 800-mg/kg-day diphenylamine via gavage for 3 days; 10 control animals dosed with peanut oil; moribund animals during the study euthanized and necropsied; surviving animals euthanized 24 hours after final dose and necropsied	4 mortalities at 400 mg/kg-day, and 100% mortality at 600 and 800 mg/kg-day; 9 incidences of splenomegaly at 400 mg/kg-day; 2 incidences of brown kidneys, 6 incidences of yellow-brown papilla, and 1 incidence of gastric ulcers at 600 mg/kg-day; 3 incidences of brown kidneys, and 5 incidences of yellow-brown papilla at 800 mg/kg-day; 4 incidences at 400 mg/kg-day, 7 incidences at 600 mg/kg-day, and 6 incidences at 800 mg/kg-day of total renal papillary necrosis	Hamsters were more sensitive than rats (see below) to nephrotoxic effects of diphenylamine	Lenz and Carlton (1990a)
Acute Oral Toxicity	10 male Sprague-Dawley rats per dose group administered 400-, 600-, or 800-mg/kg-day diphenylamine via gavage for 3 days; 10 control animals dosed with peanut oil; moribund animals during the study euthanized and necropsied; surviving animals euthanized 24 hours after final dose and necropsied	No mortalities at any dose; 1 incidence of bilateral renal cortical pallor at necropsy at 600 mg/kg-day; 2 incidences of renal papillary necrosis at 800 mg/kg-day	Rats were less sensitive than hamsters (see above) to nephrotoxic effects of diphenylamine	Lenz and Carlton (1990b)

Table 3. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Acute Oral Toxicity	10 male Mongolian gerbils per dose group administered 400-, 600-, or 800-mg/kg-day diphenylamine via gavage for 3 days; 10 control animals dosed with peanut oil; moribund animals during the study euthanized and necropsied; surviving animals euthanized 24 hours after final dose and necropsied	No mortalities at any dose; no gross or microscopic lesions at any dose	Diphenylamine did not demonstrate nephrotoxic effects in male Mongolian gerbils	Lenz and Carlton (1990c)
Acute Oral Toxicity	Male Syrian hamsters (8/dose group) administered diphenylamine in peanut oil at 0, 200, 400, or 600 mg/kg-bw via gavage; kidneys removed and the papilla, outer medulla and the cortex analyzed for GSH levels; all groups dosed at 7 hours and hamsters from each dose group sacrificed at 8.00 or 11.00 hours to ensure that the cardiac variation on tissue GSH concentrations was observable	A dose-dependent statistically significant reduction in cortical GSH observed 1 hour after diphenylamine administration; no significant changes in the outer medullar or the papilla cortical GSH levels (reduced by 53% compared to controls) in the cortex at 200 mg/kg at 4 hours; GSH in the cortex significantly reduced at 400 and 600 mg/kg at 4 hours	The study authors concluded that the reduced cortical GSH and papillotoxicty are mechanistically unrelated and that the papillotoxicity of diphenylamine is mediated by mechanisms other than oxidative cell injury	Lenz (1996)
Intraperitoneal Injection	10 male Syrian hamsters per group administered 400-, 600-, or 800-mg/kg-day diphenylamine through ip injection for 3 days; 5 control animals dosed with peanut oil alone; animals euthanized 24 hours after final dose	5 mortalities at 600 mg/kg-day and 4 at 800 mg/kg-day; 1 incidence each of pale renal cortex at 600 and 800 mg/kg-day; 1 incidence at 600 mg/kg-day and 2 incidences at 800 mg/kg-day of brown kidneys; 4 incidences at 600 mg/kg-day and 2 incidences at 800 mg/kg-day of yellow-brown papilla; 5 incidences at 600 mg/kg-day and 4 incidences at 800 mg/kg-day of total renal papillary necrosis	Diphenylamine demonstrated nephrotoxic effects in male Syrian hamsters	Lenz and Carlton (1990d)

Table 3. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Range-finding-short term study	6 pregnant New Zealand White rabbits administered 0, 200, 400, or 600 mg/kg-day of diphenylamine in 1%-methyl-cellulose via gavage for 13 days	All animals exhibited green discoloration of the urine; food consumption and body weight reduced at all doses; no treatment-related macroscopic changes	Diphenylamine caused reduction in food consumption and body weight as well as green discoloration of the urine	Edwards et al. (1983a)
Mechanistic	Pregnant Sprague-Dawley rats (number not specified) administered 2143- or 3571-mg/kg-day commercial preparation of diphenylamine via feed from GD 14 to term; pregnant Sprague-Dawley rats (number not specified) administered 0.05-mg/day three contaminants from aged diphenylamine in 70%-ethanol 7 days prior to delivery via gastric tube; control group (number not specified) received 70%-ethanol via gastric tube	Cystic lesions confined to the proximal nephron in newborn rats in contrast with the localization in the collecting ducts after long-term feeding of diphenylamine to adult rats; variations in incidence and severity of the cystic changes dependent upon the commercial source of the diphenylamine; renal lesions also produced in newborn offspring of pregnant rats fed microgram quantities of one of the components isolated from diphenylamine	Diphenylamine contaminants produced renal lesions in pups	Crocker et al. (1972)
Metabolism/Toxicokinetic	Male white rats treated with 5-mg diphenylamine in propylene glycol via ip injection and urine collected 24 hours after treatment; cannulated bile duct from anaesthetized male white rats treated with 2-mg diphenylamine in 50%-aq. ethanol injected into the femoral vein and bile collected for 6 hours and examined for metabolites; 1 male rabbit fed 1 oral dose of a suspension of 5-g diphenylamine in divided doses of 1 g over 9 days and urine collected for analysis; 2 human subjects given 100 mg of diphenylamine orally and urine collected for 24 hours; 1 cat administered 1-mM/kg aqueous suspension of diphenylamine and blood collected for methaemoglobin determination; 1 rat given a single dose of 3-mg [^{14}C]diphenylamine	Major metabolites in rats, rabbits, and humans: 4-hydroxydiphenylamine and 4,4'-dihydroxydiphenylamine; *O*-sulphate and *O*-glucuronide (as the tri-*O*-acetylmethyl ester) conjugates of 4-hydroxydiphenylamine in rabbit urine; considerable methaemoglobin formation in cat; [^{14}C]diphenylamine rapidly metabolized and excreted in rats based on excretion in the urine and bile; 75% of ip dose excreted in the urine at 48 hours; 25% of the iv dose excreted in the bile after 6 hours	Major metabolites in rats, rabbits and humans are 4-hydroxydiphenylamine and 4, 4'-dihydroxydiphenylamine; diphenylamine was rapidly excreted through both urine and bile	Alexander et al. (1965)

Table 3. Other Studies

Test	Materials and Methods	Results	Conclusions	References
	via ip injection and urine collected for 24 hours; cannulated bile ducts from male rats injected with 5-mg/kg [^{14}C]diphenylamine in 50% aqueous ethanol via iv and bile samples collected at 1-hour intervals			
Metabolism/Toxicokinetic	Female NMRI mice pretreated with 0.1%-phenobarbital in the drinking water for 5 days; liver microsomes from mice extracted and treated with 1-mM diphenylamine	Rate of metabolism approximately 40-nMol compound/mL at 10 minutes and 55-nMol compound/mL at 30 minutes (inferred from Figure 3 in article)	*N*-hydroxyl-derivate of diphenylamine may be a potential metabolite of diphenylamine	Appel et al. (1987)
Metabolism/Toxicokinetic	Holstein cow fed 5 ppm (based on daily ration of 22.7 kg) of pure recrystallized diphenylamine (in acetone) in grain for 4 days; milk sampled in the morning and evening before, during, and after dosing; total daily urine and manure samples also collected and analyzed	No diphenylamine detected in milk or urine; small amounts found in the feces (fecal excretion = 1.4% or 6.2 mg)	No conjugates of diphenylamine were observed in the hydrolyzed urine; the study authors stated that microsomal hydroxylation of diphenylamine is possible in bovine systems; however, the analytical techniques used in the study were not capable of identifying such hydrolyzed derivatives of diphenylamine	Gutenmann and Lisk (1975a)

Table 3. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Metabolism/Toxicokinetic	1 mL of diphenylamine in acetone (500 µg/mL) solution mixed with 100 mL of fresh filtered rumen fluid and later analyzed for diphenylamine using electron affinity gas chromatography; 5-ppm diphenylamine incubated with fresh beef liver fraction for 30 minutes at 37°C and analyzed using gas chromatography	Diphenylamine stable in rumen fluid for 23 hours; roughly 50% diphenylamine lost during the 30 minutes of incubation with beef liver fraction	No conjugates of diphenylamine were observed in the rumen; the study authors stated that microsomal hydroxylation of diphenylamine is possible in bovine systems; however, the analytical techniques used in the study were not capable of identifying such hydrolyzed derivatives of diphenylamine	Gutenmann and Lisk (1975b)

DERIVATION OF PROVISIONAL VALUES

Table 4 presents a summary of noncancer reference values. Table 5 presents a summary of cancer values. IRIS data are indicated in the table if available.

Table 4. Summary of Noncancer Reference Values for Diphenylamine (CASRN 122-39-4)

Toxicity Type[a] (units)	Species/Sex	Critical Effect	p-Reference Value	POD Method	POD	UF_C	Principal Study
Screening Subchronic p-RfD (mg/kg-day)	Mouse/M+F	Increased incidences of splenic hemosiderosis and splenic congestion	2×10^{-2}	NOAEL	2.1	100	Botta (1992)
Chronic RfD (IRIS) (mg/kg-day); 1993	Dog/M+F	Decreased body weight; increased liver and kidney weights	2.5×10^{-2}	NOAEL	2.5	100	Thomas et al. (1967a)
Subchronic p-RfC (mg/m^3)	None	None	None	None	None	None	None
Chronic p-RfC (mg/m^3)	None	None	None	None	None	None	None

[a]All reference values obtained from IRIS are indicated with the latest review date.

Table 5. Summary of Cancer Reference Values for Diphenylamine (CASRN 122-39-4)

Toxicity Type	Species/Sex	Tumor Type	Cancer Value	Principal Study
p-OSF	None	None	None	None
p-IUR	None	None	None	None

DERIVATION OF ORAL REFERENCE DOSES

Derivation of Subchronic Provisional RfD (Subchronic p-RfD)

No subchronic p-RfD can be derived because a nonpeer-reviewed study is selected as the principal study. However, derivation of a screening value is provided in Appendix A.

Derivation of Chronic RfD (Chronic RfD)

A chronic oral RfD of 2.5×10^{-2} mg/kg-day is included in the IRIS database (U.S. EPA, 2010a) based on a 2-year chronic-duration toxicity study in male and female beagle dogs (eight/sex/group) by Thomas et al. (1967a). The critical end point identified in the IRIS database is decreased body-weight gain and increased liver and kidney weights in beagle dogs exposed to diphenylamine in diet. A no-observed-effect-level (NOAEL) of 2.5 mg/kg-day and a lowest-effect-level (LEL) of 25 mg/kg-day were identified in the IRIS database, and the NOEL served as a point of departure (POD) for chronic RfD derivation. A composite uncertainty factor of 100 (10 for interspecies extrapolation and 10 for intraspecies extrapolation) was used to derive the chronic RfD. The IRIS value was last revised on April 1, 1993.

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

No published studies investigating the effects of subchronic- or chronic-duration inhalation exposure to diphenylamine in animals were identified. Chronic occupational studies in humans (Checkoway et al., 1981; Parkes et al, 1982) examined the effects of mixtures of chemicals, which precludes their use in the derivation of a chronic p-RfC for diphenylamine. Overall, the lack of toxicity studies for diphenylamine via inhalation exposure precludes the derivation of subchronic- and chronic-duration inhalation toxicity values.

CANCER WEIGHT-OF-EVIDENCE (WOE) DESCRIPTOR

Table 6 identifies the cancer weight-of-evidence (WOE) descriptor for diphenylamine.

Table 6. Cancer WOE Descriptor for Diphenylamine			
Possible WOE Descriptor	**Designation**	**Route of Entry (Oral, Inhalation, or Both)**	**Comments**
"Carcinogenic to Humans"	N/A	N/A	No strong human cancer data are available.
"Likely to Be Carcinogenic to Humans"	N/A	N/A	No strong animal cancer data are available.
"Suggestive Evidence of Carcinogenic Potential"	N/A	N/A	Chronic-duration toxicity/carcinogenicity studies (Botta, 1994b,c; Thomas et al., 1967b; Griswold et al., 1966; Ford et al., 1972; Holmberg et al., 1983) in various strains of rats and mice showed no increase in tumor incidence. Tumors that were observed were reportedly unrelated to diphenylamine exposure.
"Inadequate Information to Assess Carcinogenic Potential"	**Selected**	**Oral**	**Available information is inadequate to assess carcinogenic potential.**
"Not Likely to Be Carcinogenic to Humans"	N/A	N/A	No strong evidence of noncarcinogenicity in humans or animals is available.

N/A = not applicable.

DERIVATION OF PROVISIONAL CANCER POTENCY VALUES

Derivation of Provisional Oral Slope Factor (p-OSF)

The lack of carcinogenic activity in five chronic-duration toxicity/carcinogenicity studies (Botta, 1994a,b; Thomas et al., 1967b; Ford et al., 1972; Holmberg et al., 1983) following exposure to diphenylamine via the oral exposure route precludes the derivation of an oral slope factor.

Derivation of Provisional Inhalation Unit Risk (p-IUR)

The lack of quantitative data on the carcinogenicity of diphenylamine via the inhalation exposure route precludes the derivation of an inhalation unit risk.

MODE-OF-ACTION DISCUSSION

The *Guidelines for Carcinogenic Risk Assessment* (U.S. EPA, 2005) define mode of action as the following: "…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…There are many examples of possible modes of carcinogenic action, such as mutagenicity, mitogenesis, inhibition of cell death, cytotoxicity with reparative cell proliferation, and immune suppression" (p. 1–10).

Studies exploring the mutagenic potential of diphenylamine reported that it was not mutagenic to various *Salmonella typhimurium* or *E. coli* strains tested using either the traditional or modified Ames assay (Mobil Oil Corporation, 1994c; Litton Bionetics, 1994; Braun et al.,

1977; Probst et al., 1981a; Zeiger et al., 1988; Babish et al., 1983; Dolara et al., 1993). Genotoxicity results for diphenylamine were equivocal and ranged from the production of free radicals capable of genotoxic effects in rat liver cells to weakly genotoxic in human lymphocytes to nongenotoxic in an SOS chromotest in *E. coli* PQ37 (Ardito et al., 1996; Lodovici et al., 1997; von der Hude et al., 1988). Studies examining the ability of diphenylamine to cause unscheduled DNA synthesis, DNA single strand breaks, SCE, and increases in micronucleated cells were all negative (Mobil Oil Corporation, 1994b,c; Probst et al., 1981b; Görsdorf et al., 1988; Dolara et al., 1993, 1994). While these results are limited to select test systems, they suggest that diphenylamine is unlikely to be mutagenic or genotoxic in the test systems examined. In addition, the lack of carcinogenic activity following oral exposure in two animal strains (rat and mouse) precludes a more detailed discussion regarding diphenylamine carcinogenic mode of action.

APPENDIX A. PROVISIONAL SCREENING VALUES

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

DERIVATION OF SCREENING PROVISIONAL ORAL REFERENCE DOSES

Derivation of Screening Subchronic Provisional RfD (Screening Subchronic p-RfD)

The 90-day toxicity study in the mouse (Botta, 1992) is selected as the principal study for the derivation of the screening subchronic p-RfD. The critical effect is increased incidences of splenic hemosiderosis and congestion in male and female CD-1 mice. This unpublished study has not been peer reviewed, but the study was performed according to GLP principles. The study seems sufficient for the derivation of a screening subchronic p-RfD based on the number of animals and presentation of information. Details are provided in the "Review of Potentially Relevant Data" section. The number of potential toxicity end points that were examined include body and organ weights, clinical hematology, and histopathology. This study provides the most sensitive toxicological end point and the lowest POD for developing a screening subchronic p-RfD value.

As detailed in the section "Review of Potentially Relevant Data," statistically significant changes included increased incidences of splenic hemosiderosis and congestion in the three highest dose groups in female mice and in the two highest dose groups in male mice. Because these two end points (i.e., splenic hemosiderosis and congestion) were the most sensitive effects reported in this study, all of the common dichotomous models available in the EPA's Benchmark Dose Software (BMDS, version 2.1.2) were fit to the data (see Table B.11). After completion of BMD modeling, the output was reviewed for elimination of models that failed acceptability criteria (see Appendix C for BMD modeling results). BMD methods for choosing adequate model fit are described in Appendix C.

For increased incidence of splenic congestion, the Quantal-Linear model was considered the best fit and produced a BMD_{10} and $BMDL_{10}$ of 22 and 14 mg/kg-day for both male and female mice, respectively. For the increased incidence of splenic hemosiderosis, the Quantal-Linear model in male mice produced a BMD_{10} and $BMDL_{10}$ of 18 and 12 mg/kg-day, respectively. The data for increased incidence of splenic hemosiderosis in female mice are not amenable to BMD modeling because there are no data at the low-response range, which is necessary for BMD modeling. Because the data for increased incidence of splenic hemosiderosis

in female mice did not provide adequate model fit, the NOAEL of 2.1 mg/kg-day for this parameter in female mice was considered as an alternate POD.

Although splenic effects were the most sensitive data from the principal study (Botta, 1992), there were also significant changes in organ weights (i.e., kidneys, liver, spleen, and ovaries) that could be modeled by BMD for consideration of a POD because the LOAEL for some of these changes are within 10-fold of the LOAEL of 107 mg/kg-day for female mice splenic effects identified from the Botta, 1992 study. However, some of the data for these organ-weight changes (i.e., kidneys, liver, spleen, and ovaries) do not have a clear dose response, which is necessary for BMD modeling. The only organ-weight data that could be modeled were increases in both relative and absolute liver weight in male mice. For increased absolute liver weight in male mice, the Linear model was considered the best fit and produced a BMD_{10} and $BMDL_{10}$ of 456 and 349 mg/kg-day, respectively. For increased relative liver weight in male mice, the Linear model was considered the best fit and produced a BMD_{10} and $BMDL_{10}$ of 401 and 331 mg/kg-day, respectively. Both, the modeling results and methods are presented in Appendix C. Alternate PODs for the effects (e.g., decreased relative ovaries) that could not be modeled would be their respective NOAELs listed in Table A.1, which besides for relative ovary weight changes, are not lower than the BMDL values for splenic hemosiderosis and congestion. Specifically, a NOAEL of 2.1 mg/kg-day for a 17% decrease in relative ovary weights is identified from Table B.12.

After reviewing all of the modeling results listed in Appendix C, the most sensitive $BMDL_{10}$ is 11.51 mg/kg-day for increased incidence of splenic hemosiderosis in male mice. However, whereas the selection of this $BMDL_{10}$ from the splenic hemosiderosis data set as the POD would protect against both splenic congestion and organ-weight changes in male and female mice, it may not confer protection against the more sensitive endpoint of splenic hemosiderosis in female mice. The POD for increased incidence of splenic hemosiderosis in female mice is a NOAEL of 2.1 mg/kg-day. This NOAEL is identical to that indentified for decreased ovary weights in female mice. The selection of increased incidence of splenic hemosiderosis as the critical effect is supported by the observation that the spleen appears to be a target organ of diphenylamine toxicity. Specifically, not only did diphenylamine cause splenic hemosiderosis and congestion in both sexes of mice but also biologically and statistically significantly increased absolute and relative spleen weight in both sexes of mice as mentioned in the section "Review of Potentially Relevant Data." This information provides greater support for spleen being the target organ of diphenylamine toxicity rather than ovary, in which only relative ovary weights were affected by diphenylamine exposure with no accompanying histological data. **Therefore, the NOAEL of 2.1 mg/kg-day based on increased incidence of splenic hemosiderosis in female mice (Botta, 1992) was chosen as the POD to derive a screening subchronic p-RfD.**

BMD input data for these splenic data are presented in Table B.11. The curve and BMD results are provided in Appendix C.

Table A.1. Potential PODs from the Botta (1992) CD-1 Mouse Study for Derivation of a Screening Subchronic p-RfD

Critical Effect	Sex	POD Method	POD (mg/kg-day)	Comments
Increased incidence of splenic congestion	Male	BMDL	14	
Increased incidence of splenic hemosiderosis	Male	BMDL	12	
Increased incidence of splenic congestion	Female	BMDL	14	
Increased incidence of splenic hemosiderosis	**Female**	**NOAEL**	**2.1**	**Data were not amenable to BMD modeling. Chosen as the POD**.
Decreased absolute gonad weights	Male	NOAEL	926	No statistically significant effect at any dose tested.
Increased absolute kidney weights	Male	NOAEL	444	Data were not amenable to BMD modeling.
Increased absolute liver weight	Male	BMDL	349	
Increased absolute spleen weight	Male	NOAEL	94	Data were not amenable to BMD modeling.
Decreased relative gonad weights	Male	NOAEL	926	No statistically significant effect at any dose tested.
Increased relative kidney weights	Male	NOAEL	926	No biologically significant effect (≥10% change) at any dose tested.
Increased relative liver weight	Male	BMDL	331	
Increased relative spleen weight	Male	NOAEL	94	Data were not amenable to BMD modeling.
Decreased absolute ovary weights	Female	NOAEL	1101	No statistically significant effect at any dose tested.
Increased absolute kidney weights	Female	NOAEL	1101	No biologically significant effect (≥10% change) at any dose tested.
Increased absolute liver weight	Female	NOAEL	555	Data were not amenable to BMD modeling.
Decreased relative ovary weights	Female	NOAEL	2.1	Data were not amenable to BMD modeling.
Increased absolute spleen weight	Female	NOAEL	107	Data were not amenable to BMD modeling.
Increased relative kidney weights	Female	NOAEL	555	Data were not amenable to BMD modeling.

Table A.1. Potential PODs from the Botta (1992) CD-1 Mouse Study for Derivation of a Screening Subchronic p-RfD				
Critical Effect	**Sex**	**POD Method**	**POD (mg/kg-day)**	**Comments**
Increased relative liver weight	Female	NOAEL	555	Data were not amenable to BMD modeling.
Increased relative spleen weight	Female	NOAEL	555	Data were not amenable to BMD modeling.

Adjusted points for daily exposure:

The following dosimetric adjustments were made for each dose in the principal study for diet treatment in adjusting for daily exposure. Dosimetric adjustment for 2.1 mg/kg-day is presented below.

$(DOSE_{ADJ})$ = $DOSE_{BOTTA,\ 1992}$ × [conversion to daily dose]
= 2.1 mg/kg-day × (days of week dosed ÷ 7)
= 2.1 mg/kg-day × (7 ÷ 7)
= 2.1 mg/kg-day

The screening subchronic p-RfD for diphenylamine based on a $NOAEL_{ADJ}$ of 2.1 mg/kg-day in female CD-1 mice (Botta, 1992) is derived as follows:

Screening Subchronic p-RfD = $NOAEL_{ADJ}$ ÷ UF
= 2.1 mg/kg-day ÷ 100
= **2×10^{-2} mg/kg-day**

Table A.2 summarizes the uncertainty factors for the screening subchronic p-RfD for diphenylamine.

Table A.2. Uncertainty Factors for the Screening Subchronic p-RfD of Diphenylamine

UF	Value	Justification
UF_A	10	A UF_A of 10 is applied for interspecies extrapolation to account for potential toxicokinetic and toxicodynamic differences between mice and humans. There are no data to determine whether humans are more or less sensitive than mice in Botta (1992) to subchronic oral toxicity of diphenylamine.
UF_D	1	A UF_D of 1 is applied because the database includes two acceptable developmental toxicity studies (Edwards et al., 1983b; Rodwell, 1992) via the oral exposure route. In addition, one acceptable two-generation reproductive toxicity study (Rodwell, 1993) via oral exposure was also located, lending support for a relatively complete database for diphenylamine.
UF_H	10	A UF_H of 10 is applied for intraspecies differences to account for potentially susceptible individuals in the absence of information on the variability of response to humans.
UF_L	1	A UF_L of 1 is applied for using a POD based on a NOAEL identified for increased incidence of splenic hemosiderosis in female mice from a subchronic-duration study by Botta (1992).
UF_S	1	A UF_S of 1 is applied because results from a subchronic-duration study (Botta, 1992) were utilized as the principal study.
$UF_C \leq 3000$	100	

APPENDIX B. DATA TABLES

Table B.1. Average Hematological Values of Female Rats Following Dietary Exposure to Diphenylamine for 90 Days[a,b]

Endpoint		Exposure Group (Average Daily Dose [mg/kg-day])		
		0	0.3% (302)	1.0% (1007)
Sample size		5	5	5
Hematocrit		50	46 (92%)	45 (90%)
Hemoglobin (g/100 mL)		14.3	13.7 (96%)	12.1 (85%)
Total leukocytes		19.68	18.33 (93%)	19.29 (98%)
Differential count	% Neutrophils	18.2	17.2 (95%)	13.8 (76%)
	% Lymphocytes	80.4	82.2 (102%)	85.4 (106%)
	% Monocytes	0.8	0.2 (25%)	0.6 (75%)
	% Eosinophils	0.6	0.4 (67%)	0.2 (33%)

[a]Dow Chemical Company (1958).
[b]Values represent the mean (% of controls).

Table B.2. Final Average Body Weight and Relative Organ Weights of Male and Female Rats Following Dietary Exposure to Diphenylamine for 90 Days[a,b]

Endpoint	Exposure Group (Average Daily Dose [mg/kg-day])					
Males	**0**	**0.01% (9)**	**0.03% (27)**	**0.1% (90)**	**0.3% (271)**	**1.0% (902)**
Sample size	10	6	8	9	5	5
Necropsy body weight (g)	275	268 (97%)	269 (98%)	273 (99%)	256 (93%)	216 (79%)**
Lung (g/100 g-bw)	0.61	0.61 (100%)	0.60 (98%)	0.55 (90%)	0.59 (97%)	0.66 (108%)
Heart (g/100 g-bw)	0.34	0.36 (106%)	0.34 (100%)	0.38 (112%)	0.37 (109%)	0.36 (106%)
Liver (g/100 g-bw)	2.92	2.92 (100%)	2.88 (99%)	3.12 (107%)*	3.37 (115%)**	4.54 (155%)**
Kidney (g/100 g-bw)	0.76	0.77 (101%)	0.77 (101%)	0.77 (101%)	0.76 (100%)	0.88 (116%)**
Spleen (g/100 g-bw)	0.30	0.27 (90%)	0.28 (93%)	0.28 (93%)	0.28 (93%)	0.54 (180%)**
Testes (g/100 g-bw)	0.94	1.00 (106%)	1.00 (106%)	1.02 (109%)	1.06 (113%)	1.19 (127%)**
Females	**0**	**0.01% (10)**	**0.03% (30)**	**0.1% (101)**	**0.3% (302)**	**1.0% (1007)**
Sample size	9	8	8	10	9	8
Necropsy body weight (g)	165	178 (108%)	172 (104%)	181 (110%)	162 (98%)	141 (85%)**
Lung (g/100 g-bw)	0.75	0.78 (104%)	0.71 (95%)	0.74 (99%)	0.80 (107%)	0.80 (107%)
Heart (g/100 g-bw)	0.41	0.38 (93%)	0.40 (98%)	0.39 (95%)	0.42 (102%)	0.43 (105%)
Liver (g/100 g-bw)	2.93	3.13 (107%)*	3.22 (110%)**	3.22 (110%)**	3.73 (127%)**	4.60 (157%)**
Kidney (g/100 g-bw)	0.82	0.80 (98%)	0.83 (101%)	0.80 (98%)	0.87 (106%)	1.02 (124%)
Spleen (g/100 g-bw)	0.27	0.32 (119%)	0.34 (126%)**	0.34 (126%)**	0.39 (144%)**	0.63 (233%)**

[a]Dow Chemical Company (1958).
[b]Values represent the mean (% of controls).

*$p < 0.05$, test name not provided.
**$p < 0.01$, test name not provided.

Table B.3. Average Final Body Weight and Food Consumption of Albino Rats Following Dietary Exposure to Diphenylamine for 226 Days[a,b]

Endpoint	Exposure Group (Average Daily Dose [mg/kg-day])					
	0	**0.025% (21)**	**0.10% (82)**	**0.50% (410)**	**1.00% (820)**	**1.50% (1230)**
Sample size	6	6	6	6	6	6
Body weight (g)	233	226 (97%)	222 (95%)	207 (89%)	200 (86%)	174 (75%)
Food intake (g/rat/day)	11.21	11.39 (102%)	11.26 (100%)	10.37 (92.5%)	10.96 (98%)	11.45 (102%)

[a]Thomas et al. (1957).
[b]Values represent the mean (% of controls).

Table B.4. Survival and Food Consumption of Albino Male and Female Slonaker-Addis Rats Following Dietary Exposure to Diphenylamine for 2 Years[a]

Endpoint		Exposure Group (Average Daily Dose [mg/kg-day])					
Males		**0**	**0.001% (1)**	**0.01% (7)**	**0.10% (72)**	**0.50% (362)**	**1.00% (723)**
Survival[b]	Day 240	20	18 (90%)	18 (90%)	20 (100%)	17 (85%)	18 (90%)
	Day 640	13	12 (92%)	15 (115%)	15 (115%)	12 (92%)	13 (100%)
	Day 734	13	12 (92%)	15 (115%)	14 (108%)	12 (92%)	10 (77%)
Food consumption (g/rat/day)[c]		11.65	11.47 (98%)	11.68 (100%)	11.82 (101%)	10.86* (93%)	9.99* (86%)
Females		**0**	**0.001% (1)**	**0.01% (8)**	**0.10% (82)**	**0.50% (410)**	**1.00% (820)**
Survival[b]	Day 240	20	17 (85%)	19 (95%)	19 (95%)	18 (90%)	16 (80%)
	Day 640	14	12 (86%)	18 (129%)	17 (121%)	12 (86%)	12 (86%)
	Day 734	11	9 (82%)	15 (136%)	11 (100%)	9 (82%)	11 (100%)
Food consumption (g/rat/day)[c]		9.44	9.55 (101%)	9.57 (101%)	9.25 (98%)	8.77* (93%)	8.12* (86%)

[a]Thomas et al. (1967b).
[b]Values represent number of survivors (% of control).
[c]Values represent the mean (% of control).

*$p < 0.01$, Duncan method.

Table B.5. Average Hematological Values of Albino Male and Female Slonaker-Addis Rats Following Dietary Exposure to Diphenylamine for 2 Years[a,b]

Endpoint		Exposure Group (Average Daily Dose [mg/kg-day])	
Males		**0**	**1.00% (723)**
Hemoglobin (g/100 mL)	Day 126[c]	13.85	12.67
	Day 182[c,d]	13.45	12.67
	Day 230[e]	13.45	12.60
	Day 267	13.95	13.65
	Day 360	14.25	12.69
	Day 463	14.70	13.66
Red Blood cells (10^5/mm^3)	Day 126[c]	9.30	8.39
	Day 182[c,d]	8.96	8.04
	Day 230[e]	9.23	8.50
	Day 267	9.32	8.33
	Day 360	8.49	7.58
	Day 463	8.96	7.88
Females		**0**	**1.00% (820)**
Hemoglobin (g/100 mL)	Day 161[f]	14.24	14.19
	Day 264	13.48	12.33
	Day 371[g]	14.37	13.44
	Day 463	14.05	13.20
Red cells (10^5/mm^3)	Day 161[f]	9.02	8.11
	Day 264	8.81	-
	Day 371[g]	9.01	7.94
	Day 463	8.29	8.37

[a]Thomas et al. (1967b).
[b]Values represent the mean; $n = 2$ unless otherwise noted.
[c]$n = 3$.
[d]Day 177 for 1%-diphenylamine group.
[e]Day 231 for 1%-diphenylamine group.
[f]$n = 4$.
[g]Day 369 for 1%-diphenylamine group.

Table B.6. Urinary Tract Lesions of Albino Male and Female Slonaker-Addis Rats Following Dietary Exposure to Diphenylamine for 2 Years[a,b]

Endpoint		Exposure Group (Average Daily Dose [mg/kg-day])					
Males		**0**	**0.001% (1)**	**0.01% (7)**	**0.1% (72)**	**0.5% (362)**	**1.0% (723)**
Kidney	Chronic nephritis	13	12	14	13	11	10
	Tubular cysts	13	12	14	13	11	10
Bladder	Epithelial hyperplasia or metaplasia	0	1	1	0	1	5
Females		**0**	**0.001% (1)**	**0.01% (8)**	**0.1% (82)**	**0.5% (410)**	**1.0% (820)**
Kidney	Chronic nephritis	10	10	14	15	10	8
	Tubular cysts	10	10	14	15	10	8
Bladder	Epithelial hyperplasia or metaplasia	1	0	0	0	0	1

[a]Thomas et al. (1967b).
[b]Values represent number of animals observed with lesions and include animals that exhibited negligible incidences of lesions. Animals that died before Day 640 were not autopsied.

Table B.7. Benign and Malignant Tumor Incidence in Male and Female Albino Slonaker-Addis Rats Following Dietary Exposure to Diphenylamine for 2 Years[a,b]

Endpoint			Exposure Group (Average Daily Dose [mg/kg-day])					
Males			**0**	**0.001% (1)**	**0.01% (7)**	**0.1% (72)**	**0.5% (362)**	**1.0% (723)**
Adrenal	Medulla	Adenomatous hyperplasia[c]	8	0	8	5	4	5
		Adenocarcinoma	-	1	1	-	-	-
	Cortical	Adenocarcinoma	-	1	-	-	-	-
	Pheochromocytoma[c]		-	1	1	-	-	-
Abdomen	Lipoma[c]		1	-	-	1	-	-
Mammary	Adenofibroma[c]		1	2	1	0	0	0
Pituitary	Adenoma[c]		1	-	-	-	-	-
Thyroid	Adenoma[c]		-	-	1	-	-	-
Liver	Hemangioepithelioma		-	-	-	-	-	1
	Hepatoma		-	-	-	1	-	-
Lung	Lymphosarcoma		1	-	-	-	-	-
Pancreas	Adenocarcinoma		-	-	-	1	-	-
Females			**0**	**0.001% (1)**	**0.01% (8)**	**0.1% (82)**	**0.5% (410)**	**1.0% (820)**
Adrenal	Medulla	Adenomatous hyperplasia[c]	5	3	2	6	1	3
		Adenocarcinoma	1	-	-	-	-	-
Mammary	Adenofibroma[c]		2	5	6	5	2	0
Pituitary	Adenoma[c]		-	1	3	1	1	1
	Adenocarcinoma		-	1	-	-	-	-
Lung	Adenocarcinoma		-	-	-	-	1	-
Uterus	Leiomyoma[c]		-	1	-	-	-	-
Ovary	Granulosa cell[c]		1	-	-	-	-	-
Vulva	Squamous cell carcinoma		1	-	-	-	-	-

[a]Thomas et al. (1967b).
[b]Values represent number of animals observed with lesions; tumors were malignant unless otherwise noted.
[c]Tumors were benign.

Table B.8. Survival, Body Weight, Body Weight Change, and Food Consumption in Rabbits Dosed Orally with Diphenylamine from GDs 7–19[a,b]

Endpoint	Exposure Group (Average Daily Dose [mg/kg-day])[c]			
	0	33	100	300
Survival	13/16	14/16	15/18[d]	15/16
Body weight Day 1(g)	3421.9	3382.9 (99%)	3305.8 (97%)	3406.2 (100%)
Body weight Day 29 (g)	4101.2	3962.9 (97%)	4065.0 (99%)	3963.2 (97%)
Body-weight change on Day 29 relative to start of treatment on Day 7 (g)	488.9	405.8 (83%)	535.0 (109%)	389.4 (80%)
Food consumption Days 1–6 (g/animal/day)[e]	200.7	199.3 (99%)	191.3 (95%)	193.8 (97%)
Food consumption Days 7–8	204.5	201.5 (99%)	208.5 (102%)	166.5 (81%)
Food consumption Days 9–10	194.5	193.0 (99%)	194.0 (100%)	163.5 (84%)
Food consumption Days 11–14	195.5	184.0 (94%)	199.8 (102%)	161.5 (83%)
Food consumption Days 15–19	210.2	174.0 (83%)	220.2 (105%)	170.6 (81%)
Food consumption Days 20–23	199.8	178.8 (89%)	210.3 (105%)	171.8 (86%)
Food consumption Days 24–28	184.6	156.6 (85%)	168.0 (91%)	162.4 (88%)

[a]Edwards et al. (1983).
[b]Values represent the mean (% of controls).
[c]The study authors reported average daily dose as the exposure group in the study results.
[d]Includes 2 animals culled prior to start of treatment and then replaced.
[e]Food consumption means calculated for less than indicated number of animals when overt diet wastages recorded.

Table B.9. Fetal Weight, Developmental Malformations, and Anomalies in Fetuses of Rabbits Dosed Orally with Diphenylamine from GDs 7–19[a,b]

Endpoint	Exposure Group (Average Daily Dose [mg/kg-day])[c]			
	0	33	100	300
Number fetuses/total litters examined	92/12	98/12	89/12	115/13
Mean fetal weight (g)	46.6	42.8 (92%)	43.2 (93%)	42.5 (91%)
Mean % of fetuses with malformations	4.0	0.9 (23%)	1.0 (25%)	0.0 (0%)
Number fetuses with malformations	2	1 (50%)	1 (50%)	0 (0%)
Mean % of fetuses with anomalies in gross autopsy	0.8	1.7 (213%)	2.1 (263%)	1.6 (200%)
Number fetuses with anomalies in gross autopsy	1	1 (100%)	2 (200%)	2 (200%)
Mean % of fetuses with skeletal anomalies	20	16 (80%)	12 (60%)	13 (65%)
Number fetuses with skeletal anomalies	22.8	17.9 (79%)	12.4 (54%)	11.8 (52%)

[a]Edwards et al. (1983).
[b]Values represent the mean (% of controls).
[c]The study authors reported average daily dose as the exposure group in the study results.

Table B.10. Reproductive Data of Albino Male and Female Slonaker-Addis Rats Following Dietary Exposure to Diphenylamine[a,b]

Endpoint		Exposure Group (Average Daily Dose [mg/kg-day])[c]			
		0	0.1% (95)	0.25% (237)	0.5% (473)
First Mating[d]					
At birth	No. litters	12	10	10	10
	No. pups per litter	8.3	9.0	6.8	6.3*
At weaning	No. litters	10	10	9	8
	No. pups per litter	7.5	8.3	7.1	6.3
Weight at weaning (g)	Males	29.6	31.9	29.9	26.9
	Females	27.8	28.7	27.8	25.8
Second Mating[e]					
At birth	No. litters	9	12	12	11
	No. pups per litter	9.6	7.3*	7.3*	6.6**
At weaning	No. litters	9	11	12	11
	No. pups per litter	9.3	7.1	7.3	6.6
Weight at weaning (g)	Males	28.3	37.0	35.5	28.2
	Females	27.5	36.0	34.4	28.2
Second Generation[d]					
At birth	No. litters	10	11	12	11
	No. pups per litter	8.6	5.8**	7.3	7.0
At weaning	No. litters	10	11	12	11
	No. pups per litter	8.5	5.7**	7.0	6.6
Weight at weaning (g)	Males	31.0	31.1	30.0	24.9**
	Females	30.6	31.2	29.3	24.8**

[a]Thomas et al. (1967c).
[b]Values represent the mean.
[c]Adjusted Daily Doses shown as averages of values of males and females.
[d]Number of mated females = 12.
[e]Number of mated females = 12, except in the 95-mg/kg-day group where number of females = 11.

*$p < 0.05$, Duncan method.
**$p < 0.01$, Duncan method.

Table B.11. Incidence of Splenic Hemosiderosis and Congestion in the Mouse After Dietary Administration of Diphenylamine in a 90-Day Subchronic-Duration Toxicity Study[a]

Adjusted Dose Group (mg/kg-day)	Number of Mice	Incidence of Splenic Hemosiderosis	Incidence of Splenic Congestion
Males			
0	15	0	0
1.7	14	0	0
94	15	4	2
444	15	15*	15*
926	15	15*	15*
Females			
0	15	0	0
2.1	15	0	0
107	15	12*	6*
555	15	15*	14*
1101	15	15*	15*

[a]Values obtained from Botta (1992).

*$p < 0.05$ by Fisher's Exact Test.

Table B.12. Relative and Absolute Organ Weights and Body Weight of Male and Female CD-1 Mice Following Dietary Exposure to Diphenylamine for 90 Days[a]

Endpoint	Exposure Group (Adjusted Daily Dose [mg/kg-day])				
Males	**0 ppm**	**10 ppm (1.7)**	**525 ppm (94)**	**2625 ppm (444)**	**5250 ppm (926)**
Sample size	15	15	15	15	12
Necropsy body weight (g)	31.7±1.9	32.7±3.9	32.6±2.9	31.9±2.3	31.4±1.9
Absolute gonad weights (g)	0.401±0.057	0.374±0.084	0.405±0.055	0.394±0.046	0.395±0.043
Absolute kidney weights (g)	0.592±0.083	0.585±0.110	0.640±0.055	0.611±0.054	0.636±0.090
Absolute liver weight (g)	1.341±0.091	1.415±0.154	1.443±0.152	1.521±0.180**	1.669±0.173**
Absolute spleen weight (g)	0.083±0.029	0.074±0.013	0.095±0.019	0.132±0.043**	0.242±0.062**
Relative gonad weights (g/100 g-bw)	1.266±0.145	1.159±0.286	1.249±0.177	1.238±0.158	1.258±0.100
Relative kidney weights (g/100 g-bw)	1.869±0.211	1.808±0.343	1.973±0.179	1.918±0.165	2.024±0.244
Relative liver weight (g/100 g-bw)	4.248±0.340	4.349±0.348	4.441±0.404	4.765±0.393**	5.309±0.362**
Relative spleen weight (g/100 g-bw)	0.262±0.086	0.229±0.043	0.292±0.060	0.416±0.144**	0.774±0.204**
Females	**0 ppm**	**10 ppm (2.1)**	**525 ppm (107)**	**2625 ppm (555)**	**5250 ppm (1101)**
Sample size	12	15	15	15	15
Necropsy body weight (g)	26.7±2.5	25.5±2.3	26.8±2.2	25.3±2.5*	25.7±1.5**
Absolute ovary weights (g)	0.041±0.009	0.033±0.009	0.031±0.009	0.032±0.008	0.030±0.009
Absolute kidney weights (g)	0.419±0.033	0.414±0.040	0.416±0.051	0.419±0.041	0.459±0.060
Absolute liver weight (g)	1.251±0.162	1.159±0.112	1.159±0.127	1.229±0.175	1.502±0.153**
Absolute spleen weight (g)	0.079±0.018	0.076±0.017	0.098±0.016	0.163±0.046**	0.276±0.088**
Relative ovary weights (g/100 g-bw)	0.154±0.034	0.128±0.030	0.115±0.036**	0.129±0.030	0.116±0.031*
Relative kidney weights (g/100 g-bw)	1.576±0.160	1.637±0.196	1.559±0.191	1.664±0.136	1.784±0.204*
Relative liver weight (g/100 g-bw)	4.681±0.443	4.562±0.372	4.329±0.335	4.849±0.471	5.836±0.462**
Relative spleen weight (g/100 g-bw)	0.295±0.055	0.297±0.054	0.367±0.062	0.642±0.176**	1.068±0.313**

[a]Botta (1992); data are presented as means ± standard deviation.

*$p < 0.05$ by Dunnet's test.
**$p < 0.01$ by Dunnet's test.

APPENDIX C. BENCHMARK DOSE CALCULATIONS FOR THE SCREENING SUBCHRONIC p-RfD

Modeling Procedure for Dichotomous Data

The BMD modeling of dichotomous data was conducted with EPA's BMDS (version 2.1.2). For these data, all of the dichotomous models (i.e., Gamma, Multistage, Logistic, Log-logistic, Probit, Log-probit, Weibull, and Quantal-linear models) available within the software were fit using a default BMR of 10% extra risk. Adequacy of model fit was judged based on the χ^2 goodness-of-fit *p*-value ($p > 0.1$), magnitude of scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. Among all models providing adequate fit, the BMDL from the model with the lowest AIC was selected as a potential POD from which to derive the screening subchronic p-RfD.

Modeling Procedure for Continuous Data

The BMD modeling of continuous data was conducted with EPA's BMDS (version 2.1.2). For these data (e.g., increased relative liver weight), all continuous models available within the software were fit using a default BMR of 10% extra risk. An adequate fit was judged based on the χ^2 goodness-of-fit *p*-value ($p > 0.1$), magnitude of the scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. In addition to these three criteria for judging adequacy of model fit, a determination was made as to whether the variance across dose groups was homogeneous. If a homogeneous variance model was deemed appropriate based on the statistical test provided in BMDS (i.e., Test 2), the final BMD results were estimated from a homogeneous variance model. If the test for homogeneity of variance was rejected ($p < 0.1$), the model was run again while modeling the variance as a power function of the mean to account for this nonhomogeneous variance. If this nonhomogeneous variance model did not adequately fit the data (i.e., Test 3; *p*-value < 0.1), the data set was considered unsuitable for BMD modeling. Among all models providing adequate fit, the lowest BMDL was selected if the BMDLs estimated from different models varied greater than 3-fold; otherwise, the BMDL from the model with the lowest AIC was selected as a potential POD from which to derive the screening subchronic p-RfD.

INCREASED INCIDENCE OF SPLENIC HEMOSIDEROSIS IN MALE CD-1 MICE TREATED WITH DIPHENYLAMINE FOR 90 DAYS (Botta, 1992)

All dichotomous models available in BMDS (version 2.1.2) were fit to the incidence data of splenic hemosiderosis in male CD-1 mice treated with diphenylamine for 90 days (Botta, 1992; Table B.11). In the absence of a mechanistic understanding of the biological response to a toxic agent, data from exposures much higher than the study LOAEL do not provide reliable information regarding the shape of the response at low doses. Such exposures, however, can have a strong effect on the shape of the fitted model in the low-dose region of the dose-response curve. Thus, if the lack of fit is due to characteristics of the dose-response data for high doses, then the EPA Benchmark Dose Technical Guidance Document allows for data to be adjusted by eliminating the high-dose group (U.S. EPA, 2000, 052150). Because the focus of BMD analysis is on the low dose and response region, eliminating high-dose groups is deemed reasonable. For increased incidence of splenic hemosiderosis in male CD-1 mice, all modeling results shown are without the highest dose groups being included in the analysis (see Table C.1 and Figure C.1). As assessed by the χ^2 goodness-of-fit statistic and visual inspection, only the Quantal-linear model adequately fit the data (see Table C.1 and Figure C.1). Estimated doses associated with 10% extra risk and the 95% lower confidence limit on these doses (BMD_{10} values and $BMDL_{10}$ values, respectively) were 18 and 12 mg/kg-day.

Table C.1. BMD Dose-Response Modeling Results Based on the Increased Incidence of Splenic Hemosiderosis in Male CD-1 Mice Treated with Diphenylamine for 90 Days

Model[d]	χ^2 *p*-value	AIC	BMD_{10}	$BMDL_{10}$
Gamma[a]	1	21.398	75	25
Multistage[b]	0.9995	19.428	54	19
Logistic	1	21.398	88	49
Log-logistic[c]	1	21.398	86	41
Probit	1	21.398	82	44
Log-probit[c]	1	21.398	80	40
Weibull[a]	1	21.398	68	23
Quantal-linear	**0.4172**	**23.581**	**18**	**12**

[a]Restrict power ≥1.
[b]Restrict betas ≥0; degree of polynomial = 2; lowest degree polynomial with an adequate fit reported.
[c]Slope restricted to >1.
[d]All models besides Quantal–linear failed based on visual inspection due to S-shaped curves.

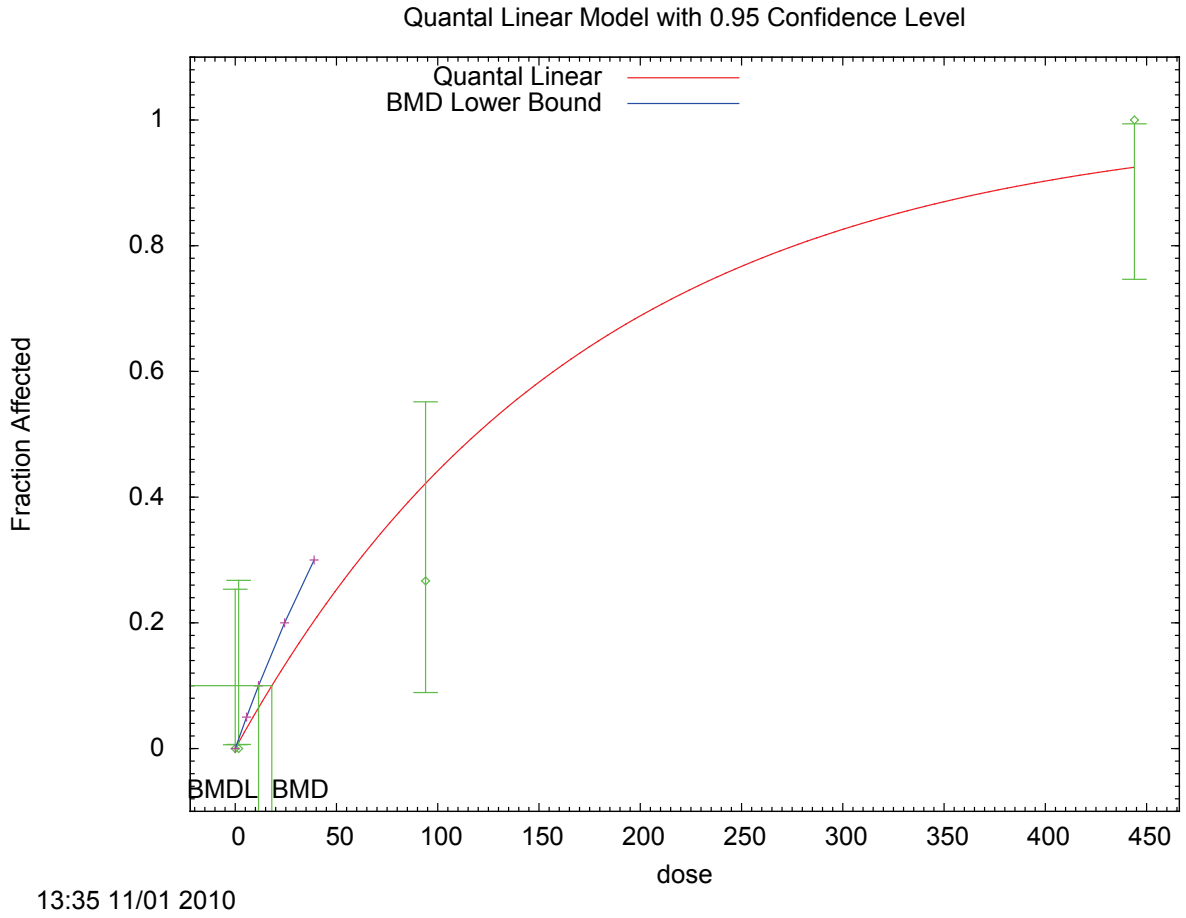

Figure C.1. Dose-Response Modeling for Increased Incidence of Splenic Hemosiderosis in Male CD-1 Mice Treated with Diphenylamine for 90 Days

INCREASED INCIDENCE OF SPLENIC CONGESTION IN MALE CD-1 MICE TREATED WITH DIPHENYLAMINE FOR 90 DAYS (Botta, 1992)

All dichotomous models available in BMDS (version 2.1.2) were fit to the incidence data of splenic congestion in male CD-1 mice treated with diphenylamine for 90 days (Botta, 1992; Table B.11). For these data, all modeling results shown are without the highest dose groups being included in the analysis (see Table C.2 and Figure C.2). As assessed by the χ^2 goodness-of-fit statistic and visual inspection, only the Quantal-linear model adequately fit the data (see Table C.2 and Figure C.2). Estimated doses associated with 10% extra risk and the 95% lower confidence limit on these doses (BMD_{10} values and $BMDL_{10}$ values, respectively) were 22 and 14 mg/kg-day.

Table C.2. BMD Dose-Response Modeling Results Based on the Incidence of Splenic Congestion in Male CD-1 Mice Treated with Diphenylamine for 90 Days

Model[d]	χ^2 *p*-value	AIC	BMD_{10}	$BMDL_{10}$
Gamma[a]	1	15.78	89	50
Multistage[b]	1	14.381	68	34
Logistic	1	15.78	92	62
Log-logistic[c]	1	15.78	92	61
Probit	1	115.78	91	57
Log-probit[c]	1	15.78	90	61
Weibull[a]	1	15.78	85	14
Quantal-linear	**0.01362**	**21.801**	**22**	**14**

[a]Restrict power ≥1.
[b]Restrict betas ≥0; degree of polynomial = 2; lowest degree polynomial with an adequate fit reported.
[c]Slope restricted to >1.
[d]All models besides Quantal–linear failed based on visual inspection due to S-shaped curves.

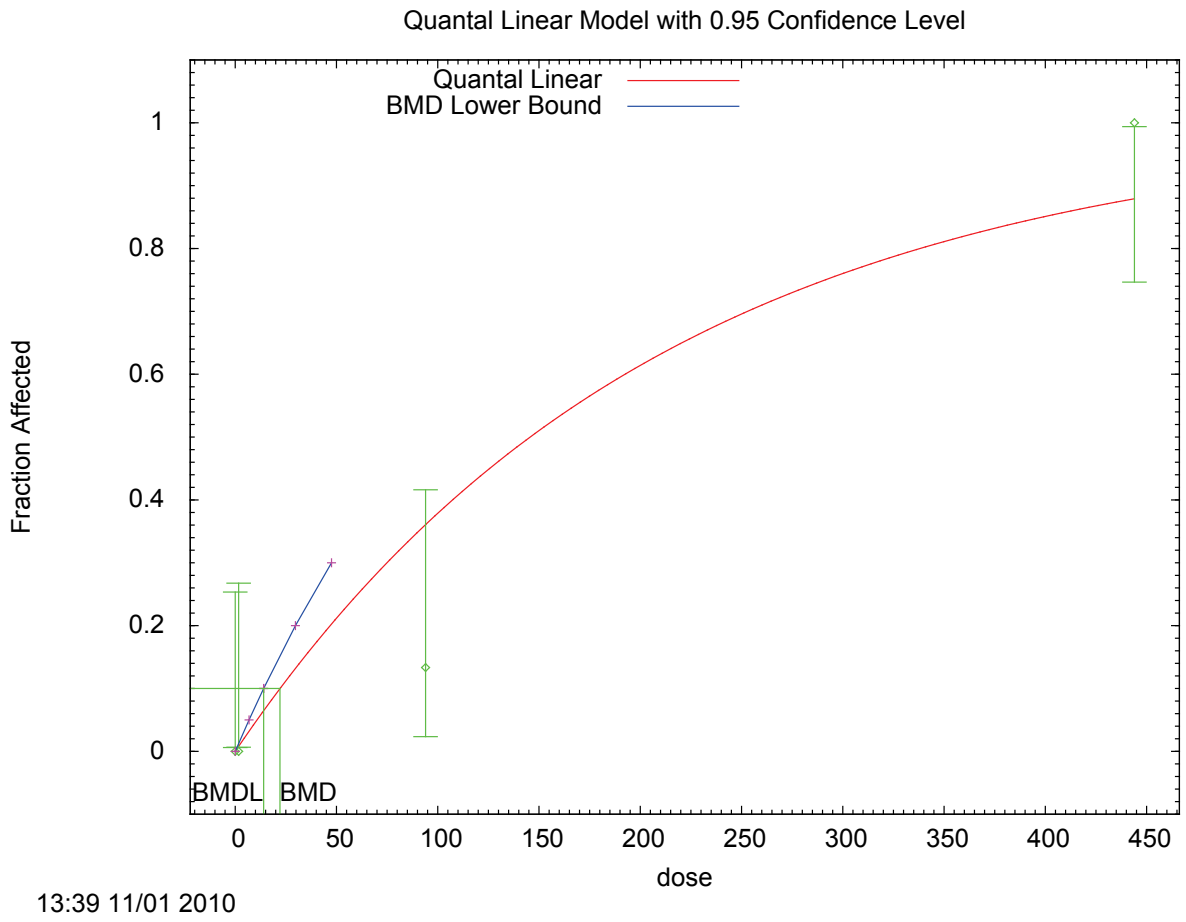

Figure C.2. Dose-Response Modeling for Increased Incidence of Splenic Congestion in Male CD-1 Mice Treated with Diphenylamine for 90 Days

INCREASED INCIDENCE OF SPLENIC CONGESTION IN FEMALE CD-1 MICE TREATED WITH DIPHENYLAMINE FOR 90 DAYS (Botta, 1992)

All dichotomous models available in BMDS (version 2.1.2) were fit to the incidence data of splenic congestion in female CD-1 mice treated with diphenylamine for 90 days (Botta, 1992; Table B.11). For these data, all modeling results shown are without the highest dose groups being included in the analysis (see Table C.3 and Figure C.3). As assessed by the χ^2 goodness-of-fit statistic and visual inspection, the Gamma, Multistage, Weibull, and the Quantal-linear models adequately fit the data (see Table C.3 and Figure C.3). Of these models, the Quantal-linear model was the best fit based on the lowest AIC and provided BMD_{10} values and $BMDL_{10}$ values of 22 and 14 mg/kg-day, respectively.

Table C.3. BMD Dose-Response Modeling Results Based on the Incidence of Splenic Congestion in Female CD-1 Mice Treated with Diphenylamine for 90 Days

Model[d]	χ^2 *p*-value	AIC	BMD_{10}	$BMDL_{10}$
Gamma[a]	0.9386	31.745	28	14
Multistage[b]	0.9276	31.832	23	14
Logistic	0.0374	39.753	83	51
Log-logistic[c]	0.9965	31.552	41	10
Probit	0.038	39.442	84	57
Log-probit[c]	1	31.538	41	24
Weibull[a]	0.933	31.783	26	14
Quantal-linear	**0.9846**	**29.843**	**22**	**14**

[a]Restrict power ≥1.
[b]Restrict betas ≥0; degree of polynomial = 2; lowest degree polynomial with an adequate fit reported.
[c]Slope restricted to >1.
[d]All models besides Gamma, Multistage, Weibull, Quantal–linear failed based on visual inspection due to S-shaped curves.

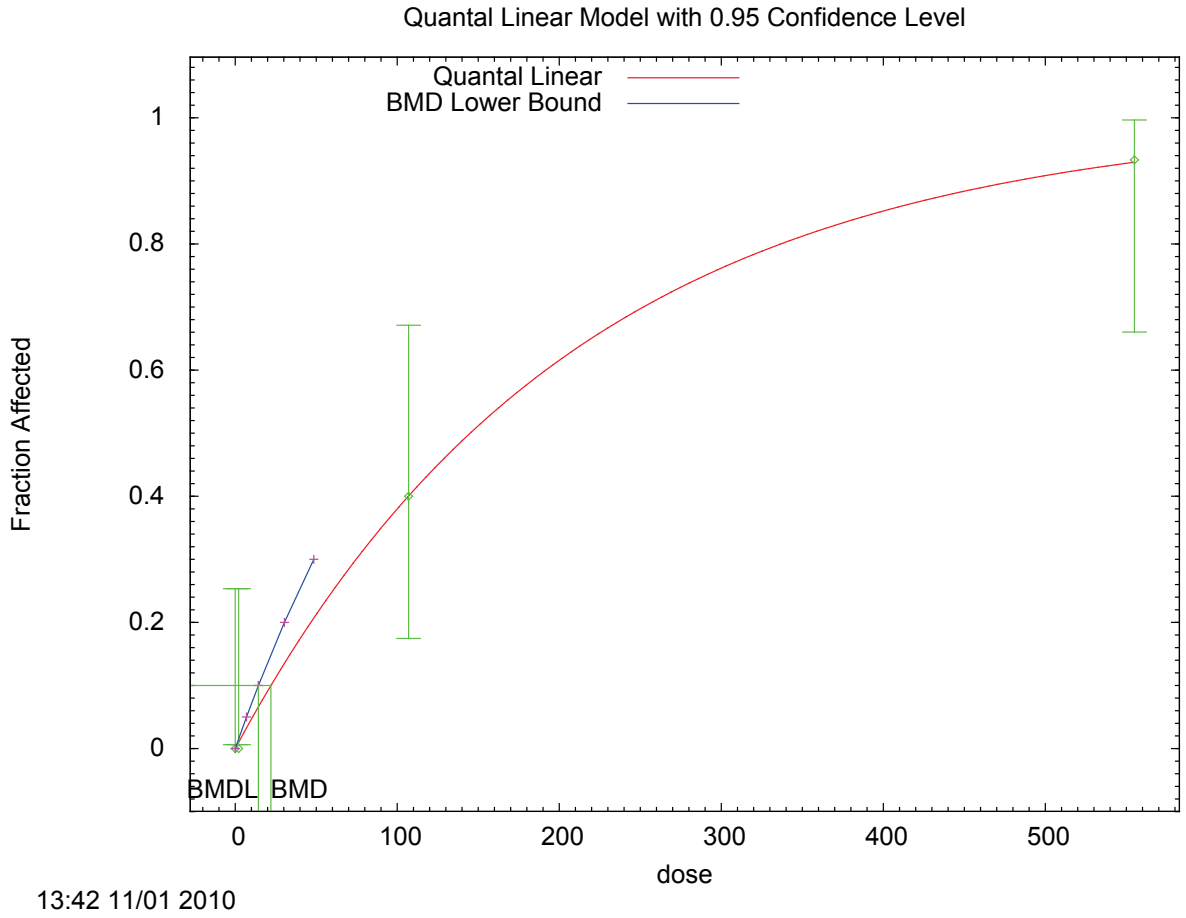

Figure C.3. Dose-Response Modeling for Increased Incidence of Splenic Congestion in Female CD-1 Mice Treated with Diphenylamine for 90 Days

INCREASED RELATIVE LIVER WEIGHT OF MALE CD-1 MICE TREATED WITH DIPHENYLAMINE FOR 90 DAYS (Botta, 1992)

All available continuous models in BMDS (version 2.1.2) were fit to the increased relative liver-weight data from male CD-1 mice exposed to diphenylamine for 90 days (Botta, 1992; Table B.12). As assessed by the χ^2 goodness-of-fit statistic and visual inspection, the Linear and Power models provided the best fit models (see Table C.4 and Figure C.4). Estimated doses associated with 10% extra risk and the 95% lower confidence limit on these doses (BMD_{10} values and $BMDL_{10}$ values, respectively) were 401 and 331 mg/kg-day.

Table C.4. BMD Modeling Results on Increased Relative Liver Weight in Male CD-1 Mice Exposed to Diphenylaminefor 90 Days

Model	Test 2	Test 3	χ^2 *p*-Value	AIC	BMD_{10}	$BMDL_{10}$
Males						
Linear	**0.9522**	**0.9522**	**0.8569**	**-69.348**	**401**	**331**
Polynomial	0.9522	0.9522	0.6834	-67.355	409	332
Power	**0.9522**	**0.9522**	**0.8569**	**-69.348**	**401**	**331**
Hill	0.9522	0.9522	0.6808	-67.348	401	331

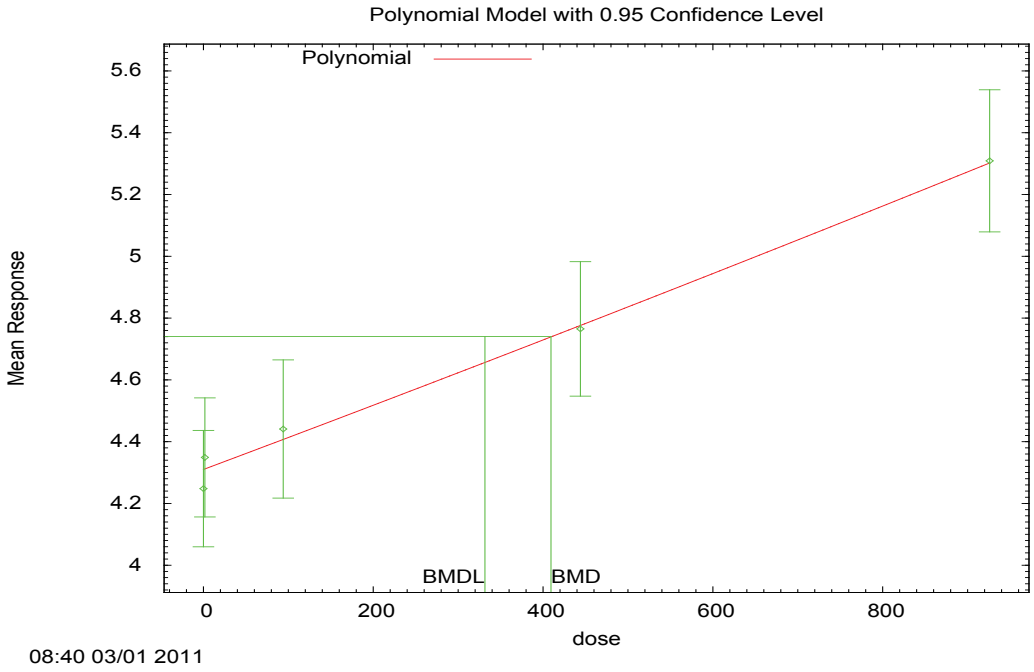

Figure C.4. Dose-Response Modeling for Increased Relative Liver Weight in Male CD-1 Mice Treated with Diphenylamine for 90 Days

INCREASED ABSOLUTE LIVER WEIGHT OF MALE CD-1 MICE TREATED WITH DIPHENYLAMINE FOR 90 DAYS (Botta, 1992)

All available continuous models in BMDS (version 2.1.2) were fit to the increased absolute liver-weight data from male CD-1 mice exposed to diphenylamine for 90 days (Botta, 1992; Table B.12). As assessed by the χ^2 goodness-of-fit statistic and visual inspection, the Linear, Power, and Polynomial models provided the best fit (see Table C.5 and Figure C.5). Estimated doses associated with 10% extra risk and the 95% lower confidence limit on these doses (BMD_{10} values and $BMDL_{10}$ values, respectively) were 456 and 349 mg/kg-day.

Table C.5. BMD Modeling Results on Increased Absolute Liver Weight in Male CD-1 Mice Exposed to Diphenylamine for 90 Days

Model	Test 2	Test 3	χ^2 *p*-Value	AIC	BMD_{10}	$BMDL_{10}$
Males						
Linear	**0.13**	**0.13**	**0.4815**	**-195.704**	**456**	**349**
Polynomial	**0.13**	**0.13**	**0.4815**	**-195.704**	**456**	**349**
Power	**0.13**	**0.13**	**0.4815**	**-195.704**	**456**	**349**
Hill	0.13	0.13	0.2989	-193.754	422	169

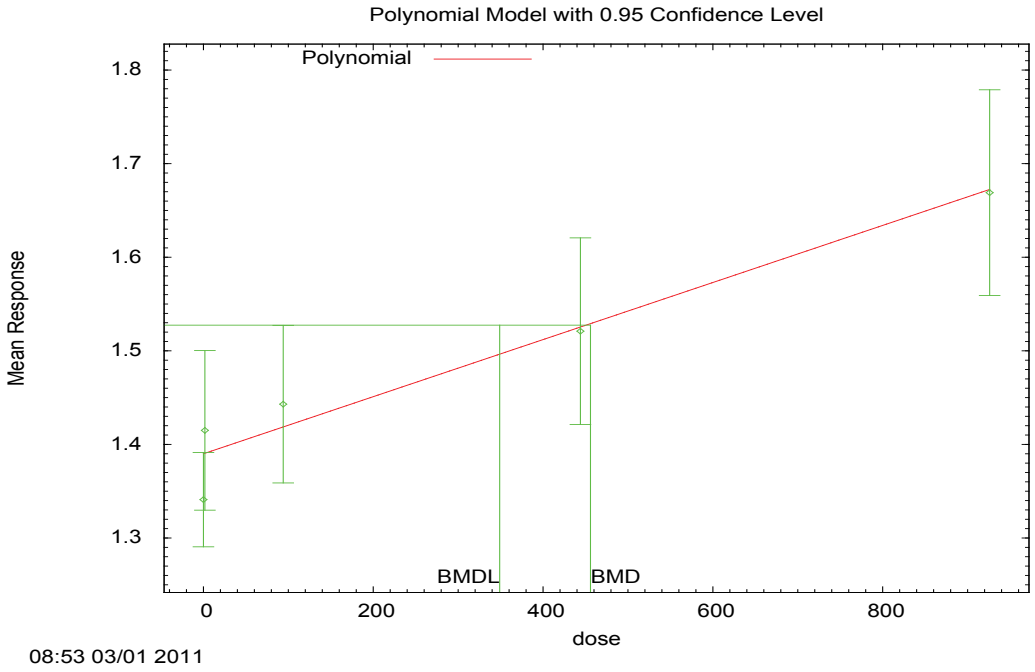

Figure C.5. Dose-Response Modeling for Increased Absolute Liver Weight in Male CD-1 Mice Treated with Diphenylamine for 90 Days

APPENDIX D. REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). (2010) Threshold limit values for chemical substances and physical agents and biological exposure indices. As cited in Hazardous Substances Data Bank, Cincinnati, Ohio. Available online at http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB. Accessed on 6/17/2010. 625688

Alexander, WE; Ryan, AJ; Wright, SE. (1965) Metabolism of diphenylamine in the rat, rabbit and man. *Food Cosmet Toxicol* 3(4):571–579. 627343

Appel, KE; Görsdorf, S; Scheper, T; Bauszus, M; Hildebrandt, AG. (1987) Enzymatic denitrosation of diphenylnitrosamine: activation or inactivation? *Arch Toxicol* 60(1−3):204–208. 627698

Ardito, G; Bramanti, B; Bigatti, P; et al. (1996) Cytogenetic effect of thiabendazole and diphenylammine on cultured human lymphocytes: sister chromatid exchanges and cell cycle delay. *Boll Soc Ital Biol Sper* 72(5−6):171–178. 627742

ATSDR (Agency for Toxic Substances and Disease Registry). (2008) Toxicological profile information sheet. U.S. Department of Health and Human Services, Public Health Service. Available online at http://www.atsdr.cdc.gov/toxpro2.html. Accessed on 6/17/2010. 595415

Babish, JG; Hotchkiss, JH; Wachs, T; et al. (1983) *N*-nitrosamines and mutagens in rubber nursing nipples. *J Toxicol Environ Health A* 11(2):167–177. 627359

Botta, JA Jr. (1992) 90 Day subchronic toxicity evaluation of diphenylamine in the mouse T.P.S., Inc., Mt Vernon, IN; unpublished report No. 426E-001-034-91. 627604

Botta, JA Jr. (1994a) One year chronic study of diphenylamine in dogs. T.P.S., Inc., Mt Vernon, IN; unpublished report No. 426B-502-044-91. 627619

Botta, JA Jr. (1994b) 24 Month combined oncogenicity/toxicity evaluation of diphenylamine in rats. T.P.S., Inc., Mt. Vernon, IN; unpublished report No. 426D-102-048-91. 627618

Botta, JA Jr. (1994c) 18 Month oncogenicity evaluation of diphenylamine in the mouse. T.P.S., Inc., Mt Vernon, IN; unpublished report No. 426H-002-646-91. 627611

Braun, R; Schoneich, J; Ziebarth, D. (1977) In vivo formation of *N*-nitroso compounds and detection of their mutagenic activity in the host-mediated assay. *Can Res* 37(12):4572–4579. 017493

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

Checkoway, H; Smith, AH; McMichael, AJ; et al. (1981) A case-control study of bladder cancer in the United States rubber and tyre industry. *Br J Ind Med* 38(3):240–246. 633839

Crocker, JFS; Brown, DM; Borch, RF; et al. (1972) Renal cystic disease induced in newborn rats by diphenylamine derivatives. *Am J Pathol* 66(3):343–350. 627385

DeEds, F. (1963a,b) Summary of toxicological data: Chronic toxicity studies on diphenylamine. *Food Cosmet Toxicol* 1:331–333. 627415

Dolara, P; Vezzani, A; Cademi, G; et al. (1993) Genetic toxicity of a mixture of fifteen pesticides commonly found in the Italian diet. *Cell Biol Toxicol* 9(4):333–43. 634998

Dolara, P; Torricelli, F; Antonelli, N. (1994) Cytogenetic effects on human lymphocytes of a mixture of fifteen pesticides commonly used in Italy. *Mutat Res* 325:47–51. 627702

Dow Chemical Co. (1958) Results of 90-day dietary feeding of diphenylamine to rats, with cover letter dated 05/10/94 (sanitized). Dow Chemical Company, Midland, MI; 86-940000831S; OTS0557241. Available online at http://www.ntis.gov/search/product.aspx?ABBR=OTS0557241. 627589

Edwards, JA; Leeming, NM; Clark, R; et al. (1983) Effect of diphenylamine on pregnancy of the New Zealand white rabbit with cover letter dated 04/13/94. Huntingdon Research Centre, Huntingdon, Cambridgeshire, England; OTS0557206; 86-940000796. Available online at http://www.ntis.gov/search/product.aspx?ABBR=OTS0557206. As cited in European Communities, 2008. 627703

Eknoyan, G; Weinman, EJ; Tsaparas, A; et al. (1976) Renal function in experimental cystic disease of the rat. *J Lab Clin Med* 88(3):402–411. 627703

European Communities. (2008) European Union risk assessment report: Diphenylamine. Office for Official Publications of the European Communities, Luxembourg. 635677

Evan, A.P., Hong, S.H., Gardner, Jr. et al. (1978) Evolution of the collecting tubular lesion in diphenylamine-induced renal disease. *Lab Invest* 38(3):244–252. As cited in European Communities, 2008. 635677.

Ferretti, JJ; Lu, W; Liu, MB. (1977) Mutagenicity of benzidine and related compounds employed in the detection of hemoglobin. *Am J Clin Pathol* 67(6):526–527. 627705

Ford, W; Abraham, R; Rockwood, W; et al. (1972) Observations consequent upon a long-term study of diphenylamine in mice. *Toxicol Appl Pharmacol* 22:302. 627472

Griswold, DP Jr; Casey, AE; Weisburger, EK; et al. (1966) On the carcinogenicity of a single intragastric dose of hydrocarbons, nitrosamines, aromatic amines, dyes, coumarins, and miscellaneous chemicals in female Sprague-Dawley rats. *Can Res* 26(4):619–625. 627530

Gutenmann, WH; Lisk, DJ. (1975a,b) A feeding study with diphenylamine in a dairy cow. *Bull Environ Contam Toxicol* 13(2):177–180. 635797

Görsdorf, S; Engeholm, C; Appel, KE. (1988) Relevance of metabolites in genotoxicity of n-nitrosodiphenylamine. *Naunyn Schmiedebergs Arch Pharmacol* 338:R74. 635011

Holmberg, B; Kronebi, T; Ackevi, S; et al. (1983) [Carcinogenicity testing of diphenylamine and gamma-butyrolactone by oral administration to male mice]. *Arbete Och Haelsa* 34:1−35. (in Swedish). 633773

HSDB (Hazardous Substances Data Bank). (2010) *N*,*N*-Diphenylamine. National Library of Medicine, Bethesda, Maryland. Available online at http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB. Accessed on 8/30/2010. 644167

IARC (International Agency for Research on Cancer). (2010) IARC monographs on the evaluation of carcinogenic risks to humans. Available online at http://monographs.iarc.fr/ENG/Monographs/PDFs/index.php. Accessed on 6/17/2010. 597416

Krohmer, RW. (1992a) 90 Day evaluation of diphenylamine in the dog. T.P.S., Inc., Mt. Vernon, IN; unpublished study No. 426C-501-034-91. 627633

Krohmer, RW. (1992b) 90 Day subchronic toxicity evaluation of diphenylamine in rats. T.P.S., Inc., Mt. Vernon, IN; unpublished study No. 426C-10-034-91. 627630

Lenz, SD; Carlton, WW. (1990a,b,c,d) Diphenylamine-induced renal papillary necrosis and necrosis of the pars recta in laboratory rodents. *Vet Pathol* 27(3):171–178. 627533

Lenz, SD. (1996) Investigation of regional glutathione levels in a model of chemically-induced renal papillary necrosis. *Food Chem Toxicol* 34(5):489–494. 635794

Litton Bionetics. (1994) Mutagenicity evaluation of AP 1185 (diphenylamine) with cover letter dated 05/06/94. Litton Bionetics Inc., Kensington, MD. 86940000374. 627729

Lodovici, M; Casalini, C; Briani, C; et al. (1997) Oxidative liver DNA damage in rats treated with pesticide mixtures. *Toxicology* 117:55–60. 627710

Masubuchi, Y; Yamada, S; Horie, T. (2000) Possible mechanism of hepatocyte injury induced by diphenylamine and its structurally related nonsteroidal anti-inflammatory drugs. *J Pharmacol Exp Ther* 292(3):982–987. 627711

Mobil Oil Corporation. (1994a) An Ames *Salmonella/*mammalian microsome mutagenesis assay for determination of potential mutagenicity of diphenylamine with cover letter dated 05/03/94. Mobil Oil Corporation, Fairfax County, VA; 86940000874. Available online at http://www.ntis.gov/search/product.aspx?ABBR=OTS0557284. 635806

Mobil Oil Corporation. (1994b) A murine lymphoma mutagenesis assay, heterozygous at the thymidine kinase locus for the determination of the potential mutagenicity of diphenylamine w/cover letter dated 04/26/94. U.S. Environmental Protection Agency, Office of Toxic Substances, Washington, DC; EPA/OTS 86940000367; OTS0572470. 635017

Mobil Oil Corporation. (1994c) Micronucleus assay of bone marrow red blood cells from rats treated via dermal administration of diphenylamine with cover letter dated 04/26/94. Mobil Oil Corporation, Fairfax County, VA; 86-940000369; OTS0572472. Available online at http://www.ntis.gov/search/product.aspx?ABBR=OTS0572472. 627537

NIOSH (National Institute for Occupational Safety and Health). (2005) Diphenylamine. NIOSH Pocket Guide to Chemical Hazards. Available online at http://www.cdc.gov/niosh/npg/npgd0240.html. Accessed on 6/21/2010.

NTP (National Toxicology Program). (2005) 11th Report on carcinogens. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC. Available online at http://ntp-server.niehs.nih.gov/index.cfm?objectid= 32BA9724-F1F6-975E-7FCE50709CB4C932. Accessed on 6/21/2010. 093207

OSHA (Occupational Safety and Health Administration). (2010) Air contaminants: occupational safety and health standards for shipyard employment, subpart Z, toxic and hazardous substances. U.S. Department of Labor, Washington, DC. OSHA Standard 1915.1000. Available online at http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=102 86. Accessed on 6/21/2010. 625691

Parkes, HG; Veys, CA; Waterhouse, JA; et al. (1982) Cancer mortality in the British rubber industry. *Br J Ind Med* 39(3):209–220. 633840

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

Rodwell, DE. (1992) Teratology study in rats with diphenylamine (DPA). Springborn Laboratories, Inc., Life Sciences Division. Spencerville, OH; unpublished study No. 3255.3. 627640

Rodwell, DE. (1993) Two-generation reproduction study in rats with diphenylamine (DPA). Springborn Laboratories, Inc., Life Sciences Division, Spencerville, OH; unpublished study No. 3255.4. 627641

Thomas, JO; Cox, AJ Jr; DeEds, F. (1957) Kidney cysts produced by diphenylamine. *Stanford Med Bull* 15(2):90–93. 627539

Thomas, JO; Ribelin, WE; Woodward, JR; et al. (1967a). The chronic toxicity of diphenylamine for dogs. *Toxicol Appl Pharmacol* 11:184–194. 627543

Thomas, JO; Ribelin, WE; Wilson, RH; et al. (1967b,c) Chronic toxicity of diphenylamine to albino rats. *Toxicol Appl Pharmacol* 10:362–374. 627541

U.S. EPA (United States Environmental Protection Agency). (1985) Health and environmental effects profile for N,N-diphenylamine. U.S. Environmental Protection Agency, Environmental Criteria and Assessment Office, Cincinnati, OH; EPA/600/X-85/393; PB88-176060. Available online at http://www.ntis.gov/search/product.aspx?ABBR=PB88176060. 627435

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

U.S. EPA (Environmental Protection Agency). (1994a) Chemical Assessments and Related Activities (CARA). Office of Health and Environmental Assessment, Washington, DC. EPA/600/R-94/904. Available online at nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=60001G8L.txt. 596444

U.S. EPA (Environmental Protection Agency). (1994b) Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. U.S. Environmental Protection Agency, Office of Research and Development, Office of Health and Environmental Assessment, Washington, DC. EPA/600/8-90/066F. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=71993. 006488

U.S. EPA (Environmental Protection Agency). (2000) Benchmark dose technical guidance document [external review draft]. EPA/630/R-00/001. Available online at http://www.epa.gov/nceawww1/pdfs/bmds/BMD-External_10_13_2000.pdf.

U.S. EPA (Environmental Protection Agency). (2005) Guidelines for carcinogen risk assessment. Risk Assessment Forum, Washington, DC; EPA/630/P-03/001F. Federal Register 70(66):17765–17817. Available online at http://www.epa.gov/raf/publications/pdfs/CANCER_GUIDELINES_FINAL_3-25-05.PDF. Accessed on 6/17/2010. 635843

U.S. EPA (Environmental Protection Agency). (2009) 2009 edition of the drinking water standards and health advisories. Office of Water, Washington, DC; EPA/822/R-09/011. Available online at http://deq.state.wy.us/wqd/groundwater/downloads/dwstandards2009%5B1%5D.pdf. Accessed on 6/21/2010.

U.S. EPA (Environmental Protection Agency). (2010a) Diphenylamine. (CASRN 122-39-4). Integrated Risk Information System (IRIS). National Center for Environmental Assessment, Washington, DC. Available online at http://www.epa.gov/iris/subst/0048.htm. Last revised 4/1/1993. Accessed on 8/23/2010. 644093

U.S. EPA (Environmental Protection Agency). (2010b) Health effects assessment summary tables (HEAST). Office of Emergency and Remedial Response, Washington, DC. Available online at http://epa-heast.ornl.gov/. Accessed on 2/25/2010. 595422

von der Hude, W; Behm, C; Gürtler, R; et al. (1988) Evaluation of the SOS chromotest. *Mutat Res* 203(2):81–94. 627708

WHO (World Health Organization). (2010) Online catalogs for the Environmental Health Criteria Series. Lyon, France: WHO. Available online at http://www.who.int/ipcs/publications/ehc/en/. Accessed on 6/21/2010. 595424

Yoshida, J; Shimoji, N; Furuta, K; et al. (1989) [Twenty-eight day repeated dose toxicity testing of diphenylamine in F344 rats]. *Eisei Shikenjo Hokoku* 56–61. 627562

Zeiger, E; Anderson, B; Haworth, S; et al. (1988) Salmonella mutagenicity tests: IV. Results from the testing of 300 chemicals. *Environ Mol Mutag* 11(Supp 12):1–158. As cited in European Communities, 2008. 635677