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Provisional Peer-Reviewed Toxicity Values for

p-Phenylenediamine
(CASRN 106-50-3)

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

AUTHORS, CONTRIBUTORS, AND REVIEWERS

CHEMICAL MANAGER

Elizabeth Owens, PhD
National Center for Environmental Assessment, Cincinnati, OH

DRAFT DOCUMENT PREPARED BY

SRC, Inc.
7502 Round Pond Road
North Syracuse, NY 13212

PRIMARY INTERNAL REVIEWER

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

This document was externally peer reviewed under contract to:

Eastern Research Group, Inc.
110 Hartwell Avenue
Lexington, MA 02421-3136

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

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COMMONLY USED ABBREVIATIONS AND ACRONYMS

α2u-g	alpha 2u-globulin
ACGIH	American Conference of Governmental Industrial Hygienists
AIC	Akaike's information criterion
ALD	approximate lethal dosage
ALT	alanine aminotransferase
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
BW	body weight
CA	chromosomal aberration
CAS	Chemical Abstracts Service
CASRN	Chemical Abstracts Service Registry Number
CBI	covalent binding index
CHO	Chinese hamster ovary (cell line cells)
CL	confidence limit
CNS	central nervous system
CPN	chronic progressive nephropathy
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DEN	diethylnitrosamine
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FEV_1	forced expiratory volume of 1 second
GD	gestation day
GDH	glutamate dehydrogenase
GGT	γ-glutamyl transferase
GSH	glutathione
GST	glutathione-S-transferase
Hb/g-A	animal blood-gas partition coefficient
Hb/g-H	human blood-gas partition coefficient
HEC	human equivalent concentration
HED	human equivalent dose
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IVF	in vitro fertilization
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
MN	micronuclei
MNPCE	micronucleated polychromatic erythrocyte
MOA	mode of action
MTD	maximum tolerated dose
NAG	N-acetyl-β-D-glucosaminidase
NCEA	National Center for Environmental Assessment
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
NZW	New Zealand White (rabbit breed)
OCT	ornithine carbamoyl transferase
ORD	Office of Research and Development
PBPK	physiologically based pharmacokinetic
PCNA	proliferating cell nuclear antigen
PND	postnatal day
POD	point of departure
POD_{ADJ}	duration-adjusted POD
QSAR	quantitative structure-activity relationship
RBC	red blood cell
RDS	replicative DNA synthesis
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	regional gas dose ratio
RNA	ribonucleic acid
SAR	structure activity relationship
SCE	sister chromatid exchange
SD	standard deviation
SDH	sorbitol dehydrogenase
SE	standard error
SGOT	glutamic oxaloacetic transaminase, also known as AST
SGPT	glutamic pyruvic transaminase, also known as ALT
SSD	systemic scleroderma
TCA	trichloroacetic acid
TCE	trichloroethylene
TWA	time-weighted average
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_H	intraspecies uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
UF_D	database uncertainty factor
U.S.	United States of America
WBC	white blood cell

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR *p*-PHENYLENEDIAMINE (CASRN 106-50-3)

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 use 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 (http://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.

This document has been reviewed in accordance with U.S. EPA policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

QUESTIONS REGARDING PPRTVS

Questions regarding the content 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

p-Phenylenediamine, CASRN 106-50-3, is used as an intermediate in the manufacture of aramid (e.g., Kevlar) textile fibers and diisocyanates for polyurethanes (Smiley, 2000). It is also used in dye mixtures for hair, leather, and fur (U.S. EPA, 2014a). *p*-Phenylenediamine is listed as a hazardous air pollutant (HAP) under the Clean Air Act as amended in 1990. This chemical is also regulated under Section 8(d) of the Toxic Substances Control Act (TSCA) (40 CFR 716.120). All handlers of this material are required to submit copies and lists of unpublished health and safety studies to the EPA. The chemical was, but is no longer, subject to testing under Section 4 of TSCA (U.S. EPA, 2014b).

p-Phenylenediamine is a solid at room temperature. As a diamine with the pKa value of 6.2 for its conjugate acid, *p*-phenylenediamine is expected to exist partially as a cation in the environment. Thus, the chemical is not expected to volatilize from moist soil or water surfaces (HSDB, 2009). In addition, the estimated Henry's law constant for the neutral form of *p*-phenylenediamine indicates low propensity for volatilization from water surfaces. Furthermore, the moderate vapor pressure of *p*-phenylenediamine's neutral form indicates that evaporation from dry soil is also not expected. However, a moderate vapor pressure suggests that *p*-phenylenediamine, if released to the air, would remain in the vapor phase (HSDB, 2009). The ability of *p*-phenylenediamine to leach from soil to groundwater is dependent on local conditions. In areas with high amounts of organic matter, leaching of *p*-phenylenediamine may be inhibited due to the high reactivity of the aromatic amine groups (HSDB, 2009). In other areas, *p*-phenylenediamine deposited on soil is likely to leach to groundwater or undergo runoff after a rain event due to its moderate water solubility and relatively low soil adsorption coefficient. The empirical formula for *p*-phenylenediamine is $C_6H_8N_2$ (see Figure 1). Synonyms include 1,4-benzenediamine, 4-aminoaniline, *p*-aminoaniline, *p*-diaminobenzene, and 1,4-phenylenediamine. A table of physicochemical properties for *p*-phenylenediamine is provided below (see Table 1).

Figure 1. *p*-Phenylenediamine Structure

Table 1. Physicochemical Properties of *p*-Phenylenediamine (CASRN 106-50-3)

Property (unit)	Value
Physical state	White to slightly red crystals[a]
Boiling point (°C)	267[a]
Melting point (°C)	145−147[a]
Density (g/cm^3)	>1[a]
Vapor pressure (mm Hg at 25°C, extrapolated)	0.005[a]
pH (unitless)	9[b]
pKa (at 25°C)	6.2 for conjugate acid[a]
Solubility in water (g/L at 23°C)	37[c]
Octanol-water partition constant (log K_{ow})	−0.25[a]
Henry's law constant (atm-m^3/mol at 25°C)	6.73×10^{-10} (estimated)[d]
Soil adsorption coefficient K_{oc} (mL/g)	33.8 (estimated)[d]
Relative vapor density (air = 1)	3.72[a]
Molecular weight (g/mol)	108.14[a]

[a]HSDB (2009).
[b]Sigma-Aldrich (2014).
[c]ChemIDplus (2015).
[d]U.S. EPA (2012b).

A summary of available toxicity values for *p*-phenylenediamine from the EPA and other agencies/organizations is provided in Table 2.

Table 2. Summary of Available Toxicity Values for *p*-Phenylenediamine (CASRN 106-50-3)

Source (parameter)[a,b]	Value (applicability)	Notes	Reference
Noncancer			
IRIS	NV	NA	U.S. EPA (2016)
HEAST (RfD)	1.9×10^{-1} mg/kg-d	Based on 2-yr rat study (NCI, 1979); "whole-body effects" (body weight); NOAEL 18.7 mg/kg-d; UF 100	U.S. EPA (2011a)
DWSHA	NV	NA	U.S. EPA (2012a)
ATSDR	NV	NA	ATSDR (2016)
IPCS	NV	NA	IPCS (2016); WHO (2016)
Cal/EPA	NV	NA	Cal/EPA (2014); Cal/EPA (2016a); Cal/EPA (2016b)
OSHA (PEL)	TWA 0.1 mg/m^3	NA	OSHA (2006)
NIOSH (REL)	TWA 0.1 mg/m^3	NA	NIOSH (2015)
ACGIH (TLV)	TWA 0.1 mg/m^3	Based on upper respiratory tract irritation, skin sensitization	ACGIH (2001); ACGIH (2015)
Cancer			
IRIS	NV	NA	U.S. EPA (2016)
HEAST	NV	NA	U.S. EPA (2011a)
DWSHA	NV	NA	U.S. EPA (2012a)
NTP	NV	NA	NTP (2014)
IARC (WOE)	Group 3—not classifiable as to its carcinogenicity to humans	Based on lack of data in humans and inadequate evidence in animals	IARC (1978); IARC (1987)
Cal/EPA	NV	NA	Cal/EPA (2011); Cal/EPA (2016a); Cal/EPA (2016b)
ACGIH (WOE)	Category A4—not classifiable as a human carcinogen	The results of nearly all of the numerous animal bioassays for carcinogenicity were negative	ACGIH (2001); ACGIH (2015)

[a]Sources: ACGIH = American Conference of Governmental Industrial Hygienists; ATSDR = Agency for Toxic Substances and Disease Registry; Cal/EPA = California Environmental Protection Agency; DWSHA = Drinking Water Standards and Health Advisories; HEAST = Health Effects Assessment Summary Tables; IARC = International Agency for Research on Cancer; IPCS = International Programme on Chemical Safety; IRIS = Integrated Risk Information System; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration.
[b]Parameters: PEL = permissible exposure level; REL = recommended exposure level; RfD = oral reference dose; TLV = threshold limit value; WOE = weight of evidence.

NA = not applicable; NOAEL = no-observed-adverse-effect level; NV = not available; TWA = time-weighted average; UF = uncertainty factor.

Literature searches were conducted in August 2013 and in March 2016 for studies published from 1900 that are relevant to the derivation of provisional toxicity values for *p*-phenylenediamine. Searches were conducted using U.S. EPA's Health and Environmental Research Online (HERO) database of scientific literature. The following databases were searched: PubMed, TOXLINE (including TSCATS1), and Web of Science. The following databases were searched outside of HERO for health-related values: ACGIH, ATSDR, Cal/EPA, U.S. EPA IRIS, U.S. EPA HEAST, U.S. EPA Office of Water (OW), U.S. EPA TSCATS2/TSCATS8e, NIOSH, NTP, and OSHA.

REVIEW OF POTENTIALLY RELEVANT DATA (NONCANCER AND CANCER)

Tables 3A and 3B provide overviews of the relevant noncancer and cancer databases for *p*-phenylenediamine and include all potentially relevant repeat-dose, short-term-, subchronic-, and chronic-duration studies, as well as reproductive and developmental toxicity studies. The principal study is identified in bold font. The phrase "statistical significance and "significant," used throughout the document, indicates a p-value of < 0.05 unless otherwise noted.

Table 3A. Summary of Potentially Relevant Noncancer Data for *p*-Phenylenediamine (CASRN 106-50-3)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[b]	BMDL/ BMCL[b]	LOAEL[b]	Reference (comments)	Notes[c]
Human								
1. Oral (mg/kg-d)								
ND								
2. Inhalation (mg/m^3)[a]								
Exposure duration cannot be determined	12 workers, evaluation of medical records of operators and operating supervisors of a phenylenediamine manufacturing plant for ≥10 yr (unknown composition of phenylenediamines)	NDr	Skin irritation, no adverse effects on blood oxygen saturation or Hb levels	ND	NDr	ND	DuPont (1984) (Study to evaluate potential for methemoglobinemia, not a comprehensive evaluation)	NPR
Animal								
1. Oral (mg/kg-d)[a]								
Short-term	10 M/10 F, Crl:CD(SD)BR rat, *p*-phenylenediamine in water by daily gavage for 14 d	0, 5, 10, 20, 40 ADD: 0, 5, 10, 20, 40	Increased LDH (≥5 mg/kg-d), increased serum ALT, AST, and CPK (≥10 mg/kg-d), increased thyroid weights (≥10 mg/kg-d), minimal myodegeneration (40 mg/kg-d), and increased (>10%) absolute and relative liver weight (40 mg/kg-d)	5	NDr	10	Toxicol Laboratories (1993)	NPR

Table 3A. Summary of Potentially Relevant Noncancer Data for *p*-Phenylenediamine (CASRN 106-50-3)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[b]	BMDL/ BMCL[b]	LOAEL[b]	Reference (comments)	Notes[c]
Subchronic	**15 M/15 F, Crl:CD(SD)BR rat, *p*-phenylenediamine in water administered by daily gavage for 13 wk**	**0, 2, 4, 8, 16** **ADD: 0, 2, 4, 8, 16**	**Increased absolute (16% at 16 mg/kg-d) and relative kidney weight (12% at 8 mg/kg-d) (females). Increased absolute (12% at 16 mg/kg-d) and relative liver weight (11% at 8 mg/kg-d) (females)**	**4**	**4 (relative liver weight)**	**8**	**Toxicol Laboratories (1995)**	**PS, NPR**
Subchronic	12 M/12 F, Crl:CD®BR rat, neurotoxicity study of *p*-phenylenediamine in water administered by daily gavage for 90 consecutive d	0, 4, 8, 16 ADD: 0, 4, 8, 16	Increased incidence of wet chins (males and females) and wet perineum or inguen (females)	8	NDr	16	Dupont Chem (1992) (Evaluations limited to body weights, food consumption, clinical signs, neuropathology, ophthalmological examinations, and neurobehavioral tests [motor activity and functional observational battery])	NPR
Subchronic	10−11 M/10−11 F, F344 rat, dose range-finding study of *p*-phenylenediamine in diet for 12 wk	0, 0.05, 0.1, 0.2, 0.4% ADD: 0, 50.0, 100, 200, 400 (M); 0, 56.8, 114, 227, 455 (F)	Body-weight decrement of ≥10% relative to controls. At the FEL, terminal body weights were 53−58% lower than controls and 9/11 males and 1/10 females died	100 (M); 56.8 (F)	88 (F)	200 (M); 114 (F) FEL: 400 (M); FEL: 455 (F)	Imaida et al. (1983) (Evaluations limited to body weights, liver and kidney weights, and histopathology)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for *p*-Phenylenediamine (CASRN 106-50-3)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[b]	BMDL/ BMCL[b]	LOAEL[b]	Reference (comments)	Notes[c]
Subchronic	5 M/5 F, F344 rat, dose range-finding study of *p*-phenylenediamine dihydrochloride in the diet for 7 wk, followed by a 1-wk observation period	0, 68, 100, 147, 215, 316, 464, 681, 1,000, 1,470, 2,150, 3,160 ppm as *p*-phenylenediamine dihydrochloride ADD: 0, 4.1, 5.97, 8.78, 12.8, 18.9, 27.7, 40.7, 59.7, 87.79, 128.4, 188.7 (M); 0, 4.6, 6.79, 9.98, 14.6, 21.5, 31.5, 46.2, 67.9, 99.82, 146.0, 214.6 (F) as *p*-phenylenediamine	Body-weight gain decreased by 10−13% at 60−66 mg/kg-d in both sexes, however terminal body weight and dietary intake data were not reported which limits interpretation of the significance of decrements in body-weight "gain"	NDr	NDr	NDr	NCI (1979) (Evaluations limited to survival, clinical signs of toxicity, and body-weight gain)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for *p*-Phenylenediamine (CASRN 106-50-3)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[b]	BMDL/ BMCL[b]	LOAEL[b]	Reference (comments)	Notes[c]
Subchronic	5 M/5 F, B6C3F$_1$ mouse, dose range-finding study of *p*-phenylenediamine dihydrochloride in the diet for 7 wk, followed by a 1-wk observation period	0, 100, 147, 215, 316, 464, 681, 1,000, 1,470, 2,150, 3,160, 4,640 ppm as *p*-phenylenediamine dihydrochloride ADD: 0, 10.8, 15.9, 23.2, 34.1, 50.1, 73.5, 108, 158.6, 232.0, 341.0, 500.7 (M); 0, 11.7, 17.3, 25.3, 37.1, 54.5, 80.0, 117, 172.7, 252.6, 371.3, 545.2 (F) as *p*-phenylenediamine	Body-weight gain decreased by 17% at 158.6 mg/kg-d in males and 13% at 252.6 mg/kg-d in females, however terminal body weight and dietary intake data were not reported which limits interpretation of the significance of decrements in body-weight "gain"	NDr	NDr	NDr	NCI (1979) (Evaluations limited to survival, clinical signs of toxicity, and body-weight gain)	PR
Chronic	63−66 M/63−66 F (exposed), 24−25 M/24−25 F (control), F344 rat, *p*-phenylenediamine in the diet for 80 wk	0, 0.05, 0.1% ADD: 0, 38.8, 77.6 (M); 0, 46.1, 92.1 (F)	Increased absolute kidney weight (23% greater than control), absolute liver weight (11% greater than control), spleen weight in females, decreased body weight in males (21% lower than control)	46.1	NDr	92.1 (F)	Imaida et al. (1983) (Effect level is uncertain due to small numbers of control animals that survived to 80 wk)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for *p*-Phenylenediamine (CASRN 106-50-3)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[b]	BMDL/ BMCL[b]	LOAEL[b]	Reference (comments)	Notes[c]
Chronic	50 M/50 F (exposed), 20 M/20 F (control), F344 rat, *p*-phenylenediamine dihydrochloride in the diet for 103 wk	0, 625, 1,250 ppm as *p*-phenylenediamine dihydrochloride ADD: 0, 29.0, 58.0 (M); 0, 34.4, 68.78 (F) as *p*-phenylenediamine	Body-weight decrement of 10% relative to controls in females, based on visual inspection of data presented graphically	34.4	NDr	68.78 (F)	NCI (1979) (Not a comprehensive evaluation of endpoints; limited to mortality, clinical signs, body weight, and gross and microscopic tissue evaluations)	PR
Chronic	50 M/50 F (exposed), 20 M/20 F (control), B6C3F$_1$ mouse, *p*-phenylenediamine dihydrochloride in the diet for 103 wk	0, 625, 1,250 ppm as *p*-phenylenediamine dihydrochloride ADD: 0, 63.7, 127.5 (M); 0, 64.9, 129.8 (F) as *p*-phenylenediamine	None	129.8	NDr	ND	NCI (1979) (Not a comprehensive evaluation of endpoints; limited to mortality, clinical signs, body weight, and gross and microscopic tissue evaluations)	PR
Developmental	0 M/25 F, Crl:OFA(SD) rat, teratogenicity study of *p*-phenylenediamine in water administered by gavage on GDs 6−19	0, 5, 10, 20 ADD: 0, 5, 10, 20	Reduced maternal body-weight gain on GDs 6−9 at 10 and 20 mg/kg-d (maternal), and a decrease in fetal body weight at 20 mg/kg-d (fetal) [Effect levels were reported in ECHA (2005) as a NOEL for maternal effects and NOAEL for fetal effects]	5 (maternal); 10 (fetal)	NDr	10 (maternal); 20 (fetal)	ECHA (2005) (Summary information as reported in a secondary source only; primary report was not available)	NPR

Table 3A. Summary of Potentially Relevant Noncancer Data for *p*-Phenylenediamine (CASRN 106-50-3)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[b]	BMDL/ BMCL[b]	LOAEL[b]	Reference (comments)	Notes[c]
Developmental	0 M/25 F, S-D rat, teratogenicity study of *p*-phenylenediamine in water administered by gavage on GDs 6−15	0, 5, 10, 15, 20, 30 ADD: 0, 5, 10, 15, 20, 30	Reduced maternal body-weight gain on GDs 0−15. 3/25 exposed dams died at 30 mg/kg-d. No fetal effects	15 (maternal); 30 (fetal)	NDr	20 (maternal); ND (fetal)	Re et al. (1981)	PR
Developmental	0 M/22 F (exposed), 0 M/26 F (control), NMRI mouse, transplacental carcinogenicity study, exposure of mothers by gavage in soy bean oil on GDs 10−19, F1 generation sacrificed 27 and 51 wk after study start, dams sacrificed 51 wk after study start	0, 30 ADD: 0, 30	No effects observed in dams or F1 offspring	30 (maternal); 30 (fetal)	NDr	ND	Holmberg et al. (1983) as translated in DuPont (1992) (Summary information as reported in DuPont translation only; EPA translation of primary report was not available)	NPR

Table 3A. Summary of Potentially Relevant Noncancer Data for *p*-Phenylenediamine (CASRN 106-50-3)								
Category[a]	**Number of Male/Female, Strain, Species, Study Type, Study Duration**	**Dosimetry[a]**	**Critical Effects**	**NOAEL[b]**	**BMDL/ BMCL[b]**	**LOAEL[b]**	**Reference (comments)**	**Notes[c]**
2. Inhalation (mg/m^3)[a]								
ND								

[a]Category (treatment/exposure duration: unless otherwise noted): short-term = repeated exposure for >24 hours ≤30 days (U.S. EPA, 2002); long-term (subchronic) = repeated exposure for >30 days ≤10% lifespan for humans (more than 30 days up to approximately 90 days in typically used laboratory animal species) (U.S. EPA, 2002); chronic = repeated exposure for >10% lifespan for humans (more than approximately 90 days to 2 years in typically used laboratory animal species) (U.S. EPA, 2002).
[b]Dosimetry: Values are presented as adjusted daily dose (ADD, in mg/kg-day) for oral noncancer effects and as human equivalent concentration (HEC, in mg/m^3) for inhalation noncancer effects. Where applicable, the dose of *p*-phenylenediamine was calculated from the dose of *p*-phenylenediamine dihydrochloride by multiplying by the ratio of the molecular weights of the two compounds (108.14 g/mol:181.08 g/mol).
[c]Notes: NPR = not peer reviewed; PR = peer reviewed; PS = principal study.

ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMCL = benchmark concentration lower confidence limit; BMDL = benchmark dose lower confidence limit; CPK = creatine phosphokinase; F = female(s); FEL = frank effect level; GD = gestation day; Hb = hemoglobin; LDH = lactate dehydrogenase; LOAEL = lowest-observed-adverse-effect level; M = male(s); ND = no data; NDr = not determined; NOAEL = no-observed-adverse-effect level; S-D = Sprague-Dawley; SD = standard deviation.

Table 3B. Summary of Potentially Relevant Cancer Data for *p*-Phenylenediamine (CASRN 106-50-3)						
Category	**Number of Male/Female, Strain, Species, Study Type, Study Duration**	**Dosimetry[a]**	**Critical Effects**	**BMDL/ BMCL[a]**	**Reference (comments)**	**Notes[b]**
Human						
1. Oral (mg/kg-d)						
ND						
2. Inhalation (mg/m^3)						
ND						
Animal						
1. Oral (mg/kg-d)						
Carcinogenicity	63−66 M/63−66 F (exposed), 24−25 M/24−25 F (control), F344 rat, *p*-phenylenediamine in the diet for 80 wk	0, 0.05, 0.1% HED: 0, 9, 19 (M); 0, 11, 22 (F)	No effects observed	NDr	Imaida et al. (1983) (This study is limited by poor reporting, inadequate numbers of control animals, survival of only 1 M and 6 F from the control group to 80 wk, and failure to achieve the MTD in males)	PR
Carcinogenicity	50 M/50 F (exposed), 20 M/20 F (control), F344 rat, *p*-phenylenediamine dihydrochloride in the diet for 103 wk	0, 625, 1,250 ppm as *p*-phenylenediamine dihydrochloride HED: 0, 7, 14 (M); 0, 8, 17 (F) as *p*-phenylenediamine	No effects observed	NDr	NCI (1979) (This study is limited by the small numbers of control animals and failure to achieve the MTD in males)	PR

Table 3B. Summary of Potentially Relevant Cancer Data for *p*-Phenylenediamine (CASRN 106-50-3)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	BMDL/ BMCL[a]	Reference (comments)	Notes[b]
Carcinogenicity	50 M/50 F (exposed), 20 M/20 F (control), B6C3F$_1$ mouse, *p*-phenylenediamine dihydrochloride in the diet for 103 wk	0, 625, 1,250 ppm as *p*-phenylenediamine dihydrochloride HED: 0, 9, 18 (M); 0, 9, 18 (F) as *p*-phenylenediamine	No effects observed	NDr	NCI (1979) (This study is limited by the small numbers of control animals and failure to achieve the MTD in either sex)	PR
Transplacental carcinogenicity	0 M/22 F (exposed), 0 M/26 F (control), NMRI mouse, transplacental carcinogenicity study, exposure of mothers by gavage on GDs 10−19, F1 generation sacrificed 27 and 51 wk after study start, dams sacrificed 51 wk after study start	0, 30 HED: 0, 4.2	No effects observed in dams or F1 offspring	NDr	Holmberg et al. (1983) as translated in DuPont (1992) (Summary information as reported in DuPont translation only; EPA translation of primary report was not available)	NPR
2. Inhalation (mg/m^3)						
ND						

[a]Dosimetry: The units for oral exposures are expressed as human equivalent dose (HED) (mg/kg-day). HED = animal dose as ADD (mg/kg-day) × default dosimetric adjustment factor (DAF) calculated as $(BW_a \div BW_h)^{1/4}$ [0.24 for rats and 0.14 for mice, U.S. EPA (2011b)]. Where applicable, the dose of *p*-phenylenediamine was calculated from the dose of *p*-phenylenediamine dihydrochloride by multiplying by the ratio of the molecular weights of the two compounds (108.14 g/mol:181.08 g/mol).
[b]Notes: NPR = not peer reviewed; PR = peer reviewed.

ADD = adjusted daily dose; BMCL = benchmark concentration lower confidence limit; BMDL = benchmark dose lower confidence limit; F = female; GD = gestation day; HED = human equivalent dose; M = male; MTD = maximum tolerated dose; ND = no data; NDr = not determined.

HUMAN STUDIES

Oral Exposures

Many case reports of human poisoning and deaths from consumption of *p*-phenylenediamine have been published. *p*-Phenylenediamine is widely used in hair dyes and henna-based skin dyes in Africa and the Indian subcontinent, and is available as a pure compound for these uses (Chaudhary et al., 2013). Its wide availability and known toxicity have made this compound a popular choice for suicide attempts, and the vast majority of poisonings reported have been from oral consumption with suicidal intent. In all but a few instances, the dose of *p*-phenylenediamine taken is unknown and not estimated.

As described in a large number of case reports (Ryoo et al., 2014; Chaudhary et al., 2013; Kumar and Patil, 2013; Gude et al., 2012; Kumar et al., 2012; Prabhakaran, 2012; Reddy et al., 2012; Abdelraheem et al., 2011; Daga et al., 2011; Jain et al., 2011; Shalaby et al., 2010; Soni et al., 2009; Mohamed et al., 2007; Kallell et al., 2005; Ashar, 2003; Shemesh et al., 1995; Ashraf et al., 1994; Averbukh et al., 1989; Baud et al., 1983; El-Ansary et al., 1983; Suliman et al., 1983; Chugh et al., 1982), the clinical presentation of acute *p*-phenylenediamine poisoning is quite consistent. Depending on the dose and time elapsed since exposure, patients may initially present with vomiting as well as dyspnea (labored breathing), stridor (grating sound), dysphasia (inability to speak or understand words), and dysphagia (difficulty in swallowing) resulting from cervicofacial/oropharyngeal edema (frequently requiring tracheostomy). Patients who survive the acute respiratory phase (typically the first 4−6 hours after exposure) may later (~12 hours after exposure) develop rhabdomyolysis (breakdown of skeletal muscle), intravascular hemolysis, and acute renal tubular necrosis/acute renal failure (Chaudhary et al., 2013), with symptoms of trismus (lockjaw), pain and stiffness in the lower limbs, dark brown or black urine (due to myoglobinuria), oliguria, or anuria. In addition, some reports of cardiotoxicity (ranging from asymptomatic myocarditis to ST segment depression to sudden cardiac death) in poisoning cases have been published (Gude et al., 2012; Jain et al., 2011; Singh et al., 2009; Soni et al., 2009; Singh et al., 2008; Brahmi et al., 2006; Ashraf et al., 1994). Fatalities generally stem from angioneurotic edema or cardiotoxicity (Chaudhary et al., 2013). One study reported the development of exophthalmia followed by permanent blindness associated with optic neuritis and atrophy in a poisoning victim (Yagi et al., 1996). Clinical chemistry findings in poisoning victims characteristically include markedly elevated creatine phosphokinase (CPK) and lactate dehydrogenase (LDH), as well as myoglobinuria (all indicative of rhabdomyolysis) and evidence of renal injury and metabolic acidosis (hyperkalemia, hyperphosphatemia, hypocalcemia, albuminuria, elevated blood urea nitrogen [BUN], and serum creatinine). Elevated serum liver enzymes (aspartate aminotransferase [AST], alanine aminotransferase [ALT]) have also been reported (Chaudhary et al., 2013; Abdelraheem et al., 2011). A case study of a suicide attempt in a young pregnant woman reported the spontaneous abortion of the 23-week-old fetus; the fetal autopsy revealed myocardial lysis (Abidi et al., 2008).

Information on the lethal dose of this compound is limited; in one fatal case of a 22-year-old male, the dose consumed was estimated to be ~20 g (Anuradha et al., 2004), but some authors suggest that the lethal dose may be in the range of 7−10 g (Chaudhary et al., 2013). In those case reports that provided an estimate of the quantity of material ingested, dye volumes ranging from 40−200 mL were consumed (Gandhe et al., 2009; Sahay et al., 2009; Soni et al., 2009; Chugh et al., 1982). Gandhe et al. (2009) reported the nonfatal case of a 19-year-old female who consumed 50 mL hair dye containing around 2 g *p*-phenylenediamine. In a case series reported by Soni et al. (2009), poisoning victims consumed 50−200 mL hair dye; those

who ingested >100 mL died, as did some of the patients who consumed 100 mL. Based on the estimate of 2 g *p*-phenylenediamine in 50 mL hair dye in Gandhe et al. (2009), the lethal quantities (≥100 mL) reported by Soni et al. (2009) may have corresponded to doses as low as 4 g *p*-phenylenediamine. Assuming a body weight of 70 kg and intakes of 4−20 g, the minimum lethal dose of *p*-phenylenediamine in humans may lie roughly in the range of 60−300 mg/kg.

Inhalation Exposures

DuPont (1984)

DuPont (1984) evaluated the frequency of methemoglobinemia in employees of a phenylenediamine manufacturing plant by reviewing employee medical records. However, the study did not identify which phenylenediamine isomer(s) the workers were exposed to. Employees in the plant provided blood samples every 6 months or whenever exposure exceeded the company-established acceptable exposure level of 0.1 mg/m^3. Records of all operators and operating supervisors working with phenylenediamine for ≥10 years were reviewed, and hemoglobin (Hb) and oxygen levels were reviewed. Neither Hb nor oxygen saturation levels among exposed employees differed from reported normal levels. Hb levels among employees averaged 15.6 g/dL, compared with a normal range of 14−17.2 g/dL; oxygen saturation averaged 93.9%, compared with normal levels ≥92.0%. The study authors reported that the medical records showed 27 cases of skin irritation associated with phenylenediamine exposure between 1975 and 1982, but that none of the affected workers exhibited blood oxygen saturation <90%.

Other Exposures

Reviewed by de Groot (2013)

p-Phenylenediamine has long been known to be a potent skin sensitizing agent, causing allergic contact dermatitis in susceptible people [reviewed by de Groot (2013)]. The literature database on sensitization in humans is extensive. However, this literature was not considered in this assessment because dermal exposure has little relevance to the derivation of oral and inhalation toxicity values.

Epidemiology studies of the association between human exposure to *p*-phenylenediamine and endpoints unrelated to allergic responses are few, and none have provided quantitative estimates of exposure. While the route of exposure is not discussed in these studies, dermal (and possibly inhalation) exposure is likely the predominant route in these populations. In addition, most studies were of hairdressers or users of hair dye, who may have had coexposure to a number of xenobiotics.

Hamdouk et al. (2011); Brown et al. (1987)

Consistent with the case reports of oral poisonings described above, there is evidence of renal toxicity in humans from chronic exposure to *p*-phenylenediamine in an occupational study (Hamdouk et al., 2011) and in a report of two cases deriving from long-term use of hair dyes (Brown et al., 1987). A cross-sectional study of renal function in Sudanese hairdressers with regular exposure (median duration of 6 years) to *p*-phenylenediamine was reported by Hamdouk et al. (2011). Seventy-two hairdressers from six salons in Khartoum, Sudan participated in the study. Each subject was interviewed about symptoms and exposures and given a physical examination; the subjects also gave blood and urine samples. Renal biopsies, as clinically warranted, were obtained from eight subjects. The study authors did not estimate doses of *p*-phenylenediamine subjects received. The prevalence of several clinically important findings were reported, including (in descending order of prevalence) black colored urine (40.3% of

participants), irritant contact dermatitis (38.9%), nail changes (31.9%), Hb below 10 g/dL (28.8%), albuminuria (26.4%), bronchitis (22%), hypertension (19.4%), hematuria (14.1%), and ocular conditions (11.1%.). The study authors used logistic regression analysis to estimate odds ratios (ORs) for use of pure forms of *p*-phenylenediamine (97% pure vs. 10% *p*-phenylenediamine in a manufactured dye preparation), duration of *p*-phenylenediamine exposure, and age. Statistically significant ORs for increased serum creatinine (defined as ≥2 mg/dL; OR 5.9 for use of pure compound; OR 1.3 for duration of exposure), proteinuria (OR 9.8 for use of pure compound; OR 1.4 for duration of exposure), and hematuria (defined as >5 erythrocytes/high-power field; OR 1.1 for duration of exposure, not significant for use of pure compound) were observed. Brown et al. (1987) reported two cases of chronic renal failure in women (51- and 62-years-old) who had habitually used hair dyes containing *p*-phenylenediamine. There were no qualitative or quantitative estimates of the dose of *p*-phenylenediamine in either case, nor was there a thorough assessment of other possible etiology of the kidney disease.

Rylander and Källén (2005)

Rylander and Källén (2005) observed an increased OR for intrauterine growth retardation in a large study of reproductive outcomes in female Swedish hairdressers who gave birth between 1983 and 2001. A total of 12,064 infants born to 8,384 women were included in the study, and compared with all other births during the study period (775,840 births to mothers working full time and 500,222 births to mothers working part time). However, no specific or quantitative information on the hairdressers' exposures was provided, so the relevance to *p*-phenylenediamine toxicity is uncertain.

Ros et al. (2012); Tavani et al. (2005)

Only two studies (Ros et al., 2012; Tavani et al., 2005) assessing the potential association between human exposure to *p*-phenylenediamine by any exposure route and cancers were located in the available literature; neither verified exposure to *p*-phenylenediamine in the study populations and neither observed a significant association between hair dye use and cancer. A case-control study comparing hair dye use in 246 women with bladder cancer (diagnosed between 1975 and 2009) with that of 2,587 control women matched on age and from the same region of the Netherlands showed that the women in these cases were no more likely to have used temporary (OR = 0.77, 95% confidence interval [CI] = 0.58, 1.02) or permanent (OR = 0.87, 95% CI = 0.65, 1.18) hair dyes than the control women (Ros et al., 2012). Stratification by duration, frequency, color, or extent of use did not alter the findings, nor did stratification by age, educational level, or smoking status. No information on the compositions of hair dyes used by participants in the study was provided. In a hospital-based case-control study, Tavani et al. (2005) compared exposure to hair dyes in patients with lymphoid neoplasms or soft tissue sarcomas with exposure among control patients hospitalized for acute non-neoplastic conditions. Cases included: 158 (91 men and 67 women) with Hodgkin's disease, 446 (256 men and 190 women) with non-Hodgkin lymphoma, 141 (70 men and 71 women) with multiple myeloma, and 221 (117 men and 104 women) with soft tissue sarcoma. A total of 1,295 (791 men and 504 women) subjects served as controls. There was no significant increase in the OR (all CIs included unity) for hair dye use among any of the conditions (ORs were 0.68, 1.03, 1.17, and 0.73 for Hodgkin's disease, non-Hodgkin lymphoma, multiple myeloma, and soft tissue sarcoma, respectively). Stratification by dye type (permanent or semipermanent) or sex did not alter the conclusions.

ANIMAL STUDIES

Oral Exposures

The effects of oral exposure of animals to *p*-phenylenediamine were evaluated in one short-term-duration study (Toxicol Laboratories, 1993), four subchronic-duration studies (Toxicol Laboratories, 1995; Dupont Chem, 1992; Imaida et al., 1983; NCI, 1979), two chronic-duration carcinogenicity studies (Imaida et al., 1983; NCI, 1979), and three developmental studies [ECHA, 2005; Holmberg et al. (1983) as translated in DuPont (1992); Re et al., 1981].

Short-Term-Duration Studies

Toxicol Laboratories (1993)

A 14-day gavage study of *p*-phenylenediamine in adult rats was performed by Toxicol Laboratories (1993) to establish doses for use in a 13-week oral toxicity study [see Toxicol Laboratories (1995)]. The study was performed according to Organisation for Economic Cooperation and Development (OECD) Guideline 408. Groups of Crl:CD(SD)BR rats (10/sex/dose) were given *p*-phenylenediamine (purity not reported) by gavage dissolved in deionized, boiled water at doses of 0, 5, 10, 20, or 40 mg/kg-day once daily for 14 days. Survival, clinical signs, body weight, food consumption, hematology, blood chemistry, organ weights (adrenals, kidneys, ovaries, thyroids, heart, liver, testes, thymus), and gross and microscopic pathology were evaluated. Histopathology was conducted on several organs and tissues (adrenals, diaphragm, duodenum, heart, kidneys, liver, skeletal muscle, ovaries, pancreas, pituitary, spleen, stomach, testes, thymus, thyroids, tongue).

No treatment-related effects on survival, food consumption, hematology, or gross necropsy were noted. No clinical signs of toxicity were observed. Males treated with ≥10 mg/kg-day gained less body weight (up to 10%) compared to controls; however, terminal body weight was comparable in all male rats (treated within 4% of controls). Females treated with 40 mg/kg-day gained less body weight (29%) compared to controls; however, this reduction was considered by the study authors to not be toxicologically relevant as it was driven by low terminal body weights of five of the animals as a result of overnight food deprivation. Increases in serum ALT, AST, and CPK were observed at doses ≥10 mg/kg-day, and LDH was increased at all doses (i.e., ≥5 mg/kg-day) (see Table B-1). Potassium levels were increased in females treated with ≥10 mg/kg-day and males treated with 40 mg/kg-day (see Table B-1).

Mean absolute and relative liver weights were increased 11 and 16%, respectively, in males treated with 40 mg/kg-day. Relative and absolute thyroid weights were statistically significantly increased in females at ≥20 mg/kg-day. Mean relative heart weights were raised in all treated male groups (see Table B-2). Microscopic lesions of the skeletal muscle (minimal myodegeneration) were noted in three female rats exposed to 40 mg/kg-day; no other histopathology findings were noted (see Table B-1). A lowest-observed-adverse-effect level (LOAEL) of 10 mg/kg-day is identified for increases in serum ALT, AST, and CPK. A no-observed-adverse-effect level (NOAEL) of 5 mg/kg-day is identified.

Subchronic-Duration Studies

Toxicol Laboratories (1995): 13 week study

Toxicol Laboratories (1995) performed a 13-week gavage study of *p*-phenylenediamine in adult rats according to OECD Guideline 408. Crl:CD(SD)BR rats (15/sex/dose) were given 0, 2, 4, 8, or 16 mg/kg-day *p*-phenylenediamine (purity not reported, in deionized, boiled water) by

daily gavage for 13 weeks. The dosing formulations were prepared daily and the test material was analyzed by high performance liquid chromatography (HPLC) once per week throughout the study to verify the dose estimates. Evaluations during the exposure period included twice daily viability checks, daily observations for clinical signs, weekly measurements of body weight and food intake, and ophthalmoscopy before treatment and during the last week of exposure. During Weeks 4 and 13, fasting blood samples were collected for hematology and clinical chemistry, and urine was collected for urinalysis during Weeks 4 and 12. Nonfasting blood samples from Week 13 were also assessed for clotting factors (i.e., prothrombin time, activated partial thromboplastin time, fibrinogen). All rats were sacrificed at the end of exposure and examined grossly. The following organs were weighed: adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes, thyroid, thymus, and pituitary. A complete set of tissues in the control and high-dose animals and all gross lesions and lungs from all animals were examined microscopically.

There were no premature deaths, clinical signs of toxicity, treatment-related body-weight changes, or ophthalmology findings throughout the treatment period. Males exposed to 8 mg/kg-day gained 8% less weight than controls, but body-weight gain of high-dose males was not altered, so this finding is not likely to be related to treatment. In addition, observed changes in serum chemistry (fluctuations in blood glucose and LDH levels, most of which were within reference ranges or highly variable) were not treatment related, and there were no significant urinalysis findings. Hematology findings were limited to decreased white blood cell (WBC) counts in some of the treated groups and red blood cell (RBC) counts in high-dose females during Week 4 and in high-dose animals of both sexes during Week 13. However, the study authors noted that the mean values were within normal reference ranges and did not consider the hematology changes to be related to treatment.

Absolute and relative liver weight in males exposed to 16 mg/kg-day were statistically significantly increased by 12% (see Table B-3). Absolute liver weight in females exposed to 16 mg/kg-day was also increased by 12%. Relative liver weight in females exposed to 8 mg/kg-day was biologically significantly increased by 11% (see Table B-3). In females, absolute and relative kidney weights were statistically significantly increased at ≥8 mg/kg-day. Absolute kidney weight increased by 8 and 16% at 8 and 16 mg/kg-day, respectively. Relative kidney weight increased by 12 and 14% at 8 and 16 mg/kg-day, respectively. In addition, absolute and relative thyroid weights were statistically significantly increased (compared with controls) in male rats of all exposure groups. However, the study authors noted that the thyroid weights of the controls were unusually low; therefore, they did not consider thyroid weight increases to be an effect of *p*-phenylenediamine exposure. There was no change in thyroid weight in female rats. There were no treatment-related increases in the incidence of gross or microscopic histopathology findings in any exposure group. Minimal myodegeneration of the skeletal muscle was observed in one male and one female high-dose rat and one male and one female control rat. EPA assigns a LOAEL of 8 mg/kg-day based on >10% increase in relative kidney weight and relative liver weight in female rats. The NOAEL is 4 mg/kg-day.

Dupont Chem (1992)

A subchronic-duration neurotoxicity study of *p*-phenylenediamine (≥98% pure, in water) administered by gavage to adult Crl:CD®BR rats was conducted by Dupont Chem (1992). The unpublished study was conducted to meet an EPA test requirement. Groups of 12 rats/sex/dose were given daily gavage doses of 0, 4, 8, or 16 mg/kg-day for 90 consecutive days. The test

material was prepared daily. Rats were examined daily for mortality, appearance, and behavior. Body weights were recorded twice weekly for 4 weeks and weekly for the remaining 8 weeks; food consumption was measured weekly. All rats received ophthalmological examinations before the beginning of the study as well as prior to sacrifice. Neurotoxicity evaluations (including motor activity and functional observational battery [FOB] assessments, as well as forelimb and hindlimb grip strength and foot splay measurements) were performed prior to the first dose and again during Weeks 4, 8, and 13. At terminal sacrifice, the following tissues were removed from control and high-dose rats for histology examination: sciatic nerve, forebrain, cerebrum, midbrain, cerebellum, pons, medulla oblongata, spinal cord, tibial nerve and gasserian ganglia, and gastrocnemius muscle.

Analysis of the test material indicated that the administered doses were within 85−100% of the target doses (Dupont Chem, 1992). All rats survived the study. Clinical signs of toxicity were observed at increased incidence in the high-dose (16 mg/kg-day) animals. These signs consisted of persistent or recurrent wet chins in both sexes and wet perineum or inguen in female rats (see Table B-4) and were not considered to be indicative of neurotoxicity. These clinical signs were considered to be "pharmacological responses" to the test substance by the study authors and are of uncertain biological significance. No treatment-related effects on body weight, weight gain, or food intake were observed at any time point. Small, not statistically significant changes in some neuromuscular measures in the FOB assessments were observed in the high-dose female rats at Week 13. Absolute and relative forelimb grip strength increased by 18% in female rats exposed to 16 mg/kg-day compared to concurrent control. Relative hindlimb grip strength was decreased by 14% in female rats exposed to 16 mg/kg-day *p*-phenylenediamine compared to control. A 20% decrease in absolute foot splay was observed in the high-dose male rats at Week 8 but did not persist to the 13-week measurement. The difference was not statistically significant and was comparable to the variability in baseline foot splay measurements (18%). Similarly, changes in motor activity levels noted in the high-dose male and female rats were not considered biologically significant because the changes were comparable to the range of variability seen in preexposure baseline tests. Due to the differing direction of effects related to neuromuscular function and the inconsistent appearance of effects across sexes and time points, these changes were not considered indicative of a neurotoxic effect. Ophthalmology examinations were unremarkable, and microscopic examination of nervous system tissues did not indicate any significant differences from controls for any lesion. A NOAEL of 8 mg/kg-day was assigned in both male and female rats; the LOAEL was 16 mg/kg-day based on clinical signs of toxicity (wet chin, perineum, or inguen).

Imaida et al. (1983): 12-week study

The toxicity of *p*-phenylenediamine (purity not reported) was tested in a 12-week range-finding study in which five groups of adult F344 rats (10-11/sex/dose) were fed diets containing 0, 0.05, 0.1, 0.2, or 0.4% *p*-phenylenediamine (Imaida et al., 1983). These concentrations correspond to estimated[1] doses of 0, 50.0, 100, 200, and 400 mg/kg-day in males and 0, 56.8, 114, 227, and 455 mg/kg-day in females. Body weights were measured weekly. The animals were sacrificed at the end of the experiment, and the main organs (not further

[1]Based on default body weight and food consumption rates for male and female F344 rats in a subchronic-duration study (U.S. EPA, 1988). Sample calculation: 0.05% *p*-phenylenediamine in food = 500 mg/kg food × (0.018 kg food/day ÷ 0.18 kg body weight) = 50 mg/kg-day.

specified, but results were presented for liver and kidney only) were weighed and grossly and microscopically examined. Statistical analyses were either not reported or not done.

Among rats receiving the highest dose (400 or 455 mg/kg-day in males and females, respectively), 9/11 males and 1/10 females died prior to study termination; no deaths were reported at lower doses. Body weights in both sexes were lower than controls over the course of the study and decreased in a dose-dependent manner. At termination, body-weight decrements of at least 10% were observed in males at ≥200 mg/kg-day and females at ≥114 mg/kg-day (see Table B-5). At the highest dose, terminal body weights were 53−58% lower than controls in the surviving rats. Liver and kidney weights were similar to controls in all treated groups with the exception of the highest dose group; in this group, absolute liver weights were decreased (see Table B-5), while relative liver weights (data not shown) were increased as a consequence of the markedly lower body weights in this group. The only histopathology change noted by the authors was fatty degeneration in the livers of males and females "at the highest doses" (incidences and statistical significance at specific doses were not reported). The highest dose (400 mg/kg-day in males and 455 mg/kg-day in females) is a frank effect level (FEL) based on mortality and profound (>50% compared with controls) body-weight decrements. The LOAEL is 200 mg/kg-day in males and 114 mg/kg-day in females based on body-weight decrease of at least 10% relative to controls. NOAEL values are 50.0 and 56.8 mg/kg-day in males and females, respectively; however, this value should be viewed with caution due to the limited toxicological evaluations performed in the study. The study authors set the top dose of the chronic-duration study at 0.1% (100 to 114 mg/kg-day), a concentration associated with body-weight decrements of 10% in both males and females.

NCI (1979): 7-week study

In a dose range-finding study for a chronic-duration carcinogenicity study, *p*-phenylenediamine dihydrochloride (purity not provided) was administered in the diet for 7 weeks to both rats and mice (NCI, 1979). Groups of adult F344 rats and groups of adult $B6C3F_1$ mice (five/sex/dose) were fed *p*-phenylenediamine dihydrochloride in the diet at concentrations of 0, 68 (rats only), 100, 147, 215, 316, 464, 681, 1,000, 1,470, 2,150, 3,160, or 4,640 (mice only) ppm. The animals were observed for one untreated week following dosing. During the study, the animals were observed for clinical signs of toxicity and mortality, and individual body weights and food consumption rates were recorded twice weekly. At study termination, gross necropsies were conducted on all survivors. No statistical analyses were reported.

Doses to rats were estimated for this assessment[2] to be 0, 6.8, 10.0, 14.7, 21.5, 31.6, 46.4, 68.1, 100, 147.0, 215.0, and 316.0 mg/kg-day *p*-phenylenediamine dihydrochloride in males and 0, 7.7, 11.4, 16.7, 24.4, 35.9, 52.8, 77.4, 114, 167.2, 244.5, and 359.3 mg/kg-day in females. Equivalent[3] doses of *p*-phenylenediamine were 0, 4.1, 5.97, 8.78, 12.8, 18.9, 27.7, 40.7, 59.7,

[2]Based on default body weight and food consumption rates for male and female F344 rats in a subchronic-duration study (U.S. EPA, 1988). Default body weights for F344 rats in subchronic-duration study: 0.180 kg (males), 0.124 kg (females). Default food intake for F344 rats in subchronic-duration study: 0.018 kg/day (males), 0.014 kg/day (females).

[3]The following equation was used to calculate dose of *p*-phenylenediamine (*p*-PD) from administered dose of *p*-phenylenediamine dihydrochloride (*p*-PD2HCl): mg *p*-PD2HCl/kg-day × (molecular weight *p*-PD ÷ molecular weight *p*-PD2HCl) = mg *p*-PD/kg-day. Sample calculation:
6.8 mg *p*-PD2HCl/kg-day × (108.14 g/mol ÷ 181.08 g/mol) = 4.1 mg *p*-PD/kg-day.

87.79, 128.4, and 188.7 mg/kg-day in males and 0, 4.6, 6.79, 9.98, 14.6, 21.5, 31.5, 46.2, 67.9, 99.82, 146.0, and 214.6 mg/kg-day in females. No deaths occurred among rats during the course of the study (NCI, 1979). Clinical signs of toxicity included arched backs and rough coats in all males and females at the high dose of 3,160 ppm (188.7 and 214.6 mg *p*-phenylenediamine/kg-day, respectively); these signs were not seen at lower doses or in controls. Body-weight data were not reported directly, but only as percent change in body-weight gain. Animals given doses ≤681 ppm (40.7−46.2 mg *p*-phenylenediamine/kg-day) gained more weight than controls (9−48%), while those exposed to doses ≥1,000 ppm (59.7−67.9 mg *p*-phenylenediamine/kg-day) gained less weight than controls (−1 to −41%). Based on these results, NCI (1979) set the top dose of the chronic-duration study at 1,250 ppm. Due to the lack of body weight and food intake data, a LOAEL and NOAEL could not be determined based on decreased body-weight gain.

Doses to mice were estimated for this assessment[4] to be 0, 18.1, 26.6, 38.8, 57.1, 83.8, 123, 181, 265.6, 388.5, 571.0, and 838.4 mg/kg-day *p*-phenylenediamine dihydrochloride in males and 0, 19.7, 28.9, 42.3, 62.2, 91.3, 134, 197, 289.2, 423.0, 621.7, and 912.9 mg/kg-day *p*-phenylenediamine dihydrochloride in females. Equivalent doses of *p*-phenylenediamine were 0, 10.8, 15.9, 23.2, 34.1, 50.1, 73.5, 108, 158.6, 232.0, 341.0, and 500.7 mg/kg-day in males and 0, 11.7, 17.3, 25.3, 37.1, 54.5, 80.0, 117, 172.7, 252.6, 371.3, and 545.2 mg/kg-day in females. No mice died during the study, and no clinical signs of toxicity attributable to treatment were observed. Body-weight data were not reported directly, but only as percent change in body-weight gain. Mean body-weight gains among males were lower than controls in all dose groups, with the largest deficits (−9 to −18%) at doses ≥1,470 ppm (158.6 mg *p*-phenylenediamine/kg-day). Among females, deficits in body-weight gain (−8 to −13%) compared to controls occurred at doses ≥2,150 ppm (252.6 mg *p*-phenylenediamine/kg-day). NCI (1979) set the top dose of the chronic-duration study in mice at 1,250 ppm. Due to the lack of body weight and food intake data, a LOAEL and NOAEL could not be determined based on decreased body-weight gain.

Chronic-Duration/Carcinogenicity Studies

Imaida et al. (1983): 80-week study

p-Phenylenediamine was administered in the diet to adult F344 rats of both sexes for 80 weeks in a chronic-duration toxicity and carcinogenicity study (Imaida et al., 1983). Groups of rats (63−66/sex/dose) were fed diets containing 0.05 or 0.1% *p*-phenylenediamine (purity not reported); control group comprised 24−25 rats/sex. These dietary concentrations correspond to estimated[5] doses of 0, 38.8, and 77.6 mg/kg-day in males and 0, 46.1, and 92.1 mg/kg-day in females. Body weights and food intakes were recorded weekly. At the end of the exposure duration (or when moribund), each animal was necropsied. Evaluations were not reported in detail, but included hematological analysis and gross and histological examination of "all organs" in animals surviving to Week 80. Statistical analyses were not described.

[4]Based on default body weight and food consumption rates for male and female $B6C3F_1$ mice in a subchronic-duration study (U.S. EPA, 1988). Default body weights for $B6C3F_1$ mice in subchronic-duration study: 0.0316 kg (males), 0.0246 kg (females). Default food intake for $B6C3F_1$ mice in subchronic-duration study: 0.0057 kg/d (males), 0.0048 kg/d (females).

[5]Based on default body weight and food consumption rates for male and female F344 rats in a chronic-duration study (U.S. EPA, 1988). Sample calculation: 0.1% *p*-phenylenediamine in food = 1,000 mg/kg food × (0.0211 kg food/day ÷ 0.229 kg body weight) = 92.1 mg/kg-day.

Survival data were reported graphically as number of animals surviving each week, without statistical analysis (Imaida et al., 1983). Visual inspection of the survival curves for treated and control groups showed that they were comparable, and no dose-related differences in survival patterns were evident; the study authors did not discuss any dose-related effects on survival. However, because of the small number of animals in the control group, only one male and six female rats survived to 80 weeks in this group. Weekly body weights were reported graphically. Based on visual inspection of the graph, body weights of exposed males were similar to or higher than controls through most of the study, while body weights of high-dose females were consistently lower than controls by a small (≤10%) amount. Terminal body weights were reported only for the small numbers of animals that survived to Week 80 (see Table B-6). Among animals surviving to Week 80, high-dose females receiving 92.1 mg/kg-day exhibited 21% lower body weight than the six concurrent control females ($p < 0.001$), and low-dose males receiving 38.8 mg/kg-day exhibited 14% lower body weight than the single surviving control (terminal body weight in the high-dose male group was similar to the control). Because only a single male control survived to Week 80, no meaningful comparison to concurrent control animals can be made for any male data. The study authors provided terminal body-weight data from F344 rats in another 78-week study performed earlier for comparison. However, mean terminal body weights of these controls were markedly (28−46%) higher than those of the controls or treated animals in the study of *p*-phenylenediamine. Further, the control data presented from the other study were based on only 10 male and 10 female rats, and thus, are not considered sufficient historical control data. Food intake was not altered by treatment.

Hematology data did not indicate any statistically significant dose-related effects in the animals surviving to Week 80, although erythrocyte counts in high-dose males and females were ~20% lower than concurrent and alternate controls. Dose-related, biologically significant increases in absolute liver and kidney weights (compared to concurrent controls) were seen in both sexes (see Table B-6); increases of 14 and 32% were seen in livers of high-dose males and females, respectively. High-dose males had 5 and 13% increased absolute weight of left and right kidneys, and high-dose females had 39 and 11% increased absolute weight of left and right kidneys. Given the small numbers of surviving controls, caution should be taken when interpreting this finding. Statistically significant declines in absolute spleen weight were observed in female rats, but not in male rats, exposed to both doses of *p*-phenylenediamine (see Table B-6). The study authors reported that relative organ weights were not significantly different from concurrent or alternative control values, but relative kidney weights did increase similarly to absolute kidney weights in female rats (data reported on relative organ weights lacked variability measures, precluding independent statistical evaluation of these data).

The study authors reported the incidences of preneoplastic and neoplastic lesions based on the numbers of rats surviving at the time the first tumor appeared (Week 60; group sizes ranged between 19 and 42 animals). The only non-neoplastic histopathology data reported were forestomach hyperplasia and ductal hyperplasia of the pancreas (these were considered preneoplastic), and neither of these endpoints was significantly increased by exposure in either sex of rat. The authors reported that the incidences of other non-neoplastic lesions were not significantly altered by exposure (data not shown). A LOAEL of 92.1 mg/kg-day *p*-phenylenediamine is identified for this study based on significantly reduced (21% less than concurrent controls) terminal body weight among females surviving to 80 weeks. The NOAEL is 46.1 mg/kg-day.

Tumor incidences of all types were low (0−2 animals per group) apart from the incidences of adrenal gland pheochromocytomas in male rats, which were not significantly increased by exposure to *p*-phenylenediamine (incidences were 6/19, 8/35, and 10/36 in control, low-, and high-dose males, respectively). One strength of this study is the adequate number of animals used in the exposed groups. However, the utility of this study for evaluating carcinogenicity is limited due to poor reporting (e.g., limited information on experimental design, no specific information on organs evaluated for histopathology, inadequate reporting of relative organ-weight data), the small number of control animals (24−25/sex), and the small number of animals surviving to termination (between 1 and 32 animals/sex/dose). It appears that the maximum tolerated dose (MTD) was achieved for females in this study, based on decreased body weight at the high dose, but the MTD may not have been achieved for males.

NCI (1979): 2-year study

Groups of adult male and female F344 rats and B6C3F$_1$ mice (50/sex/dose) were exposed to *p*-phenylenediamine dihydrochloride (purity not provided) in the diet at concentrations of 625 or 1,250 ppm for 103 weeks. Groups of male and female rats and mice (20/sex/species) receiving unaltered feed served as controls (NCI, 1979). Doses of *p*-phenylenediamine dihydrochloride estimated[6,7] for this review were 48.5 and 97.04 mg/kg-day in male rats, 57.6 and 115.2 mg/kg-day in female rats, 107 and 213.5 mg/kg-day in male mice, and 109 and 217.4 mg/kg-day in female mice. Equivalent doses of *p*-phenylenediamine are 29.0 and 58.0 mg/kg-day in male rats, 34.4 and 68.78 mg/kg-day in female rats, 63.7 and 127.5 mg/kg-day in male mice, and 64.9 and 129.8 mg/kg-day in female mice. At the end of exposure, rats were observed untreated for 2 weeks, and mice were observed untreated for 1 week prior to sacrifice. All animals were monitored twice daily, and body weights were measured monthly. Food intake for one-fifth of the animals in each group was measured monthly. All animals were necropsied at death or terminal sacrifice, and gross and microscopic examinations of tissues (skin, subcutaneous tissue, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder [mice], pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, brain, uterus, mammary gland, and ovary) from each animal were performed. Hematology and clinical chemistry were not examined, and organ weights were not recorded. Skeletal muscle (a tissue affected in human poisoning incidents and in short-term-duration exposure studies in rats) was not examined microscopically. Kaplan-Meier survival probabilities were analyzed using Cox and Tarone's methods. Fisher's exact test and the Cochran-Armitage trend test were used to analyze tumor incidences.

Survival of rats was not significantly affected by treatment (NCI, 1979). Among males, 13/20, 38/50, and 34/50 control, low-, and high-dose rats, respectively, survived to termination; among females, 14/20, 39/50, and 39/50 rats, respectively, survived. No clinical signs were reported. Body-weight data were presented graphically; the study authors reported slightly lower

[6]Based on default body weight and food consumption rates for male and female F344 rats in a chronic-duration study (U.S. EPA, 1988). Default body weights for F344 rats in chronic-duration study: 0.38 kg (males), 0.229 kg (females). Default food intake for F344 rats in chronic-duration study: 0.0295 kg/day (males), 0.0211 kg/day (females).

[7]Based on default body weight and food consumption rates for male and female B6C3F$_1$ mice in a chronic-duration study (U.S. EPA, 1988). Default body weights for B6C3F$_1$ mice in chronic-duration study: 0.0373 kg (males), 0.0353 kg (females). Default food intake for B6C3F$_1$ mice in chronic-duration study: 0.00637 kg/day (males), 0.00614 kg/day (females).

body weight in high-dose males and dose-related reductions in female body weight. Based on visual inspection of the graphs, terminal body weight of males at the high dose was <10% lower than controls and not biologically significant; however, terminal body weight of females at the high dose was biologically significantly decreased by ~15% compared to controls. Treatment with *p*-phenylenediamine dihydrochloride did not significantly increase the incidence of any non-neoplastic lesion in rats of either sex. A LOAEL of 68.78 mg/kg-day is identified for >10% lower terminal body weight in female rats; the NOAEL is 34.4 mg/kg-day.

Survival of mice was also not significantly affected by treatment (NCI, 1979). Among males, 16/20, 38/50, and 42/50 control, low-, and high-dose mice, respectively, survived to termination; among females, 17/20, 44/50, and 41/49 mice, respectively, survived. No clinical signs were reported. Body weights of male mice were not affected by treatment, while mean body weights of female mice were slightly lower than controls. Based on visual inspection of data presented graphically, terminal body weight of high-dose female mice was <10% different from controls. Treatment with *p*-phenylenediamine dihydrochloride did not significantly increase the incidence of any non-neoplastic lesion in mice of either sex. A LOAEL is not identified in the study of mice; the highest dose (129.8 mg/kg-day) is the NOAEL.

There were no significant increases in the incidence of any neoplastic lesion in rats or mice of either sex. A small increase in the incidence of leukemia or malignant lymphoma in female mice was observed (2/20 [10%], 10/50 [20%], 10/49 [20%]), and these lesions were observed earlier in dosed mice (low-dose: 67 weeks, high-dose: 31 weeks) compared to the control group (98 weeks). The study authors conducted an additional life-table analysis and reported no significant difference between the probability of survival without a known leukemia or malignant lymphoma in dosed and control groups. Strengths of this study for evaluating carcinogenicity include adequate reporting, adequate numbers of animals in the exposed groups, comprehensive histopathology examinations, and appropriate statistical analysis. The study is limited by the small number of control animals (20/sex). The MTD appears to have been reached for female rats, based on the decreased terminal body weight, but not for male rats. However, the MTD does not appear to have been reached for male or female mice, based on the lack of non-neoplastic effects or biologically significant body-weight changes.

Developmental Studies

ECHA (2005)

In a study available only in the ECHA (1995) database, time-mated female Crl:OFA(SD) 10−13-week-old rats (25/dose) were given *p*-phenylenediamine (99.8% pure, in water) by daily gavage on Gestation Days (GDs) 6−19 at doses of 0, 5, 10, or 20 mg/kg-day. Concentrations in the dosing solutions were verified analytically by HPLC. Daily examinations for clinical signs were performed, and maternal animals were weighed on GDs 0, 6, 9, 12, 15, 18, and 20. The amounts of food taken in between body-weight measurements were recorded. The dams were sacrificed on GD 20 for examination of ovaries, uteri, and placentae. The gravid uterine weight; numbers of corpora lutea, implantations, and early and late resorptions; and fetal weight and sex were recorded. All fetuses were examined externally, and half of the fetuses in each litter were examined for skeletal, visceral, and cranial malformations and variations. Statistical analysis of litter data included analysis of variance (ANOVA) with Dunnett's test (for homogeneous variance data) or Kruskal-Wallis with Dunn's test (for nonhomogeneous variance data) for continuous data and χ^2 followed by Fisher's exact test with Bonferroni correction for incidence data.

None of the dams died during the study, and no clinical signs of toxicity were noted. (ECHA, 2005). Maternal-weight gain of the 10- and 20-mg/kg-day dams was lower than controls during the first 3 days of exposure; the magnitude of difference was not reported in the ECHA (1995) database. Body-weight gain was not affected at 5 mg/kg-day. Food intake levels did not differ among the groups in the study, and there were no gross pathology findings attributable to treatment in the dams. In each of the study groups, at least 23 of 25 rats were pregnant. One dam in each of the control and high-dose groups delivered no viable fetuses. An "equivocal" increase in the incidence of early resorptions was observed in the high-dose group (data were not shown). There were no treatment-related differences in the mean live litter sizes or fetal sex ratio. In the high-dose group, gravid uterine weight and fetal body weights were slightly, but not statistically significantly, lower than controls (magnitude of difference not reported). None of the fetuses in any group were malformed, and the incidences of anomalies and variations did not differ with treatment. The ECHA (1995) database entry identified a maternal NOEL of 5 mg/kg-day (based on transient body-weight gain decreases at higher doses) and a developmental NOAEL of 10 mg/kg-day (based on the nonsignificant decrease in fetal body weight at the high dose). The study and effect level designations could not be independently evaluated, as the study report was not available and secondary sources were relied upon.

Re et al. (1981)

The teratogenic potential of *p*-phenylenediamine was evaluated in groups of 25 female Sprague-Dawley (S-D) rats given daily gavage doses of 0, 5, 10, 15, 20, or 30 mg/kg-day on GDs 6−15. *p*-Phenylenediamine (99.78% pure) was administered in water. Dosing solutions were prepared within 2 hours prior to dosing, as analysis of the solutions showed that the compound was stable for this duration. Dams were examined daily, and body weight was measured on GDs 0, 6, 9, 12, 15, and 20. Food consumption was measured daily beginning on GD 6. In addition to a vehicle control group, a pair-fed control group received the same food quantity as consumed by the group receiving 30 mg/kg-day. Dams were sacrificed on GD 20 for examination of uterine contents and ovaries, and gross examination of internal organs of the thorax. Numbers of resorptions and live and dead fetuses were recorded, along with live fetal weight and sex. All fetuses were examined for external malformations. From each litter, one of three of the fetuses was examined for visceral anomalies, and the rest were examined for skeletal anomalies. Statistical analyses employed ANOVA followed by *t*-test (for weights, food intake, corporal lutea, implantations, and numbers of live fetuses per dam). Sex ratio and number of litters with malformed fetuses were analyzed using the χ^2 and/or Fisher's exact tests.

Three rats (two pregnant and one nonpregnant) in the 30-mg/kg-day group died during the first 4 days of dosing (see Table B-7); there were no other deaths (Re et al., 1981). The authors indicated that necropsy of the decedents did not suggest that the deaths resulted from dosing error, but causes of the death were not reported. Dams in the 20- and 30-mg/kg-day dose groups and in the pair-fed control group exhibited significantly lower body-weight gain than vehicle controls when assessed on Days 0−12 and 0−15. In addition, the dams in the 30-mg/kg-day group and in the pair-fed control group gained significantly less weight on Days 0−9 (see Table B-7). Rats of all exposure groups gained weight after the dosing period, such that overall body-weight change (GDs 0−20) was not statistically significantly different from controls. The pattern of lower food intake in dams exposed to 20 or 30 mg/kg-day mirrored that of the decreased body-weight gain in terms of timing; the food consumption on GD 10 is shown in Table B-7 as an example of the magnitude of change. Slight, but statistically

significant, decreases in food intake occurred in the group given 15 mg/kg-day on GD 11 and GD 15, but not on other days. There were no treatment-related statistically or biologically significant alterations in uterine parameters (number of corpora lutea or implantation sites per dam, number of resorptions, sex ratio, or number of live fetuses per litter). Likewise, there were no treatment-related increases in the incidence of any malformation or variation. This study identifies a maternal LOAEL of 20 mg/kg-day for significantly lower body-weight gain and reduced feed intake on GDs 0−15; the NOAEL is 15 mg/kg-day. The highest dose (30 mg/kg-day) is a FEL for maternal mortality. The highest dose is also a NOAEL for fetal effects; no LOAEL is identified for fetal effects.

Holmberg et al. (1983) as translated in DuPont (1992)

In a transplacental carcinogenicity study, a group of 22 pregnant 6−8-week-old NMRI albino mice were administered 30 mg/kg-day *p*-phenylenediamine (≥99% pure) in soybean oil by daily gavage on GDs 10−19 [Holmberg et al. (1983) as translated from Swedish to English in DuPont (1992)]. A control group of 26 pregnant mice served as negative controls, and a positive control group of 18 mice received urethane (300 mg/kg-day). The F1 generation was comprised of 190 mice (95 male and 95 female) in the *p*-phenylenediamine group, 209 mice (110 males and 99 females) in the urethane group, and 158 mice (77 males and 81 females) in the vehicle control group. After parturition, the mice (maternal and offspring) were observed daily and weighed and palpated every 6 weeks. Subgroups of offspring were sacrificed 27 weeks (10 exposed mice, 10 negative controls, and 5 positive controls) or 51 weeks (23 exposed mice, 20 negative controls, and 16 positive controls) after the start of the experiment. In addition, five exposed mothers, seven negative control mothers, and five positive control mothers were sacrificed after 51 weeks. The remaining mothers and offspring were allowed to die naturally or were sacrificed moribund—the last after 137 weeks of observation. All animals were necropsied, and 30 tissues (including brain, lung, liver, kidneys, spleen, and endocrine and reproductive organs) were sampled and examined microscopically. Statistical analysis of survival data employed the Cox test.

Survival of *p*-phenylenediamine-treated dams was lower than negative controls (mean age at death was 70 weeks, vs. 83 weeks in controls); however, the difference was not statistically significant [Holmberg et al. (1983) as translated in DuPont (1992)]. Survival of *p*-phenylenediamine-exposed offspring did not differ from controls. Clinical observations and body weights of dams and offspring were not affected by treatment with *p*-phenylenediamine, indicating a maternal and fetal NOAEL of 30 mg/kg-day, albeit based on limited observations and information available from the DuPont (1992) translation.

There was a slight, statistically significant increase in the incidence of alveolar adenoma in female offspring of mice exposed to *p*-phenylenediamine (18/88 vs. 12/86 in negative controls, $p = 0.04$); this tumor type was not increased in male offspring or in maternal animals. The incidences of other neoplastic and non-neoplastic lesions did not differ significantly between the negative control and *p*-phenylenediamine-treated groups. In contrast, a significant increase in tumor incidence (predominantly alveolar adenomas) was observed in the urethane-treated positive control group.

Inhalation Exposures

No studies examining effects of *p*-phenylenediamine in animals exposed via inhalation have been identified, with the exception of an acute inhalation lethality study discussed below.

OTHER DATA (SHORT-TERM TESTS, OTHER EXAMINATIONS)

Table 4A provides an overview of genotoxicity studies of *p*-phenylenediamine, and Table 4B provides an overview of other supporting studies on *p*-phenylenediamine, including acute oral lethality and toxicity studies and an acute inhalation lethality study.

Table 4A. Summary of *p*-Phenylenediamine (CASRN 106-50-3) Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
Genotoxicity studies in prokaryotic organisms						
Mutation	*Salmonella typhimurium* strain TA102	5, 125 µM	−	NA	Plate incorporation assay with and without light irradiation. Authors reported that *p*-phenylenediamine was not mutagenic with or without light irradiation.	Mosley-Foreman et al. (2008)
Mutation	*S. typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537	0, 200, 1,000, 5,000 µg/plate (plate incorporation); 0, 625, 1,250, 2,500, 5,000 µg/plate (preincubation)	−	+ (TA98) − (TA100, TA102, TA1535, TA1537)	Plate incorporation and preincubation assays. *p*-Phenylenediamine was mutagenic in TA98 in the presence of S9 at ≥1,000 µg/plate in the plate incorporation assay and at ≥625 µg/plate in the preincubation assay.	Garrigue et al. (2006)
Mutation	*S. typhimurium* strains TA98 and TA100	67, 135, 269, 538, 1,076 µg/plate	−	+ (TA98) − (TA100)	Plate incorporation assay. Mutagenic in TA98 at 67 and 135 µg/plate, with inverse dose response due to toxicity. Authors reported that tests with lower doses (4−32 µg/plate) confirmed mutagenic response (data not shown).	Assmann et al. (1997)
Mutation	*S. typhimurium* strains TA98, TA100, TA1535, and TA1538	100, 333, 666, 1,000, 3,333, 5,000, 6,666 µg/plate	−	+ (TA98, TA100, TA1535, and TA1538)	Plate incorporation assay. Mutagenic in TA98, TA100, TA1535, and TA1538 in the presence of Aroclor-induced mouse or rat liver S9 at all doses.	Dunkel and Simmon (1980)
Mutation	*S. typhimurium* strains TA98 and TA100 and their nitroreductase-deficient mutants, TA98NR and TA100NR	1, 10, 30, 100, 300, 1,000, 3,000 µg/plate	+ (TA100NR) − (TA98, TA98NR TA100)	+ (TA98NR) − (TA98, TA100, TA100NR)	Preincubation assay. *p*-Phenylenediamine was mutagenic to TA98NR at ≥30 µg/plate with S9 added and in strain TA100NR at 3,000 µg/plate without S9.	Chung et al. (1996); Chung et al. (1995)
Mutation	*S. typhimurium* strains TA98 and TA100	0, 1, 10, 100, 1,000, 10,000 µg/plate	−	−	Plate incorporation and preincubation assays. Cytotoxicity was observed at 10,000 µg/plate (highest concentration tested).	Gentile et al. (1987)

Table 4A. Summary of *p*-Phenylenediamine (CASRN 106-50-3) Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
Mutation	*S. typhimurium* strain TA98	0, 25, 50, 75, 100 µg/plate	−	+	Plate incorporation assay. *p*-Phenylenediamine was mutagenic at all doses tested.	Lee et al. (1986)
Mutation	*S. typhimurium* strain TA98	0, 250, 1,000 µg/plate	−	+	Plate incorporation assay. *p*-Phenylenediamine dihydrochloride was mutagenic at ≥500 µg/plate.	Rojanapo et al. (1986)
Mutation	*S. typhimurium* strains TA100, TA1535, TA1537, and TA1538	1, 10, 50, 100, 250, 500, 750, 1,000 µg/plate	−	−	Plate incorporation assay.	Brady and Troll (1977); also reported in SRI (1975)
Mutation	*S. typhimurium* strains C3076, D3052, G46, TA98, TA 100, TA1535, TA1537, and TA1538 and *Escherichia coli* strains WP2 and WP2uvrA-	0.1−1,000 µg/mL	–	+ (TA98, TA1538,) – (C3076, D3052, G46, TA 100, TA1535, TA1537, and *E. coli* strains WP2 and WP2uvrA-)	Modified Ames gradient plate test. *p*-Phenylenediamine was mutagenic in strains TA1538 and TA98, with positive results at ≥0.6 µg/mL.	Thompson et al. (1983)
Mutation	*S. typhimurium* strains TA98 and TA1538; tested effects of DMSO aging (0, 1, 2, and 4 hr) as well as dose response	0, 25, 50, 100, 250 µg	NA	−	Plate incorporation assay comparing effects of solvents (DMSO or distilled water) and aging of solution prior to testing. *p*-Phenylenediamine was not mutagenic at Time 0 in DMSO or at any time in distilled water. Mutagenicity was reported in tests conducted after aging in DMSO solution for 1−4 hr. The reaction product(s) responsible for the mutagenic response were not identified.	Burnett et al. (1982)
Mutation	*S. typhimurium* strain TA98	20 µg/plate	NA	−	Plate incorporation assay with and without light irradiation. *p*-Phenylenediamine was not mutagenic in the dark, but was mutagenic when exposed to visible light for 1 hr.	Nishi and Nishioka (1982)

Table 4A. Summary of *p*-Phenylenediamine (CASRN 106-50-3) Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
Mutation	*S. typhimurium* strain TA98	0, 0.25, 0.5, 1.0, 2.0 mg/plate (1 chemically pure sample and 2 commercial samples tested)	−	−	Plate incorporation assay. The chemically pure sample was nonmutagenic. The two commercial samples (compositions not reported) were mutagenic in the presence of S9.	Crebelli et al. (1981)
Mutation	*S. typhimurium* strains TA98 and TA100	0, 0.5, 1.0, 2.0 µmol/plate	−	+ (TA98) − (TA100)	Modified plate incorporation assay. Mutagenic in TA98 at ≥0.5 µmol/plate with metabolic activation.	Degawa et al. (1979)
Mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538	0, 5, 10, 20, 50, 100, 250, 500, 1,000 µg/plate	−	−	Plate incorporation assay in the absence and presence of uninduced and Aroclor-induced rat-liver homogenate. *p*-Phenylenediamine induced a slight increase in revertants (<2-fold compared with controls) in strains TA98 and TA1538 at ≥250 µg/plate with Aroclor-induced activation, but not with the uninduced activation system.	Shahin et al. (1979)
Mutation	*S. typhimurium* strain TA97, TA98, TA100, and TA1538	1 mg	−	−	Spot test (results shown) and plate incorporation assay. In plate incorporation assay, *p*-phenylenediamine was not mutagenic; however, after oxidation with equal volume hydrogen peroxide (to mimic use in hair dye), *p*-phenylenediamine was strongly mutagenic in strain TA1538 when S9 was present (data not shown).	Ames et al. (1975)
DNA damage	*S. typhimurium* strain TA1535/pSK1002	200, 500, 1,000, 2,000, 5,000 µg/mL	−	+	Umu test of bacterial SOS response system. *p*-Phenylenediamine induced positive response (doubling of activity ratio) at 5,000 µg/mL with activation.	Yasunaga et al. (2006)

Table 4A. Summary of *p*-Phenylenediamine (CASRN 106-50-3) Genotoxicity						
Endpoint	**Test System**	**Doses/ Concentrations Tested**	**Results without Activation[a]**	**Results with Activation[a]**	**Comments**	**References**
Genotoxicity studies in nonmammalian eukaryotic organisms						
Mutation	*Saccharomyces cerevisiae* D3	0.05%	−	−	Preliminary experiments indicated toxicity at a concentration of 0.1% (not further specified).	SRI (1975)
Mutation	*Drosophila melanogaster*, strains UZ, [$(w^i)_4$], multiple wing hairs, and flare-3	0, 0.10, 0.50 mM (zeste white); 0, 0.50, 1.0, 5.0 mM (white ivory); 0, 0.10, 1.0, 2.0 mM (wing spot)	+	+	Three assays: zeste white, white ivory, and wing spot. *p*-Phenylenediamine produced a significant increase in the frequency of mutant clones in the zeste-white assay at 0.5 mM, in the white-ivory assay at ≥0.50 mM, and in the wing-spot assay at ≥1.0 mM.	Batiste-Alentorn et al. (1995)
Sex-linked recessive lethal mutation	Male *D. melanogaster* exposed by injection or feeding for 2−3 d, followed by mating with Basc females	0, 5.1, 15.5 mM (Blijleven, 1977); 0, 2.5, 5, 10, 15.5 mM (Blijleven, 1981)	−	−	Purified *p*-phenylenediamine did not produce a significant increase in the mutation frequency compared to controls. Doses ≥15 mM produced toxicity and sterility. In the Blijleven (1977) study, *p*-phenylenediamine produced an increase in mutation frequency; however, Blijleven (1981) concluded that the positive results were attributable to impurities in the sample used in that study.	Blijleven (1981); Blijleven (1977)
Genotoxicity studies in mammalian cells in vitro						
Mutation	L5178Y mouse lymphoma cells	0, 2.5, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 80 µg/mL (without activation); 0, 25, 50, 75, 100, 175, 200, 250, 375, 400, 500, 600, 625, 750, 900, 1,000 µg/mL (with activation)	−	−	Doses ≥35 µg/mL without activation ≥900 µg/mL with activation were toxic.	Garrigue et al. (2006)

Table 4A. Summary of *p*-Phenylenediamine (CASRN 106-50-3) Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
Mutation	L5178Y mouse lymphoma cells, TK$^{\pm}$ heterozygote	0, 0.625, 1.25, 2.5, 3.75, 5, 7.5, 10 µg/mL (Four trials without activation); 0, 15.6, 31.3, 50, 62.5, 100, 125, 150, 175, 200, 250, 300, 400, 500 µg/mL (Three trials with activation) *p*-phenylenediamine dihydrochloride	+	+	Increases in mutant frequency with and without Aroclor-induced rat liver S9 activation. Statistically significant mutant frequency increase at doses ≥1.25 µg/mL without activation and ≥31.3 µg/mL with activation in Trial 1 and ≥100 µg/mL in Trials 2 and 3. Moderate toxicity at 5 µg/mL, high toxicity at 7.5 µg/mL, and lethality at 10 µg/mL without activation. Toxicity at 31.3 µg/mL and lethality at 250 µg/mL with activation.	Myhr and Caspary (1988)
Mutation	L5178Y mouse lymphoma cells, TK$^{\pm}$ heterozygote	0, 2.1, 2.6, 3.3, 4.1, 5.1, 6.4 µg/mL for Trial 1; 0, 2.1, 2.62, 3.28, 4.1, 5.12 µg/mL for Trial 2; 0, 2.1, 2.7, 3.3, 4.2, 5.2, 6.5 µg/mL for Trial 3 (Three trials without activation); 0, 7, 11.7, 19.4, 32.4, 54, 90, 150, 192, 240, 250, 300 µg/mL for Trials 1 and 2; 0, 33.2, 55.3, 92.2, 154, 240, and 300 for Trial 3 (Three trials with activation) *p*-phenylenediamine dihydrochloride	+	+	Inconsistent among trials. Without Aroclor-induced rat liver S9 activation, mutant frequencies increased in two of three trials at doses ≥3.28 µg/mL and ≥5.2 µg/mL. Concentration-dependent toxicity at doses ≥2.6 µg/mL. With S9 activation, statistically significant increases in mutant frequency were reported in two of three trials at doses ≥192 µg/mL. Concentration-dependent toxicity at doses ≥19.4 µg/mL.	Mitchell et al. (1988)

Table 4A. Summary of *p*-Phenylenediamine (CASRN 106-50-3) Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
Mutation	CHO/HGPRT	0, 5, 10, 20, 30 µg/mL *p*-phenylenediamine dihydrochloride (without activation); 100, 250, 500, 600, 700 µg/mL *p*-phenylenediamine dihydrochloride (with activation)	−	−		Oshiro et al. (1991)
DNA damage	Mardin-Darby canine kidney cells	0, 12.5, 25, 37.5 or 50 µg/mL for up to 24-hr incubation time	+	+	DNA damage was detected by both the comet and TUNEL assays at a concentration and time that was not cytotoxic. *p*-Phenylenediamine decreased cell viability in a dose- and time-dependent manner, with reduction to 50% viability at concentrations ≥37.5 µg/mL for 24 hr or 50 µg/mL for 12 hr.	Chen et al. (2010)
DNA damage	SV40 immortalized human uroepithelial cells	0, 2, 5, 10, 20 40 µg/mL	+	+	Comet assay. Increased mean migration length and percentage of cells with tails at ≥2 µg/mL. Severity of DNA damage increased dose dependently. Cell viability was reduced to ≤50% of controls at concentrations ≥10 µg/mL. Immunocytochemistry of exposed cells showed that *p*-phenylenediamine induced overexpression of a mutant form of p53.	Huang et al. (2007)
DNA strand breaks	HaCaT cells	0, 5, 15, 30, 40, 100 µg/mL	+	+	Comet assay. DNA strand breaks induced at 100 µg/mL *p*-phenylenediamine with and without 10.5 µg/mL hydrogen peroxide.	Zanoni et al. (2015)
Single strand DNA breaks	Human lymphocytes	0, 50, 100, 200, 500 µM	+	NA	*p*-Phenylenediamine caused single strand DNA breaks at ≥50 µM.	Chye et al. (2008)
Unscheduled DNA synthesis	Primary rat hepatocytes	0.5−1,000 nmol/mL	−	NA	Concentrations >100 nmol/mL were cytotoxic.	Thompson et al. (1983)

Table 4A. Summary of *p*-Phenylenediamine (CASRN 106-50-3) Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
CAs	CHO-K1 cells	15, 29, 58, 87 µg/mL	+	NA	Increased percentage of aberrant cells at ≥15 µg/mL. TC_{50} (concentration cytotoxic to 50% of cells) was 29 ± 4 µg/mL.	Chung et al. (1996); Chung et al. (1996)
MN	Female human lymphocytes	Experiment 1 (exposure 24 hr after mitogen [PHA] stimulation): 0, 5, 30, 80 µg/mL without activation and 0, 500, 900, 1,600 µg/mL with activation; Experiment 2 (exposure 48 hr after PHA stimulation): 0, 50, 100, 125 µg/mL without activation and 0, 400, 1,400, 2,000 µg/mL with activation	+ (48 hr after PHA stimulation) − (24 hr after PHA stimulation)	+	Experiment 1: Significant increase in frequency of MN at 1,600 µg/mL with activation; no increase in frequency of MN without activation. Experiment 2: Significant increase in frequency of MN at ≥50 µg/mL with and without activation.	Garrigue et al. (2006)
MN	CHO	0, 5, 10, 20, 30 µg/ml *p*-phenylenediamine dihydrochloride (without activation); 100, 250, 500, 600, 700 µg/mL *p*-phenylenediamine dihydrochloride (with activation)	+	−	*p*-Phenylenediamine dihydrochloride significantly increased frequency of MN at ≥20 µg/mL.	Oshiro et al. (1991)

Table 4A. Summary of *p*-Phenylenediamine (CASRN 106-50-3) Genotoxicity						
Endpoint	**Test System**	**Doses/ Concentrations Tested**	**Results without Activation[a]**	**Results with Activation[a]**	**Comments**	**References**
SCE	CHO	0.2, 0.4, 0.8, 1 mM	+	+	In the absence of S9 activation, treatment with *p*-phenylenediamine increased the mean number of SCE per cell by 71, 92, 140, and 156% at concentrations of 0.2, 0.4, 0.8, and 1 mM, respectively. In the presence of S9, the mean number of SCE per cell was increased by 54, 66, 106, and 131% at concentrations of 0.2, 0.4, 0.8, and 1 mM, respectively.	Lee et al. (1986)
Oxidative DNA adducts	HaCaT cells	Experiment 1: 100 µg/mL Experiment 2: 0, 20, 80, 100 µg/mL	+	NA	Experiment 1: *p*-Phenylenediamine increased M1dG adducts with and without addition of H_2O_2. Experiment 2: *p*-Phenylenediamine increased 8-oxo-dG at 20 µg/mL (not 80 or 100 µg/mL), but not with H_2O_2 cotreatment.	Zanoni et al. (2015)
Genotoxicity studies—in vivo						
Dominant lethal mutagenicity	Male Charles River CD rats (20/group) treated with *p*-phenylenediamine in 0.2% aqueous solution by i.p. injection 3 times/wk for 8 wk, and then mated to untreated females; females sacrificed after 17 d and uteri examined	20 mg/kg	−	−	No significant increase in postimplantation fetal loss.	Burnett et al. (1977)

Table 4A. Summary of *p*-Phenylenediamine (CASRN 106-50-3) Genotoxicity						
Endpoint	**Test System**	**Doses/ Concentrations Tested**	**Results without Activation[a]**	**Results with Activation[a]**	**Comments**	**References**
MN	CFY rats (5/sex/dose) administered *p*-phenylenediamine in gum tragacanth/sodium sulfite via gavage as 2 equal doses 24 hr apart, and sacrificed 24 hr later for analysis of bone marrow smears	0, 300 mg/kg	−	−	Authors reported clinical signs of toxicity including agitation, convulsions, and/or lethargy.	Hossack and Richardson (1977)
MN	Male CD-1 mice (5/dose) were administered *p*-phenylenediamine dihydrochloride as a single i.p. injection and sacrificed 24, 48, or 72 hr after dosing for analysis of bone marrow smears	0, 25, 50, 100 mg/kg *p*-phenylenediamine dihydrochloride	−	−		Soler-Niedziela et al. (1991)

Table 4A. Summary of *p*-Phenylenediamine (CASRN 106-50-3) Genotoxicity						
Endpoint	**Test System**	**Doses/ Concentrations Tested**	**Results without Activation[a]**	**Results with Activation[a]**	**Comments**	**References**
DNA damage	Male S-D rats (5/dose) administered *p*-phenylenediamine dihydrochloride by gavage for 3 d, sacrificed 3 hr after final dose, and liver and stomach sampled	0, 25, 50, 100 mg/kg-d *p*-phenylenediamine dihydrochloride	−	−	Comet assay of isolated liver and stomach cells. No difference in DNA migration (median % tail intensity and % hedgehogs). Two of five animals in the 100-mg/kg-d group died prior to testing.	De Boeck et al. (2015)
Genotoxicity in cell-free systems						
DNA cleavage	ΦX174 phage DNA	0−1,000 μM with light irradiation	−	−		Mosley-Foreman et al. (2008)

[a]+ = positive; − = negative; NA = not applicable.

8-oxo-dG = 8-oxo-7,8-dihydro-2′-deoxyguanosine; CA = chromosomal aberration; CHO = Chinese hamster ovary; DMSO = dimethylsulfoxide; DNA = deoxyribonucleic acid; HaCaT cells = human immortalized keratinocytes; HGPRT = Hypoxanthine-guanine phosphoribosyltransferase; i.p. = intraperitoneal; M1dG = malondialdehyde-DNA adducts; MN = micronuclei; MNBN = micronucleated binucleate cells; PHA = phytohemagglutinin; SCE = sister chromatid exchange; S-D = Sprague-Dawley; TUNEL = Terminal deoxynucleotidyl transferase dUTP nick end labeling.

Table 4B. Other Studies				
Test	**Materials and Methods**	**Results**	**Conclusions**	**References**
Acute toxicity (oral/inhalation)				
Acute oral lethality rats	S-D albino rats (2−3/sex/dose) were administered *p*-phenylenediamine in a 5% aqueous solution as single oral doses of 126, 158, 200, 251, or 316 mg/kg. Animals were monitored for clinical signs of toxicity and mortality for 14 d after dosing. Study reported in tabular form with few details.	Mortality occurred within 24 hr in all exposure groups: 1/5, 2/5, 3/5, 5/5, and 5/5 deaths at 126, 158, 200, 251, and 316 mg/kg, respectively. Clinical signs of toxicity in surviving rats included weight loss, weakness, tremors, and collapse. Gross necropsy of decedents revealed hemorrhagic lungs, liver hyperemia, and gastrointestinal inflammation.	Oral LD_{50} = 180 mg/kg (95% CI 150−220 mg/kg)	Younger Laboratories (1978); Litton Bionetics (1976)
Acute oral lethality rats	Fasted CFY rats (5/sex/dose) were administered *p*-phenylenediamine in a 1% aqueous solution (0.05% Na_2SO_3) via gavage. Animals were monitored for clinical signs of toxicity and mortality for 14 d after dosing.	Incidence of mortality not reported. Study tested 12 compounds, and reported clinical signs and necropsy findings without specifying the compound(s) eliciting the effects. Clinical signs of toxicity seen with all tested compounds included lethargy and piloerection. Gross necropsy findings clearly associated with *p*-phenylenediamine administration could not be discerned from the report.	Oral LD_{50} = 98 mg/kg (95% CI 84−114 mg/kg)	Lloyd et al. (1977)
Acute oral lethality rats	Rats (strain and sex unspecified; 10/dose) were administered *p*-phenylenediamine via oral doses of 56.2, 59.6, 63.1, 66.8, 70.79, 75.0, 79.4, 84.1, 89.1, 94.4, or 100 mg/kg. Observation time following exposure, clinical signs, and necropsy findings were not reported. Study reported data in tabular form with few details.	Mortality occurred at doses ≥59.6 mg/kg: 6/10, 1/10, 6/10, 8/10, 6/10, 4/10, 8/10, 10/10, 10/10, and 7/10 deaths at 59.6, 63.1, 66.8, 70.79, 75.0, 79.4, 84.1, 89.1, 94.4, and 100 mg/kg, respectively.	Oral LD_{50} = 75 mg/kg	Rhone-Poulenc (1951); Woodard (1951)
Acute oral lethality mice	Mice (strain and sex unspecified; 10/dose) were administered *p*-phenylenediamine via oral doses of 79.4, 100, 126, 141, 159, 176, 199, 224, or 251 mg/kg. Observation time following exposure, clinical signs, and necropsy findings were not reported. Study reported data in tabular form with few details.	Mortality occurred in all exposure groups: 1/10, 4/10, 4/10, 4/10, 4/10, 7/10, 7/10, 7/10, and 9/10 deaths at 79.4, 100, 126, 141, 159, 176, 199, 224, and 251 mg/kg, respectively.	Oral LD_{50} = 180 mg/kg	Rhone-Poulenc (1951); Woodard (1951)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Acute oral systemic toxicity	Groups of six rats (strain and sex unspecified) received oral doses of 10 or 20 mg *p*-phenylenediamine (method of administration not specified). At sacrifice 24 hr later, blood was analyzed for hematology and clinical chemistry, and liver and kidney were examined microscopically.	Significant increases in plasma AST, ALT, and leukocyte count occurred at both doses. The authors reported liver lesions, including vacuolated cytoplasm, irregular and deeply stained nuclei in hepatocytes with vascular congestion, and lymphocyte infiltration. No other significant effects were reported in the abstract.	*p*-Phenylenediamine induced liver lesions (effective dose not specified), increased liver enzymes (at ≥10 mg/kg), and increased leukocyte count after a single dose.	Ahmed (2011) (abstract only)
Acute oral systemic toxicity	Groups of three male Wistar rats were given phenylenediamine at single doses of 0, 20, 40, or 80 mg/kg (method not specified). Exposed groups were sacrificed after 6 d, 3 d, and 3 hr (respectively) for kidney histopathology evaluation.	All 3 exposure groups exhibited glomerular congestion, tubular necrosis, and intertubular hemorrhages, with dose-dependent increases in severity (incidences were not reported but effects were not seen in controls).	*p*-Phenylenediamine induced renal lesions after single doses ≥20 mg/kg.	Reddy et al. (2012)
Acute oral systemic toxicity	Groups of 15 Beit Dagan mice (sex not specified) were given single doses of 0, 35, or 70 mg/kg *p*-phenylenediamine in water administered by nasogastric tube. Subgroups of five each were sacrificed after 24, 72, and 120 hr for analysis of urea, uric acid, aldolase, and CPK in blood; muscle, liver, and kidney were examined microscopically.	Dose-related increases in CPK and aldolase were observed at each time point, with statistically significantly higher values seen at the high dose in the groups sacrificed 24 and 72 hr after dosing. Results of urea and uric acid measurements were not reported. Histopathology of the muscle revealed similar effects in both dose groups: acute rhabdomyolysis with segmental necrosis of myofibers after 24 hr, necrosis with infiltration of macrophages and phagocytosis at 72 hr, and regeneration of myofibers at 120 hr (incidences not reported). No lesions were seen in liver or kidney.	A single dose of ≥35 mg/kg *p*-phenylenediamine exposure caused rhabdomyolysis in mice exposed orally.	Averbukh et al. (1989)
Acute oral systemic toxicity	A total of 14 hybrid dogs received single oral doses of 50, 80, or 100 mg/kg (group sizes not reported in abstract). Serum enzymes and muscle histology were evaluated. No other information on study design was available in the abstract.	Exposed animals exhibited swelling of the face, limbs, and external genitalia, as well as "painful" muscle rigor. Serum levels of CPK were markedly increased in nearly all animals, with the highest increase in animals receiving 80 mg/kg. Liver enzymes (serum AST and ALT) were not altered by exposure. "Massive" necrosis of the skeletal muscles was observed, with the most pronounced effects seen at 80 mg/kg.	Exposure to *p*-phenylenediamine resulted in rhabdomyolysis in dogs, including increased CPK and skeletal muscle necrosis at ≥50 mg/kg.	Yabe et al. (1991) (abstract only)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Acute oral systemic toxicity	Female beagle dogs (2/dose) were administered *p*-phenylenediamine in distilled water via single gavage doses of 1.0, 3.0, or 10.0 mg/kg (no control). Animals were monitored for clinical signs of toxicity for 24 hr, and blood samples were drawn at 6 and 24 hr and analyzed for methemoglobin.	No deaths occurred. Clinical signs of toxicity included lacrimation (all doses), redness of conjunctiva (≥3 mg/kg), and swelling of the conjunctiva (10 mg/kg). Results of the study are inconclusive in the absence of a vehicle control group. Methemoglobin levels were within the normal range at both postdosing measurement time points.	*p*-Phenylenediamine did not induce methemoglobinemia in beagle dogs at doses up to 10 mg/kg.	Clairol (1980)
Acute oral neuro-toxicity	After preexposure baseline motor activity and FOB assessments, single doses of 0, 20, 40, or 80 mg/kg were administered by gavage in water to groups of 12 male and 12 female Crl:CD®BR rats. Body weights and clinical signs were recorded prior to exposure, 1 hr after dosing, and on 1, 4, and 7 d after dosing. Food consumption measurements were taken on the day of dosing as well as 1, 3, 4, and 7 d after dosing. Neurotoxicity assessments consisted of motor activity, FOB, forelimb and hindlimb grip strength, and foot splay assessments conducted at 1.5 and 24 hr after dosing and again 4 d after dosing.	One male died due to dosing error. Most high-dose females exhibited stained fur, and one high-dose female exhibited palpebral closure and another high-dose female exhibited body shakes. A high-dose male exhibited head shaking. Significantly lower body-weight gains from D 0 (day of dosing) to D 4 were seen in high-dose animals only; all rats resumed gaining weight after D 4. Mean body weight of the high-dose animals was significantly lower than controls on D 4; other differences were not statistically significant. Food intake measures followed the general pattern of body weight. FOB assessment in female rats showed significant and dose-related increases in general malaise, postural changes, palpebral closure, and decreased arousal. In males, similar effects were seen, but the differences from control were not statistically significant. Forelimb and hindlimb grip strength and foot splay were not altered by exposure to *p*-phenylenediamine. All three dose groups exhibited significantly lower horizontal and vertical activity on the day of dosing; at doses ≥40 mg/kg, activity was decreased relative to controls through postdosing D 4.	The study authors concluded that the effects on motor activity and FOB assessment parameters reflected overall systemic toxicity that led to general malaise and decreased arousal, and not neurotoxicity.	Haskell Laboratories (1990)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Acute inhalation lethality	Male Crl:CD rats (10/group) were exposed to *p*-phenylenediamine vapor via inhalation (nose only) as a single 4 hr exposure at mean concentrations of 0.07, 0.30, 0.54, 0.94, or 1.8 mg/L (70, 300, 540, 940, or 1,800 mg/m^3). Animals were monitored for clinical signs of toxicity and mortality for 14 d after dosing. Study reported data in tabular form with few details.	Authors reported that vapors in chamber condensed into aerosol at concentrations ≥540 mg/m^3. Mortality occurred at ≥300 mg/m^3: 1/10, 4/10, 5/10, and 7/10 deaths at 300, 540, 940, and 1,800 mg/m^3, respectively. During exposure, red nasal discharge was seen at ≥300 mg/m^3, and cyanosis was observed at 1,800 mg/m^3. Dose-dependent, slight to severe weight loss occurred for 3 d after dosing. During the postexposure observation period, all exposure groups showed red ocular discharge or brown-stained fur around the eyes. Rats exposed to ≥940 mg/m^3 exhibited pallor, diarrhea, loss of righting reflex, and tremors.	Inhalation LC_{50} = 920 mg/m^3 (95% CI 590−1,900 mg/m^3)	Haskell Laboratories (1982)
Mechanistic	Male F344 rats pretreated with an initiating dose of diethylnitrosamine (200 mg/kg) via i.p. injection were administered *p*-phenylenediamine in the diet (1,000 ppm) for 6 wk. Livers were examined for GST-P+ foci.	Administration of *p*-phenylenediamine did not increase the number or size of GST-P+ foci.	Administration of *p*-phenylenediamine did not induce GST-positive foci.	Ito et al. (1988)
Mechanistic	Male F344 rats pretreated with an initiating dose of diethylnitrosamine (200 mg/kg) via i.p. injection were administered ^{14}C ring-labelled *p*-phenylenediamine in the diet (1,000 ppm) for 6 wk. Livers were examined for GGT-positive liver foci.	Administration of *p*-phenylenediamine did not increase the number or size of GGT-positive foci.	Administration of *p*-phenylenediamine did not induce GGT-positive foci.	Ogiso et al. (1984) (abstract only)

ALT = alanine aminotransferase; AST = aspartate aminotransferase; CI = confidence interval; CPK = creatine phosphokinase; FOB = functional observational battery; GGT = γ-glutamyl transferase; GST = glutathione-*S*-transferase; GST-P+ = glutathione *S*-transferase placental form-positive; i.p. = intraperitoneal; LC_{50} = median lethal concentration; LD_{50} = median lethal dose; S-D = Sprague-Dawley.

Genotoxicity

Genotoxicity of *p*-phenylenediamine has been tested in a wide variety of systems, as shown in Table 4A. Results of bacterial testing have been largely negative. In tests for mutation of *Salmonella typhimurium* strains, *p*-phenylenediamine gave negative results in the absence of metabolic activation (Mosley-Foreman et al., 2008; Garrigue et al., 2006; Assmann et al., 1997; Chung et al., 1996; Chung et al., 1995; Gentile et al., 1987; Lee et al., 1986; Rojanapo et al., 1986; Thompson et al., 1983; Crebelli et al., 1981; Dunkel and Simmon, 1980; Degawa et al., 1979; Shahin et al., 1979; Brady and Troll, 1977; Ames et al., 1975). The only exception was a positive finding in testing of the nitroreductase-deficient mutant of TA100 (Chung et al., 1996; Chung et al., 1995). In tests for mutation of *S. typhimurium* with the addition of metabolic activation, *p*-phenylenediamine gave positive results in some, but not all, tests with strains TA98 (Garrigue et al., 2006; Assmann et al., 1997; Lee et al., 1986; Rojanapo et al., 1986; Thompson et al., 1983; Dunkel and Simmon, 1980; Degawa et al., 1979) and TA98NR (nitroreductase-deficient mutant) (Chung et al., 1996), TA1538 (Thompson et al., 1983; Dunkel and Simmon, 1980), and in a single test of TA1535 (Dunkel and Simmon, 1980). Some studies suggested that positive tests could have resulted from the reaction of *p*-phenylenediamine with aged (1−4 hours) dimethylsulfoxide (DMSO) (Burnett et al., 1982), chemical contaminants (Crebelli et al., 1981), light irradiation (Mosley-Foreman et al., 2008), or hydrogen peroxide (Ames et al., 1975). Yasunaga et al. (2006) observed a positive response when *p*-phenylenediamine was tested for induction of the bacterial SOS response system in *S. typhimurium* strain TA1535/pSK1002.

In genotoxicity assays using *Drosophila*, uncontaminated *p*-phenylenediamine did not induce sex-linked recessive lethal mutations (Blijleven, 1981), but did increase the frequency of mutant clones in zeste-white (UZ), white-ivory, and wing-spot assays (Batiste-Alentorn et al., 1995). In mammalian cell systems, *p*-phenylenediamine gave mixed results in tests of mutagenicity, but did consistently produce deoxyribonucleic acid (DNA) damage and clastogenic effects. No increase in the frequency of mutations was seen in Chinese hamster ovary (CHO) cells incubated with *p*-phenylenediamine dihydrochloride (Oshiro et al., 1991) with or without metabolic activation. Two studies of mouse lymphoma (L5178Y) cells incubated with *p*-phenylenediamine reported increased frequency of mutations (Mitchell et al., 1988; Myhr and Caspary, 1988). One study (Garrigue et al., 2006) reported no increase. *p*-Phenylenediamine was shown to induce DNA damage in comet and TUNEL assays using Mardin-Darby canine kidney cells (Chen et al., 2010), SV40 immortalized human urothelial cells (Huang et al., 2007), human lymphocytes (Chye et al., 2008), and HaCaT immortalized human keratinocytes (Zanoni et al., 2015). *p*-Phenylenediamine was observed to increase the percentage of CHO-K1 cells with chromosomal aberrations (CAs) in the absence of S9 (Chung et al., 1996; Chung et al., 1995) and the mean number of sister chromatid exchanges (SCEs) in CHO cells tested both with and without metabolic activation (Lee et al., 1986). An increased frequency of micronuclei was observed in CHO cells treated with *p*-phenylenediamine dihydrochloride without metabolic activation (Oshiro et al., 1991) and in mitogen-stimulated human lymphocytes with and without S9 (Garrigue et al., 2006).

In in vivo studies, *p*-phenylenediamine did not induce dominant lethal mutations in male Charles River CD rats exposed to intraperitoneal (i.p.) doses of 20 mg/kg, three times per week for 8 weeks prior to mating (Burnett et al., 1977). In contrast to the increased frequencies of micronuclei seen in mammalian cells in vitro (Garrigue et al., 2006; Oshiro et al., 1991), no increase in micronuclei was observed in bone marrow smears obtained from CFY rats exposed to

two gavage doses of 300 mg/kg *p*-phenylenediamine (Hossack and Richardson, 1977), or in CD-1 mice given a single i.p. injection of up to 100 mg/kg *p*-phenylenediamine dihydrochloride (Soler-Niedziela et al., 1991). Similarly, no increase in DNA damage assessed by comet assay was observed in isolated liver and stomach cells of S-D rats exposed to three gavage doses of 25–100 mg/kg-day *p*-phenylenediamine dihydrochloride (De Boeck et al., 2015).

Metabolism/Toxicokinetic Studies

The absorption, distribution, metabolism, and excretion of ^{14}C ring-labelled *p*-phenylenediamine dihydrochloride was evaluated in male and female rats and mice given single gavage doses of 60 or 600 µmol/kg or an intravenous (i.v.) dose of 600 µmol/kg (Ioannou and Matthews, 1985). Based on radioactivity in urine and feces collected over 72 hours postdosing, absorption of *p*-phenylenediamine dihydrochloride was nearly complete after oral dosing, with no indication of species or sex differences. Dose-dependence was noted, with a larger proportion of the lower dose excreted in feces; the authors suggested that this could be due to adsorption of the chemical to stomach contents or alterations in the metabolite profile that led to greater excretion in bile or lesser enterohepatic recycling. Evaluation of biliary excretion after i.v. dosing with 6, 60, or 600 µmol/kg *p*-phenylenediamine dihydrochloride showed that biliary excretion was inversely proportional to dose (59.3, 38.5, and 26.7% of administered dose excreted in the bile at 6, 60, and 600 µmol/kg, respectively), suggesting the possibility that metabolism may be saturated at higher doses.

Radioactivity was distributed to major tissues (blood, liver, kidney, skin, and muscle) in proportion to their volume (adipose tissue was an exception, as it contained a lower proportion than predicted by its volume) (Ioannou and Matthews, 1985). After i.v. administration, clearance from tissues was rapid in the first 2 hours after dosing, and slower thereafter. Small species- and sex-related differences in distribution were observed, most notably higher residual radioactivity in all female rat tissues at 72 hours compared to male rats; higher radioactivity in female mouse muscle compared with male at 72 hours; higher radioactivity in male mouse liver than in female at 72 hours; and lower radioactivity in mouse muscle compared with rats at virtually all time points.

Both rats and mice excreted most (62−87%) of the administered radioactivity in urine, and the remainder in feces, after both oral and i.v. dosing (see Table B-8). Most (~90%) of the excretion occurred during the first 24 hours postdosing. Metabolites in tissues, urine, and feces differed by sex and species (Ioannou and Matthews, 1985). Table B-9 shows the fractions of radioactivity in urine and feces that were excreted as parent compound or various metabolites. The table shows marked species differences in the excretion of metabolites B, C, H, and J, as well as sex differences (especially in mice) in the excretion of metabolites A, B, E, and F. The study authors determined that at least four of the urine or biliary metabolites were hydrolyzed by sodium hydroxide or hydrochloric acid (F, G, H, and K), indicating that the metabolites (not further specified) were conjugants of the parent compound. HPLC analysis of radioactivity in rat and mouse tissues after i.v. dosing showed that a number of metabolites (C, E, F, H, J, and K) were present in significant proportions in liver, muscle, skin, adipose, kidney, and blood.

The absorption, plasma kinetics, metabolism, and excretion of *p*-phenylenediamine has been evaluated in humans exposed via a normal salon hair coloring procedure. Nohynek et al. (2015) and Hueber-Becker et al. (2004) exposed human subjects (n = 28 males and 4 females; n = 8 males, respectively) to [^{14}C]-*p*-phenylenediamine-containing hair dye. Mean plasma C_{max}

values were 132.6 and 97.4 ng/mL, and mean $AUC_{0-\infty}$ values were 1,415 and 966 *p*-phenylenediamine$_{eq}$/mL-hour in subjects dermally exposed to dye containing 2.0 and 1.0% *p*-phenylenediamine, respectively (Nohynek et al., 2015). The predominant metabolite detected in plasma and urine was *N,N′*-diacetylated-*p*-phenylenediamine. Total urinary excretion over 48 hours was 0.72 and 0.88% of the applied radioactivity in hair dye containing 2.0 and 1.0% *p*-phenylenediamine, respectively, but mainly occurred during the first 24 hours following exposure (Nohynek et al., 2015). Minimal excretion occurred through feces (0.04%) (Hueber-Becker et al., 2004). No major differences were noted between sexes. The mean elimination half-life ($T_{1/2}$) was 7.8 hours in subjects dermally exposed to dye containing either 1.0 or 2.0% *p*-phenylenediamine.

Mode-of-Action/Mechanistic Studies

Modes of action (MOAs) leading to *p*-phenylenediamine-induced toxicity are uncertain and have not been described in detail. In vitro studies suggest *p*-phenylenediamine treatment increases oxidative damage through the induction of reactive oxygen species (ROS). Elyoussoufi (2013) found increased lipid peroxidation (measured as malondialdehyde, 23 µM) in neutrophils treated with *p*-phenylenediamine. Chen et al. (2010) also reported increased ROS in *p*-phenylenediamine treated canine kidney cells (37.5 µg/mL), which could be decreased by pretreatment with Vitamin C or E. Depletion of glutathione levels and increases in malondialdehyde have been reported in serum samples from *p*-phenylenediamine poisoning patients (Srinivas et al., 2010).

Administration of ^{14}C ring-labelled *p*-phenylenediamine in the diet (1,000 ppm) for 6 weeks did not increase the number or size of glutathione-*S*-transferase placental form-positive ($GST\text{-}P^+$) foci (Ito et al., 1988) or γ-glutamyl transferase (GGT)-positive liver foci (Ogiso et al., 1984, abstract only) in male F344 rats pretreated with an initiating dose of diethylnitrosamine (200 mg/kg) via i.p. injection. These results suggest *p*-phenylenediamine is not a liver tumor promoter, which is consistent with the absence of liver carcinogenicity in rats or mice exposed chronically to *p*-phenylenediamine in the diet (Imaida et al., 1983; NCI, 1979).

Acute Toxicity

The acute lethality of *p*-phenylenediamine administered orally has been examined in rats (Lloyd et al., 1977; Litton Bionetics, 1976; Rhone-Poulenc, 1951; Woodard, 1951) and mice (Rhone-Poulenc, 1951; Woodard, 1951); the median lethal doses (LD_{50}) values were estimated to be 180 mg/kg in mice and between 75 and 189 mg/kg in rats. The lowest doses at which deaths occurred in these studies were 59.6 mg/kg in rats and 79.4 mg/kg in mice (Rhone-Poulenc, 1951; Woodard, 1951). The 4-hour inhalation median lethal concentration (LC_{50}) for *p*-phenylenediamine was estimated to be 920 mg/m^3; however, condensation of the *p*-phenylenediamine test material was observed at concentrations ≥540 mg/m^3, rendering the exposure concentrations uncertain (Haskell Laboratories, 1982). The lowest concentration associated with mortality was 300 mg/m^3 (Haskell Laboratories, 1982).

As shown in Table 4B, overt rhabdomyolysis was observed in mice given 35 mg/kg by nasogastric tube (Averbukh et al., 1989) and in dogs exposed to oral doses ≥50 mg/kg (Yabe et al., 1991). These observations are consistent with the effects seen in human *p*-phenylenediamine poisonings and the effects reported in the short-term-duration exposure study in rats (Toxicol Laboratories, 1993).

Liver and kidney toxicity were also reported in rats after acute exposure to *p*-phenylenediamine. Ahmed (2011) (abstract only) reported that single oral doses of 10 and 20 mg (~54−110 mg/kg assuming body weight of 0.18 kg) resulted in increased plasma liver enzymes (AST and ALT) and liver lesions consisting of vacuolation, irregular nuclear staining, vascular congestion, and lymphocyte infiltration. In Wistar rats, single oral doses ≥20 mg/kg resulted in glomerular congestion with tubular necrosis and intertubular hemorrhages evident within 3 hours after exposure (Reddy et al., 2012).

Haskell Laboratories (1990) found no evidence of neurotoxicity in rats assessed for motor activity, FOB, forelimb and hindlimb grip strength, and foot splay after single oral doses of *p*-phenylenediamine up to 80 mg/kg.

Other Routes

Similar to the effects seen in the companion oral study, renal lesions consisting of glomerular congestion, intertubular hemorrhage, tubular necrosis, mononuclear infiltration, and tubular epithelial cell proliferation were found in Wistar rats given single i.p. doses of 18 or 37 mg/kg and sacrificed 3 days or 3 hours later, respectively (Reddy et al., 2012).

Bharali and Dutta (2012a), Bharali et al. (2012), and Bharali and Dutta (2012b) conducted short-term- and subchronic-duration studies of S-D rats exposed to *p*-phenylenediamine via skin painting. In rats exposed for 60 days to 1, 2, or 3 mg/kg, body weights were decreased (9−13%) compared with controls, but the changes were not statistically significant. Relative, but not absolute, kidney weight was increased at the highest dose, potentially as a function of the decreased body weight. Hematology findings were indicative of hemolytic anemia (decreased RBC count, Hb, and mean corpuscular hemoglobin [MCH], and increased reticulocyte count) (Bharali and Dutta, 2012a). Serum CPK was increased at all doses, and serum creatinine was increased at the high dose. Microscopic lesions in the kidneys of all treated rats (but no controls) included extensive tubular necrosis with cytoplasmic vacuolation and desquamation of the epithelium from the surrounding basement membrane, as well as extensive hemosiderin deposits in the renal cortex (Bharali et al., 2012). At the highest dose, tubular interstitial inflammation with infiltration of hyperchromic leukocytes was noted. In male rats exposed by skin painting to 1, 2, or 3 mg/kg-day for 90 days, decreased sperm count, increased abnormal sperm morphology, and decreased testicular weight were observed at doses ≥2 mg/kg-day. Testicular histopathology findings were noted at the high dose and included increased germ cell apoptosis and sloughing of testicular cellular layers (Bharali and Dutta, 2012b). A similar study evaluated effects following 30 days of continuous topical application in rats and reported hepatotoxicity including dose-dependent increased in serum ALT, AST, and ALP, histopathological changes, and statistically significantly increased absolute and relative liver weight (Bharali and Dutta, 2009). Dermal exposure of *p*-phenylenediamine to male albino guinea pigs for 5 and 7 days resulted in increases in activity of serum AST, ALT, tyrosinase, GGT, and β-glucuronidase (Mathur et al., 1990).

Several other studies examined nephrotoxicity and hepatotoxicity associated with dermal exposure to *p*-phenylenediamine in rats. Alalwani (2013), Hummadi (2012), and Hummdi (2012) reported statistically significant increases in body weight and absolute and relative kidney and liver weight in female rats after 6 months of daily topical application of 0.5, 1, 3, or 6 mg/kg *p*-phenylenediamine. These changes were accompanied by significantly increased serum creatinine and BUN and histopathological changes in the kidney, such as glomerular

hypertrophy, necrosis, and damaged proximal convoluted tubules (Hummadi, 2012; Hummdi, 2012). Histopathological changes were also observed in the liver, including hepatic necrosis and congestion of the blood sinusoids and central vein (Alalwani, 2013). These studies report increased mortality of 10% in the low-dose group and 55% in the high-dose group.

DERIVATION OF PROVISIONAL VALUES

Tables 5 and 6 present summaries of noncancer and cancer references values, respectively.

Table 5. Summary of Noncancer Reference Values for *p*-Phenylenediamine (CASRN 106-50-3)

Toxicity Type (units)	Species/Sex	Critical Effect	*p*-Reference Value	POD Method	POD (HED)	UF_C	Principal Study
Screening subchronic p-RfD (mg/kg-d)	Rat/F	Increased relative kidney and liver weight	1×10^{-2}	NOAEL and $BMDL_{10}$	0.96	100	Toxicol Laboratories (1995)
Screening chronic p-RfD (mg/kg-d)	Rat/F	Increased relative kidney and liver weight	1×10^{-3}	NOAEL and $BMDL_{10}$	0.96	1,000	Toxicol Laboratories (1995)
Subchronic p-RfC (mg/m^3)	NDr						
Chronic p-RfC (mg/m^3)	NDr						

$BMDL_{10}$ = 10% benchmark dose lower confidence limit; F = female(s); HED = human equivalent dose; NDr = not determined; NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfC = provisional reference concentration; p-RfD = provisional reference dose; UF_C = composite uncertainty factor.

Table 6. Summary of Cancer Reference Values for *p*-Phenylenediamine (CASRN 106-50-3)

Toxicity Type (units)	Species/Sex	Tumor Type	Cancer Value	Principal Study
p-OSF $(mg/kg\text{-}d)^{-1}$	NDr			
p-IUR $(mg/m^3)^{-1}$	NDr			

NDr = not determined; p-IUR = provisional inhalation unit risk; p-OSF = provisional oral slope factor.

DERIVATION OF ORAL REFERENCE DOSES

Human studies of oral exposure to *p*-phenylenediamine are limited to case reports of poisonings by suicide or attempted suicide. Available animal studies of *p*-phenylenediamine include an unpublished 14-day gavage study in rats (Toxicol Laboratories, 1993); 7- and 12-week dose range-finding dietary studies of rats and mice (Imaida et al., 1983; NCI, 1979) that

evaluated limited endpoints, an unpublished 90-day gavage neurotoxicity study in rats (Dupont Chem, 1992); an unpublished 13-week gavage study in rats (Toxicol Laboratories, 1995); two chronic-duration dietary studies of carcinogenicity in rats (Imaida et al., 1983; NCI, 1979), a chronic-duration dietary study of carcinogenicity in mice (NCI, 1979); a published teratogenicity study in rats exposed by gavage (Re et al., 1981); an unpublished teratogenicity study in rats exposed by gavage, available only as summarized in a secondary source (ECHA, 2005); and a transplacental carcinogenicity study in rats, available only as translated by Dupont Chem (1992).

Derivation of Subchronic or Chronic Provisional RfD (p-RfD)

Available information on the toxicity of *p*-phenylenediamine is not considered to be sufficiently reliable for use in derivation of subchronic and chronic provisional reference doses (p-RfDs) because the lowest effect levels in the database are from unpublished subchronic-duration rat studies (Toxicol Laboratories, 1995; Dupont Chem, 1992). Other subchronic-duration studies evaluated very limited endpoints, as did the chronic-duration studies, which were designed to evaluate carcinogenicity. However, the unpublished study by (Toxicol Laboratories, 1995) is suitable for the derivation of a "screening level" value for subchronic and chronic oral exposure. Appendix A provides details on the screening subchronic and chronic p-RfDs.

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

No studies of humans or animals exposed to *p*-phenylenediamine via inhalation have been identified in the available literature (other than an acute inhalation lethality study in rats), precluding derivation of provisional reference concentrations (p-RfCs).

CANCER WEIGHT-OF-EVIDENCE (WOE) DESCRIPTOR

Table 7 provides the cancer WOE descriptor for *p*-phenylenediamine.

Table 7. Cancer WOE Descriptor for *p*-Phenylenediamine (CASRN 106-50-3)

Possible WOE Descriptor	Designation	Route of Entry (oral, inhalation, or both)	Comments
"Carcinogenic to Humans"	NS	NA	There are no human data to support this descriptor.
"Likely to Be Carcinogenic to Humans"	NS	NA	There are no human or animal data to support this descriptor.
"Suggestive Evidence of Carcinogenic Potential"	NS	NA	There are no human or animal data to support this descriptor.
"Inadequate Information to Assess Carcinogenic Potential"	**Selected**	**Both**	**There is little pertinent information available to assess the carcinogenic potential of *p*-phenylenediamine.**
"Not Likely to Be Carcinogenic to Humans"	NS	NA	The available human and animal data do not support this descriptor.

NA = not applicable; NS = not selected; WOE = weight-of-evidence.

Under the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), there is *"Inadequate Information to Assess Carcinogenic Potential"* for *p*-phenylenediamine by both oral and inhalation exposure (see Table 7).

The few studies available to assess the potential for carcinogenicity due to exposure to *p*-phenylenediamine are inadequate due to poor study quality, design, and reporting. Available human data on the potential carcinogenicity of *p*-phenylenediamine are limited to two case-control epidemiology studies of hair dye users (Ros et al., 2012; Tavani et al., 2005). Neither study verified exposure to *p*-phenylenediamine in the study populations nor observed a significant association between hair dye use and cancer. Animal studies of carcinogenicity include two chronic-duration dietary studies in rats (Imaida et al., 1983; NCI, 1979), a chronic-duration dietary study in mice (NCI, 1979), and a transplacental carcinogenicity study in rats [Holmberg et al. (1983) as translated in Dupont Chem (1992)]. Chronic (≥2 years) dietary exposure of rats to *p*-phenylenediamine (Imaida et al., 1983) or rats and mice to *p*-phenylenediamine dihydrochloride (NCI, 1979) did not significantly increase the incidence of any tumor type. However, these studies were limited by small control-group sizes. The study by Imaida et al. (1983) was further limited by poor reporting and low survival in all groups. The NCI (1979) studies failed to achieve the MTD in male rats, male mice, and female mice. There was a slight increase in the frequency of alveolar adenomas (18/88 vs. 12/86 in negative controls, $p = 0.04$) in the female offspring of mice exposed to *p*-phenylenediamine (30 mg/kg-day) via gavage during gestation that was statistically significant with respect to the latency time ("time to tumor appearance") [Holmberg et al. (1983) as translated in Dupont Chem (1992)]. However, the increase in alveolar adenomas was not statistically significant when analyzed "among groups, regardless of the time factor." In addition, there was no increase in alveolar adenomas observed in male offspring or in maternal animals.

p-Phenylenediamine has been tested in a number of in vitro and in vivo genotoxicity tests (see Table 4A) with mixed results. Results of tests for mutation of *S. typhimurium* strains have largely been negative in the absence of metabolic activation and inconsistent in tests with activation. There is some indication of increased mutation following treatment with *p*-phenylenediamine in *Drosophila* and in mammalian cell systems. *p*-Phenylenediamine consistently produced DNA damage and clastogenic effects (i.e., CAs, SCEs, micronuclei [MN]) in mammalian cells. However, *p*-phenylenediamine did not induce dominant lethal mutagenicity, MN, or DNA damage in in vivo animal tests. The inconsistent results from genotoxicity tests do not support the potential for carcinogenicity due to exposure to *p*-phenylenediamine.

DERIVATION OF PROVISIONAL CANCER POTENCY VALUES

As described above, there is inadequate information to assess the carcinogenic potential of *p*-phenylenediamine; thus, provisional cancer potency values are not derived.

APPENDIX A. SCREENING PROVISIONAL VALUES

For the reasons noted in the main document, provisional toxicity values for *p*-phenylenediamine could not be derived. 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 main documents to ensure their appropriateness within the limitations detailed in the document. Users of screening toxicity values in an appendix to a provisional peer-reviewed toxicity value (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 Heath Risk Technical Support Center.

DERIVATION OF SCREENING SUBCHRONIC PROVISIONAL REFERENCE DOSE (p-RfD)

The unpublished subchronic-duration study in adult rats exposed via gavage to *p*-phenylenediamine is considered the principal study for use in deriving the screening subchronic provisional reference dose (p-RfD) (Toxicol Laboratories, 1995). The critical effects from this study include increased relative liver and relative kidney weight in female rats.

The subchronic-duration study by Toxicol Laboratories (1995) reported daily administration of *p*-phenylenediamine by gavage to Crl:CD(SD)BR rats (15/sex/dose) for 13 weeks. This study was included in an unpublished technical report conducted according to Good Laboratory Practice (GLP) standards. It is a well-conducted study with comprehensive assessment of body weight, hematology, serum chemistry, urinalysis, organ weights, and gross and microscopic pathology of various organs. The short-term-duration gavage study in rats was not selected as the principal study because of the brief exposure duration (14 days). Other subchronic-duration studies evaluated limited endpoints and did not report effects at lower levels of exposure than the principal study. The developmental studies (ECHA, 2005; Re et al., 1981) were also not chosen as the principal study because the reported effects resulted from higher doses than those producing organ-weight changes in adult rats. In the published developmental toxicity study by Re et al. (1981), a lowest-observed-adverse-effect level (LOAEL) of 20 mg/kg-day and a no-observed-adverse-effect level (NOAEL) of 15 mg/kg-day were identified based on reduced body-weight gain in rat dams; maternal mortality occurred at the next higher dose of 30 mg/kg-day (3/25 dams died). In the developmental toxicity study available only as reported in the ECHA (2005) database, a fetal LOAEL and NOAEL of 20 and 10 mg/kg-day, respectively, were identified for a statistically nonsignificant decrease in fetal body weight; however, the biological significance is uncertain because the magnitude of change was not reported.

Endpoints reported by Toxicol Laboratories (1995) to be significantly different from controls (either statistically significant or of such magnitude to be considered biologically significant) include increased liver weight (absolute and relative) in male and female rats, increased thyroid weight (absolute and relative) in male rats, and increased kidney weight (absolute and relative) in female rats (see Table A-1). The study authors noted that the thyroid

weights of the controls were unusually low and the EPA notes no apparent dose-response relationship; thus, the changes in thyroid weight were not considered to be indicative of an effect of *p*-phenylenediamine exposure. The EPA identified a LOAEL of 8 mg/kg-day based on >10% increase in relative kidney weight and relative liver weight in female rats and a NOAEL of 4 mg/kg-day.

Table A-1. Selected Non-neoplastic Effects in Male and Female Crl:CD(SD)BR Rats Exposed to *p*-Phenylenediamine (CASRN 106-50-3) via Gavage for 13 Weeks[a]

Dose (mg/kg-d)	0	2	4	8	16
Male					
Number of animals	15	15	15	15	15
Absolute liver weight (g)	21.76 ± 3.6[b]	21.29 ± 1.9 (−2%)[c]	20.84 ± 2.7 (−4%)	21.58 ± 3.2 (−1%)	24.43 ± 3.4* (12%)
Relative liver weight (% body weight)	4.20 ± 0.3	4.25 ± 0.4 (1%)	4.19 ± 0.4 (−0.2%)	4.47 ± 0.4* (6%)	4.72 ± 0.4* (12%)
Absolute thyroid weight (mg)	17 ± 2.7	21 ± 3.3*	23 ± 2.6*	21 ± 3.0*	23 ± 3.2*
Relative thyroid weight (% body weight × 1,000)	3.28 ± 0.4	4.14 ± 0.7*	4.66 ± 0.5*	4.42 ± 0.5*	4.44 ± 0.8*
Female					
Number of animals	15	15	15	15	15
Absolute liver weight (g)	10.22 ± 1.1	10.66 ± 1.4 (4%)	10.55 ± 1.5 (3%)	10.94 ± 1.2 (7%)	11.46 ± 0.9 (12%)
Relative liver weight (% body weight)	3.53 ± 0.2	3.64 ± 0.3 (3%)	3.76 ± 0.3 (7%)	3.91 ± 0.7 (11%)	3.90 ± 0.4 (10%)
Absolute kidney weight (g)	2.12 ± 0.2	2.13 ± 0.2 (0.5%)	2.19 ± 0.3 (3%)	2.30 ± 0.2* (8%)	2.45 ± 0.2* (16%)
Relative kidney weight (% body weight)	0.73 ± 0.06	0.73 ± 0.06 (0%)	0.78 ± 0.05 (7%)	0.82 ± 0.13* (12%)	0.83 ± 0.08* (14%)

[a]Toxicol Laboratories (1995).
[b]Mean ± standard deviation.
[c]Percent change from control.

*Significantly different from control at $p \leq 0.05$, as reported by the study authors.

Potential points of departure (PODs) from Toxicol Laboratories (1995) were modeled using the EPA's Benchmark Dose Software (BMDS, Version 2.6) (see Table A-2). The results are summarized in Table A-2. Benchmark dose (BMD) modeling did not result in a suitable model fit for increased relative kidney weight in female rats.

Table A-2. Potential Subchronic PODs in Male and Female Rats Exposed to *p*-Phenylenediamine (CASRN 106-50-3) via Gavage for 13 Weeks[a]

Endpoint	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Animal POD[b] (mg/kg-d)
Male			
Absolute liver weight	8	16	$BMDL_{10}$ = 10
Relative liver weight	8	16	$BMDL_{10}$ = 8
Female			
Absolute liver weight	8	16	$BMDL_{10}$ = 9
Relative liver weight	**4**	**8**	**$BMDL_{10}$ = 4[c]**
Absolute kidney weight	8	16	$BMDL_{10}$ = 5
Relative kidney weight	**4**	**8**	**NOAEL = 4[c]**

[a]Toxicol Laboratories (1995).
[b]BMD modeling results are described in detail in Appendix C.
[c]Chosen as the critical effect for derivation of the screening subchronic p-RfD.

BMD = benchmark dose; $BMDL_{10}$ = 10% benchmark dose lower confidence limit; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfD = provisional reference dose.

The lowest PODs following subchronic exposure to *p*-phenylenediamine are for >10% increase in relative liver weight (10% benchmark dose lower confidence limit [$BMDL_{10}$] = 4 mg/kg-day) and relative kidney weight (NOAEL = 4 mg/kg-day) in female rats. This POD is protective of other effects observed following *p*-phenylenediamine exposure including absolute liver- and kidney-weight changes in female rats and liver-weight (absolute and relative) changes in male rats. In addition, these effects are consistent with effects observed in other studies of *p*-phenylenediamine exposure and coherent with other *p*-phenylenediamine-induced effects. Renal failure is a hallmark of human exposure to *p*-phenylenediamine (see discussion in "Human Studies" section) and was seen in rats following acute exposure (Reddy et al., 2012), supporting inferences that effects on the kidney are treatment related and relevant to humans. In addition, similar effects on kidney weight were accompanied by functional and pathological changes following administration of *p*-phenylenediamine to rats by other routes of exposure (i.e., dermal) (Bharali et al., 2012), strengthening the evidence that the increase in kidney weight from oral exposure is treatment related and biologically significant. Further evidence also supports the biological significance of changes in the liver. Elevated serum liver enzymes have been reported in human studies and in rats following acute and short-term exposure to *p*-phenylenediamine (Ahmed, 2011; Toxicol Laboratories, 1993). In addition, consistent with effects following subchronic exposure in females, absolute and relative liver weight increased by >10% after short-term and subchronic exposure to *p*-phenylenediamine in male rats. The organ-weight changes reported in Toxicol Laboratories (1995) were not accompanied by significant changes in body weight that would confound the increase in relative organ weight, reducing the uncertainty in these organ changes (see Table B-3). Based on the consistency and coherence in these effects across studies and biological significance of the changes, **the NOAEL for increased relative kidney weight and**

$BMDL_{10}$ for increased relative liver weight in female rats (4 mg/kg-day) is selected as the POD for derivation of the screening subchronic p-RfD.

Dosimetric Adjustment

In *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b), the Agency endorses a hierarchy of approaches to derive human equivalent oral exposures from data from laboratory animal species, with the preferred approach being physiologically based toxicokinetic modeling. Other approaches may include using some chemical-specific information, without a complete physiologically based toxicokinetic model. In lieu of chemical-specific models or data to inform the derivation of human equivalent oral exposures, the EPA endorses body-weight scaling to the 3/4 power (i.e., $BW^{3/4}$) as a default to extrapolate toxicologically equivalent doses of orally administered agents from all laboratory animals to humans for the purpose of deriving an RfD under certain exposure conditions. More specifically, the use of $BW^{3/4}$ scaling for deriving an RfD is recommended when the observed effects are associated with the parent compound or a stable metabolite but not for portal-of-entry effects.

A validated human physiologically based toxicokinetic model for *p*-phenylenediamine is not available for use in dose extrapolation from animals to humans. Furthermore, kidney-weight changes are not portal-of-entry effects. Therefore, scaling by $BW^{3/4}$ is relevant for deriving human equivalent doses (HEDs) for this effect.

Following U.S. EPA (2011b) guidance, the NOAEL and $BMDL_{10}$ of 4 mg/kg-day in adult rats is converted to an HED through application of a dosimetric adjustment factor (DAF)[8] derived as follows:

$$DAF = (BW_a^{1/4} \div BW_h^{1/4})$$

where:

DAF = dosimetric adjustment factor
BW_a = animal body weight
BW_h = human body weight

Using a BW_a of 0.25 kg for rats and a BW_h of 70 kg for humans (U.S. EPA, 1988), the resulting DAF is 0.24. Applying this DAF to the POD identified for the critical effect in rats yields a POD (HED) as follows:

$$\begin{aligned} \text{POD (HED)} &= \text{POD (mg/kg-day)} \times \text{DAF} \\ &= 4 \text{ mg/kg-day} \times 0.24 \\ &= 0.96 \text{ mg/kg-day} \end{aligned}$$

The screening subchronic p-RfD for *p*-phenylenediamine is derived as follows:

[8]As described in detail in *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b), rate-related processes scale across species in a manner related to both the direct ($BW^{1/1}$) and allometric scaling ($BW^{3/4}$) aspects such that $BW^{3/4} \div BW^{1/1} = BW^{-1/4}$, converted to a $DAF = BW_a^{1/4} \div BW_h^{1/4}$.

Screening Subchronic p-RfD = POD (HED) ÷ UF_C
= 0.96 mg/kg-day ÷ 100
= **1×10^{-2} mg/kg-day**

The composite uncertainty (UF_C) for the screening subchronic p-RfD for *p*-phenylenediamine is 100, as summarized in Table A-3.

Table A-3. Uncertainty Factors for the Screening Subchronic p-RfD for *p*-Phenylenediamine (CASRN 106-50-3)

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied to account for uncertainty in characterizing the toxicokinetic or toxicodynamic differences between rats and humans following oral *p*-phenylenediamine treatment. The toxicokinetic uncertainty has been accounted for by calculating an HED through application of a DAF as outlined in the EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b).
UF_H	10	A UF_H of 10 is applied for intraspecies variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess toxicokinetic and toxicodynamic variability of *p*-phenylenediamine in humans.
UF_D	3	A UF_D of 3 is applied to account for deficiencies and uncertainties in the database, specifically the lack of reproductive toxicity studies.
UF_L	1	A UF_L of 1 is applied because the POD is a NOAEL, not a LOAEL.
UF_S	1	A UF_S of 1 is applied because the POD comes from a subchronic-duration exposure study.
UF_C	100	Composite UF = $UF_A \times UF_H \times UF_D \times UF_L \times UF_S$.

DAF = dosimetric adjustment factor; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfD = provisional reference dose; UF = uncertainty factor.

DERIVATION OF SCREENING CHRONIC PROVISIONAL REFERENCE DOSE (p-RfD)

The unpublished subchronic-duration study (Toxicol Laboratories, 1995) used to derive the screening subchronic p-RfD, was also selected for use in deriving the screening chronic p-RfD.

The available chronic-duration studies of *p*-phenylenediamine were conducted as cancer bioassays with limited assessment of noncancer endpoints (Imaida et al., 1983; NCI, 1979). LOAELs in these chronic-duration dietary studies (i.e., 68.78−92.1 mg/kg-day) for decreases in body weight, were consistent with subchronic-duration dietary range-finding studies (i.e., 59.7−200 mg/kg-day) performed in advance of these studies, but high in relation to effect levels in subchronic-duration and acute studies performed by gavage (e.g., increased liver and kidney weight at 8 mg/kg-day).

In addition, the chronic-duration studies were of poor quality and reporting (Imaida et al., 1983; NCI, 1979). The NCI (1979) studies did not include a comprehensive evaluation of noncancer effects; hematology, clinical chemistry, and organ weights were not examined or recorded. The study by Imaida et al. (1983) was also limited by low survival in all groups,

including controls. Rats of both sexes exhibited dose-related and biologically significant (>10%) increases in absolute liver and kidney weights, with no NOAEL identified in female rats (LOAEL = 46 mg/kg-day) (see Table B-6). These increases are consistent with the increased kidney and liver weight from the subchronic-duration rat gavage study (Toxicol Laboratories, 1995); however, due to the small numbers of surviving controls, this comparison is difficult to interpret. Even though the study by Imaida et al. (1983) is not appropriate for derivation of the chronic p-RfD, it provides qualitative evidence for effects in the kidney and liver. Due to the poor study quality and incomplete endpoint examination, the chronic-duration studies were not selected for derivation of the screening chronic p-RfD.

The developmental studies (ECHA, 2005; Re et al., 1981) were also not chosen as the principal study because the reported effects resulted from higher doses than the dose producing organ-weight changes in adult rats following subchronic exposure. In the published developmental toxicity study by Re et al. (1981), a LOAEL of 20 mg/kg-day and NOAEL of 15 mg/kg-day were identified based on reduced body-weight gain in rat dams; maternal mortality occurred at the next higher dose of 30 mg/kg-day (3/25 dams died). In the developmental toxicity study available only as reported in the ECHA (2005) database, a fetal LOAEL and NOAEL of 20 and 10 mg/kg-day, respectively, were identified in the database for a statistically nonsignificant decrease in fetal body weight; however, the biological significance is uncertain because the magnitude of change was not reported.

Therefore, the NOAEL and $BMDL_{10}$ of 4 mg/kg-day (POD [HED] = 0.96 mg/kg-day) for increased relative kidney and liver weight in female rats from the subchronic-duration rat gavage study by Toxicol Laboratories (1995), used as the POD for the screening subchronic p-RfD, is used as the POD for the screening chronic p-RfD, which is derived as follows:

$$\begin{aligned} \textbf{Screening Chronic p-RfD} &= \text{POD (HED)} \div UF_C \\ &= 0.96 \text{ mg/kg-day} \div 1{,}000 \\ &= \mathbf{1 \times 10^{-3} \text{ mg/kg-day}} \end{aligned}$$

The UF_C for the screening chronic p-RfD for *p*-phenylenediamine is 1,000, as summarized in Table A-4.

Table A-4. Uncertainty Factors for the Screening Chronic p-RfD for *p*-Phenylenediamine (CASRN 106-50-3)

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied to account for uncertainty in characterizing the toxicokinetic or toxicodynamic differences between rats and humans following oral *p*-phenylenediamine treatment. The toxicokinetic uncertainty has been accounted for by calculating an HED through application of a DAF as outlined in the EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b).
UF_H	10	A UF_H of 10 is applied for intraspecies variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess toxicokinetic and toxicodynamic variability of *p*-phenylenediamine in humans.
UF_D	3	A UF_D of 3 is applied to account for deficiencies and uncertainties in the database, including limited assessment of noncancer endpoints in the dietary studies and lack of reproductive toxicity studies.
UF_L	1	A UF_L of 1 is applied because the POD is a NOAEL, not a LOAEL.
UF_S	10	A UF_S of 10 is applied to account for the extrapolation from less than chronic exposure.
UF_C	1,000	Composite UF = $UF_A \times UF_H \times UF_D \times UF_L \times UF_S$.

DAF = dosimetric adjustment factor; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfD = provisional reference dose; UF = uncertainty factor.

APPENDIX B. DATA TABLES

Table B-1. Selected Effects in Male and Female Crl:CD(SD)BR Rats Exposed to *p*-Phenylenediamine (CASRN 106-50-3) via Gavage for 14 Days[a]

Dose (mg/kg-d)	0	5	10	20	40
Male					
Number of animals	10	10	10	10	10
ALT (U/L)	48 ± 7.8[b]	49 ± 8.0	51 ± 7.7	62 ± 7.6*	58 ± 9.8*
AST (U/L)	84 ± 4.8	90 ± 11	95 ± 9.8*	112 ± 20*	96 ± 19*
CPK (U/L)	337 ± 86.2	327 ± 73.1	475 ± 147*	581 ± 115*	556 ± 276*
LDH (mg%)	283 ± 87	426 ± 85*	557 ± 208*	1,642 ± 903*	936 ± 805*
K (mmol/L)	3.9 ± 0.3	3.6 ± 0.4	4.1 ± 0.3	4.1 ± 0.2	4.3 ± 0.3*
Skeletal muscle myodegeneration	0/10	0/10	0/10	0/10	0/10
Female					
Number of animals	9[c]	10	10	10	10
ALT (U/L)	37 ± 9.9	39 ± 7.1	47 ± 8.0	41 ± 7.9	42 ± 7.3
AST (U/L)	80 ± 9.2	83 ± 7.7	98 ± 12*	91 ± 16*	93 ± 17*
CPK (U/L)	334 ± 193	320 ± 136	344 ± 76	357 ± 80	383 ± 195
LDH (mg%)	249 ± 60	301 ± 70	488 ± 299	596 ± 438	583 ± 520
K (mmol/L)	3.7 ± 0.3	3.9 ± 0.3	4.2 ± 0.5*	4.3 ± 0.6*	4.5 ± 0.4*
Skeletal muscle myodegeneration	0/10	0/10	0/10	0/10	3/10

[a]Toxicol Laboratories (1993).
[b]Mean ± standard deviation.
[c]One control female died during blood withdrawal on Day 13.

*Significantly different from control at $p \leq 0.05$, as reported by the study authors.

ALT = alanine aminotransferase; AST = aspartate aminotransferase; CPK = creatinine phosphokinase; K = potassium; LDH = lactate dehydrogenase.

Table B-2. Selected Effects in Male and Female Crl:CD(SD)BR Rats Exposed to *p*-Phenylenediamine (CASRN 106-50-3) via Gavage for 14 Days[a]

Dose (mg/kg-d)	0	5	10	20	40
Male					
Number of animals	10	10	10	10	10
Terminal body weight (g)	286 ± 27.7[b]	290 ± 18.2 (1%)[c]	279 ± 19.0 (−2%)	274 ± 19.2 (−4%)	275 ± 21.5 (−4%)
Absolute liver weight (g)	15.3 ± 1.75	15.2 ± 1.60 (−1%)	15.8 ± 1.72 (3%)	14.7 ± 1.48 (−4%)	17.0 ± 2.25 (11%)
Relative liver weight (% body weight)	5.34 ± 0.40	5.24 ± 0.38 (−2%)	5.66 ± 0.42 (6%)	5.38 ± 0.31 (0.7%)	6.19 ± 0.53* (16%)
Absolute thyroid weight (mg)	16 ± 2.5	17 ± 2.9	13 ± 1.8	16 ± 3.2	15 ± 3.7
Relative thyroid weight (% body weight × 1,000)	5.67 ± 1.08	5.68 ± 0.82	4.79 ± 0.81	5.79 ± 1.03	5.63 ± 1.40
Absolute heart weight (g)	1.13 ± 0.12	1.22 ± 0.11	1.14 ± 0.07	1.13 ± 0.14	1.18 ± 0.13
Relative heart weight (% body weight)	0.39 ± 0.02	0.42 ± 0.02*	0.41 ± 0.02*	0.41 ± 0.03	0.43 ± 0.03*
Female					
Number of animals	9[d]	10	10	10	10
Terminal body weight (g)	193 ± 12.4	195 ± 16.6 (1%)	187 ± 15.0 (−3%)	198 ± 19.7 (3%)	188 ± 16.7 (−3%)
Absolute liver weight (g)	9.46 ± 0.77	9.64 ± 1.25 (2%)	9.15 ± 0.90 (−3%)	9.92 ± 1.05 (5%)	9.81 ± 1.23 (4%)
Relative liver weight (% body weight)	4.89 ± 0.30	4.95 ± 0.48 (1%)	4.89 ± 0.34 (0%)	5.03 ± 0.41 (3%)	5.20 ± 0.26 (6%)
Absolute thyroid weight (mg)	11 ± 1.7	12 ± 1.6	12 ± 1.5	16 ± 2.1*	14 ± 1.2*
Relative thyroid weight (% body weight × 1,000)	5.96 ± 1.04	5.93 ± 0.82	6.57 ± 1.02	8.04 ± 1.17*	7.64 ± 0.69*
Absolute heart weight (g)	0.88 ± 0.08	0.90 ± 0.12	0.91 ± 0.21	0.90 ± 0.10	0.86 ± 0.09
Relative heart weight (% body weight)	0.46 ± 0.03	0.46 ± 0.5	0.48 ± 1.0	0.46 ± 0.4	0.46 ± 0.4

[a]Toxicol Laboratories (1993).
[b]Mean ± standard deviation.
[c]Percent change from control.
[d]One control female died during blood withdrawal on Day 13.

*Significantly different from control at $p \leq 0.05$, as reported by the study authors.

Table B-3. Selected Effects in Male and Female Crl:CD(SD)BR Rats Exposed to *p*-Phenylenediamine (CASRN 106-50-3) via Gavage for 13 Weeks[a]

Dose (mg/kg-d)	0	2	4	8	16
Male					
Number of animals	15	15	15	15	15
Terminal body weight (g)	516 ± 65.5[b]	502 ± 22.5 (−3%)[c]	497 ± 27.6 (−4%)	482 ± 50.6 (−7%)	516 ± 45.0 (0%)
Absolute liver weight (g)	21.76 ± 3.6	21.29 ± 1.9 (−2%)	20.84 ± 2.7 (−4%)	21.58 ± 3.2 (−1%)	24.43 ± 3.4* (12%)
Relative liver weight (% body weight)	4.20 ± 0.3	4.25 ± 0.4 (1%)	4.19 ± 0.4 (−0.2%)	4.47 ± 0.4* (6%)	4.72 ± 0.4* (12%)
Absolute thyroid weight (mg)	17 ± 2.7	21 ± 3.3*	23 ± 2.6*	21 ± 3.0*	23 ± 3.2*
Relative thyroid weight (% body weight × 1,000)	3.28 ± 0.4	4.14 ± 0.7*	4.66 ± 0.5*	4.42 ± 0.5*	4.44 ± 0.8*
Absolute kidney weight (g)	3.68 ± 0.4	3.63 ± 0.3 (−1%)	3.47 ± 0.2 (−6%)	3.48 ± 0.4 (−5%)	3.87 ± 0.4 (5%)
Relative kidney weight (% body weight)	0.71 ± 0.05	0.73 ± 0.07 (3%)	0.70 ± 0.04 (−1%)	0.73 ± 0.07 (3%)	0.75 ± 0.07 (6%)
Female					
Number of animals	15	15	15	15	15
Terminal body weight (g)	290 ± 26.8	292 ± 20.6 (1%)	280 ± 27.6 (−3%)	284 ± 33.9 (−2%)	297 ± 35.2 (2%)
Absolute liver weight (g)	10.22 ± 1.1	10.66 ± 1.4 (4%)	10.55 ± 1.5 (3%)	10.94 ± 1.2 (7%)	11.46 ± 0.9 (12%)
Relative liver weight (% body weight)	3.53 ± 0.2	3.64 ± 0.3 (3%)	3.76 ± 0.3 (7%)	3.91 ± 0.7 (11%)	3.90 ± 0.4 (10%)
Absolute thyroid weight (mg)	19 ± 2.9	19 ± 3.8	19 ± 2.3	18 ± 4.2	18 ± 3.8
Relative thyroid weight (% body weight × 1,000)	6.66 ± 1.0	6.40 ± 1.2	6.3 ± 0.9	6.52 ± 1.6	6.13 ± 1.3
Absolute kidney weight (g)	2.12 ± 0.2	2.13 ± 0.2 (0.5%)	2.19 ± 0.3 (3%)	2.30 ± 0.2* (8%)	2.45 ± 0.2* (16%)
Relative kidney weight (% body weight)	0.73 ± 0.06	0.73 ± 0.06 (0%)	0.78 ± 0.05 (7%)	0.82 ± 0.13* (12%)	0.83 ± 0.08* (14%)

[a]Toxicol Laboratories (1995).
[b]Mean ± standard deviation.
[c]Percent change from control.

*Significantly different from control at $p \leq 0.05$, as reported by the study authors.

Table B-4. Selected Effects in Male and Female Crl:CD®BR Rats Exposed to *p*-Phenylenediamine (CASRN 106-50-3) via Gavage for 90 Days[a]

Dose (mg/kg-d)	0	4	8	16
Male				
Incidence of wet chin[b]	4/12	6/12	6/12	11/12*
Female				
Incidence of wet chin	3/12	4/12	6/12	12/12*
Incidence of wet inguen/perineum	0/12	0/12	0/12	9/12*

[a]Dupont Chem (1992).
[b]Number affected/number exposed.

*Significantly different from control at $p \leq 0.05$, as reported by the study authors.

Table B-5. Selected Effects in Male and Female F344 Rats Exposed to *p*-Phenylenediamine (CASRN 106-50-3) via Diet for 12 Weeks[a]

Male					
Dose (mg/kg-d)	**0**	**50.0**	**100**	**200**	**400**
Mortality[b]	0/10	0/10	0/10	0/10	9/11
Terminal body weight (g)	307.9 ± 21.2[c]	291.7 ± 19.2 (−5.3%)[d]	278.0 ± 6.8** (−9.7%)	247.0 ± 14.2** (−20%)	130.0 ± 7.7** (−58%)
Absolute liver weight (g)	7.3 ± 0.7	7.1 ± 0.7 (−2.7%)	7.3 ± 0.4 (0%)	7.2 ± 0.9 (−1.4%)	4.3 ± 0.8** (−41%)
Female					
Dose (mg/kg-d)	**0**	**56.8**	**114**	**227**	**455**
Mortality	0/10	0/11	0/11	0/10	1/10
Terminal body weight (g)	170.6 ± 4.0	160.9 ± 6.0** (−5.7%)	150.7 ± 5.3** (−12%)	129.5 ± 8.3** (−24%)	81.0 ± 6.2** (−53%)
Absolute liver weight (g)	4.0 ± 0.1	3.9 ± 0.2 (−2.5%)	4.1 ± 0.5 (2.5%)	3.9 ± 0.5 (−2.5%)	3.0 ± 0.2** (−25%)

[a]Imaida et al. (1983).
[b]Number dead/number exposed.
[c]Mean ± SD.
[d]Percent change from control.

**Significantly different from control at $p < 0.001$ based on *t*-test performed for this review.

SD = standard deviation.

Table B-6. Selected Effects in Male and Female F344 Rats Exposed to *p*-Phenylenediamine (CASRN 106-50-3) via Diet for 80 Weeks[a]

Dose (mg/kg-d)		Other Control[b]	Concurrent Control	38.8	77.6
Male					
Number of animals		10	1	11	16
Terminal body weight (g)		309.9 ± 27.6	241.7	207.8 ± 29.5[c] (−14%)[d]	243.8 ± 46.6 (0.87%)
RBC count (10^4/mm^3)		1,078 ± 160[e]	1,016.0	1,099.2 ± 82.0 (8.2%)	823.6 ± 167.1 (−19%)
Absolute liver weight (g)		9.70 ± 0.55	5.02	5.29 ± 0.80 (5.4%)	5.74 ± 0.91 (14%)
Relative liver weight (% body weight)		2.4	2.1	2.5	2.3
Absolute kidney weight (g)	Left	1.36 ± 0.11	1.02	1.00 ± 0.09 (−2%)	1.07 ± 0.10 (5%)
	Right	1.31 ± 0.12	0.96	0.99 ± 0.09 (3%)	1.08 ± 0.11 (13%)
Relative kidney weight (% body weight)	Left	0.4	0.3	0.4	0.6
	Right	0.3	0.4	0.5	0.4
Absolute spleen weight (g)		0.87 ± 0.09	0.41	0.39 ± 0.08 (−4.9%)	0.50 ± 0.11 (22%)
Relative spleen weight (% body weight)		0.2	0.2	0.2	0.2
Female					
Number of animals		10	6	32	11
Terminal body weight (g)		254.6 ± 33.7	174.4 ± 2.5	171.5 ± 31.8 (−1.7%)	138.5 ± 20.6** (−21%)
RBC count (10^4/mm^3)		976 ± 169[f]	1,077.9 ± 155.9	883.0 ± 158.0 (−18%)	802.6 ± 103.9 (−26%)
Absolute liver weight (g)		6.14 ± 0.86	3.57 ± 0.24	3.97 ± 0.65 (11%)	4.71 ± 0.89 (32%)
Relative liver weight (% body weight)		2.4	2.0	2.3	3.4
Absolute kidney weight (g)	Left	0.89 ± 0.11	0.56 ± 0.16	0.69 ± 0.05 (23%)	0.78 ± 0.05 (39%)
	Right	0.87 ± 0.11	0.71 ± 0.01	0.68 ± 0.04 (−4%)	0.79 ± 0.09 (11%)
Relative kidney weight (% body weight)	Left	0.4	0.3	0.4	0.6
	Right	0.4	0.4	0.4	0.6

Table B-6. Selected Effects in Male and Female F344 Rats Exposed to *p*-Phenylenediamine (CASRN 106-50-3) via Diet for 80 Weeks[a]

Dose (mg/kg-d)	Other Control[b]	Concurrent Control	38.8	77.6
Female				
Absolute spleen weight (g)	0.63 ± 0.15	0.63 ± 0.02	0.46 ± 0.14* (−27%)	0.44 ± 0.12* (−30%)
Relative spleen weight (% body weight)	0.2	0.4	0.3	0.3

[a]Imaida et al. (1983). Hematology and organ-weight data available only for animals surviving to Week 80.
[b]Results from control F344 rats in another 78-week experiment as reported by the study authors.
[c]Mean ± SD.
[d]Percent change from concurrent control.
[e]Number of animals for this endpoint was 34.
[f]Number of animals for this endpoint was 32.

*Significantly different from control at $p < 0.05$, as reported by the study authors.
**Significantly different from control at $p < 0.001$, as reported by the study authors.

SD = standard deviation.

Table B-7. Selected Effects in Pregnant S-D Rats Exposed to *p*-Phenylenediamine (CASRN 106-50-3) via Gavage on GDs 6-15[a]

Dose (mg/kg-d)	Vehicle Controls	Pair-Fed Controls[b]	5	10	15	20	30
Number of animals	25	25	25	25	25	25	25
Number of dead animals	0	0	0	0	0	0	3
Number of term pregnancies	24	23	25	25	25	24	21
Mean body-weight change on GDs 0−15 (g)	62.6 ± 9.9[c]	50.7 ± 15.7** (−19%)[d]	61.3 ± 10.4 (−2.1%)	60.8 ± 9.4 (−2.9%)	61.0 ± 10.0 (−2.6%)	51.6 ± 11.9* (−18%)	44.7 ± 11.8*** (−29%)
Mean body-weight change on GDs 0−20 (g)	129.0 ± 19.2	120.4 ± 13.8 (−6.7%)	126.4 ± 14.7 (−2.0%)	124.2 ± 11.6 (−3.7%)	127.7 ± 18.3 (−1.0%)	121.6 ± 15.5 (−5.7%)	123.7 ± 17.6 (−4.1%)
Mean food consumption on GD 10 (g)	24.1 ± 4.0	19.7 ± 4.3*** (−18%)	23.5 ± 5.1 (−2.5%)	23.2 ± 2.5 (−3.7%)	22.3 ± 4.5 (−7.5%)	21.6 ± 3.1* (−10%)	19.8 ± 7.6* (−18%)

[a]Re et al. (1981).
[b]Fed the average amount of food consumed by animals in 30-mg/kg-day-group on the previous day.
[c]Mean ± SD.
[d]Percent change from vehicle control.

*Significantly different from vehicle control at $p < 0.05$, as reported by the study authors.
**Significantly different from vehicle control at $p < 0.01$, as reported by the study authors.
***Significantly different from vehicle control at $p < 0.001$, as reported by the study authors.

GD = gestation day; SD = standard deviation; S-D = Sprague-Dawley.

Table B-8. Mean Cumulative Percent of Administered Radioactivity Excreted in Urine and Feces over 72 Hours Postdosing with ^{14}C-*p*-Phenylenediamine[a] (CASRN 106-50-3)

	Rat		Mouse	
	Male	**Female**	**Male**	**Female**
After i.v. dosing with 600 µmol/kg				
Urine	85.5	74.2	68.5	67.7
Feces	11.1	10.2	19.4	15.9
After oral dosing with 60 µmol/kg				
Urine	81.5	65.0	61.5	78.3
Feces	33.4	32.1	25.1	26.1
After oral dosing with 600 µmol/kg				
Urine	75.7	68.6	73.5	87.4
Feces	13.6	14.6	15.0	18.5

[a]Ioannou and Matthews (1985).

i.v. = intravenous.

Table B-9. Relative Amount of *p*-Phenylenediamine (CASRN 106-50-3) or Metabolites (A−K) Excreted in Urine, Feces, and Bile[a]

	Rat[b]		Mouse[b]	
	Male	**Female**	**Male**	**Female**
Urine				
p-Phenylenediamine	3.7	2.6	2.5	1.3
A	4.7	7.7	1.3	7.9
B	21.1	23.9	1.9	7.7
C	1.8	1.4	6.9	8.0
D	1.0	5.7	9.5	13.4
E	NDt	0.8	11.1	4.6
F	17.6	11.9	16.0	1.3
G	1.7	NDt	NDt	2.6
H	13.7	14.1	27.7	32.8
I	0.9	1.5	1.6	1.0
J	34	30.3	20.2	20.3
Feces				
p-Phenylenediamine	30	25	100	100
I	70	75	NDt	NDt
Bile				
p-Phenylenediamine	1.7	NDt	NDt	NDt
A	23.6	NDt	NDt	NDt
C	4.4	NDt	NDt	NDt
E	4.1	NDt	NDt	NDt
F	10.8	NDt	NDt	NDt
G	18.7	NDt	NDt	NDt
H	20.6	NDt	NDt	NDt
J	6.2	NDt	NDt	NDt
K	10.2	NDt	NDt	NDt

[a]Ioannou and Matthews (1985).
[b]Percent mean ± SD.

NDt = not detected; SD = standard deviation.

APPENDIX C. BENCHMARK DOSE MODELING RESULTS

MODELING OF NONCANCER ENDPOINTS

As discussed in Appendix A under the "Derivation of Screening Subchronic Provisional Reference Dose" section, the endpoints selected for benchmark dose (BMD) modeling were: (1) increased liver weight (absolute and relative) in male and female rats and (2) increased kidney weight (absolute and relative) in female rats (Toxicol Laboratories, 1995). The animal doses in the study, converted to equivalent doses of *p*-phenylenediamine, were used in the BMD modeling; the data are shown in Tables A-1 and B-3.

Modeling Procedure for Continuous Noncancer Data

BMD modeling of continuous noncancer data was conducted with the EPA's Benchmark Dose Software (BMDS, Version 2.5). For these data, all continuous models available within the software were fit using a benchmark response (BMR) of 10% extra risk or 1 standard deviation (SD). Adequacy of model fit was judged based on the χ^2 goodness-of-fit *p*-value ($p > 0.1$), magnitude of the scaled residuals at the data point (except the control) closest to the predefined benchmark response (absolute value <2.0), and visual inspection of the model fit. In addition to these three criteria for judging the 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 the 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; $p < 0.1$), the data set was considered unsuitable for BMD modeling. In the cases where no best model was found to fit to the data, a reduced data set without the high-dose group was further attempted for modeling and the result was presented along with that of the full data set. Among all of the models providing adequate fit, the benchmark dose lower confidence limit (BMDL) from the model with the lowest Akaike's information criterion (AIC) was selected as a potential point of departure (POD) when BMDL values were sufficiently close. Otherwise, the lowest BMDL was selected as a potential POD.

Model Predictions for Absolute Liver Weight in Male Rats

The procedure outlined above was applied to the data (see Table A-1) on absolute liver weight in male rats exposed to *p*-phenylenediamine via gavage for 13 weeks (Toxicol Laboratories, 1995). All models provided adequate fit to the data set when assessed by the overall goodness-of-fit ($p > 0.1$) (see Table C-1). A homogeneous variance model was accepted (Test 2; $p > 0.1$). The 10% benchmark dose lower confidence limit ($BMDL_{10}$) from all models were sufficiently close; therefore, the Polynomial3 model providing the lowest AIC was selected as the best fitting. The 10% benchmark dose (BMD_{10}) and $BMDL_{10}$ values for absolute liver weight in male rats from this model were 14.03 and 10.85 mg/kg-day, respectively.

Table C-1. BMD Modeling Results on Absolute Liver Weight in Male Rats[a]

Model Name	*p*-Value Test 2: Constant Variance?	χ^2 Goodness-of-Fit *p*-Value[b]	AIC	BMD_{10} (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)
Exponential2	0.16	0.28	245.25	11.11	7.31
Exponential3	0.16	0.68	244.23	14.58	9.85
Exponential4	0.16	0.13	247.47	11.10	6.99
Exponential5	0.16	0.38	246.23	14.52	9.81
Hill	0.16	0.69	244.20	9.48	8.18
Linear	0.16	0.26	245.47	11.10	6.99
Polynomial2	0.16	0.71	242.82	13.05	9.24
Polynomial3[c]	**0.16**	**0.84**	**242.29**	**14.03**	**10.85**
Power	0.16	0.68	244.23	14.52	9.81

[a]Toxicol Laboratories (1995).
[b]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[c]Selected model. All models provided adequate fit to the data. BMDL values estimated from models providing adequate fit were sufficiently close; therefore, the model with the lowest AIC was selected (Polynomial3).

AIC = Akaike's information criterion; BMD = maximum likelihood estimate of the dose associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = dose associated with 10% extra risk).

Model Predictions for Relative Liver Weight in Male Rats

The procedure outlined above was applied to the data (see Table A-1) on relative liver weight in male rats exposed to *p*-phenylenediamine via gavage for 13 weeks (Toxicol Laboratories, 1995). All models provided adequate fit to the data set when assessed by the overall goodness-of-fit ($p > 0.1$) (see Table C-2). A homogeneous variance model was accepted (Test 2; $p > 0.1$). The $BMDL_{10}$ from all models were sufficiently close; therefore, the Exponential5 model providing the lowest AIC was selected as the best fitting. The BMD_{10} and $BMDL_{10}$ values for absolute liver weight in male rats from this model were 8.42 and 7.75 mg/kg-day, respectively.

Table C-2. BMD Modeling Results on Relative Liver Weight in Male Rats[a]					
Model Name	***p*-Value Test 2: Constant Variance?**	**χ^2 Goodness-of-Fit *p*-Value[b]**	**AIC**	**BMD_{10} (mg/kg-d)**	**$BMDL_{10}$ (mg/kg-d)**
Exponential2	0.60	0.64	−72.05	12.15	9.04
Exponential3	0.60	0.48	−70.25	13.05	9.14
Exponential4	0.60	0.42	−69.99	11.96	7.18
Exponential5[c]	**0.60**	**0.62**	**−69.48**	**8.42**	**7.75**
Hill	0.60	0.89	−71.48	8.73	7.81
Linear	0.60	0.63	−71.99	11.96	8.69
Polynomial2	0.60	0.46	−70.17	12.95	8.78
Polynomial3	0.60	0.46	−70.17	12.95	8.78
Power	0.60	0.49	−70.28	13.01	8.83

[a]Toxicol Laboratories (1995).
[b]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[c]Selected model. All models provided adequate fit to the data. BMDL values estimated from models providing adequate fit were sufficiently close; therefore, the model with the lowest AIC was selected (Exponential5).

AIC = Akaike's information criterion; BMD = maximum likelihood estimate of the dose associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = dose associated with 10% extra risk).

Model Predictions for Absolute Kidney Weight in Female Rats

The procedure outlined above was applied to the data (see Table A-1) on absolute kidney weight in female rats exposed to *p*-phenylenediamine via gavage for 13 weeks (Toxicol Laboratories, 1995). All models provided adequate fit to the data set when assessed by the overall goodness-of-fit ($p > 0.1$) (see Table C-3). A homogeneous variance model was accepted (Test 2; $p > 0.1$). The $BMDL_{10}$ from all models were sufficiently close; therefore, the Exponential5 model providing the lowest AIC was selected as the best fitting. The BMD_{10} and $BMDL_{10}$ values for absolute kidney weight in female rats from this model were 8.97 and 4.58 mg/kg-day, respectively.

Table C-3. BMD Modeling Results on Absolute Kidney Weight in Female Rats[a]

Model Name	*p*-Value Test 2: Constant Variance?	χ^2 Goodness-of-Fit *p*-Value[b]	AIC	BMD_{10} (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)
Exponential2	0.85	0.95	−145.17	10.03	7.51
Exponential3	0.85	0.95	−145.17	10.03	7.51
Exponential4	0.85	0.86	−143.21	9.34	4.40
Exponential5[c]	**0.85**	**0.86**	**−141.47**	**8.97**	**4.58**
Hill	0.85	0.87	−141.48	9.04	4.54
Linear	0.85	0.96	−145.20	9.69	7.05
Polynomial2	0.85	0.96	−145.20	9.69	7.05
Polynomial3	0.85	0.96	−145.20	9.69	7.05
Power	0.85	0.86	−143.20	9.77	7.05

[a]Toxicol Laboratories (1995).
[b]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[c]Selected model. All models provided adequate fit to the data. BMDL values estimated from models providing adequate fit were sufficiently close; therefore, the model with the lowest AIC was selected (Exponential5).

AIC = Akaike's information criterion; BMD = maximum likelihood estimate of the dose associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = dose associated with 10% extra risk).

Model Predictions for Relative Kidney Weight in Female Rats

The procedure outlined above was applied to the data (see Table A-1) on relative kidney weight in female rats exposed to *p*-phenylenediamine via gavage for 13 weeks (Toxicol Laboratories, 1995). However, data of relative kidney weight in female rats failed to meet the modeling criteria (see Table C-4). Initial test determined that constant and nonhomogeneous variance was invalid for modeling these data (Tests 2 and 3; $p < 0.1$). The initial modeling including all dose groups was found to be unsuitable for BMD modeling. After excluding the highest-dose group (16 mg/kg-day), the same results were obtained. Table C-4 presents the BMD modeling results for relative kidney weight in female rats using the nonhomogeneous variance models excluding the highest dose.

Table C-4. BMD Modeling Results on Relative Kidney Weight in Female Rats[a,b]

Model Name	*p*-Value Test 2: Constant Variance?	*p*-Value Test 3: Good Variance Model?	χ^2 Goodness-of-Fit *p*-Value[c]	AIC	BMD_{10} (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)
Exponential2	0.0003	0.0971	0.28	−248.83	6.21	4.16
Exponential3	0.0003	0.0971	0.58	−249.05	6.70	4.80
Exponential4	0.0003	0.0971	0.09	−246.55	6.27	4.07
Exponential5	0.0003	0.0971	NA	−247.36	4.49	4.06
Hill	0.0003	0.0971	NA	−247.36	4.45	4.08
Linear	0.0003	0.0971	0.25	−248.55	6.27	4.07
Polynomial2	0.0003	0.0971	0.57	−249.03	6.74	4.86
Polynomial3	0.0003	0.0971	0.57	−249.03	6.74	4.86
Power	0.0003	0.0971	0.59	−249.06	6.67	4.76

[a]Toxicol Laboratories (1995).
[b]Modeling results excluding the high dose group (16 mg/kg-day).
[c]Values <0.1 fail to meet conventional goodness-of-fit criteria.

AIC = Akaike's information criterion; BMD = maximum likelihood estimate of the dose associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = dose associated with 10% extra risk); NA = not applicable.

Model Predictions for Absolute Liver Weight in Female Rats

The procedure outlined above was applied to the data (see Table A-1) on absolute liver weight in female rats exposed to *p*-phenylenediamine via gavage for 13 weeks (Toxicol Laboratories, 1995). All models provided adequate fit to the data set when assessed by the overall goodness-of-fit ($p > 0.1$) (see Table C-5). A homogeneous variance model was accepted (Test 2; $p > 0.1$). The $BMDL_{10}$ from all models were sufficiently close; therefore, the models providing the lowest AIC were selected as the best fitting. The Linear, Polynomial2, Polynomial3, and Power models provided identical outputs, so were selected. The BMD_{10} and $BMDL_{10}$ values for absolute liver weight in female rats from these models were 14.48 and 9.00 mg/kg-day, respectively.

Table C-5. BMD Modeling Results on Absolute Liver Weight in Female Rats[a]

Model Name	*p*-Value Test 2: Constant Variance?	χ^2 Goodness-of-Fit *p*-Value[b]	AIC	BMD_{10} (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)
Exponential2	0.32	0.90	108.49	14.62	9.38
Exponential3	0.32	0.90	108.49	14.62	9.38
Exponential4	0.32	0.78	110.42	13.85	4.05
Exponential5	0.32	0.78	110.42	13.85	4.05
Hill	0.32	0.78	110.42	13.82	3.54
Linear[c]	**0.32**	**0.91**	**108.47**	**14.48**	**9.00**
Polynomial2[c]	**0.32**	**0.91**	**108.47**	**14.48**	**9.00**
Polynomial3[c]	**0.32**	**0.91**	**108.47**	**14.48**	**9.00**
Power[c]	**0.32**	**0.91**	**108.47**	**14.48**	**9.00**

[a]Toxicol Laboratories (1995).
[b]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[c]Selected model. All models provided adequate fit to the data. BMDL values estimated from models providing adequate fit were sufficiently close; therefore, the models with the lowest AIC were selected.

AIC = Akaike's information criterion; BMD = maximum likelihood estimate of the dose associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = dose associated with 10% extra risk).

Model Predictions for Relative Liver Weight in Female Rats

The procedure outlined above was applied to the data (see Table A-1) on relative liver weight in female rats exposed to *p*-phenylenediamine via gavage for 13 weeks (see Table C-6) (Toxicol Laboratories, 1995). Initial test determined that constant variance was invalid for modeling these data (Test 2; $p < 0.1$). Nonhomogeneous variance models were appropriate for the Hill, Linear, Polynomial2, and Polynomial3 models (Test 3; $p > 0.1$). The initial modeling including all dose groups failed to provide an adequate fit to the data, as assessed by the χ^2 goodness-of-fit test. After excluding the highest-dose group (16 mg/kg-day), the Hill, Linear, Polynomial2, and Polynomial3 models adequately fit the data (see Table C-6). The $BMDL_{10}$ from all models that provided an adequate fit were sufficiently close; therefore, the Hill model providing the lowest AIC was selected as the best fitting model. The BMD_{10} and $BMDL_{10}$ values for relative liver weight in female rats from this model were 6.13 and 4.27 mg/kg-day, respectively. Figure C-1 shows the model fit to the data.

Table C-6. BMD Modeling Results on Relative Liver Weight in Female Rats[a,b]

Model Name	*p*-Value Test 2: Constant Variance?	*p*-Value Test 3: Good Variance Model?	χ^2 Goodness-of-Fit *p*-Value[c]	AIC	BMD_{10} (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)
Exponential2	<0.0001	<0.0001	NA	−65.61	7.49	4.36
Exponential3	<0.0001	<0.0001	NA	−65.61	7.49	4.36
Exponential4	<0.0001	<0.0001	NA	−31.04	NA	NA
Exponential5	<0.0001	<0.0001	NA	−29.04	NA	NA
Hill[d]	**<0.0001**	**0.55**	**0.42**	**−63.52**	**6.13**	**4.27**
Linear	<0.0001	0.55	0.87	−67.43	5.98	4.24
Polynomial2	<0.0001	0.55	0.76	−65.62	6.22	4.30
Polynomial3	<0.0001	0.55	0.79	−65.71	6.33	4.32
Power	<0.0001	<0.0001	<0.0001	−65.53	6.13	4.27

[a]Toxicol Laboratories (1995).
[b]Modeling results excluding the high dose group (16 mg/kg-day).
[c]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[d]Selected model. The Hill, Linear, Polynomial2, and Polynomial3 models provided adequate fit to the data. BMDL values estimated from models providing adequate fit were sufficiently close; therefore, the model with the lowest AIC was selected (Hill).

AIC = Akaike's information criterion; BMD = maximum likelihood estimate of the dose associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., $_{10}$ = dose associated with 10% extra risk); NA = not applicable.

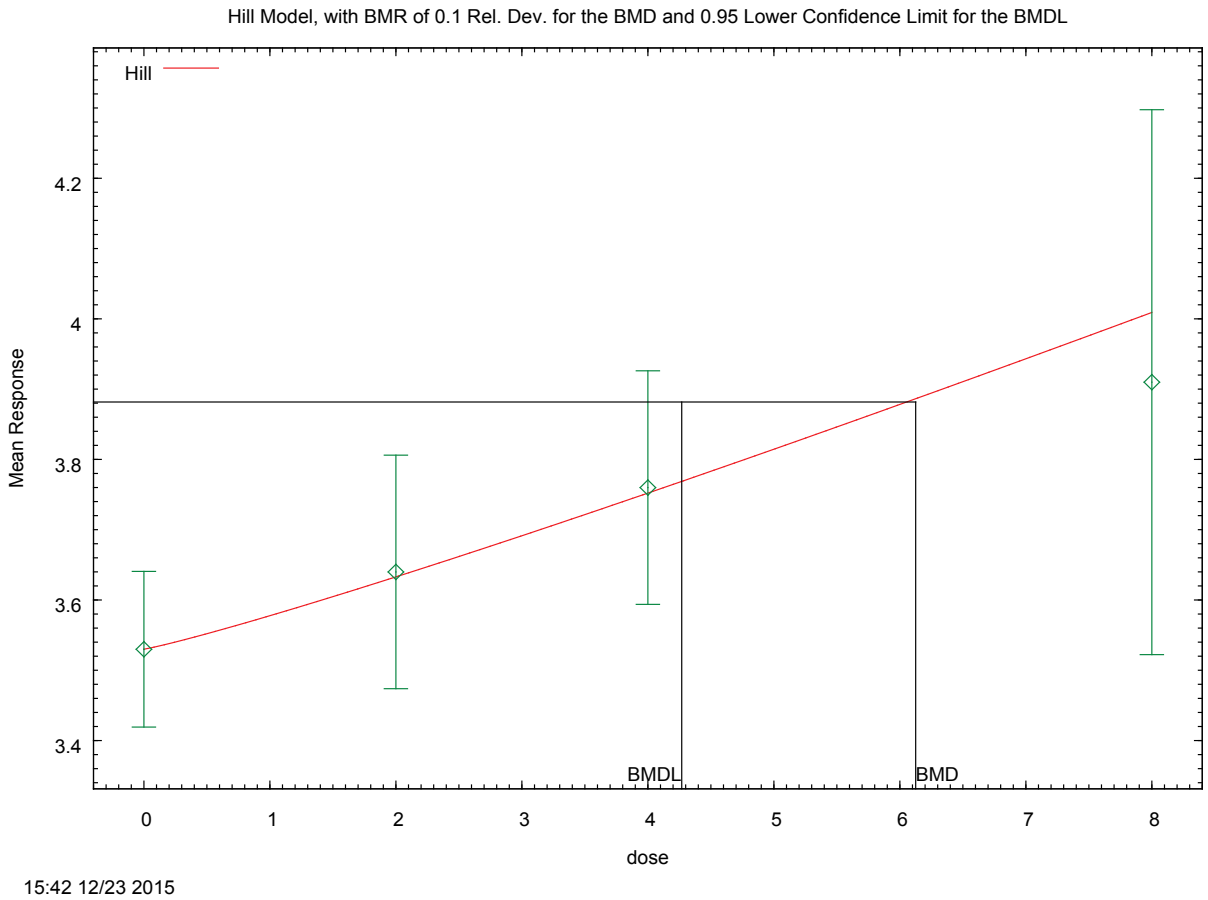

Figure C-1. Fit of Nonhomogeneous Variance Hill Model to Relative Liver Weight in Female Rats after Dropping the Highest Dose

Text Output for Nonhomogeneous Variance Hill Model to Relative Liver Weight in Female Rats after Dropping the Highest Dose (Toxicol Laboratories, 1995)

```
 ================================================================
        Hill Model. (Version: 2.17;  Date: 01/28/2013)
        Input Data File: C:/Users/bowens/BMDS2601/Data/hil_Continuous1_Opt.(d)
        Gnuplot Plotting File:  C:/Users/bowens/BMDS2601/Data/hil_Continuous1_Opt.plt
                                          Wed Dec 23 15:42:25 2015
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
   Independent variable = Dose
   Power parameter restricted to be greater than 1
   The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))
```

```
   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 500
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                         lalpha =     -1.72878
                            rho =            0
                      intercept =         3.53
                              v =         0.38
                              n =     0.529885
                              k =      4.66667


           Asymptotic Correlation Matrix of Parameter Estimates

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

                 lalpha    intercept            v            n            k

    lalpha            1        -0.39      -0.0045      -0.0092      -0.0019

 intercept        -0.39            1       0.0065         0.55       -0.028

         v      -0.0045       0.0065            1       -0.059            1

         n      -0.0092         0.55       -0.059            1        -0.12

         k      -0.0019       -0.028            1        -0.12            1


                                Parameter Estimates

                                                          95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
         lalpha         -25.8908
0.274355            -26.4285             -25.353
            rho               18              NA
      intercept          3.52887        0.0470492             3.43665
3.62108
              v          362.061          12858.1            -24839.3
25563.5
              n          1.11144         0.390807             0.34547
1.8774
              k          3133.18           100843             -194515
200781

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.


     Table of Data and Estimated Values of Interest
```

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	15	3.53	3.53	0.2	0.203	0.0217
2	15	3.64	3.63	0.3	0.262	0.138
4	15	3.76	3.75	0.3	0.349	0.125
8	15	3.91	4	0.7	0.631	-0.574

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	23.933326	5	-37.866652
A2	37.680663	8	-59.361326
A3	37.084408	6	-62.168816
fitted	36.762186	5	-63.524372
R	20.522150	2	-37.044300

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	34.317	6	<.0001
Test 2	27.4947	3	<.0001
Test 3	1.19251	2	0.5509
Test 4	0.644443	1	0.4221

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears

```
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


        Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =     Relative deviation

Confidence level =           0.95

             BMD =        6.12535

            BMDL =       4.26872
```

APPENDIX D. REFERENCES

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