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Provisional Peer-Reviewed Toxicity Values for

o-Phenylenediamine
(CASRN 95-54-5)

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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 *o*-PHENYLENEDIAMINE (CASRN 95-54-5)

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 Environmental 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

o-Phenylenediamine, CASRN 95-54-5, is primarily used as a chemical intermediate, especially in the production of benzimidazole-derived agricultural fungicides, such as benomyl, and substituted benzimidazoles used as a veterinary anthelmintic (HSDB, 2013). *o*-Phenylenediamine is also used in the manufacture of dyes, although its use in hair dyes was banned by the European Union in September of 2007 (HSDB, 2013). *o*-Phenylenediamine is also used as a fluorescence indicator, a photographic developing agent, and a laboratory reagent (HSDB, 2013).

o-Phenylenediamine is solid at room temperature. As a diamine with pKa values of <2 and 4.47, *o*-phenylenediamine is expected to exist partially as a cation in the environment. As a result, it is not expected to volatilize from moist soil or water surfaces (HSDB, 2013). In addition, the estimated Henry's law constant for the neutral form of *o*-phenylenediamine indicates a low propensity to volatilize from water surfaces. Furthermore, the moderate vapor pressure of *o*-phenylenediamine's neutral form indicates that evaporation from dry soil is not expected. However, a moderate vapor pressure suggests that *o*-phenylenediamine, if released to the air, would remain in the vapor phase (HSDB, 2013). *o*-Phenylenediamine's ability to leach from soil to groundwater is dependent on local conditions. In areas with high amounts of organic matter, leaching of *o*-phenylenediamine may be inhibited due to the high reactivity of the aromatic amine groups (HSDB, 2013). In other areas, *o*-phenylenediamine deposited on soil is likely to leach to groundwater or undergo runoff after a rain event, due to its high water solubility and relatively low soil adsorption coefficient. The empirical formula for *o*-phenylenediamine is $C_6H_8N_2$ (see Figure 1). Synonyms include 2-aminoaniline, 1,2-benzenediamine, *o*-benzenediamine, *o*-diaminobenzene, 1,2-diaminobenzene, and 1,2-phenylenediamine. A table of physicochemical properties for *o*-phenylenediamine is provided below (see Table 1).

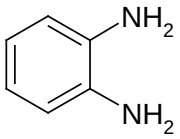

Figure 1. *o*-Phenylenediamine Structure

Table 1. Physicochemical Properties of *o*-Phenylenediamine (CASRN 95-54-5)

Property (unit)	Value
Physical state	White crystals[a]
Boiling point (°C)	257[a]
Melting point (°C)	102.1[a]
Density (g/cm^3)	0.6[b]
Vapor pressure (mm Hg at 25°C)	2.06×10^{-3} [a]
pH (unitless)	9.7 (saturated aqueous solution at 24°C)[b]
pKa (at 25°C)	pKa1 = <2; pKa2 = 4.47[a]
Solubility in water (g/L at 20°C)	31[b]
Octanol-water partition constant (log K_{ow})	0.15[a]; 0.12[b]
Henry's law constant (atm-m^3/mol at 25°C)	7.20×10^{-9} (estimated)[c]
Soil adsorption coefficient (K_{oc}) (mL/g)	34.5 (estimated)[c]
Relative vapor density (air = 1)	3.7[d]
Molecular weight (g/mol)	108.14[a]

[a]HSDB (2013).
[b]ECHA (2015).
[c]U.S. EPA (2012b).
[d]Sigma-Aldrich (2015).

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

Table 2. Summary of Available Toxicity Values for *o*-Phenylenediamine (CASRN 95-54-5)

Source (parameter)[a,b]	Value (applicability)	Notes	Reference
Noncancer			
IRIS	NV	NA	U.S. EPA (2016)
HEAST	NV	NA	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	NV	NA	OSHA (2011); OSHA (2006)
NIOSH	NV	NA	NIOSH (2016)
ACGIH (TLV-TWA)	0.1 mg/m^3	Based on the potential for "blood dyscrasia (e.g., reduction of RBCs), as well as eye and skin irritation"	ACGIH (2015)
Cancer			
IRIS	NV	NA	U.S. EPA (2016)
HEAST (OSF, WOE)	OSF: 4.7×10^{-2} (mg/kg-d)$^{-1}$; WOE: B2	Based on liver tumors in rats treated orally with *o*-phenylenediamine dihydrochloride	U.S. EPA (1985); U.S. EPA (2011b)
DWSHA	NV	NA	U.S. EPA (2012a)
NTP	NV	NA	NTP (2014)
IARC	NV	NA	IARC (2015)
Cal/EPA (cancer potency value)	0.027 (mg/kg-d)$^{-1}$	Based on liver tumors in male rats and female mice after oral exposure to *o*-phenylenediamine dihydrochloride	Cal/EPA (2002)
ACGIH (WOE)	A3—confirmed animal carcinogen with unknown relevance to humans	Based on the "production of hepatocellular carcinomas and hepatomas" in rat and mouse bioassays	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: OSF = oral slope factor; TLV = threshold limit value; TWA = time-weighted average; WOE = weight-of-evidence.

NA = not applicable; NV = not available; RBC = red blood cell.

Literature searches were conducted in July 2013 and in March 2016 for studies published from 1900 that are relevant to the derivation of provisional toxicity values for *o*-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 an overview of the relevant noncancer and cancer databases for *o*-phenylenediamine, respectively, and include all potentially relevant repeat-dose, short-term-, subchronic-, and chronic-duration studies, as well as reproductive and developmental toxicity studies. Principal studies are identified in bold font. The phrases "statistical significance" and "significant," used throughout the document, indicate a *p*-value of < 0.05 unless otherwise noted.

Table 3A. Summary of Potentially Relevant Noncancer Data for *o*-Phenylenediamine (CASRN 95-54-5)								
Category[a]	**Number of Male/Female, Strain, Species, Study Type, Study Duration**	**Dosimetry[b]**	**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)[b]								
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)[b]								
Short-term	7−14 M/0 F, ChR-CD® rat, palatability study of *o*-phenylenediamine administered in drinking water, 4 wk	0, 2,240 (Group B1), 2,070 (Group B2) ppm ADD: 0, 167 (Group B1), 132 (Group B2)	Decreased body weight and water intake	ND	NDr	ND	Haskell Laboratories (1980) (Not a comprehensive evaluation of endpoints; limited to clinical signs, body weight, and water consumption)	NPR

Table 3A. Summary of Potentially Relevant Noncancer Data for *o*-Phenylenediamine (CASRN 95-54-5)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[b]	Critical Effects	NOAEL[b]	BMDL/ BMCL[b]	LOAEL[b]	Reference (comments)	Notes[c]
Subchronic	10 M/10 F, Crl:CD®BR rat, neurotoxicity study of *o*-phenylenediamine administered by gavage (suspended in aqueous methyl cellulose) for 90 consecutive d	0, 20, 40, 80 ADD: 0, 20, 40, 80	Increased incidence of slight palpebral closure (M and F); increased incidence of yellow staining of abdomen, perineum, inguen (groin), and/or underbody (F)	40	NDr	80	DuPont (1992a) (Not a comprehensive evaluation of endpoints; limited to mortality, clinical signs, body weight, food consumption, and neurotoxicity endpoints, including neurological histology)	NPR
Chronic	**50 M/50 F, F344/DuCrj rat, *o*-phenylenediamine dihydrochloride administered in drinking water for 2 yr**	**0, 500, 1,000, 2,000 (M); 0, 250, 500, 1,000 (F) ppm as *o*-phenylenediamine dihydrochloride** **ADD: 0, 13, 25, 51 (M); 0, 11, 20, 35 (F) as *o*-phenylenediamine**	**Increased incidences of renal papillary mineralization and basophilic cell foci of the liver (M); increased incidences of urothelial hyperplasia of the renal pelvis and basophilic foci of the liver (F)**	**ND**	**4.8 (papillary mineralization, M)**	**13 (M); 11 (F)**	**Matsumoto et al. (2012)**	**PR, PS**
Chronic	50 M/50 F, Crj:BDF_1 mouse, *o*-phenylenediamine dihydrochloride administered in drinking water for 2 yr	0, 500, 1,000, 2,000 (M); 0, 1,000, 2,000, 4,000 (F) ppm as *o*-phenylenediamine dihydrochloride ADD: 0, 27, 56, 106 (M); 0, 63.3, 119, 234 (F) as *o*-phenylenediamine	Increased ALP and relative liver weight (M and F); decreased terminal body weight and increased incidence of papillary hyperplasia of the gall bladder (M); increased incidences of eosinophilic change of the nasal cavity respiratory epithelium and hydronephrosis of the kidney (F)	ND	23 (hydronephrosis, F)	27 (M); 63.3 (F)	Matsumoto et al. (2012) (Note: Liver tumor incidences were significantly increased at all doses in both male and female mice)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for *o*-Phenylenediamine (CASRN 95-54-5)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[b]	Critical Effects	NOAEL[b]	BMDL/ BMCL[b]	LOAEL[b]	Reference (comments)	Notes[c]
2. Inhalation (mg/m^3)								
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 an ADD (mg/kg-day) for oral noncancer effects and as an HEC (mg/m^3) for inhalation noncancer effects. Where applicable, the dose of *o*-phenylenediamine was calculated from the dose of *o*-phenylenediamine dihydrochloride by multiplying by the ratio of the molecular weights of the two compounds (108.14 g/mol:181.062 g/mol).

[c]Notes: NPR = not peer reviewed; PR = peer reviewed; PS = principal study.

ADD = adjusted daily dose; ALP = alkaline phosphatase; BMCL = benchmark concentration lower confidence limit; BMDL = benchmark dose lower confidence limit; F = female(s); Hb = hemoglobin; HEC = human equivalent concentration; LOAEL = lowest-observed-adverse-effect level; M = male(s); ND = no data; NDr = not determined; NOAEL = no-observed-adverse-effect level.

Table 3B. Summary of Potentially Relevant Cancer Data for *o*-Phenylenediamine (CASRN 95-54-5)						
Category	**Number of Male/Female, Strain, Species, Study Type, Study Duration**	**Dosimetry[a]**	**Critical Effects**	**BMDL[a]**	**Reference (comments)**	**Notes[b]**
Human						
1. Oral (mg/kg-d)						
ND						
2. Inhalation (mg/m^3)						
ND						
Animal						
1. Oral (mg/kg-d)[a]						
Carcinogenicity	50 M/50 F, F344/DuCrj rat, *o*-phenylenediamine dihydrochloride administered in drinking water for 2 yr	0, 500, 1,000, 2,000 (M); 0, 250, 500, 1,000 (F) ppm as *o*-phenylenediamine dihydrochloride HED: 0, 3.2, 6.1, 13 (M); 0, 2.6, 4.8, 8.5 (F) as *o*-phenylenediamine	Significantly increased incidences of hepatocellular adenomas and/or carcinomas in males and females (≥6.1 and 4.8 mg/kg-d, respectively); significantly increased incidence of urinary bladder transitional cell papilloma or carcinoma in males (13 mg/kg-d); significant dose-related trend for thyroid follicular adenoma in males	2.5 (M) (combined tumors); 2.2 (F) (liver tumors)	Matsumoto et al. (2012)	PR
Carcinogenicity	**50 M/50 F, Crj:BDF$_1$ mouse, *o*-phenylenediamine dihydrochloride administered in drinking water for 2 yr**	**0, 500, 1,000, 2,000 (M); 0, 1,000, 2,000, 4,000 (F) ppm as *o*-phenylenediamine dihydrochloride HED: 0, 3.8, 7.7, 14.5 (M); 0, 8.70, 16.4, 32.1 (F) as *o*-phenylenediamine**	**Significantly increased incidences of hepatocellular adenomas and/or carcinomas (≥3.8 and 8.70 mg/kg-d in males and females, respectively) and papillary adenomas of the gall bladder (at 14.5 mg/kg-d in males and 16.4 mg/kg-d in females)**	**0.84 (M) (combined tumors); 1.56 (F) (combined tumors)**	**Matsumoto et al. (2012)**	**PS, PR**

Table 3B. Summary of Potentially Relevant Cancer Data for *o*-Phenylenediamine (CASRN 95-54-5)						
Category	**Number of Male/Female, Strain, Species, Study Type, Study Duration**	**Dosimetry[a]**	**Critical Effects**	**BMDL[a]**	**Reference (comments)**	**Notes[b]**
Carcinogenicity	25 M/0 F, Charles River CD rat, *o*-phenylenediamine dihydrochloride administered in diet for 18 mo	0, 2,000, 4,000 ppm as *o*-phenylenediamine dihydrochloride HED: 0, 20.1, 40.1 as *o*-phenylenediamine	Significantly increased incidence of liver tumors at 40.1 mg/kg-d	NDr	Weisburger et al. (1978) (Note: Animals were not exposed over a lifetime [only for 18 mo] and then were allowed to recover for 6 mo after the exposure period. All endpoints were only measured at study termination of 24 mo)	PR
Carcinogenicity	25 M/25 F, CD-1 mouse, *o*-phenylenediamine dihydrochloride administered in diet for 18 mo	0, 4,000, 8,000 ppm for 5 mo; 0, 8,000, 16,000 ppm for 13 mo as *o*-phenylenediamine dihydrochloride HED: 0, 98.5, 200 (M); 0, 100, 204 (F) as *o*-phenylenediamine	Significantly increased incidences of hepatomas in males at 98.5 mg/kg-d and females at ≥100 mg/kg-d	NDr	Weisburger et al. (1978) (Note: Animals were not exposed over a lifetime [only for 18 mo] and then were allowed to recover for 3 mo after the exposure period. All endpoints were only measured at study termination of 21 mo)	PR
2. Inhalation (mg/m^3)						
ND						

[a]Dosimetry: The units for oral exposures are expressed as human equivalent dose (HED, mg/kg-day). HED = ADD (mg/kg-day) × default dosimetric adjustment factor (DAF) (U.S. EPA, 2011c). Where applicable, the dose of *o*-phenylenediamine was calculated from the dose of *o*-phenylenediamine dihydrochloride by multiplying by the ratio of the molecular weights of the two compounds (108.14 g/mol:181.062 g/mol).
[b]Notes: PR = peer reviewed; PS = principal study.

ADD = adjusted daily dose; BMDL = benchmark dose lower confidence limit; F = female(s); HED = human equivalent dose; M = male(s); ND = no data; NDr = not determined.

HUMAN STUDIES

Oral Exposures

No studies examining possible associations between health effects in humans and oral exposure to *o*-phenylenediamine have been identified.

Inhalation Exposures

DuPont (1984)

The frequency of methemoglobinemia in employees of a phenylenediamine manufacturing plant was evaluated by reviewing employee medical records. The phenylenediamine isomer(s) that the workers were exposed to were not provided. Employees in the plant provided blood samples every 6 months or whenever exposure excursions above the company-established acceptable exposure level of 0.1 mg/m^3 occurred. Records of all operators and operating supervisors working with phenylenediamines for ≥10 years were reviewed, and hemoglobin (Hb) and oxygen levels were examined for the period of potential phenylenediamine exposure. Neither Hb nor oxygen saturation levels among 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 none of the affected workers exhibited blood oxygen saturation <90%.

ANIMAL STUDIES

Oral Exposures

The effects of oral exposure of animals to *o*-phenylenediamine were evaluated in one short-term-duration study (Haskell Laboratories, 1980), one subchronic-duration neurotoxicity study (DuPont, 1992a), and two chronic-duration carcinogenicity studies (Matsumoto et al., 2012; Weisburger et al., 1978).

Short-Term-Duration Studies

Haskell Laboratories (1980)

The palatability of *o*-phenylenediamine (purity 99%) in drinking water was tested in an unpublished 4-week study conducted by Haskell Laboratories (1980). Two groups of seven male ChR-CD® rats received 4,000 ppm *o*-phenylenediamine (purity 99%) in drinking water on Days 0−7, and then untreated water on Days 7−10. One group (B1) then received 400 ppm on Days 10−14, 1,000 ppm on Days 14−17, 2,000 ppm on Days 17−24, and 4,000 ppm on Days 24−28; the other group (B2) received 1,000 ppm on Days 10−24 and 4,000 ppm on Days 24−28. These exposure concentrations corresponded to time-weighted average (TWA) concentrations of 2,240 ppm (Group B1) and 2,070 ppm (Group B2); doses estimated for this review using TWA-measured body weight and water intake values were 167 and 132 mg/kg-day in Groups B1 and B2, respectively. A control group of 14 rats received tap water. Toxicological evaluations were limited to daily observations for clinical signs and twice-weekly body-weight and water consumption measurements. None of the animals were necropsied. Statistical analysis was not reported or performed.

There were no deaths among rats of any group. Some rats reportedly exhibited brown facial discoloration, but no other clinical signs were noted. In both groups of treated rats, the initial 1-week exposure to 4,000 ppm *o*-phenylenediamine in drinking water resulted in body-weight losses of >10% of their initial body weight after only 3 days and 18% after 7 days.

Both groups resumed gaining weight when exposed to concentrations up to 2,000 ppm, but lost weight again during the final 3 days of exposure to 4,000 ppm. Terminal body weights in both groups of treated rats were much lower (~24%) than controls. Treated rats drank less water compared to controls over the 28-day study; TWA daily water intake values were 22 and 19 mL/day in Groups B1 and B2 (65 and 56% of controls, respectively), compared with 34 mL/day in controls. The observed body-weight decreases in this study may have been attributable to the markedly reduced water intake, possibly due to poor palatability of the treated water. Given the uncertainty in the role of water intake on body-weight decrements, and the lack of evaluations of other toxicological endpoints, effect levels cannot be determined from this study.

Subchronic-Duration Studies

DuPont (1992a)

A subchronic-duration neurotoxicity study of *o*-phenylenediamine (purity ≥98%, suspended in aqueous methyl cellulose) administered by gavage to Crl:CD®BR rats was conducted by DuPont (1992a). The study was not published. Groups of 10 rats/sex/dose were given daily doses of 0, 20, 40, or 80 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 study commencement and before sacrifice. Neurotoxicity evaluations (including motor activity and functional observational battery [FOB] assessments, 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 sacrifice at the end of exposure, the following tissues were removed from the control and high-dose rats for histology examination (sciatic nerve, forebrain, cerebrum, midbrain, cerebellum, pons, medulla oblongata, spinal cord, tibial nerve and Gasserian ganglia, dorsal root ganglia, dorsal and ventral root fibers, and gastrocnemius muscle).

High performance liquid chromatography (HPLC) analysis showed that concentrations of *o*-phenylenediamine in the test suspensions were within 85−100% of the target levels. One male rat exposed to 40 mg/kg-day was removed from the study for poor health apparently unrelated to toxicity (severe mouth injury, black discharge from the eye, and irregular respiration). All remaining rats survived the study. Clinical signs of toxicity consisting of yellow staining of the perineum, inguen, abdomen, and/or underbody were observed at increased incidence in female rats exposed to the high dose (see Table B-1). But only the increased incidences of perineum and inguen staining were statistically significant compared with the controls. In contrast, clinical signs attributed to toxicity were not observed in male rats. Male rats in the high-dose group exhibited significant decreases in body-weight gain during the sixth week of treatment, and statistically nonsignificant decreases were also seen during Weeks 4, 9, and 12. In addition, a significant decrease (13% less than controls) in body-weight gain calculated over the entire exposure period was observed in the 40-mg/kg-day male rats (see Table B-1). In female rats, a significant decrease in body-weight gain occurred during Week 4 only. However, average body weights in all exposed groups of male and female rats did not differ significantly from controls throughout the exposure period. Likewise, food consumption rates were not affected by treatment, although there were sporadic reductions in feed efficiency (weight gain/food consumption) in the 40- and 80-mg/kg-day males and 80-mg/kg-day females, reflecting the reduced weight-gain values noted above.

Among the FOB endpoints assessed, only the response to tail pinch showed any evidence of an effect of treatment. Depending on the time of assessment, two or three male rats in the 80-mg/kg-day group displayed enhanced response to tail pinch, versus zero or one in the control and lower dose groups; however, the incidence was not statistically significantly different from control at any time and there was no similar observation in females (see Table B-1). Forelimb and hindlimb grip strength and foot splay were not altered by exposure at any dose in males or females. An increase in relative foot splay in high-dose males during Week 4 was attributed to unusually low values obtained at baseline. Both male and female rats exposed to the highest dose exhibited statistically significantly increased incidences of slight palpebral closure (i.e., rats with their eyelids slightly closed) during Week 13 (see Table B-1). Motor activity measures were comparable among control and treated groups of rats. There were no exposure-related ocular effects in either male or female rats. Microscopic examination of nervous system tissues did not indicate any significant differences from controls for any lesion. The study authors identified the 40-mg/kg-day dose as a no-effect level in both male and female rats. At this dose, a significant decrease in body-weight gain (calculated over the study duration) was observed in male rats, but the absolute body weights did not differ at any time and no such decrease in overall body-weight gain was seen in high dose males. The high dose (80 mg/kg-day) is a lowest-observed-adverse-effect level (LOAEL) for increased incidence of slight palpebral closure (males and females). Similar clinical signs were seen in an acute oral neurotoxicity study conducted by the same laboratory, in which single gavage doses of 225−900 mg/kg were administered to rats [DuPont (1990), see discussion in the "Neurotoxicity" section], providing support for this effect level. At this dose, incidences of persistent or recurrent yellow staining of perineum, abdomen, inguen, and/or underbody in female rats were significantly increased, while these were not observed in the males. However, the biological significance of these observations is unclear because they could represent changes in grooming behavior, rather than to the direct toxic effect of *o*-phenylenediamine. The no-observed-adverse-effect level (NOAEL) is 40 mg/kg-day.

Chronic-Duration/Carcinogenicity Studies

Matsumoto et al. (2012) (Rat Study)

A chronic-duration carcinogenicity study of *o*-phenylenediamine dihydrochloride (99.5% pure) was conducted in rats exposed via drinking water (Matsumoto et al., 2012). Groups of 50 male and 50 female F344/DuCrj rats were exposed for 2 years to drinking water concentrations of 0, 250 (females only), 500, 1,000, or 2,000 ppm (males only). Exposure solutions were prepared twice weekly, and the concentrations determined analytically every 3 months during the study. The study authors calculated *o*-phenylenediamine dihydrochloride doses of 0, 22, 42, and 86 mg/kg-day in male rats and 0, 18, 33, and 58 mg/kg-day in female rats. Equivalent doses of *o*-phenylenediamine[1] are 0, 13, 25, and 51 mg/kg-day in male rats and 0, 11, 20, and 35 mg/kg-day in female rats. The authors reported that the doses were chosen based on their 13-week unpublished study that showed that female rats were more sensitive than male rats. In that experiment, 2/10 female rats given 3,000 ppm *o*-phenylenediamine dihydrochloride died; however, timing of deaths and additional information were not reported. A complete report of this subchronic-duration study could not be located for further review.

[1]Calculated as the product of dose (mg/kg-day) *o*-phenylenediamine dihydrochloride and the ratio of molecular weights (108.14 g/mol for *o*-phenylenediamine:181.062 g/mol for *o*-phenylenediamine dihydrochloride).

The investigators performed daily observations of the animals for death and clinical signs of toxicity. Food and water intake and body weights were measured weekly for 14 weeks and monthly thereafter. At sacrifice, blood was collected for hematology and clinical chemistry, and urine samples were collected and analyzed. The measured hematological parameters included Hb, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelets. Serum samples were analyzed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), and blood urea nitrogen (BUN). Urinalysis measured occult blood and pH. All animals (including those that died or were sacrificed moribund) received gross necropsy. Organ-weight measurements and histopathology examinations were performed, but the study authors did not provide details of the selected tissues.

Survival was not significantly altered by treatment with *o*-phenylenediamine dihydrochloride (see Table B-2); the 2-year survival rates for all groups ranged between 72 and 88% and exhibited no dose-related reductions in males or females. The only clinical signs of toxicity were observed in the high-dose males, which exhibited hematuria (presence of blood in urine) after the first year on treatment (incidence and frequency not reported). Biological and/or statistically significant dose-related reductions in body weight (compared with controls) were evident in both sexes (see Table B-2). In males, terminal body weights were significantly reduced by 7, 14, and 30% (compared with controls) at 13-, 25-, and 51-mg/kg-day *o*-phenylenediamine, respectively. In females, terminal body weights were 6, 8, and 19% lower than controls at 11-, 20-, and 35-mg/kg-day *o*-phenylenediamine, respectively; the differences were significant at the two higher doses. All treated male rats exhibited significantly lower water intake than controls (10, 17, and 25% less in the low-, medium-, and high-dose groups), as did females in the mid- and high-dose groups (15 and 30% less than controls, respectively). Food intake was also reduced in high-dose males and females (compared with controls) throughout the study, and in all dosed males in the beginning and end of the study (data not shown).

Among male rats, small (≤4% difference from controls) reductions in MCV and MCH occurred, but the changes were not dose-related (see Table B-3). Among females, statistically significant, albeit modest (≤3% difference from controls), reductions in Hb, MCV, MCH, and MCHC were seen at the high dose (see Table B-3). In addition, platelet count was statistically significantly increased (21% higher than controls) in high-dose females. Serum chemistry changes indicative of liver toxicity (see Table B-3) occurred in high-dose male and female rats, including marked, statistically significant, increases in AST and ALT (both sexes) and in ALP and GGT (females only). In males, AST and ALT were increased by >18- and >4-fold, respectively, compared with controls; in females, AST and ALT were both increased by >3-fold. Increases in ALP and GGT in high-dose females were 48% and 6-fold, respectively, compared with controls. Finally, both male and female rats exposed to the highest doses exhibited statistically significantly higher BUN than controls (36 and 9% higher than controls, respectively; see Table B-3). Statistically significant increases in relative liver weights were observed in both male and female rats at the high dose (16 and 75% higher than controls, respectively) and in males exposed to 25-mg/kg-day *o*-phenylenediamine (8% higher) (see Table B-2). Absolute liver weight was increased in high-dose females (41% higher than controls), while male rats exhibited dose-related declines in absolute liver weight, likely related to the declines in body weight. Weights of organs other than liver were either not measured or not reported.

In both male and female rats exposed to *o*-phenylenediamine dihydrochloride, non-neoplastic histopathology findings occurred in the liver, urinary bladder, and kidney. The incidences of these lesions are shown in Table B-4. All exposed male groups exhibited statistically significant increases in the incidences of basophilic cell foci of the liver, and males of the low- and mid-dose groups also exhibited increased incidences of clear cell foci. Exposed females at all doses exhibited statistically significantly increased incidences of basophilic cell foci, but not clear cell foci. Renal lesions occurring at statistically significantly increased incidences at all doses included papillary mineralization in males and renal pelvis urothelial hyperplasia in females (see Table B-4). Male rats exposed to ≥25-mg/kg-day *o*-phenylenediamine also exhibited urothelial hyperplasia of the renal pelvis, and high-dose males exhibited an increased incidence of papillary necrosis. In females, other renal lesions (papillary necrosis and mineralization) occurred at statistically significantly increased incidence at the high dose only. Only high-dose male rats exhibited a statistically significant increase in the incidence of bladder lesions (papillary and/or nodular hyperplasia of the transitional epithelium). The lowest dose in this study is a LOAEL for increased incidences of renal papillary mineralization and basophilic cell foci of the liver in males (13-mg/kg-day *o*-phenylenediamine) and increased incidences of urothelial hyperplasia of the renal pelvis and basophilic cell foci of the liver in females (11-mg/kg-day *o*-phenylenediamine). At this dose, male rats also exhibited a significantly lower terminal body weight than controls, although the decrease was <10% and was possibly attributed to concomitant decreases in food and water consumption rather than a direct adverse effect of *o*-phenylenediamine. A NOAEL is not identified.

Tumors of the liver, including hepatocellular adenomas and carcinomas, were observed at significantly increased incidences in both male and female rats (see Table B-5). Statistically significant increases occurred at doses ≥25-mg/kg-day *o*-phenylenediamine in males and ≥20-mg/kg-day *o*-phenylenediamine in females. High-dose male rats also exhibited a significantly increased incidence of transitional cell papilloma and/or carcinoma of the urinary bladder; this tumor did not occur at increased incidence in females. Finally, a significant dose-related trend was seen in the incidence of follicular adenoma of the thyroid in male rats; pairwise comparisons with the control incidence were not significant at any dose. Treatment with *o*-phenylenediamine dihydrochloride did not alter the incidence of thyroid tumors in female rats.

Matsumoto et al. (2012): Mouse Study

A chronic-duration carcinogenicity study of *o*-phenylenediamine dihydrochloride (purity 99.5%) was conducted in mice exposed via drinking water (Matsumoto et al., 2012). Groups of 50 male and 50 female Crj:BDF_1 mice were exposed for 2 years to drinking water concentrations of 0, 500 (males only), 1,000, 2,000, or 4,000 (females only) ppm. Exposure solutions were prepared twice weekly, and the concentrations determined analytically every 3 months during the study. The study authors calculated *o*-phenylenediamine dihydrochloride doses of 0, 46, 94, and 177 mg/kg-day in male mice and 0, 106, 200, and 391 mg/kg-day in female mice. Equivalent doses of *o*-phenylenediamine[2] are 0, 27, 56, and 106 mg/kg-day in male mice and 0, 63.3, 119, and 234 mg/kg-day in female mice. The study authors reported that the doses were chosen based on the authors' 13-week unpublished study that showed that male

[2]Calculated as the product of dose (mg/kg-day) *o*-phenylenediamine dihydrochloride and the ratio of molecular weights (108.14 g/mol for *o*-phenylenediamine:181.062 g/mol for *o*-phenylenediamine dihydrochloride).

mice were more sensitive than female mice. A complete report of this 13-week, subchronic-duration study could not be located for further review.

As in the rat study above, daily observations for death and clinical signs of toxicity were performed. Food and water intake and body weights were measured weekly for 14 weeks and monthly thereafter. At sacrifice, blood was collected for hematology and clinical chemistry, and urine samples were collected and analyzed; red and white blood cells were measured in mice, while all other parameters were measured in both mice and rats. All of the mice (including those that died or were sacrificed moribund) received gross necropsy. Organ-weight measurements and histopathology examinations were performed, but the study authors did not provide details of the selected tissues.

Survival of mice was not affected by treatment with *o*-phenylenediamine dihydrochloride (see Table B-6); 2-year survival rates ranged between 76 and 84% in males and between 48 and 68% in females. No exposure-related clinical signs of toxicity were noted at any dose. Terminal body weight was significantly decreased at all doses in male mice (16−36% lower than controls) and at ≥119 mg/kg-day in female mice (15−31% lower than controls). Intake of water was significantly reduced in all treated animals (7−35% lower than controls); food consumption rates were also significantly decreased in male and female mice at all doses (data not shown).

Hematology analyses showed statistically significant reductions in red blood cells (RBCs) (males only), white blood cells (WBCs) (males only), Hb (males and females), and MCHC (males and females) in mice receiving the highest dose of *o*-phenylenediamine dihydrochloride (see Table B-7); the decreases (relative to controls) ranged between 1 and 5% for the erythrocyte parameters and between 9−55% for total leukocyte count. In addition, MCV and platelet counts were statistically significantly increased in both sexes at the high dose. The only hematology parameter with a statistically significant change at the middle dose was MCHC, which was decreased by 2% relative to controls in females receiving 119-mg/kg-day *o*-phenylenediamine. Statistically significant, marked increases in serum levels of liver enzymes occurred in both male and female mice. In males, ALT was statistically significantly increased (>2-fold) at doses ≥56 mg/kg-day, and ALP was significantly increased (by 77−172%) at all doses. Serum AST and GGT increased from controls at all doses in male mice and all doses except the lowest dose for GGT in female mice, but did not reach statistical significance. In female mice, serum ALP was significantly increased at all doses (from a 49% increase over controls at the low dose to a 3.5-fold increase at the high dose). Serum ALT was statistically significantly increased (>2-fold compared to controls) at 234-mg/kg-day *o*-phenylenediamine in females. In both male and female mice, statistically significantly increased BUN was noted at the middle and high doses; these changes ranged between 17−32% above controls.

Relative liver weights were significantly increased in male mice at all doses (26−57%) and in females at the two higher doses (61−93%) compared with controls (see Table B-6). Absolute liver weights increased >10% compared to control in male mice exposed to 56 mg/kg-day and female mice ≥119 mg/kg-day, but not statistically significantly. Weights of organs other than the liver were either not measured or not reported. As was seen with the rats, histopathology lesions were noted in the livers and kidneys of mice; however, mice also developed microscopic lesions in the gall bladder, nasal cavity, and nasopharynx (see Table B-8). In male mice, statistically significant increases in the incidences of basophilic cell foci of the liver (≥56-mg/kg-day *o*-phenylenediamine), acidophilic cell foci of the liver

(27 and 56 mg/kg-day), papillary hyperplasia of the gall bladder (at all doses), eosinophilic changes in the olfactory epithelium (27 mg/kg-day), and eosinophilic changes in the respiratory epithelium of the nasal cavity (at 106 mg/kg-day) were noted. Females exhibited statistically significant increased incidences of clear cell (at 234 mg/kg-day), acidophilic cell of the liver (at 234 mg/kg-day), and basophilic cell foci of the liver (at 63.3 and 234 mg/kg-day); papillary hyperplasia of the gall bladder (at ≥119 mg/kg-day); hydronephrosis of the kidney (at all doses); inflammatory polyps of the renal pelvis (at 63.3 and 119 mg/kg-day; increase was not statistically significant at 234 mg/kg-day); eosinophilic change of the respiratory epithelium in the nasal cavity (at all doses); glandular respiratory metaplasia in the nasal cavity (at ≥119 mg/kg-day); and eosinophilic change of the nasal olfactory epithelium and the nasopharynx (at the highest dose).

The lowest dose (27-mg/kg-day *o*-phenylenediamine in males and 63.3-mg/kg-day *o*-phenylenediamine in females) is a LOAEL based on increased serum ALP (both sexes), increased incidence of papillary hyperplasia of the gall bladder (males), and increased incidences of eosinophilic change of the nasal cavity respiratory epithelium and hydronephrosis of the kidney (females). At this dose, male mice also exhibited a significantly lower terminal body weight (>10%) compared to controls; however, this decrease may be the result of concomitant decreases in food and water intake. In addition, male and female mice exposed to the lowest dose exhibited an increase in relative liver weight (>10%); however, there is uncertainty whether the liver weight measurements were affected by significant increases in liver tumor incidences at all doses or the reduction in terminal body weight. A NOAEL is not identified.

Exposure to *o*-phenylenediamine dihydrochloride for 2 years resulted in increased incidences of hepatocellular adenoma and/or carcinoma in male and female mice at all doses; the data are shown in Table B-9. In addition, significant increases in the incidence of papillary adenoma of the gall bladder were noted in high-dose male and mid-dose female mice (incidences were 0/46, 2/50, 4/49, and 5/47 in control, low-, mid-, and high-dose males and 0/50, 1/50, 5/50, and 3/50 in the respective female groups) (see Table B-9). This finding is remarkable because spontaneous papillary adenoma of the gall bladder is a rare tumor in mice. The researchers reported that papillary adenomas of the gall bladder occurred in only 9 of the 60,000 control and chemically treated $B6C3F_1$ mice in the NTP database through 1998, and in none of the almost 2,600 historical control BDF_1 mice in the Japanese Bioassay Research Center.

Weisburger et al. (1978) (Rat Study)

In a chronic-duration carcinogenicity study of 21 aromatic amines, Sprague-Dawley (S-D)−derived Charles River CD rats (25 males/group) were exposed to *o*-phenylenediamine dihydrochloride (97−99% pure; purity of individual test compounds not specified) at concentrations of 0, 2,000, or 4,000 ppm in the diet for 18 months (Weisburger et al., 1978). Doses of 0, 140, or 279 mg/kg-day as *o*-phenylenediamine dihydrochloride[3] are calculated for this review; these doses correspond to equivalent doses of 0, 83.6, or 167 mg/kg-day *o*-phenylenediamine. The rats were observed for up to 6 months after the end of the treatment period and monitored daily for mortality and clinical signs of toxicity. Body weights were consistently recorded. Complete necropsies were conducted on all animals that died after

[3]Based on chronic reference values for food consumption (0.036 kg/day) and body weight (0.523 kg) in male S-D rats (U.S. EPA, 1988). Reference values for CD rats are not available; in the absence of strain-specific information, data for S-D rats were used because CD rats are derived from the S-D strain.

≥6 months of treatment or at study termination. Histological examinations of grossly abnormal organs, tumor masses, lung, liver, kidneys, spleen, adrenal, heart, bladder, stomach, intestines, reproductive organs, and pituitaries were performed.

The results reported in the study were limited to neoplastic changes; no data on mortality, clinical signs of toxicity, body weights, or non-neoplastic findings were provided. The authors indicated that their protocol called for doses to be decreased if a body-weight-gain difference of ≥10% from controls was observed or if animals died due to toxicity, but apparently neither mortality nor significant body-weight-gain differences were observed because the doses were unchanged over the treatment period. Male rats exposed to 167-mg/kg-day *o*-phenylenediamine exhibited a significant ($p < 0.025$) increase in hepatocellular carcinomas when compared with either simultaneous controls or controls pooled across the experiments with all 21 compounds (see Table B-10). No liver tumors occurred at the low dose, and no other tumor types were reported.

Weisburger et al. (1978) (Mouse Study)

In a companion mouse study, HaM/ICR-derived albino CD-1 mice (25/sex/group) were treated with concentrations of 0, 4,000, or 8,000 ppm in the diet for 5 months, after which the dietary concentrations were increased to 8,000 and 16,000 ppm, respectively, for the remaining 13 months on treatment. The study authors did not indicate why the exposure concentrations were increased, but presumably, toxicity was low at the initial concentrations. These concentrations yielded TWA concentrations of 6,900 and 14,000 ppm for the low and high doses, respectively, corresponding to doses of 0-, 1,180-, or 2,390-mg/kg-day and 0-, 1,200-, or 2,440-mg/kg-day *o*-phenylenediamine dihydrochloride for male and female mice, respectively.[4] These doses correspond to equivalent doses of 0-, 704-, or 1,430-mg/kg-day and 0-, 717-, or 1,450-mg/kg-day *o*-phenylenediamine for male and female mice, respectively. Mice in all groups were given control diets for 3 months after the end of exposure. The same toxicological parameters that were evaluated in rats were also evaluated in mice, and the same tissues were subjected to histological examination, except that pituitaries were not examined.

No data regarding mortality, clinical signs of toxicity, or body weights were reported; however, as noted above, the increase in exposure concentration after 5 months suggests that the mice tolerated the initial doses. As seen with rats, both male and female mice exhibited significant ($p < 0.05$) increases in hepatocellular carcinomas (see Table B-10). In male mice, the increase was statistically significant only at the low dose (704-mg/kg-day *o*-phenylenediamine), and not at the high dose. In female mice, the increase was statistically significant at both doses, but the incidence at the low dose was significant only when compared with the pooled control group. This carcinogenicity study is limited in that small sample sizes were used, only two doses were tested, exposure duration was less than lifetime, and data reporting was incomplete (growth and survival data were not reported).

[4]Based on chronic reference values for food consumption (0.0064 and 0.0061 kg/day in males and females, respectively) and body weight (0.0373 and 0.0353 kg in males and females, respectively) in $B6C3F_1$ mice (U.S. EPA, 1988). Reference values for CD-1 mice are not available; in the absence of strain-specific information, U.S. EPA recommends using data for $B6C3F_1$ mice.

The lack of data on noncancer endpoints (other than the inference that toxicity was low based on the authors' decision to increase the doses) precludes the identification of noncancer effect levels for rats or mice.

Inhalation Exposures

No studies have been identified.

OTHER DATA (SHORT-TERM TESTS, OTHER EXAMINATIONS)

Table 4A provides an overview of genotoxicity studies of *o*-phenylenediamine. Table 4B provides an overview of acute oral and inhalation lethality studies in rats. Acute dermal lethality and toxicity studies, skin and eye irritation studies, and a skin sensitization study are described briefly below in the "Other Routes" section. Dermal absorption of *o*-phenylenediamine was evaluated in a single in vitro study described below in the "Metabolism/Toxicokinetic Studies" stection; no other studies were pertinent to the toxicokinetics of *o*-phenylenediamine.

Table 4A. Summary of *o*-Phenylenediamine (CASRN 95-54-5) 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* strains TA98 and TA100 and their nitroreductase-deficient mutants, TA98NR and TA100NR	1, 10, 30, 300, 1,000, 3,000 µg/plate	−	+ (TA98, TA98NR) − (TA100, TA100NR)	Preincubation assay. *o*-Phenylenediamine was mutagenic to TA98NR at ≥10 µg/plate with S9 added. Study authors reported that *o*-phenylenediamine was a potent mutagen in strain TA98 in the presence of S9, but data were not provided. Cytotoxic to TA98NR and TA100NR at 3,000 µg/plate.	Chung et al. (1996)
Mutation	*S. typhimurium* strains TA98 and TA100	0, 1, 10, 100, 1,000, 10,000 µg/plate	−	+ (TA98, TA100)	Plate incorporation and preincubation assays. *o*-Phenylenediamine was mutagenic at ≥10 µg/plate. Mutagenic in TA98 at lower concentrations than in TA100. Cytotoxicity observed at 10,000 µg/plate (highest concentration tested).	Gentile et al. (1987)
Mutation	*S. typhimurium* strain TA1538	0, 20, 40, 60, 80, 100 µg/plate	NT	+ (TA1538)	Plate incorporation assay. *o*-Phenylenediamine was mutagenic to TA1538 at ≥50 µg/plate with S9 added (effective concentration estimated from data presented graphically).	Ames et al. (1975)
Mutation	*S. typhimurium* strains C3076, D3052, G46, TA98, TA 100, TA1535, TA1537, and TA1538	0.1−100 µg/mL	−	+ (D3052, G46, TA98, TA100, TA1537, TA1538) – (C3076, TA1535)	Modified Ames gradient plate test. TA1538 and TA98 showed the greatest sensitivity to *o*-phenylenediamine, with positive results at ≥0.1 µg/mL.	Thompson et al. (1983)

Table 4A. Summary of *o*-Phenylenediamine (CASRN 95-54-5) Genotoxicity						
Endpoint	**Test System**	**Doses/ Concentrations Tested**	**Results without Activation[a]**	**Results with Activation[a]**	**Comments**	**References**
Mutation	*S. typhimurium* strains TA100, TA1535, TA1537, and TA1538	1, 10, 50, 100, 250, 500, 750, 1,000 µg/plate	–	+ (TA1538) – (TA100, TA1535, TA1537)	Plate incorporation assay. Mutagenic in strain TA1538 only, at ≥250 µg/plate.	Zeiger et al. (1988)
Mutation	*S. typhimurium* strains TA98, TA100, and TA1537	0.01, 0.1, 0.5, 1, 2, 5 g/L	± (TA98) – (TA100, TA1537)	+ (TA98) – (TA100, TA1537)	Plate incorporation assay. Mutagenic in TA98 at ≥0.01 g/L in the presence of S9. Mutagenic without S9 only at a concentration of 1 g/L, but not at higher concentrations.	Voogd et al. (1980)
Mutation	*S. typhimurium* strains TA98 and TA100	68, 135, 269, 539, 1,077 µg/plate	–	+ (TA98) – (TA100)	Plate incorporation assay. Mutagenic in TA98 at ≥269 µg/plate.	Assmann et al. (1997)
Mutation	*S. typhimurium* strains TA98, TA100, and TA1537	NR	See comments	See comments	Preincubation assay. *o*-Phenylenediamine was reportedly positive, but dose, presence or absence of S9, and affected strain(s) were not specified.	Ishidate and Yoshikawa (1980)
Mutation	*S. typhimurium* strain TA98	3 µg/plate	–	– (without H_2O_2) + (with H_2O_2)	Suspension assay. Mutagenicity tested before and after addition of H_2O_2. H_2O_2 oxidation products of *o*-phenylenediamine were mutagenic with S9.	Watanabe et al. (1989)
Mutation	*Escherichia coli* strains WP2 and WP2uvrA-	0.1−100 µg/mL	–	–	Modified Ames gradient plate test	Thompson et al. (1983)

Table 4A. Summary of *o*-Phenylenediamine (CASRN 95-54-5) Genotoxicity						
Endpoint	**Test System**	**Doses/ Concentrations Tested**	**Results without Activation[a]**	**Results with Activation[a]**	**Comments**	**References**
Mutation	*Klebsiella pneumoniae*	0.5 g/L	−	−	Fluctuation test	Voogd et al. (1980)
Genotoxicity studies in nonmammalian eukaryotic organisms						
Mutation	*Saccharomyces cerevisiae* D3	1−5%	−	−	Toxicity observed at concentration between 1 and 5% (not further specified).	Zeiger et al. (1988)
Genotoxicity studies in mammalian cells in vitro						
Mutation	Mouse lymphoma cells (L5178Y TK ±)	0.04, 0.08, 0.12, 0.15, 1.0 mM	+	+	Mutagenic at ≥0.08 mM	Asgård et al. (2013)
CAs	CHO-K1 cells	187, 374, 748, 1,122 µg/mL	+	NA	Increased percentage of aberrant cells at ≥187 µg/mL. The TC_{50} (concentration cytotoxic to 50% of cells) was 374 ± 30 µg/mL. S9 mix was not included because nitroreductase in the mix affects the direct-acting mutagenic activity of the test compound.	Chung et al. (1996)
CAs	Chinese hamster lung fibroblasts	NR	+	+	CAs detected in 20% of metaphase cells at concentrations between 10^{-2} and 10^{-3} mg/mL (results shown graphically).	Ishidate and Yoshikawa (1980)
CAs	Human lymphocytes	5, 10, 15 mM	+	NA	A twofold increase in total aberration frequency/cell, including gaps, was observed at ≥5 mM *o*-phenylenediamine.	Cebulska-Wasilewska et al. (1998)
SCE	Human lymphocytes	5, 10, 15 mM	+	NA	A twofold increase in SCE/cell was observed at ≥10 mM *o*-phenylenediamine.	Cebulska-Wasilewska et al. (1998)
Unscheduled DNA synthesis	Primary rat hepatocytes	NR (see comments)	NA	+	50−500 nM/mL (effective concentration range)	Thompson et al. (1983)
DNA damage	Mouse lymphoma cells (L5178Y TK ±)	0.04, 0.08, 0.12, 0.15, 1.0 mM	+	+	General DNA damage (frank strand breaks and alkali-labile sites, comet assay) at 0.08 mM without S9 and 0.12 mM with S9. Oxidative DNA damage (modified comet assay with enzyme hOGG1) at 0.08 mM with and without S9.	Asgård et al. (2013)

Table 4A. Summary of *o*-Phenylenediamine (CASRN 95-54-5) Genotoxicity						
Endpoint	**Test System**	**Doses/ Concentrations Tested**	**Results without Activation[a]**	**Results with Activation[a]**	**Comments**	**References**
DNA damage	Human lymphocytes	5, 10, 15 mM	+	NA	Comet assay; increased tail moment at 15 mM (effective concentration defined as that yielding mean tail moment ≥control +2 SD).	Cebulska-Wasilewska et al. (1998)
Genotoxicity studies—in vivo						
Dominant lethal mutagenicity	Male Charles River CD rats (20/group) treated with *o*-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)
Somatic mutation	Mouse spot test; female C57BL/6JHan mice (unspecified number/group) mated with T-stock males and subsequently given i.p. injection of *o*-phenylenediamine in saline on GD 10; offspring examined for spots from Wk 2−4 after birth	NR (see comments)	−	−	Pre and postnatal mortality increased at 108 and 215 mg/kg; negative for spot test at "weighted" dose of 196 mg/kg (as reported by study authors).	Gocke et al. (1983)
Mouse bone marrow MN test (oral)	NMRI mice (two/sex/group); 2 doses orally in 0.9% NaCl at 1 and 24 hr; sacrifice 6 hr after second exposure	108, 216, 324 mg/kg/dose	+	+	Significant increase in frequency of MNPCEs at ≥108 mg/kg/dose.	Wild et al. (1980)

Table 4A. Summary of *o*-Phenylenediamine (CASRN 95-54-5) Genotoxicity						
Endpoint	**Test System**	**Doses/ Concentrations Tested**	**Results without Activation[a]**	**Results with Activation[a]**	**Comments**	**References**
Bone marrow micronuclei	NMRI mice (two/sex/group); 2 doses i.p. in 0.9% NaCl at 1 and 24 hr; sacrifice 6 hr after second exposure	27, 54, 108, 216, 324 mg/kg/ injection	+	+	Significant increase in frequency of MNPCEs at ≥108 mg/kg/injection.	Wild et al. (1980)
Bone marrow micronuclei	Chinese hamster (two/sex/group); 2 doses i.p. in 0.9% NaCl at 1 and 24 hr; sacrifice 6 hr after second exposure	54, 108, 216, 324 mg/kg/ injection	+	+	Significant increase in frequency of MNPCEs at ≥216 mg/kg/injection.	Wild et al. (1980)
Bone marrow micronuclei	Male and female albino guinea pigs (three/group; unspecified strain); 2 doses i.p. in 0.9% NaCl at 1 and 24 hr; sacrifice 6 hr after second exposure	108, 216, 324 mg/kg/ injection	+	+	Significant increase in frequency of MNPCEs at ≥108 mg/kg/injection.	Wild et al. (1980)

Table 4A. Summary of *o*-Phenylenediamine (CASRN 95-54-5) Genotoxicity						
Endpoint	**Test System**	**Doses/ Concentrations Tested**	**Results without Activation[a]**	**Results with Activation[a]**	**Comments**	**References**
Inhibition of testicular DNA synthesis	Male mice (three to four/group, unspecified strain), *o*-phenylenediamine administered in DMSO orally; ^{3}H-thymidine incorporation measured in testicular DNA	200 mg/kg	+	+	Significant inhibition of testicular DNA synthesis relative to controls.	Seiler (1977)
Genotoxicity studies in subcellular systems						
DNA damage	λDNA	250 µM	+	NA	Yielded DNA fragments >4 × 10^{6} daltons in size.	Yamada et al. (1985)

[a]+ = positive; − = negative.

CA = chromosomal aberration; CHO = Chinese hamster ovary; DMSO = dimethylsulfoxide; DNA = deoxyribonucleic acid; GD = gestation day; i.p. = intraperitoneal; MN = micronuclei; MNPCE = micronucleated polychromatic erythrocyte; NA = not applicable; NR = not reported; NT = not tested; SCE = sister chromatid exchange; SD = standard deviation.

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Acute toxicity (oral/inhalation)				
Acute oral lethality rats	Rats (strain and sex unspecified; 10/dose) were administered *o*-phenylenediamine via gavage at doses of 501, 562, 631, 708, 800, 891, 1,000, 1,122, or 1,259 mg/kg. Observation time following exposure, clinical signs, and necropsy findings not reported. Study reported in tabular form with few details.	Mortality: 1/10, 0/10, 0/10, 2/10, 3/10, 4/10, 8/10, 8/10, and 9/10 deaths at 501, 562, 631, 708, 800, 891, 1,000, 1,122, and 1,259 mg/kg, respectively	Oral LD_{50} = 900 mg/kg	Rhone-Poulenc (1951); Woodard (1951)
Acute oral lethality mice	Mice (strain and sex unspecified; 10/dose) were administered *o*-phenylenediamine via gavage at doses of 794, 841, 891, 944, 1,000, 1,059, 1,122, or 1,259 mg/kg. Observation time following exposure, clinical signs, and necropsy findings not reported. Study reported in tabular form with few details.	Mortality: 5/10, 0/10, 1/10, 2/10, 5/10, 4/10, 9/10, and 10/10 deaths at 794, 841, 891, 944, 1,000, 1,059, 1,122, and 1,259 mg/kg, respectively	Oral LD_{50} = 1,000 mg/kg	Rhone-Poulenc (1951); Woodard (1951)
Acute oral lethality	Individual male ChR-CD rats received *o*-phenylenediamine in peanut oil via single gavage doses of 450, 670, 1,000, 1,500, or 2,250 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.	The rat exposed to 2,250 mg/kg died 1 hr after dosing. There were no other deaths. Clinical signs in surviving rats included irregular breathing, pallor, dark red-brown urine, poor muscle tone, restlessness, lethargy, and initial weight loss.	The study authors reported the ALD as 2,250 mg/kg.	DuPont (1967a)
Acute oral lethality	Individual male ChR-CD rats received *o*-phenylenediamine in peanut oil or in acetone:peanut oil (1:10) via single gavage doses of 0, 300, 450, 670, 1,000, 1,500, 2,250, or 3,400 mg/kg. Clinical signs of toxicity and body weight were monitored and animals were observed for 14 d after dosing. At sacrifice, all animals were subjected to gross and histopathological examination. Study reported in tabular form with few details.	Rats exposed to ≥1,500 mg/kg died within 2 d after dosing. Pathological changes in animals that died included fatty changes and congestion in the liver. Clinical signs of toxicity in surviving animals included weight loss and discolored urine at ≥450 mg/kg. Fatty changes and congestion of the liver were also seen at sacrifice of the rat exposed to 1,000 mg/kg. No pathology changes attributed to dosing were reported at doses ≤670 mg/kg.	The study authors reported the ALD as 1,500 mg/kg.	DuPont (1967b)
Acute oral lethality	Seven individual female Charles River rats were given single gavage doses of *o*-phenylenediamine in aqueous solution or methylcellulose suspension (10, 30, 100, 300, 1,000, 3,000, or 10,000 mg/kg). Clinical signs of toxicity and body weight were monitored, and animals were observed for 14 d following dosing. Gross necropsies were conducted on all animals. Study was reported in tabular form with few details.	Mortality occurred at 3,000 and 10,000 mg/kg. Surviving rats exhibited hypoactivity and roughed fur (≥100 mg/kg), ptosis (≥300 mg/kg), and hyperpnea (1,000 mg/kg). Gross necropsy of decedents revealed hemorrhaging in the gastrointestinal tract and mottled kidneys. No gross pathological findings were noted in surviving animals.	*o*-Phenylenediamine was lethal to rats at oral doses of ≥3,000 mg/kg. This conclusion should be viewed with caution due to issues with reliability of the performing laboratory.[a]	IBT Labs (1983)

Table 4B. Other Studies				
Test	**Materials and Methods**	**Results**	**Conclusions**	**References**
Acute inhalation lethality	Male ChR-CD rats (six/group) were exposed to *o*-phenylenediamine aerosol for 4 hr at analytical TWA concentrations of 1.0, 1.3, 2.1, 3.1, 3.4, or 9.2 mg/L (1,000−9,200 mg/m^3). Exposures were generated by heating the *o*-phenylenediamine and forming aerosols by blowing compressed nitrogen through a nebulizer immersed in the molten test material. The "pure" *o*-phenylenediamine concentrations were measured by ultraviolet spectroscopy in order to account for the known presence of oxidation products. The aerosol mass median diameters varied with concentration, ranging from 1.8−3.3 µm. The study was reported in tabular form and details of the toxicological assessments performed were not provided. The timing of sacrifice varied both among and within groups.	Mortality occurred in all exposure groups: 1/6, 2/6, 1/6, 3/6, 3/6, and 6/6 deaths at 1,000, 1,300, 2,100, 3,100, 3,400, and 9,200 mg/m^3, respectively. Irregular breathing was observed in all rats during exposure, and rats exposed to 1,300 and 2,100 mg/m^3 did not respond to sound stimulation. Clinical signs of toxicity observed after the exposure period included hypersensitivity, aggression, and yellowed fur at 1,300 mg/m^3; colored urine, tremors, weakness, and loss of balance or incoordination were seen at higher exposures. Hindquarter paralysis was seen in one surviving rat exposed to 2,100 mg/m^3 and in two surviving rats in each of the 3,100- and 3,400-mg/m^3 groups. Weight loss of ~10% was noted in rats exposed to ≤2,100 mg/m^3, while at ≥3,100 mg/m^3, weight losses were ~10−15%. Histopathological findings included evidence of respiratory tract irritation and decreased sperm formation (data and details not provided). Damage to testicular germinal epithelium was observed in animals sacrificed 7, 14, 18, and 46 d after exposure (affected groups and further details not provided).	A 4-hr LC_{50} of 3,600 mg/m^3 (95% CI 2,500−5,200 mg/m^3) was estimated for *o*-phenylenediamine aerosol. Exposure to concentrations of ≥1,300 mg/m^3 resulted in signs of neurotoxicity.	DuPont (1969)

Table 4B. Other Studies				
Test	**Materials and Methods**	**Results**	**Conclusions**	**References**
Acute inhalation lethality	Male and female S-D rats (10/sex) were exposed to a measured concentration of 2.46-mg/L (2,460 mg/m^3) *o*-phenylenediamine aerosol (equivalent aerodynamic diameter of 7.2 µm ± 2.43) for 1 hr. The aerosol was generated by delivery of *o*-phenylenediamine powder mixed with dry filtered air into the exposure chamber; concentrations were measured by collecting material on a filter and weighing it. Animals were observed for clinical signs of toxicity during exposure and daily thereafter for 14 d. Body weights were recorded prior to exposure, and after 7 and 14 d. Gross necropsies were performed on all animals.	No deaths occurred. Clinical signs of toxicity were more pronounced or at higher incidence in males than in females. All exposed males exhibited decreased activity on D 2 and hyperactivity on D 2−5. Increased aggression was noted in both males and females. Other clinical signs included labored breathing, salivation, and body tremors. Male rats (6/10) developed alopecia in the second wk. At 10 d postexposure, 7/10 male rats and 3/10 females exhibited loss of muscle coordination in the hind limbs; one female became paraplegic. Mean body-weight gain for both sexes was normal for the duration of the study. Gross findings at necropsy included gelatinous areas on the lung surfaces in approximately 1/3 of the males and females, a “slight incidence” of enlarged cervical lymph nodes in both sexes, and enlarged adrenal glands in 4/10 females.	Exposure to 2,460-mg/m^3 *o*-phenylenediamine aerosol for 1 hr was not lethal to rats, but resulted in clinical signs of neurotoxicity as well as grossly visible lesions of the lung and enlarged lymph nodes and adrenal glands.	Sherwin Williams (1992)

Table 4B. Other Studies				
Test	**Materials and Methods**	**Results**	**Conclusions**	**References**
Acute inhalation toxicity	Male and female BLU:(LE)BR rats (five/sex/group) were exposed to 1,340-mg/m^3 (nominal concentration) *o*-phenylenediamine vapor for 4 hr. Clinical signs of toxicity and body weight were monitored for 14 d following exposure. Gross necropsy was performed on all animals. The study was reported in tabular form with few details.	All animals survived exposure and no clinical signs of toxicity were noted. Body-weight gain was within normal limits for all animals. There were no gross pathological findings at necropsy.	A 4-hr exposure to 1,340-mg/m^3 *o*-phenylenediamine vapor was not lethal to 10 rats. This conclusion should be viewed with caution due to issues with reliability of the performing laboratory.[a]	IBT Labs (1975c)

[a]A total of 618/867 nonacute toxicity studies conducted by Industrial Bio-Test Laboratories (including subacute-duration, subchronic-duration, carcinogenicity, reproductive toxicity, genotoxicity, and neurotoxicity studies) were found to be invalid during a post hoc audit program conducted by U.S. EPA and the Canadian Health and Welfare Department (OECD, 2007). Discrepancies and deficiencies were also identified in acute studies, but the focus of the investigation was on repeated exposure studies that formed the basis of regulatory decisions. The laboratory closed in 1978. OECD (2007) outlined specific criteria for using data generated by Industrial Bio-Test Laboratories, and recommended rejecting a study when either a regulatory or internal audit revealed problems impacting the reliability of the findings, or when the findings of unaudited studies are inconsistent with data collected later by reputable laboratories. OECD (2007) recommended that studies that have not been audited should be used with caution and only as weak evidence if supported by later data from reputable laboratories. No information was available on internal or external auditing of this study.

ALD = approximate lethal dosage; CI = confidence interval; LC_{50} = median lethal concentration; LD_{50} = median lethal dose; S-D = Sprague-Dawley; TWA = time-weighted average.

Genotoxicity

o-Phenylenediamine has been tested in a number of in vitro and in vivo genotoxicity tests (see Table 4A for details), with predominantly positive results. *o*-Phenylenediamine induced mutations in *Salmonella typhimurium* in the presence of metabolic activation (Assmann et al., 1997; Chung et al., 1996; Zeiger et al., 1988; Gentile et al., 1987; Thompson et al., 1983; Ishidate and Yoshikawa, 1980; Voogd et al., 1980; Ames et al., 1975); results without activation were uniformly negative. Mutagenicity was greatly enhanced in *S. typhimurium* with the addition of hydrogen peroxide treatment (applied to mimic conditions of *o*-phenylenediamine exposure in hair dye) (Watanabe et al., 1989). Mutagenicity assays in *Escherichia coli* (Thompson et al., 1983), *Klebsiella pneumoniae* (Voogd et al., 1980), and *Saccharomyces cerevisiae* (Zeiger et al., 1988) were negative both with and without metabolic activation.

In mammalian cells, *o*-phenylenediamine induced mutations and deoxyribonucleic acid (DNA) damage in mouse lymphoma cells (L5178Y TK±) (Asgård et al., 2013). Increased frequencies of chromosomal aberrations (CAs) were reported in Chinese hamster cells (Chung et al., 1996) and Chinese hamster lung fibroblasts (Ishidate and Yoshikawa, 1980) treated with *o*-phenylenediamine. Furthermore, unscheduled DNA synthesis was seen in primary rat hepatocytes incubated with *o*-phenylenediamine (Thompson et al., 1983). *o*-Phenylenediamine also induced CAs, sister chromatid exchanges (SCEs), and DNA damage in human lymphocytes (Cebulska-Wasilewska et al., 1998). Incubation of λDNA with *o*-phenylenediamine resulted in increased DNA damage (Yamada et al., 1985).

In in vivo animal tests, *o*-phenylenediamine did not induce dominant lethal mutagenicity (to assess mutation in the germinal cells) in male rats following intraperitoneal (i.p.) administration (Burnett et al., 1977) or somatic mutations of fetal cells in a spot test in female mice treated intraperitoneally (Gocke et al., 1983). *o*-Phenylenediamine induced increased frequencies of bone marrow micronuclei in mice following oral and i.p. administration, and in hamsters and guinea pigs following i.p. administration (Wild et al., 1980). Treatment of male mice with *o*-phenylenediamine resulted in significant inhibition of testicular DNA synthesis compared with control mice; the study authors reported that a positive response (e.g., inhibition) in this test was seen with many chemical carcinogens and mutagens, but not with compounds that are not carcinogenic or mutagenic (Seiler, 1977).

Acute Toxicity

The acute oral and inhalation toxicity studies of *o*-phenylenediamine are detailed in Table 4B. Oral median lethal dose (LD_{50}) values of 900 and 1,000 mg/kg in rats and mice, respectively, were estimated by Rhone-Poulenc (1951) and Woodard (1951). In two studies using individual male ChR-CD rats receiving single gavage doses of *o*-phenylenediamine, DuPont (1967a, 1967b) estimated the approximate lethal dose of *o*-phenylenediamine to be 1,500 mg/kg (DuPont, 1967b) or 2,250 mg/kg (DuPont, 1967a). Industrial Bio-Test Laboratories (IBT Labs, 1983) assessed the lethality of single gavage doses of *o*-phenylenediamine in individual female Charles River rats; the authors reported deaths at doses of ≥3,000-mg/kg *o*-phenylenediamine. The latter study should be viewed with caution, as studies by the

performing laboratory were found to have a number of deficiencies in an audit conducted by the U.S. EPA and the Canadian Health and Welfare Department.[5]

No deaths occurred in an acute inhalation study in which male and female S-D rats were exposed for 1 hour to *o*-phenylenediamine aerosol at a concentration of 2,460 mg/m^3; however, signs of neurotoxicity, including loss of muscle coordination and limb paralysis were observed (Sherwin Williams, 1992). Exposure of male ChR-CD rats to *o*-phenylenediamine aerosol for 4 hours at analytically determined TWA concentrations of 1,000−9,200 mg/m^3 resulted in deaths at all exposure levels; the 4-hour median lethal concentration (LC_{50}) in rats was estimated to be 3,600 mg/m^3 (DuPont, 1969). All exposure levels ≥1,300 mg/m^3 resulted in signs of neurotoxicity, with hindlimb paralysis seen at concentrations ≥2,100 mg/m^3 (DuPont, 1969). There were no deaths, and no evidence of toxicity (based on clinical observations, body-weight measurement, and gross necropsy) when male and female BLU:(LE)BR rats were exposed for 4 hours to *o*-phenylenediamine vapor at a nominal concentration of 1,340 mg/m^3 in a study performed by Industrial Bio-Test Laboratories (IBT Labs, 1975c); however, results from this laboratory must be viewed with caution.[5]

Other Routes

Studies by Haskell Laboratories (1970b) and DuPont (1968), in which *o*-phenylenediamine was applied to intact skin for 24 hours under occlusion and animals were observed for 2 weeks, indicated that the dermal approximate lethal dose (ALD) of *o*-phenylenediamine was ~1,500 mg/kg in male albino rabbits. No mortality occurred within the 14-day observation period when *o*-phenylenediamine was applied as a slurry in water to the abraded skin of female New Zealand white (NZW) rabbits at doses as high as 3,000 mg/kg (IBT Labs, 1975a, b); however, results from this laboratory may not be reliable.[5] At a dermal dose of 200 mg/kg (administered to intact skin under occlusion), *o*-phenylenediamine did not affect the 48-hour survival of six male albino rabbits; weight loss was noted on the day after treatment (details not provided) (Haskell Laboratories, 1982).

Haskell Laboratories (1981) reported that *o*-phenylenediamine was slightly irritating, but not corrosive, to the skin of albino rabbits (sex not specified) following application of 0.5 g to clipped skin under occlusive wrapping for 4 hours. Solid *o*-phenylenediamine (~0.04−0.09 g) applied as a paste in hydrophilic ointment to the intact or abraded skin of male guinea pigs produced mild skin irritation (Haskell Laboratories, 1970a). Application of *o*-phenylenediamine in acetone: dioxane containing 13% guinea pig fat at exposure concentrations between 5−25% resulted in increased incidence and severity of erythema (compared with controls) in male guinea pigs (Haskell Laboratories, 1967). Mild to moderate sensitization was noted in male guinea pigs following the first challenge test after treatment with nine applications of *o*-phenylenediamine at

[5]A total of 618/867 nonacute toxicity studies conducted by Industrial Bio-Test Laboratories (including subacute, subchronic-duration, carcinogenicity, reproductive toxicity, genotoxicity, and neurotoxicity studies) were found to be invalid during a post hoc audit program conducted by U.S. EPA and the Canadian Health and Welfare Department (OECD, 2007). Discrepancies and deficiencies were also identified in acute studies, but the focus of the investigation was on repeated exposure studies that formed the basis of regulatory decisions. The laboratory closed in 1978. OECD (2007) outlined specific criteria for using data generated by Industrial Bio-Test Laboratories, and recommended rejecting a study when either a regulatory or internal audit revealed problems impacting the reliability of the findings, or when the findings of unaudited studies are inconsistent with data collected later by reputable laboratories. OECD (2007) recommended that studies that have not been audited should be used with caution and only as weak evidence if supported by later data from reputable laboratories. No information was available on internal or external auditing of this study.

concentrations between 1−25% (Haskell Laboratories, 1967). The second challenge test in this study produced fewer sensitized animals, and there was no evidence of cross-sensitization with *p*-phenylenediamine.

o-Phenylenediamine was mildly to moderately irritating to the eyes of rabbits when applied as a solid or dissolved in propylene glycol (see Footnote 5) (IBT Labs, 1975c; Haskell Laboratories, 1970a). Haskell Laboratories (1970a) also reported corneal haziness and slight iris congestion after exposure to the solid. In general, ocular effects were resolved within 7 days.

Metabolism/Toxicokinetic Studies

As a solid chemical or in solution, *o*-phenylenediamine is readily oxidized; however, little is known on the metabolism of *o*-phenylenediamine. When used as a chromogenic laboratory substrate, *o*-phenylenediamine is oxidized to 2,3-diaminophenazine (Tarcha et al., 1987), which also has been identified following treatment of *o*-phenylenediamine with hydrogen peroxide (Watanabe et al., 1989).

Bronaugh and Congdon (1984) measured the percutaneous absorption of *o*-phenylenediamine in excised human abdominal skin under alkaline conditions designed to mimic the pH of hair dye. A permeability constant of 4.5×10^{-4} cm/hour was estimated. Concentration-dependent binding of *o*-phenylenediamine to the stratum corneum was observed, with Km (bound/free) values ranging from 6.86−45 at concentrations from 77.2−0.45 M × 10^5.

Mode-of-Action/Mechanistic Studies

Methemoglobin formation was measured in five male rats given *o*-phenylenediamine intraperitoneally at a dose of 100 µmol/kg and sacrificed 5 hours later (Watanabe et al., 1976). The methemoglobin level in treated rats was 10.8 ± 3.5%; the level in control rats was not reported. Serum liver enzyme levels (AST and ALT) were not significantly altered by treatment. In vitro incubation of *o*-phenylenediamine (0.5 µmol) with Hb (0.1 µmol) for 5 hours resulted in a significant increase in the percent methemoglobin (5.9 vs. 4.2% in untreated, $p < 0.01$). The importance of this finding is uncertain, given the small increase in vitro and the fact that cyanosis has not been reported in animals exposed to *o*-phenylenediamine at high oral and inhalation doses.

Neurotoxicity

DuPont (1990) conducted an acute oral neurotoxicity study of *o*-phenylenediamine suspended in aqueous methyl cellulose, administered by gavage to groups of 12 male and 12 female Crl:CD®BR rats. After pre-exposure baseline motor activity and FOB assessments, single doses of 0, 225, 450, or 900 mg/kg were administered. Body weights and clinical signs were recorded before dosing, on the day of dosing, and on 1, 4, and 7 days after dosing; clinical signs were also recorded 1 hour after dosing. Food consumption measurements were taken on the day of dosing as well as 1, 3, 4, and 7 days after dosing. Neurotoxicity assessments consisted of motor activity, FOB, forelimb and hindlimb grip strength, and foot splay assessments conducted at 1 and 24 hours after dosing and repeated 4 days after dosing.

Six rats (two males and four females) exposed to 900-mg/kg *o*-phenylenediamine died between Days 1 and 4; the cause(s) of death were not reported (DuPont, 1990). Clinical signs of toxicity occurred from dosing through Day 4 postdosing, and were increased in a dose-related fashion. These signs included bright yellow urine, abnormal respiration (≥450 mg/kg), and

staining on the body (900 mg/kg). Significant, dose-related body-weight losses occurred in both sexes of rat at all doses of *o*-phenylenediamine during the first day after dosing and when body-weight change was assessed from Days 0 (day of dosing) to 4; all rats resumed gaining weight after Day 4. Mean body weights of high-dose males and females, as well as females exposed to 450 mg/kg, were significantly lower than controls on Day 4. FOB assessment showed significant increases in palpebral closure (males and females) and altered posture (females) at all doses of *o*-phenylenediamine; decreased arousal in both sexes at >450 mg/kg; and labored breathing (both sexes), salivation (males), increased ease of removal from home cage (males), and altered fur appearance (females) at the highest dose. Forelimb and hindlimb grip strength and foot splay were not altered by exposure to *o*-phenylenediamine. Motor activity was significantly decreased at all doses when tested on the day of dosing. Dose-related decrements in activity persisted at later measurements, with gradual recovery toward normal levels of activity occurring at the lower doses. The study authors concluded that the effects of *o*-phenylenediamine on motor activity and FOB assessment parameters reflected an overall systemic toxicity that led to general malaise and decreased arousal in the exposed animals, rather than a specific neurotoxic effect of *o*-phenylenediamine.

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 *o*-Phenylenediamine (CASRN 95-54-5)

Toxicity Type (units)	Species/Sex	Critical Effect	p-Reference Value	POD Method	POD (HED)	UF_C	Principal Study
Subchronic p-RfD (mg/kg-d)	NDr						
Chronic p-RfD (mg/kg-d)	Rat/M	Increased incidence of renal papillary mineralization	4×10^{-3}	$BMDL_{10}$	1.2	300	Matsumoto et al. (2012)
Subchronic p-RfC (mg/m^3)	NDr						
Chronic p-RfC (mg/m^3)	NDr						

$BMDL_{10}$ = 10% benchmark dose lower confidence limit; HED = human equivalent dose; M = male(s); NDr = not determined; 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 *o*-Phenylenediamine (CASRN 95-54-5)

Toxicity Type (units)	Species/Sex	Tumor Type	Cancer Value	Principal Study
p-OSF $(mg/kg\text{-}d)^{-1}$	Mouse/M	Combined tumors	1.2×10^{-1}	Matsumoto et al. (2012)
p-IUR $(mg/m^3)^{-1}$	NDr			

M = male(s); NDr = not determined; p-IUR = provisional inhalation unit risk; p-OSF = provisional oral slope factor.

DERIVATION OF ORAL REFERENCE DOSES

The database of oral studies in experimental animals includes one short-term-duration study (Haskell Laboratories, 1980), one subchronic-duration neurotoxicity study (DuPont, 1992a), and two chronic-duration carcinogenicity studies (Matsumoto et al., 2012; Weisburger et al., 1978). A chronic provisional reference dose (p-RfD) was derived based on the available studies.

Derivation of a Subchronic Provisional Reference Dose

Available data on oral exposure to *o*-phenylenediamine are not sufficient to derive a subchronic p-RfD that is different from the chronic p-RfD. A single, unpublished, subchronic-duration study of *o*-phenylenediamine in rats, conducted by DuPont (1992a), is available. The study was designed to examine the potential neurotoxic effects of oral exposure

to *o*-phenylenediamine based on neurotoxicity seen in acute inhalation toxicity studies (Sherwin Williams, 1992; DuPont, 1969). Although clinical signs of toxicity and neurobehavioral evaluations were thoroughly investigated, neither clinical chemistry nor hematology were assessed and necropsy investigations were limited to histopathology of nervous system tissues. Findings in the study did not suggest that neurotoxicity was a sensitive endpoint in rats after subchronic oral exposure. A chronic-duration study of rats exposed to *o*-phenylenediamine via drinking water (Matsumoto et al., 2012) at doses lower than the subchronic-duration neurotoxicity study (DuPont, 1992a) identified the liver, kidney, and urinary bladder as target organs. Because the available subchronic-duration study (DuPont, 1992a) provides no information on potential toxicity to organs identified as targets in the chronic-duration study (Matsumoto et al., 2012), it was not considered suitable to use in deriving a subchronic p-RfD. In the absence of relevant subchronic data, the chronic p-RfD of 4×10^{-3} mg/kg-day (described below) can be adopted as the subchronic p-RfD.

Derivation of a Chronic Provisional Reference Dose

The chronic-duration study in adult rats exposed to *o*-phenylenediamine in drinking water is considered the principal study for deriving the chronic p-RfD (Matsumoto et al., 2012). The critical effect from this study is increased incidence of renal papillary mineralization in male rats.

The study conducted by Matsumoto et al. (2012) reported administration of *o*-phenylenediamine (as *o*-phenylenediamine dihydrochloride) in drinking water to F344/DuCrj rats and Crj:BDF_1 mice (50/sex/dose) for 2 years. This study followed GLP procedures, was published in a peer-reviewed journal, had adequate reporting and consideration for appropriate study design, methods, and conduct, including appropriate numbers of animals per group and three treatment doses in each of two species. The study includes comprehensive assessment of body weight, hematology, serum chemistry, urinalysis, liver weight, and gross and microscopic pathology of various organs. No other chronic-duration exposure study of *o*-phenylenediamine assessed noncancer endpoints. Thus, the study by Matsumoto et al. (2012) was considered suitable for derivation of a chronic p-RfD.

Statistically significant effects were observed following exposure in all treatment groups of mice or rats; thus, NOAELs were not identified in the Matsumoto et al. (2012) study. The LOAELs in male and female rats were 13 and 11 mg/kg-day, respectively (see Table 3A). Endpoints that were significantly different from controls (either statistically significantly altered, or of such magnitude of change to be considered biologically significant) at the lowest dose in male or female rats and exhibited a dose-related change included increased incidence of renal papillary mineralization in males, increased incidence of renal pelvis urothelial hyperplasia in females, and increased incidences of basophilic cell foci of the liver in males and females (see Table B-4).

The LOAELs in male and female mice were 27 and 63.3 mg/kg-day, respectively (see Table 3A). Endpoints that were significantly different from controls (either statistically significantly altered, or of such magnitude of change to be considered biologically significant [e.g., >10% change]) at the lowest dose in male or female mice and exhibited a dose-related change included decreased terminal body weight in males, increased relative liver weight and serum ALP in males and females, increased incidence of papillary hyperplasia of the gall bladder in males, increased incidences of eosinophilic change of the nasal cavity respiratory epithelium in females, and hydronephrosis in females (see Tables B-6 to B-8).

Several endpoints that were altered in rats and mice following exposure to the lowest dose of *o*-phenylenediamine were not considered as potential noncancer points of departure (PODs). Basophilic cell foci in the liver of male and female rats was not considered due to uncertain toxicological significance. These common lesions were not accompanied by other endpoints indicative of liver toxicity, such as changes in serum chemistry enzymes (i.e., ALT or AST) or further pathological lesions. In mice, terminal body weight (males) and relative liver weight (males and females) were not considered for potential POD selection because the changes were not considered to be directly related to the toxicity of *o*-phenylenediamine or biologically significant (i.e., the change was <10%). Terminal (2-year) body-weight changes were likely a result of decreased food and water consumption. Multiple factors confound the interpretation of increased 2-year relative liver weight in treated male and female mice. Significant increases in proliferative liver lesions were observed at all treatment doses, potentially causing the increased organ weight. In addition, as absolute liver weight was not significantly increased at the lowest dose, reduced terminal body weight may skew interpretation of increases in the organ-to-body weight ratio (i.e., relative weight) at this dose level. Measurements of serum ALP were highly variable, lacked a dose response, and were not accompanied by other non-neoplastic endpoints characteristic of gall bladder toxicity. Thus, increased serum ALP was not judged to be biologically relevant to the toxicity of *o*-phenylenediamine. Eosinophilic change of the nasal cavity respiratory epithelium (female mice) was also not considered because the effect may have resulted from accidental sipping of drinking water containing *o*-phenylenediamine via the nose. Thus, the biological significance of the pathological effect observed in the nasal cavity following exposure via the oral route is unclear. Finally, papillary hyperplasia in the gall bladder of male mice was not considered because it may be considered a preneoplastic lesion. Increased incidences of rare papillary adenomas of the gall bladder were reported in male and female mice following exposure to *o*-phenylenediamine (Matsumoto et al., 2012) (see Table B-9). These lesions were accompanied by increased incidences of papillary hyperplasia in the gall bladder which may act as a precursor to further carcinogenesis. The remaining data for effects occurring at the lowest doses tested were selected for benchmark dose (BMD) modeling and are provided in Table 7.

Table 7. Selected Non-neoplastic Endpoints in Male and Female F344/DuCrj Rats and Crj:BDF$_1$ Mice Administered *o*-Phenylenediamine Dihydrochloride in Drinking Water for 2 Years[a]

Male Rats				
Dose, mg/kg-d (as *o*-phenylenediamine)	**0**	**13**	**25**	**51**
Kidney—Papillary mineralization	7/50[b]	18/50[c]	16/50	26/50
Female Rats				
Dose, mg/kg-d (as *o*-phenylenediamine)	**0**	**11**	**20**	**35**
Kidney—Urothelial hyperplasia: pelvis	2/50	12/50	10/50	17/50
Female Mice				
Dose, mg/kg-d (as *o*-phenylenediamine)	**0**	**63.3**	**119**	**234**
Kidney—Hydronephrosis	2/50	12/50	13/50	11/50

[a]Matsumoto et al. (2012).
[b]Number of animals affected/number of animals examined.
[c]All treatment groups were significantly different from control at $p < 0.05$ by Fisher's exact test performed by the study authors.
[d]Mean ± standard deviation.

All dichotomous models in the U.S. EPA's Benchmark Dose Software (BMDS, Version 2.5) were fit to the data of each potential dichotomous endpoint occurring at the lowest doses tested from the study conducted by Matsumoto et al. (2012) (see Table 7). Appendix C presents the details of the modeling procedure and results for all endpoints. The results are summarized in Table 8.

Table 8. Potential Chronic PODs in Male and Female Rats and Mice Exposed Orally to *o*-Phenylenediamine (CASRN 95-54-5) for 2 Years[a]

Endpoint	NOAEL mg/kg-d	LOAEL mg/kg-d	Animal POD[b] mg/kg-d	POD (HED)[c] mg/kg-d
Male Rats				
Kidney—Papillary mineralization[d]	**ND**	**13**	**$BMDL_{10}$ = 4.8**	**$BMDL_{10}$ (HED) = 1.2**
Female Rats				
Kidney—Urothelial hyperplasia: pelvis	ND	11	$BMDL_{10}$ = 5.6	$BMDL_{10}$ (HED) = 1.3
Female Mice				
Kidney—Hydronephrosis	ND	63.3	$BMDL_{10}$ = 23.1	$BMDL_{10}$ (HED) = 3.23

[a]Matsumoto et al. (2012).
[b]BMD modeling results are described in detail in Appendix C.
[c]POD (HED) = Animal POD (mg/kg-day) × DAF of 0.24 for rats or 0.14 for mice (U.S. EPA, 2011c).
[d]Chosen as the critical effect for derivation of the chronic p-RfD.

BMD = benchmark dose; $BMDL_{10}$ = 10% benchmark dose lower confidence limit; DAF = dosimetric adjustment factor; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; ND = no data; NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfD = provisional reference dose.

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, 2011c), the Agency endorses a hierarchy of approaches to derive human equivalent oral exposures from data derived 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, 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 administrated 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 effect endpoints.

A validated human physiologically based toxicokinetic model for *o*-phenylenediamine is not available for use in extrapolating doses from animals to humans. Furthermore, the selected lesions of the kidney in rats or mice are not portal-of-entry effects. Therefore, scaling by $BW^{3/4}$ is relevant for deriving human equivalent doses (HEDs) for these effects.

Following U.S. EPA (2011c) guidance, all potential PODs are converted to HEDs by application of dosimetric adjustment factors (DAFs)[6] 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 0.025 kg for mice, and a BW_h of 70 kg for humans, the resulting default DAFs are 0.24 and 0.14 for rats and mice, respectively (U.S. EPA, 2011c). Each POD candidate is multiplied by the appropriate species-specific DAF to obtain a POD (HED) (see Table 8).

The lowest POD (HED) following chronic exposure to *o*-phenylenediamine is increased incidence of renal papillary mineralization in male rats ($BMDL_{10}$ (HED) = 1.2 mg/kg-day). This POD is protective of other effects observed following *o*-phenylenediamine exposure and increased renal papillary mineralization is consistently observed across sexes and coherent with other *o*-phenylenediamine-induced renal effects. Renal papillary mineralization not only exhibited a dose-response relationship in male rats, but was also increased in female rats at all doses with statistical significance at the highest dose. In addition, increased papillary mineralization was accompanied by other adverse *o*-phenylenediamine-induced events indicating kidney toxicity; male and female rats exhibited increased renal papillary necrosis, urothelial hyperplasia in the renal pelvis, and increased blood urea nitrogen. Based on the available data

[6]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, 2011c), 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 $DAF = BW_a^{1/4} \div BW_h^{1/4}$.

supporting effects on the kidney, **the $BMDL_{10}$ (HED) for renal papillary mineralization in male rats (1.2 mg/kg-day) is selected as the POD for derivation of the chronic p-RfD.**

The chronic p-RfD for *o*-phenylenediamine is derived as follows:

$$\textbf{Chronic p-RfD} = BMDL_{10}\text{ (HED)} \div UF_C$$
$$= 1.2\text{ mg/kg-day} \div 300$$
$$= \mathbf{4 \times 10^{-3}\text{ mg/kg-day}}$$

The composite uncertainty factor (UF_C) for the chronic p-RfD for *o*-phenylenediamine is 300, as summarized in Table 9.

Table 9. Uncertainty Factors for the Chronic p-RfD for *o*-Phenylenediamine (CASRN 95-54-5)

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 *o*-phenylenediamine exposure. The toxicokinetic uncertainty has been accounted for by calculation of 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, 2011c).
UF_D	10	A UF_D of 10 is applied to account for deficiencies and uncertainties in the database, specifically the lack of data on reproductive or developmental toxicity.
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 information to assess toxicokinetic and toxicodynamic variability of *o*-phenylenediamine in humans.
UF_L	1	A UF_L of 1 is applied because the POD is a BMDL, not a LOAEL.
UF_S	1	A UF_S of 1 is applied because the POD comes from a chronic-duration study of rats.
UF_C	300	Composite Uncertainty Factor = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$.

BMDL = benchmark dose lower confidence limit; DAF = dosimetric adjustment factor; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; POD = point of departure; UF = uncertainty factor.

The confidence in the chronic p-RfD for *o*-phenylenediamine is low as described in Table 10.

Table 10. Confidence Descriptors for the Chronic p-RfD for *o*-Phenylenediamine (CASRN 95-54-5)

Confidence Categories	Designation	Discussion
Confidence in study	M	Confidence in the principal study is medium. Factors contributing to medium confidence in the principal study include: (1) appropriate numbers of animals in exposure and control groups for meaningful statistical analyses; (2) adequate numbers of exposure groups to establish dose-response relationships; and (3) assessment of a wide range of toxicological endpoints (body weight, hematology, serum chemistry, liver weight, and gross and microscopic pathology). The major factor restricting confidence in the principal study is the lack of a dose low enough to permit identification of a NOAEL.
Confidence in database	L	Confidence in the database is low. The oral database for noncancer effects of *o*-phenylenediamine consists of a short-term-duration rat drinking water palatability study assessing limited endpoints (Haskell Laboratories, 1980), a subchronic-duration neurotoxicity study in rats (DuPont, 1992a), and a chronic-duration study in rats and mice exposed via drinking water (Matsumoto et al., 2012). There are no reproductive or developmental toxicity studies in animals. The database deficiencies restrict confidence in the determination of the critical effect from oral exposure to *o*-phenylenediamine.
Confidence in chronic p-RfD[a]	L	The overall confidence in the chronic p-RfD for *o*-phenylenediamine is low.

[a]The overall confidence cannot be greater than the lowest entry in the table (low).

L = low; M = medium; NOAEL = no-observed-adverse-effect level; p-RfD = provisional reference dose.

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

No studies of humans or animals exposed to *o*-phenylenediamine via inhalation have been identified in the available literature, precluding derivation of provisional inhalation reference concentrations (p-RfCs).

CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR

Table 11 provides the cancer weight-of-evidence (WOE) descriptor for *o*-phenylenediamine.

Table 11. Cancer WOE Descriptor for *o*-Phenylenediamine (CASRN 95-54-5)

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.
"Likely to Be Carcinogenic to Humans"	**Selected**	**Oral**	**There are no human data on the potential carcinogenicity of *o*-phenylenediamine by any exposure route. Chronic (≥18 mo) exposure to *o*-phenylenediamine dihydrochloride in the drinking water or diet resulted in significantly increased incidences of liver tumors in male and female rats and in male and female mice in two studies (Matsumoto et al., 2012; Weisburger et al., 1978), a significantly increased incidence of urinary bladder tumors and a significant dose-related trend for thyroid follicular adenoma in male rats (Matsumoto et al., 2012), and significantly increased incidences of rare papillary adenomas of the gall bladder in male and female mice (Matsumoto et al., 2012). The induction of tumors at multiple sites and in both sexes and species tested, as well as the induction of rare tumors in mice, support this cancer WOE for *o*-phenylenediamine.**
"Suggestive Evidence of Carcinogenic Potential"	NS	NA	The available evidence is more than suggestive of carcinogenicity and is judged sufficient for a stronger conclusion.
"Inadequate Information to Assess Carcinogenic Potential"	NS	NA	Adequate data are available to assess carcinogenic potential.
"Not Likely to Be Carcinogenic to Humans"	NS	NA	Evidence of the carcinogenic potential of *o*-phenylenediamine is available in animals.

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

Under the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), exposure to *o*-phenylenediamine is *"Likely to Be Carcinogenic to Humans"* based on evidence of carcinogenicity in orally treated male and female rats and mice (see Table 11).

There are no human data on the potential carcinogenicity of *o*-phenylenediamine by any exposure route. The carcinogenicity of *o*-phenylenediamine has been tested in two chronic-duration studies of rats and mice exposed orally (Matsumoto et al., 2012; Weisburger et al., 1978). Chronic (≥18 months) exposure to *o*-phenylenediamine dihydrochloride in the drinking water or diet resulted in significantly increased incidences of tumors in all studies, including increased liver tumors in male and female rats and mice in all studies (Matsumoto et al., 2012; Weisburger et al., 1978), increased incidence of urinary bladder tumors and thyroid follicular adenoma in male rats (Matsumoto et al., 2012), and increased incidences of rare papillary adenomas of the gall bladder in male and female mice (Matsumoto et al., 2012)

(see Table 12). The papillary adenomas of the gall bladder observed in male and female mice following exposure to *o*-phenylenediamine are considered a rare tumor in mice. Matsumoto et al. (2012) reported that papillary adenomas of the gall bladder occurred in only 9 of the 60,000 control and chemically treated $B6C3F_1$ mice in the NTP database through 1998, and in none of the almost 2,600 historical control BDF_1 mice in the Japanese Bioassay Research Center.

Table 12. Incidences of Selected Neoplastic Lesions in Male and Female F344/DuCrj Rats and Crj:BDF$_1$ Mice Administered *o*-Phenylenediamine Dihydrochloride in Drinking Water for 2 Years[a]

Male Rats				
Dose, mg/kg-d (as *o*-phenylenediamine, ADD [HED])[b]	**0 (control)**	**13 (3.2)**	**25 (6.1)**	**51 (13)**
Hepatocellular adenoma and/or carcinoma—liver	4/50[#c] (8)	3/50 (6)	16/50* (32)	22/50* (44)
Transitional cell papilloma and/or carcinoma—urinary bladder	2/50[#] (4)	0/50 (0)	0/50 (0)	10/50* (20)
Follicular adenoma—thyroid	0/50[#] (0)	1/50 (2)	0/50 (0)	4/50 (8)
Female Rats				
Dose, mg/kg-d (as *o*-phenylenediamine)[b]	**0 (control)**	**11 (2.6)**	**20 (4.8)**	**35 (8.5)**
Hepatocellular adenoma and/or carcinoma—liver	1/50[#] (2)	3/50 (6)	19/50* (38)	44/50* (88)
Male Mice				
Dose, mg/kg-d (as *o*-phenylenediamine)[b]	**0 (control)**	**27 (3.8)**	**56 (7.7)**	**106 (14.5)**
Hepatocellular adenoma and/or carcinoma—liver	18/50[#] (36)	29/50* (58)	39/50* (78)	38/50* (76)
Papillary adenoma—gall bladder	0/46[#] (0)	2/50 (4)	4/49 (8)	5/47* (10)
Female Mice				
Dose, mg/kg-d (as *o*-phenylenediamine)[b]	**0 (control)**	**63.3 (8.70)**	**119 (16.4)**	**234 (32.1)**
Hepatocellular adenoma and/or carcinoma—liver	6/50[#] (12)	23/50* (46)	31/50* (62)	41/50* (82)
Papillary adenoma—gall bladder	0/50 (0)	1/50 (2)	5/50* (10)	3/50 (6)

[a]Matsumoto et al. (2012).
[b]Dose expressed as average daily animal dose of *o*-phenylenediamine (mg/kg-day) and as an HED in parentheses. HED = Dose × $(BW_a \div BW_h)^{1/4}$ where Dose = average daily animal dose of *o*-phenylenediamine, BW_a = reference animal body weight, BW_h = 70 kg, reference human body weight (U.S. EPA, 1988). Example calculation: HED = 13 × $(0.25/70)^{1/4}$ = 3.2 mg/kg-day.
[c]Number of animals affected/total number of animals (% of animals affected); % calculated by EPA.
*Statistically different from controls, $p < 0.05$ by Fisher's exact test performed by study authors.
[#]Statistically dose-related positive trend at $p < 0.05$ by Peto's test performed by study authors.

ADD = adjusted daily dose; HED = human equivalent dose.

As stated in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), examples of supporting data to conclude that a chemical is *"Likely to Be Carcinogenic to Humans"* include (1) "an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans"; (2) "a rare animal tumor response in a single experiment that is assumed to be relevant to humans." Based on these examples from the U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) and the data available in male and female rats and mice, exposure to *o*-phenylenediamine is *"Likely to Be Carcinogenic to Humans."*

MODE-OF-ACTION DISCUSSION

The *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) defines mode of action (MOA) "as a sequence of key events and processes, starting with the interaction of an agent with a cell, proceeding through operational and anatomical changes and resulting in cancer formation." Examples of possible modes of carcinogenic action include mutagenic, mitogenic, anti-apoptotic (inhibition of programmed cell death), cytotoxic with reparative cell proliferation, and immunologic suppression. The available data support a determination that *o*-phenylenediamine may be carcinogenic by a mutagenic MOA. There are no data to suggest alternative MOAs. Elements to consider in determining whether a carcinogen acts by a mutagenic MOA are provided in Section 5 of the 2005 Cancer Supplementary Guidance (U.S. EPA, 2005b), which states that "determinations of chemicals that are operating by a mutagenic MOA entails evaluation of test results for genotoxic endpoints, metabolic profiles, physiochemical properties, and structure-activity relationships." No data on the metabolism of *o*-phenylenediamine were identified in the available literature.

Key Events—The proposed MOA for *o*-phenylenediamine carcinogenicity consists of the following key events: (1) metabolism to DNA-reactive metabolite(s), (2) covalent binding with DNA, (3) mutation of critical genes such as oncogenes, and (4) proliferation of initiated cells.

o-Phenylenediamine has been tested in a number of in vitro and in vivo genotoxicity tests (see Table 4A), with predominantly positive results. *o*-Phenylenediamine induced mutations in *S. typhimurium* in the presence of metabolic activation (Assmann et al., 1997; Chung et al., 1996; Zeiger et al., 1988; Gentile et al., 1987; Thompson et al., 1983; Ishidate and Yoshikawa, 1980; Voogd et al., 1980; Ames et al., 1975), but not without metabolic activation. Mutagenicity assays in *E. coli* (Thompson et al., 1983), *K. pneumoniae* (Voogd et al., 1980), and *S. cerevisiae* (Zeiger et al., 1988) were negative both with and without metabolic activation. In mammalian cells, *o*-phenylenediamine induced mutations and DNA damage in mouse lymphoma cells (Asgård et al., 2013), increased frequencies of CAs in Chinese hamster ovary cells (Chung et al., 1996) and Chinese hamster lung fibroblasts (Ishidate and Yoshikawa, 1980), induced unscheduled DNA synthesis in primary rat hepatocytes (Thompson et al., 1983), and induced CAs, SCEs, and DNA damage in human lymphocytes (Cebulska-Wasilewska et al., 1998).

In in vivo animal tests, *o*-phenylenediamine did not induce dominant lethal mutagenicity (Burnett et al., 1977) or somatic mutations in a spot test (Gocke et al., 1983), but did induce increased frequencies of bone marrow micronuclei in mice, hamsters, and guinea pigs (Wild et al., 1980).

Strength, Consistency, Specificity of Association—There is consistent evidence from a variety of different in vitro and in vivo genotoxicity tests to suggest *o*-phenylenediamine exposure induces mutations. These tests include mutagenicity and DNA damage assays in bacteria and eukaryotic cells. Less evidence is available to assess genotoxicity and mutagenicity in in vivo studies, although bone marrow micronuclei were consistently induced in several experimental species. The strength, consistency, or specificity of the association between *o*-phenylenediamine exposure and subsequent key events (i.e., specific critical gene mutation and cell proliferation) cannot be evaluated due to the lack of data.

Analogy—Data on the carcinogenicity of structurally related compounds are limited, and suggest that *o*-phenylenediamine may differ toxicologically from the compounds most similar to it. *m*-Phenylenediamine was not carcinogenic in C3H or C57BL/6 mice exposed by skin application three times per week for 24 months at doses of 0.6 or 3 mg/week (Holland et al., 1979). Chronic (≥2 years) exposure of rats to *p*-phenylenediamine (Imaida et al., 1983) or rats and mice to *p*-phenylenediamine dihydrochloride (NCI, 1979) in the diet did not significantly increase the incidence of any tumor type. There was a slight, statistically significant increase in the incidence of alveolar adenoma (18/88 vs. 12/86 in negative controls, $p = 0.04$) in female offspring of mice exposed to *p*-phenylenediamine via gavage during gestation [Holmberg et al. (1983) as translated in DuPont (1992b)].

Dose-Response Concordance—There are no data on genotoxic events in any tissue of animals exposed to *o*-phenylenediamine at doses below those associated with tumor formation [≥20 mg/kg-day in rats and ≥27 mg/kg-day in mice; Matsumoto et al. (2012)]. Significant increases in the incidences of non-neoplastic proliferative lesions (clear cell foci and basophilic foci) were seen at all doses in the livers of rats in the study by Matsumoto et al. (2012), with increased tumor incidences occurring at the mid and high doses. In mice, increased incidences of liver tumors (as well as foci of alterations) were seen at all doses (Matsumoto et al., 2012), so there is no information on events occurring at lower doses. There were no significantly increased incidences of proliferative lesions in the urinary bladders of male rats at doses not associated with increased tumor formation (Matsumoto et al., 2012). Male, but not female, mice exhibited significantly increased incidences of proliferative lesions in the gall bladder (papillary hyperplasia) at all doses, with an increased incidence of papillary adenomas in high-dose male mice.

Temporal Relationships—No data are available with which to evaluate the temporal relationship between mutagenesis and tumor formation after *o*-phenylenediamine exposure. Matsumoto et al. (2012) did not perform any interim sacrifices, and available short-term- and subchronic-duration studies (DuPont, 1992a; Haskell Laboratories, 1980) did not evaluate histopathology in any of the tissues in which tumor formation was seen in the chronic-duration study by Matsumoto et al. (2012).

Biological Plausibility and Coherence—Supporting evidence for the association between mutagenesis and tumor formation comes from the observation that *o*-phenylenediamine exposure produced increased incidences of tumors in a wide variety of tissues: liver tumors in male and female rats and in male and female mice (Matsumoto et al., 2012; Weisburger et al., 1978), urinary bladder tumors and thyroid follicular adenoma in male rats (Matsumoto et al., 2012), and rare papillary adenomas of the gall bladder in male and female mice

(Matsumoto et al., 2012). Induction of tumors at multiple sites and in different species is characteristic of carcinogens acting via mutagenesis (U.S. EPA, 2005a).

Available data do not suggest nongenotoxic MOAs for the liver or urinary bladder. Matsumoto et al. (2012) noted in their chronic cancer bioassay in rats and mice that no pathology changes suggestive of nongenotoxic MOAs (e.g., necrosis, hypertrophy, regenerative hyperplasia, or inflammation in the liver; or calculus formation in the urinary bladder) were observed in the liver or urinary bladder. Papillary hyperplasia was observed in the gall bladders of mice at lower doses than those inducing adenomas, suggesting the possibility that a nonmutagenic MOA could apply to these tumors.

Early-Life Susceptibility—According to the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), those exposed to carcinogens with a mutagenic MOA are assumed to have increased early-life susceptibility. Data on *o*-phenylenediamine are not sufficient to develop separate risk estimates for childhood exposure. There are no data comparing the tumorigenicity of *o*-phenylenediamine after exposure during early life with tumorigenicity after exposure during adulthood. In the carcinogenicity bioassays of *o*-phenylenediamine, exposure of the animals began at 6 weeks of age (Matsumoto et al., 2012) or between 6−8 weeks of age (Weisburger et al., 1978) and continued through adulthood.

Conclusions—Available data on the tumorigenicity of *o*-phenylenediamine support a mutagenic MOA. Three lines of evidence provide support for the conclusion of a mutagenic MOA. First, *o*-phenylenediamine was capable of eliciting genotoxic effects, including mutations, in both bacteria and eukaryotic cells and in vivo tests. Second, administration of *o*-phenylenediamine to rats and mice resulted in the induction of tumors at multiple sites, a hallmark of a mutagenic MOA. Third, pathology changes suggestive of nongenotoxic MOAs were not observed in liver or urinary bladder in chronic cancer bioassays in rats and mice (although potentially nongenotoxic preneoplastic lesions were seen in the gall bladder). Therefore, a mutagenic MOA for carcinogenicity is proposed for *o*-phenylenediamine and a linear approach would be appropriate to extrapolate from the POD in the derivation of the provisional oral slope factor (p-OSF) (U.S. EPA, 2005a).

DERIVATION OF PROVISIONAL CANCER POTENCY VALUES

Derivation of a Provisional Oral Slope Factor

As shown in Table 13, a provisional oral slope factor (p-OSF) for *o*-phenylenediamine was derived from the combined incidences of hepatocellular adenomas and/or carcinomas and papillary adenomas of the gall bladder in male mice reported by Matsumoto et al. (2012).

As noted in Table 11, EPA concluded that exposure to *o*-phenylenediamine is *"Likely to Be Carcinogenic to Humans."* The chronic-duration oral carcinogenicity study by Matsumoto et al. (2012) was selected as the primary study for derivation of the p-OSF for *o*-phenylenediamine. Of the two available publications, the study by Matsumoto et al. (2012) used lower doses for a longer duration (2 years), tested larger groups of animals, included male and female rats and mice, and was reported more completely than Weisburger et al. (1978) (18 months), which tested both species, but only male rats. In the study by Matsumoto et al. (2012), there was a significant increase in the incidence of hepatocellular adenomas and carcinomas in male and female rats and mice, urinary bladder tumors and thyroid follicular adenomas in male rats, and papillary

adenomas of the gall bladder in male and female mice at several doses compared to controls (see Table 12). The incidences of the various tumor types were modeled using BMDS and the modeling results are presented in Table 13. Based on the BMD modeling results, the calculated cancer slope factors for the various tumor types in male and female mice or rats were calculated and also presented in Table 13. Because treatment with *o*-phenylenediamine produced multiple types of tumors in different tissues within a single study (i.e., within a single sex and species), the overall oral cancer slope factor for *o*-phenylenediamine exposure was derived based on the incidence data for combined tumors assuming that different tumor types are independent from each other. The overall tumor incidence was fit with the MS_Combo multiple tumor model (see BMDS, Version 2.6; Appendix C for details), and the estimated 10% benchmark dose (BMD_{10}), 10% benchmark dose lower confidence limit ($BMDL_{10}$), and calculated cancer slope factors are presented in Table 13. This modeling provides an estimate of composite risk for developing any combination of tumors at any site within a single study. Modeling procedures and results are described in detail in Appendix C.

Prior to dose-response modeling, doses administered to the animals in the studies by Matsumoto et al. (2012) were converted to HEDs according to the equation below:

$$\text{Dose (HED)} = \text{Dose} \times (BW_a \div BW_h)^{1/4}$$

where:

Dose = average daily animal dose of *o*-phenylenediamine
BW_a = reference animal body weight[7]
BW_h = 70 kg, reference human body weight (U.S. EPA, 1988)

Using a BW_a of 0.25 kg for rats and 0.025 kg for mice, and a BW_h of 70 kg for humans, the resulting default DAFs are 0.24 and 0.14 for rats and mice, respectively (U.S. EPA, 2011c, 2005a). The animal doses, calculated HED values, and associated tumor incidences are provided in Table 12.

[7]Time-weighted body weight was not reported by the study authors or calculable from reported study data. Default animal body weights (0.25 kg for rats and 0.025 kg for mice) were used in calculating HED values.

Table 13. Goodness-of-Fit Statistics and BMD_{10} and $BMDL_{10}$ Values for Tumors and Combined Tumors in Male and Female F344/DuCrj Rats and Crj:BDF_1 Mice Administered *o*-Phenylenediamine Dihydrochloride in Drinking Water for 2 Years[a,c]

	Multistage-Cancer Model Degree of Polynomial	Goodness-of-Fit *p*-Value[b]	BMD_{10} (HED) mg/kg-d	$BMDL_{10}$ (HED) mg/kg-d	Cancer Slope Factor $(mg/kg\text{-}d)^{-1}$
Male Rats					
Hepatocellular adenoma and/or carcinoma—liver	2-degree (high dose dropped)	0.1	3.8	2.8	0.036
Transitional cell papilloma and/or carcinoma—urinary bladder	3-degree	0.08	11	9.1	0.011
Follicular adenoma—thyroid	1-degree	0.58	23	12	0.0083
Combined tumors			3.5	2.5	0.040
Female Rats					
Hepatocellular adenoma and/or carcinoma—liver	3-degree	0.42	3.1	2.2	0.045
Male Mice					
Hepatocellular adenoma and/or carcinoma—liver	1-degree	0.13	1.3	0.90	0.11
Papillary adenoma—gall bladder	1-degree	0.94	12	7.4	0.014
Combined tumors			**1.1**	**0.84**	**0.12**
Female Mice					
Hepatocellular adenoma and/or carcinoma—liver	1-degree	0.93	2.04	1.64	0.0610
Papillary adenoma—gall bladder	1-degree	0.33	32.1	19.4	0.00515
Combined tumors			1.92	1.56	0.0641

[a]Matsumoto et al. (2012).
[b]Values >0.05 meet conventional goodness-of-fit criteria.
[c]BMD modeling results are described in detail in Appendix C.

BMD_{10} (HED) = 10% benchmark dose human equivalent dose; $BMDL_{10}$ (HED) = 10% benchmark dose lower confidence limit human equivalent dose.

The lowest $BMDL_{10}$ (HED) of 0.84 mg/kg-day, obtained from data on the combined tumors in male mice, was selected as the POD for calculation of the p-OSF. Because the Matsumoto et al. (2012) study was conducted for the full lifetime of the mice (2 years), no adjustment for less-than-lifetime observation was necessary. Because a mutagenic MOA has been implicated for *o*-phenylenediamine-induced tumors, a linear extrapolation to low dose was used to obtain the slope from the POD. The **p-OSF of 1.2×10^{-1} $(mg/kg\text{-}day)^{-1}$** was derived as follows:

p-OSF	=	$BMR \div BMDL_{10}$ (HED)
	=	$0.1 \div 0.84$ mg/kg-day
	=	**1.2×10^{-1} $(mg/kg\text{-}day)^{-1}$**

The p-OSF should not be used with exposure exceeding the POD ($BMDL_{10}$ [HED] = 0.84 mg/kg-day) because at doses higher than this value, the fitted dose-response model better characterizes the dose-response relationship.

A WOE evaluation has concluded that *o*-phenylenediamine may be carcinogenic by a mutagenic MOA. According to the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b) those exposed to carcinogens with a mutagenic MOA are assumed to have increased early-life susceptibility. Data on *o*-phenylenediamine are not sufficient to develop separate risk estimates for childhood exposure. The p-OSF of 1.2×10^{-1} $(mg/kg\text{-}day)^{-1}$ calculated from data from adult exposure does not reflect presumed early-life susceptibility for this chemical, and age-dependent adjustment factors (ADAFs) should be applied to this parameter when assessing cancer risks. The current ADAFs and their age groupings are 10 for <2 years, 3 for 2 to <16 years, and 1 for 16 years and above (U.S. EPA, 2005b). The adjusted slope factors associated with these ADAFs are 1.2 $(mg/kg\text{-}day)^{-1}$ for <2 years, 0.36 $(mg/kg\text{-}day)^{-1}$ for 2 to <16 years, and 0.12 $(mg/kg\text{-}day)^{-1}$ for 16 years and above. These slope factors are to be combined with age-specific exposure estimates when estimating cancer risks from early-life (<16 years of age) exposure to *o*-phenylenediamine. A cancer risk is derived for each age group and these are summed across age groups to obtain the total risk for the exposure period of interest.

Derivation of a Provisional Inhalation Unit Risk

No carcinogenicity studies of humans or animals exposed to *o*-phenylenediamine via inhalation have been identified in the available literature, precluding derivation of inhalation cancer potency values.

APPENDIX A. SCREENING PROVISIONAL VALUES

No screening values for *o*-phenylenediamine are identified.

APPENDIX B. DATA TABLES

Table B-1. Selected Effects on Male and Female Crl:CD®BR Rats Exposed to *o*-Phenylenediamine (CASRN 95-54-5) via Gavage for 90 Days[a]

Endpoint	Exposure Group, mg/kg-d			
	0	20	40	80
Males				
Stained underbody	0/10[b]	0/10	1/10	1/10
Body weight on D 92 (g)	553.1 ± 66.2	575.9 ± 25.8 (4%)	526.1 ± 65.4 (−5%)	529.5 ± 47.9 (−4%)
Body-weight gain D 1−92 (g)	340.3 ± 66.5[c]	356.8 ± 27.2 (5%)	296.3 ± 47.5* (−13%)	312.8 ± 49.3 (−8%)
Slight palpebral closure in home cage, Wk 13	0/10	0/10	0/9	3/10
Slight palpebral closure in open field, Wk 13	0/10	0/10	0/9	4/10**
Increased response to tail pinch, Wk 4	1/10	0/10	0/10	2/10
Increased response to tail pinch, Wk 6	1/10	1/10	0/10	3/10
Increased response to tail pinch, Wk 13	0/10	0/10	0/10	3/10
Females				
Stained abdomen	0/10	0/10	0/10	2/10
Stained inguen	0/10	0/10	1/10	7/10**
Stained perineum	0/10	0/10	0/10	5/10**
Stained underbody	0/10	0/10	0/10	2/10
Body weight on D 92 (g)	290.4 ± 25.7	292.7 ± 38.2 (0.8%)	288.0 ± 20.5 (−0.8%)	270.9 ± 28.3 (−7%)
Body-weight gain D 1−92 (g)	132.8 ± 16.3	133.5 ± 29.7 (0.5%)	125.1 ± 18.3 (−6%)	116.5 ± 19.8 (−12%)
Slight palpebral closure in home cage, Wk 13	0/10	0/10	1/10	5/10**
Slight palpebral closure in open field, Wk 13	0/10	0/10	0/10	2/10
Increased response to tail pinch, Wk 13	0/10	1/10	0/10	0/10
Slightly soiled fur	0/10	0/10	0/10	5/10**

[a]DuPont (1992a).
[b]Number affected/number examined.
[c]Mean ± standard deviation.
*Significantly different from control ($p < 0.05$) based on Dunnett's test, as reported by the study authors.
**Significantly different from control ($p < 0.05$) based on Fisher's exact test performed for this review.

Table B-2. Survival, Terminal Body Weights, and Liver Weights in Male and Female F344/DuCrj Rats Administered *o*-Phenylenediamine Dihydrochloride in Drinking Water for 2 Years[a]

Males				
Dose, mg/kg-d (as *o*-phenylenediamine)	**0**	**13**	**25**	**51**
Survival to termination[b]	41/50	36/50	42/50	42/50
Terminal body weight (g)	382 ± 33[c]	355 ± 47* (−7%)[d]	330 ± 22** (−14%)	269 ± 29** (−30%)
Absolute liver weight (g)	11.37 ± 2.69	10.61 ± 1.29 (−7%)	10.65 ± 2.41 (−6%)	9.27 ± 3.41** (−18%)
Relative liver weight (%)	2.99 ± 0.75	3.01 ± 0.34 (0.67%)	3.24 ± 0.82** (8%)	3.46 ± 1.34** (16%)
Females				
Dose, mg/kg-d (as *o*-phenylenediamine)	**0**	**11**	**20**	**35**
Survival to termination[b]	41/50	38/50	44/50	41/50
Terminal body weight (g)	253 ± 23	237 ± 30 (−6%)	234 ± 24** (−8%)	204 ± 19** (−19%)
Absolute liver weight (g)	6.69 ± 0.95	6.58 ± 1.10 (−2%)	6.81 ± 1.32 (2)%	9.41 ± 3.63** (41%)
Relative liver weight (%)	2.65 ± 0.34	2.81 ± 0.54 (6%)	2.93 ± 0.63 (11%)	4.65 ± 1.85** (75%)

[a]Matsumoto et al. (2012).
[b]Number of animals survived/number of animals examined.
[c]Mean ± standard deviation.
[d]Percent change from control.
*Significantly different from control at $p < 0.05$ by Dunnett's test performed by the study authors.
**Significantly different from control at $p < 0.01$ by Dunnett's test performed by the study authors.

Table B-3. Hematology and Serum Chemistry in Male and Female F344/DuCrj Rats Administered *o*-Phenylenediamine Dihydrochloride in Drinking Water for 2 Years[a]

Males				
Dose, mg/kg-d (as *o*-phenylenediamine)	**0**	**13**	**25**	**51**
Number of animals examined	40	36	42	42
Hematology				
Hb (g/dL)	13.2 ± 2.5[b]	13.2 ± 3.4 (0%)[c]	13.6 ± 2.4 (3%)	13.7 ± 2.6 (4%)
MCV (fL)	50.1 ± 7.7	48.4 ± 2.3 (−3%)	48.8 ± 4.4* (−3%)	50.0 ± 3.7 (−0.2%)
MCH (pg)	16.8 ± 2.0	16.2 ± 1.5* (−4%)	16.4 ± 1.3* (−2%)	16.8 ± 1.2 (0%)
MCHC (g/dL)	33.5 ± 1.8	33.3 ± 2.5 (−0.6%)	33.7 ± 1.6 (0.6%)	33.6 ± 1.4 (0.3%)
Platelet (10^3/μL)	923 ± 238	869 ± 171 (−6%)	889 ± 226 (−4%)	822 ± 238 (−11%)
Serum chemistry[b]				
AST (IU/L)	97 ± 49	76 ± 25 (−22%)	167 ± 270 (72%)	1,887 ± 10,973* (1,845%)
ALT (IU/L)	45 ± 23	41 ± 17 (−9%)	90 ± 168 (100%)	256 ± 1,059* (469%)
ALP (IU/L)	228 ± 87	212 ± 68 (−7%)	202 ± 49 (−11%)	231 ± 111 (1%)
GGT (IU/L)	12 ± 6	14 ± 6 (17%)	23 ± 36* (92%)	16 ± 12 (33%)
BUN (mg/dL)	19.1 ± 2.0	20.0 ± 2.8 (5%)	19.6 ± 3.7 (3%)	26.0 ± 19.2** (36%)
Urinalysis				
Positive occult blood[d]	1/40	4/36	7/42	34/43[##]
Urinary pH[e]	7.2	7.2	7.0	6.9[#]
Females				
Dose, mg/kg-d (as *o*-phenylenediamine)	**0**	**11**	**20**	**35**
Number of animals examined	39	38	43	41
Hematology				
Hb (g/dL)	14.5 ± 1.8	14.1 ± 3.2 (−3%)	13.9 ± 2.8 (−4%)	14.0 ± 1.8* (−3%)
MCV (fL)	53.2 ± 3.4	55.5 ± 14.1 (4%)	54.4 ± 10.0 (2%)	52.1 ± 5.8** (−2%)
MCH (pg)	18.4 ± 0.8	18.8 ± 2.7 (2%)	18.5 ± 2.5 (0.5%)	17.9 ± 1.9** (−3%)
MCHC (g/dL)	34.7 ± 1.0	34.3 ± 2.2 (−1%)	34.2 ± 1.9 (−1%)	34.4 ± 0.8** (−0.9%)
Platelet (10^3/μL)	644 ± 115	578 ± 154 (−10%)	661 ± 156 (3%)	777 ± 168** (21%)

Table B-3. Hematology and Serum Chemistry in Male and Female F344/DuCrj Rats Administered *o*-Phenylenediamine Dihydrochloride in Drinking Water for 2 Years[a]

Females				
Dose, mg/kg-d (as *o*-phenylenediamine)	**0**	**11**	**20**	**35**
Number of animals examined	39	38	43	41
Serum chemistry				
AST (IU/L)	127 ± 82	179 ± 263 (41%)	179 ± 325 (41%)	596 ± 928** (369%)
ALT (IU/L)	54 ± 26	62 ± 54 (15%)	78 ± 218 (44%)	254 ± 322** (370%)
ALP (IU/L)	139 ± 81	193 ± 313 (39%)	141 ± 128 (1%)	206 ± 128** (48%)
GGT (IU/L)	6 ± 5	7 ± 6 (17%)	9 ± 13 (50%)	42 ± 56** (600%)
BUN (mg/dL)	17.2 ± 5.3	17.1 ± 2.7 (−0.6%)	18.8 ± 11.6 (9%)	18.7 ± 3.2** (9%)
Urinalysis				
Positive occult blood[d]	1/41	4/39	4/44	16/41[##]
Urinary pH[e]	7.4	7.4	7.0[#]	6.7[##]

[a]Matsumoto et al. (2012).
[b]Mean ± standard deviation.
[c]Percent change from control.
[d]Number of animals having positive occult blood/number of animals examined.
[e]Number of animals in calculation of average pH was same as number of animals examined for positive occult blood.
*Significantly different from control at $p < 0.05$ by Dunnett's test performed by the study authors.
**Significantly different from control at $p < 0.01$ by Dunnett's test performed by the study authors.
[#]Significantly different from control at $p < 0.05$ by χ^2 test with severity for urinalysis, performed by the study authors.
[##]Significantly different from control at $p < 0.01$ by χ^2 test with severity for urinalysis, performed by the study authors.

ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen; GGT = γ-glutamyl transferase; Hb = hemoglobin; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume.

Table B-4. Incidences of Selected Non-neoplastic Lesions in Male and Female F344/DuCrj Rats Administered *o*-Phenylenediamine Dihydrochloride in Drinking Water for 2 Years[a]

Males				
Dose, mg/kg-d (as *o*-phenylenediamine)	**0**	**13**	**25**	**51**
Liver				
Clear cell foci	2/50[b]	9/50*	12/50**	3/50
Basophilic cell foci	19/50	31/50*	37/50**	38/50**
Urinary bladder				
Simple hyperplasia: transitional epithelium	1/50	1/50	3/50	6/50
Papillary and/or nodular hyperplasia: transitional epithelium	0/50	2/50	1/50	7/50**
Kidney				
Necrosis: papilla	0/50	0/50	0/50	15/50**
Mineralization: papilla	7/50	18/50**	16/50*	26/50**
Urothelial hyperplasia: pelvis	8/50	10/50	18/50*	22/50**
Females				
Dose, mg/kg-d (as *o*-phenylenediamine)	**0**	**11**	**20**	**35**
Liver				
Clear cell foci	1/50	0/50	2/50	3/50
Basophilic cell foci	8/50	21/50**	39/50**	33/50**
Urinary bladder				
Simple hyperplasia: transitional epithelium	0/50	1/50	0/50	2/50
Papillary and/or nodular hyperplasia: transitional epithelium	0/50	0/50	0/50	1/50
Kidney				
Necrosis: papilla	2/50	1/50	1/50	11/50**
Mineralization: papilla	7/50	9/50	12/50	24/50**
Urothelial hyperplasia: pelvis	2/50	12/50**	10/50*	17/50**

[a]Matsumoto et al. (2012).
[b]Number of animals affected/number of animals examined.
*Significantly different from control at $p < 0.05$ by Fisher's exact test performed by the study authors.
**Significantly different from control at $p < 0.01$ by Fisher's exact test performed by the study authors.

Table B-5. Incidences of Selected Neoplastic Lesions in Male and Female F344/DuCrj Rats Administered *o*-Phenylenediamine Dihydrochloride in Drinking Water for 2 Years[a]

Males				
Dose, mg/kg-d (as *o*-phenylenediamine)	**0**	**13**	**25**	**51**
Liver				
Hepatocellular adenoma	3/50[##b]	2/50	12/50*	15/50**
Hepatocellular carcinoma	1/50[##]	1/50	6/50	10/50**
Hepatocellular adenoma and/or carcinoma	4/50[##]	3/50	16/50**	22/50**
Urinary bladder				
Transitional cell papilloma	1/50[##]	0/50	0/50	6/50
Transitional cell carcinoma	1/50[#]	0/50	0/50	4/50
Transitional cell papilloma and/or carcinoma	2/50[##]	0/50	0/50	10/50*
Thyroid				
Follicular adenoma	0/50[##]	1/50	0/50	4/50
Follicular adenocarcinoma	1/50	0/50	1/50	1/50
Females				
Dose, mg/kg-d (as *o*-phenylenediamine)	**0**	**11**	**20**	**35**
Liver				
Hepatocellular adenoma	1/50[##]	3/50	15/50**	36/50**
Hepatocellular carcinoma	0/50[##]	0/50	4/50	18/50**
Hepatocellular adenoma and/or carcinoma	1/50[##]	3/50	19/50**	44/50**
Urinary bladder				
Transitional cell papilloma	1/50	0/50	1/50	1/50
Transitional cell carcinoma	0/50	0/50	0/50	0/50
Transitional cell papilloma and/or carcinoma	1/50	0/50	1/50	1/50
Thyroid				
Follicular adenoma	1/50	0/50	1/50	0/50
Follicular adenocarcinoma	0/50	0/50	1/50	0/50

[a]Matsumoto et al. (2012).
[b]Number of animals affected/number of animals examined.
*Significantly different from control at $p < 0.05$ by Fisher's exact test performed by the study authors.
**Significantly different from control at $p < 0.01$ by Fisher's exact test performed by the study authors.
#Significant dose-related positive trend at $p < 0.05$ by Peto's test performed by the study authors.
##Significant dose-related positive trend at $p < 0.01$ by Peto's test performed by the study authors.

Table B-6. Survival, Terminal Body Weights, and Liver Weights in Male and Female Crj:BDF$_1$ Mice Administered *o*-Phenylenediamine Dihydrochloride in Drinking Water for 2 Years[a]

Males				
Dose, mg/kg-d (as *o*-phenylenediamine)	**0**	**27**	**56**	**106**
Survival to termination[b]	38/50	38/50	42/50	39/50
Terminal body weight (g)	48.1 ± 7.0[c]	40.3 ± 5.3* (−16%)[d]	35.1 ± 4.1* (−27%)	31.0 ± 2.6* (−36%)
Absolute liver weight (g)	1.79 ± 0.60	1.90 ± 0.63 (6%)	2.09 ± 0.96 (17%)	1.87 ± 0.50 (4%)
Relative liver weight (%)	3.92 ± 2.26	4.95 ± 2.63* (26%)	6.16 ± 3.22* (57%)	6.12 ± 1.98* (56%)
Females				
Dose, mg/kg-d (as *o*-phenylenediamine)	**0**	**63.3**	**119**	**234**
Survival to termination[b]	24/50	29/50	28/50	34/50
Terminal body weight (g)	31.1 ± 3.4	28.4 ± 4.4 (−9%)	26.4 ± 5.6* (−15%)	21.5 ± 2.1* (−31%)
Absolute liver weight (g)	1.49 ± 0.30	1.58 ± 0.44 (6%)	1.99 ± 1.21 (34%)	2.02 ± 0.98 (36%)
Relative liver weight (%)	4.89 ± 1.35	5.61 ± 1.53 (15%)	7.83 ± 5.04* (61%)	9.45 ± 4.63* (93%)

[a]Matsumoto et al. (2012).
[b]Number of animals survived/number of animals examined.
[c]Mean ± standard deviation.
[d]Percent change from control.
*Significantly different from control at $p < 0.01$ by Dunnett's test performed by the study authors.

Table B-7. Hematology and Serum Chemistry in Male and Female Crj:BDF$_1$ Mice Administered *o*-Phenylenediamine Dihydrochloride in Drinking Water for 2 Years[a]

Males				
Dose, mg/kg-d (as *o*-phenylenediamine)	**0**	**27**	**56**	**106**
Hematology				
Number of animals examined	36	38	42	39
RBC (10^6/μL)	9.60 ± 0.91[b]	9.59 ± 1.28 (−0.1%)[c]	9.43 ± 1.92 (−2%)	9.10 ± 0.84** (−5%)
Hb (g/dL)	13.8 ± 1.1	13.6 ± 1.5 (−1%)	13.5 ± 2.3 (−2%)	13.3 ± 1.2** (−4%)
MCV (fL)	45.8 ± 1.9	45.3 ± 2.9 (−1%)	46.9 ± 6.3 (2%)	46.9 ± 1.2** (2%)
MCHC (g/dL)	31.5 ± 0.7	31.4 ± 1.0 (−0.3%)	31.1 ± 1.3 (−1%)	31.1 ± 0.6** (−1%)
Platelet (10^3/μL)	1,911 ± 411	1,985 ± 418 (4%)	2,087 ± 470 (9%)	2,279 ± 303** (19%)
WBC (10^3/μL)	4.47 ± 8.86	2.96 ± 1.61 (−34%)	3.05 ± 2.90 (−32%)	2.00 ± 1.31** (−55%)
Serum chemistry				
Number of animals examined	37	38	42	39
AST (IU/L)	88 ± 167	127 ± 224 (44%)	174 ± 355 (98%)	119 ± 264 (35%)
ALT (IU/L)	49 ± 77	135 ± 355 (176%)	178 ± 514** (263%)	119 ± 300** (143%)
ALP (IU/L)	124 ± 28	220 ± 244** (77%)	337 ± 396** (172%)	279 ± 182** (125%)
GGT (IU/L)	1 ± 1	2 ± 1 (100%)	2 ± 1 (100%)	2 ± 1 (100%)
BUN (mg/dL)	23.4 ± 9.4	22.8 ± 3.3 (−3%)	28.4 ± 12.1** (21%)	30.9 ± 11.3** (32%)
Females				
Dose, mg/kg-d (as *o*-phenylenediamine)	**0**	**63.3**	**119**	**234**
Hematology				
Number of animals examined	22	27	25	28
RBC (10^6/μL)	9.82 ± 1.99	9.30 ± 1.22 (−5%)	8.94 ± 1.90 (−9%)	9.37 ± 0.85 (−5%)
Hb (g/dL)	14.1 ± 2.3	13.6 ± 1.7 (−4%)	12.9 ± 2.7 (−9%)	13.4 ± 1.2** (−5%)
MCV (fL)	45.7 ± 2.3	46.7 ± 2.8 (2%)	47.4 ± 5.1 (4%)	46.7 ± 2.1** (2%)
MCHC (g/dL)	31.6 ± 1.1	31.5 ± 1.1 (−0.3%)	30.9 ± 1.6* (−2%)	30.7 ± 0.6** (−3%)
Platelet (10^3/μL)	1,210 ± 273	1,329 ± 374 (10%)	1,399 ± 453 (16%)	1,641 ± 454** (36%)
WBC (10^3/μL)	2.13 ± 1.63	4.50 ± 11.15 (111%)	4.17 ± 4.18 (96%)	1.94 ± 1.51 (−9%)

Table B-7. Hematology and Serum Chemistry in Male and Female Crj:BDF$_1$ Mice Administered *o*-Phenylenediamine Dihydrochloride in Drinking Water for 2 Years[a]

Females				
Dose, mg/kg-d (as *o*-phenylenediamine)	**0**	**63.3**	**119**	**234**
Serum chemistry				
Number of animals examined	23	27	26	31
AST (IU/L)	87 ± 36	130 ± 203 (49%)	133 ± 151 (53%)	190 ± 224 (118%)
ALT (IU/L)	40 ± 23	74 ± 123 (85%)	120 ± 189 (200%)	207 ± 316** (418%)
ALP (IU/L)	171 ± 53	254 ± 88* (49%)	443 ± 468** (159%)	598 ± 559** (250%)
GGT (IU/L)	2 ± 1	2 ± 1 (0%)	3 ± 3 (50%)	4 ± 6 (100%)
BUN (mg/dL)	23.6 ± 24.3	24.5 ± 10.6 (4%)	27.5 ± 11.9** (17%)	30.7 ± 13.9** (30%)

[a]Matsumoto et al. (2012).
[b]Mean ± standard deviation.
[c]Percent change from control.
*Significantly different from control at $p < 0.05$ by Dunnett's test performed by the study authors.
**Significantly different from control at $p < 0.01$ by Dunnett's test performed by the study authors.

ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen; GGT = γ-glutamyl transferase; Hb = hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; RBC = red blood cell; WBC = white blood cell.

Table B-8. Incidences of Selected Non-neoplastic Lesions in Male and Female Crj:BDF$_1$ Mice Administered *o*-Phenylenediamine Dihydrochloride in Drinking Water for 2 Years[a]

Males				
Dose, mg/kg-d (as *o*-phenylenediamine)	**0**	**27**	**56**	**106**
Liver				
Clear cell foci	3/50[b]	6/50	2/50	0/50
Acidophilic cell foci	2/50	10/50*	9/50*	5/50
Basophilic cell foci	2/50	5/50	9/50*	9/50*
Gall bladder[c]				
Papillary hyperplasia	0/46	13/50**	8/49**	8/47**
Nasal cavity				
Eosinophilic change: olfactory epithelium	22/50	11/50*	22/50	24/50
Eosinophilic change: respiratory epithelium	32/50	27/50	28/50	45/50**
Respiratory metaplasia: gland	31/50	25/50	25/50	34/50
Nasopharynx				
Eosinophilic change	2/50	2/50	1/50	4/50
Kidney				
Hydronephrosis	3/50	2/50	2/50	4/50
Inflammatory polyp: pelvis	2/50	2/50	2/50	3/50
Females				
Dose, mg/kg-d (as *o*-phenylenediamine)	**0**	**63.3**	**119**	**234**
Liver				
Clear cell foci	0/50	4/50	3/50	8/50**
Acidophilic cell foci	2/50	4/50	3/50	18/50**
Basophilic cell foci	1/50	7/50*	4/50	10/50**
Gall bladder				
Papillary hyperplasia	0/50	2/50	14/50**	10/50**
Nasal cavity				
Eosinophilic change: olfactory epithelium	7/50	1/50	11/50	18/50*
Eosinophilic change: respiratory epithelium	37/50	45/50**	48/50**	48/50**
Respiratory metaplasia: gland	14/50	19/50	27/50**	34/50**

Table B-8. Incidences of Selected Non-neoplastic Lesions in Male and Female Crj:BDF$_1$ Mice Administered *o*-Phenylenediamine Dihydrochloride in Drinking Water for 2 Years[a]

Females				
Dose, mg/kg-d (as *o*-phenylenediamine)	**0**	**63.3**	**119**	**234**
Nasopharynx				
Eosinophilic change	3/50	5/50	3/50	13/50**
Kidney				
Hydronephrosis	2/50	12/50**	13/50**	11/50**
Inflammatory polyp: pelvis	2/50	9/50*	10/50*	6/50

[a]Matsumoto et al. (2012).
[b]Number of animals affected/number of animals examined.
[c]Number of males examined for non-neoplastic lesions of the gall bladder varied by dose group.
*Significantly different from control at $p < 0.05$ by Fisher's exact test performed by the study authors.
**Significantly different from control at $p < 0.01$ by Fisher's exact test performed by the study authors.

Table B-9. Selected Neoplastic Lesions in Male and Female Crj:BDF$_1$ Mice Administered *o*-Phenylenediamine Dihydrochloride in Drinking Water for 2 Years[a]

Males				
Dose, mg/kg-d (as *o*-phenylenediamine)	**0**	**27**	**56**	**106**
Liver				
Hepatocellular adenoma	12/50[##b]	25/50**	34/50**	35/50**
Hepatocellular carcinoma	6/50	9/50	12/50	10/50
Hepatocellular adenoma and/or carcinoma	18/50[##]	29/50**	39/50**	38/50**
Gall bladder				
Papillary adenoma	0/46[#]	2/50	4/49	5/47*
Females				
Dose, mg/kg-d (as *o*-phenylenediamine)	**0**	**63.3**	**119**	**234**
Liver				
Hepatocellular adenoma	6/50[##]	22/50**	23/50**	34/50**
Hepatocellular carcinoma	1/50[##]	4/50	11/50**	17/50**
Hepatocellular adenoma and/or carcinoma	6/50[##]	23/50**	31/50**	41/50**
Gall bladder				
Papillary adenoma	0/50	1/50	5/50*	3/50

[a]Matsumoto et al. (2012).
[b]Number of animals affected/number of animals examined.
*Significantly different from control at $p < 0.05$ by Fisher's exact test performed by the study authors.
**Significantly different from control at $p < 0.01$ by Fisher's exact test performed by the study authors.
[#]Significant dose-related positive trend ($p < 0.05$) by Peto's test performed by the study authors.
[##]Significant dose-related positive trend ($p < 0.01$) by Peto's test performed by the study authors.

Table B-10. Incidences of Tumors in Male CD Rats and Male and Female CD-1 HaM/ICR Mice Exposed to *o*-Phenylenediamine Dihydrochloride in Food for 18 Months[a]

	Exposure Group, mg/kg-d (as *o*-phenylenediamine)			
	Simultaneous control	**Pooled control**	**Low**	**High**
Male rats	**0**	**0**	**83.6**	**167**
Hepatocellular carcinoma	0/16[b]	2/111	0/14	5/16*
Male mice	**0**	**0**	**704**	**1,430**
Hepatocellular carcinoma	0/14	7/99	5/17**	3/14
Female mice	**0**	**0**	**717**	**1,450**
Hepatocellular carcinoma	1/15	1/102	6/18***	6/15**

[a]Weisburger et al. (1978).
[b]Number of animals affected/number of animals examined.
*Significantly different from simultaneous and pooled controls ($p < 0.025$), as reported by the study authors.
**Significantly different from simultaneous and pooled controls ($p < 0.05$), as reported by the study authors.
***Significantly different from pooled controls ($p < 0.025$), as reported by the study authors.

APPENDIX C. BENCHMARK DOSE MODELING RESULTS

MODELING OF NONCANCER ENDPOINTS

As discussed in the body of the report in the "Derivation of a Chronic Provisional Reference Dose" section, the endpoints selected for benchmark dose (BMD) modeling were incidence of renal papillary mineralization in male rats, incidence of renal pelvis urothelial hyperplasia in female rats, and incidence of renal hydronephrosis in female mice (Matsumoto et al., 2012). The animal doses in the study, converted to equivalent doses of *o*-phenylenediamine, were used in the BMD modeling; the data are shown in Tables 7, B-4, B-7, and B-8.

Modeling Procedure for Dichotomous Noncancer Data

BMD modeling of dichotomous noncancer data was conducted with the EPA's Benchmark Dose Software (BMDS, Version 2.5). For these data, the Gamma, Logistic, Log-Logistic, Log-Probit, Multistage, Probit, and Weibull dichotomous models available within the software were fit using a benchmark response (BMR) of 10% extra risk. The Multistage model is run for all polynomial degrees up to $n - 1$, where n is the number of dose groups including control. Adequacy of model fit was judged based on the χ^2 goodness-of-fit p-value ($p > 0.1$), 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 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 Incidence of Renal Papillary Mineralization in Male Rats

The procedure outlined above was applied to the data (see Table 7) on renal papillary mineralization in male rats exposed chronically to *o*-phenylenediamine dihydrochloride via drinking water for 2 years (Matsumoto et al., 2012). All models provided adequate fit to the data set when assessed by the overall goodness-of-fit ($p > 0.1$) and scaled residuals (absolute value <2.0) (see Table C-1). The 10% benchmark dose lower confidence limit ($BMDL_{10}$) from all models were sufficiently close; therefore, the Log-Logistic model providing the lowest AIC was selected as the best fitting. The BMD_{10} and $BMDL_{10}$ values for incidence of renal papillary mineralization in male rats from this model were 7.60 and 4.80 mg/kg-day, respectively. Figure C-1 shows the Log-Logistic model fit to the data.

Table C-1. Modeling Results for Renal Papillary Mineralization in Male Rats[a]

Model Name	AIC	χ^2 Goodness-of-Fit *p*-Value[b]	BMD_{10} mg/kg-d	$BMDL_{10}$ mg/kg-d	Scaled Residual
Gamma[c]	244.12	0.30	9.49	6.52	1.30
Logistic	245.13	0.18	14.86	11.68	1.49
Log-Logistic[d,e]	**243.81**	**0.35**	**7.60**	**4.80**	**1.10**
Log-Probit[c]	246.32	0.10	17.09	11.82	1.78
Multistage (2-degree)[f]	244.12	0.30	9.49	6.52	1.30
Multistage (3-degree)[f]	244.12	0.30	9.49	6.52	1.30
Probit	245.02	0.19	14.27	11.19	1.48
Weibull[c]	244.12	0.30	9.49	6.52	1.30
Quantal-Linear	244.12	0.30	9.49	6.52	1.30

[a]Matsumoto et al. (2012).
[b]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[c]Power restricted to ≥1.
[d]Slope restricted to ≥1.
[e]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 (Log-Logistic).
[f]Betas restricted to ≥0.

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]).

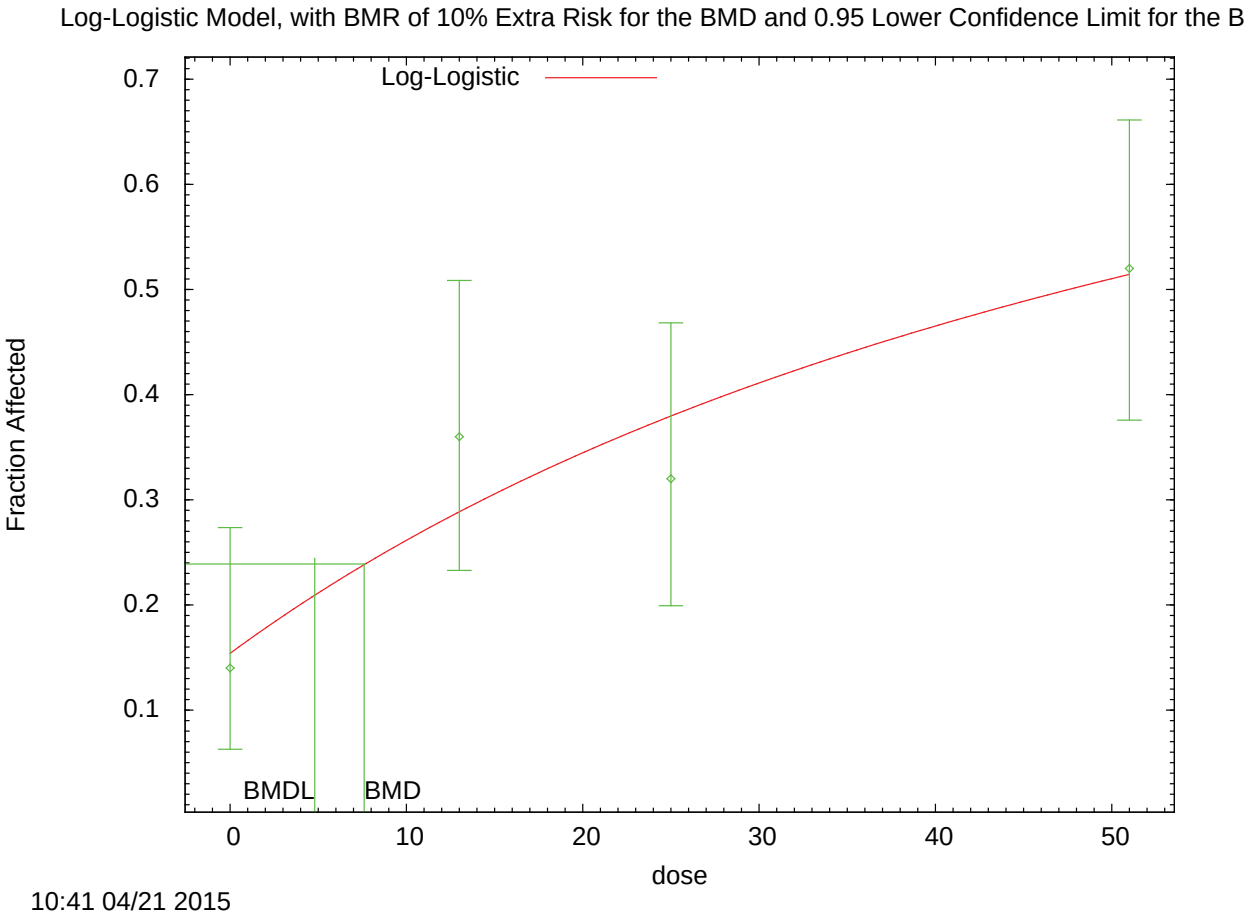

Figure C-1. Fit of Log-Logistic Model to Incidence of Renal Papillary Mineralization in Male Rats (Matsumoto et al., 2012)

Text Output for Log-Logistic Model to Incidence of Renal Papillary Mineralization in Male Rats (Matsumoto et al., 2012)

```
 ================================================================
        Logistic Model. (Version: 2.14; Date: 2/28/2013)
         ================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = Effect
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 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

User has chosen the log transformed model

Default Initial Parameter Values
background = 0.14
intercept = -4.11546
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

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

	background	intercept
background	1	-0.67
intercept	-0.67	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0.154391	0.0498843	0.0566197	0.252162
intercept	-4.22567	0.322865	-4.85847	-3.59286
slope	1	NA		

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

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-118.88	4			
Fitted model	-119.903	2	2.04645	2	0.3594
Reduced model	-127.533	1	17.3055	3	0.0006115

AIC: 243.806

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1544	7.720	7.000	50	-0.282
51.0000	0.5155	25.776	26.000	50	0.063
25.0000	0.3807	19.034	16.000	50	-0.884
13.0000	0.2894	14.470	18.000	50	1.101

Chi^2 = 2.08 d.f. = 2 P-value = 0.3542

```
   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        7.60222

            BMDL =        4.79952
```

Model Predictions for Incidence of Renal Pelvis Urothelial Hyperplasia in Female Rats

The procedure outlined above was applied to the data (see Table 7) on renal pelvis urothelial hyperplasia in female rats exposed chronically to *o*-phenylenediamine dihydrochloride via drinking water for 2 years (Matsumoto et al., 2012). The Gamma, Log-Logistic, Multistage, Weibull, and Quantal-Linear models provided adequate fit to the data when assessed by the overall goodness-of-fit ($p > 0.1$) and scaled residuals (absolute value <2.0) (see Table C-2). The $BMDL_{10}$ from models providing adequate fit were sufficiently close; therefore, the Log-Logistic model providing the lowest AIC was selected as the best fitting. The BMD_{10} and $BMDL_{10}$ values for incidence of renal pelvis urothelial hyperplasia in female rats from this model were 8.32 and 5.58 mg/kg-day, respectively. Figure C-2 shows the Log-Logistic model fit to the data.

Table C-2. Modeling Results for Renal Pelvis Urothelial Hyperplasia in Female Rats[a]

Model Name	AIC	χ^2 Goodness-of-Fit *p*-Value[b]	BMD_{10} mg/kg-d	$BMDL_{10}$ mg/kg-d	Scaled Residual
Gamma[c]	192.81	0.23	9.49	6.68	1.49
Logistic	195.11	0.08	16.17	12.93	−0.25
Log-Logistic[d,e]	**192.40**	**0.29**	**8.32**	**5.58**	**1.31**
Log-Probit[c]	196.30	0.04	15.59	11.04	−0.45
Multistage (2-degree)[f]	192.81	0.23	9.49	6.68	1.49
Multistage (3-degree)[f]	192.81	0.23	9.49	6.68	1.49
Probit	194.82	0.09	15.27	12.12	1.76
Weibull[c]	192.81	0.23	9.49	6.68	1.49
Quantal-Linear	192.81	0.23	9.49	6.68	1.49

[a]Matsumoto et al. (2012).
[b]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[c]Power restricted to ≥1.
[d]Slope restricted to ≥1.
[e]Selected model. All models, except Logistic, Log-Probit, and Probit 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 (Log-Logistic).
[f]Betas restricted to ≥0.

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]).

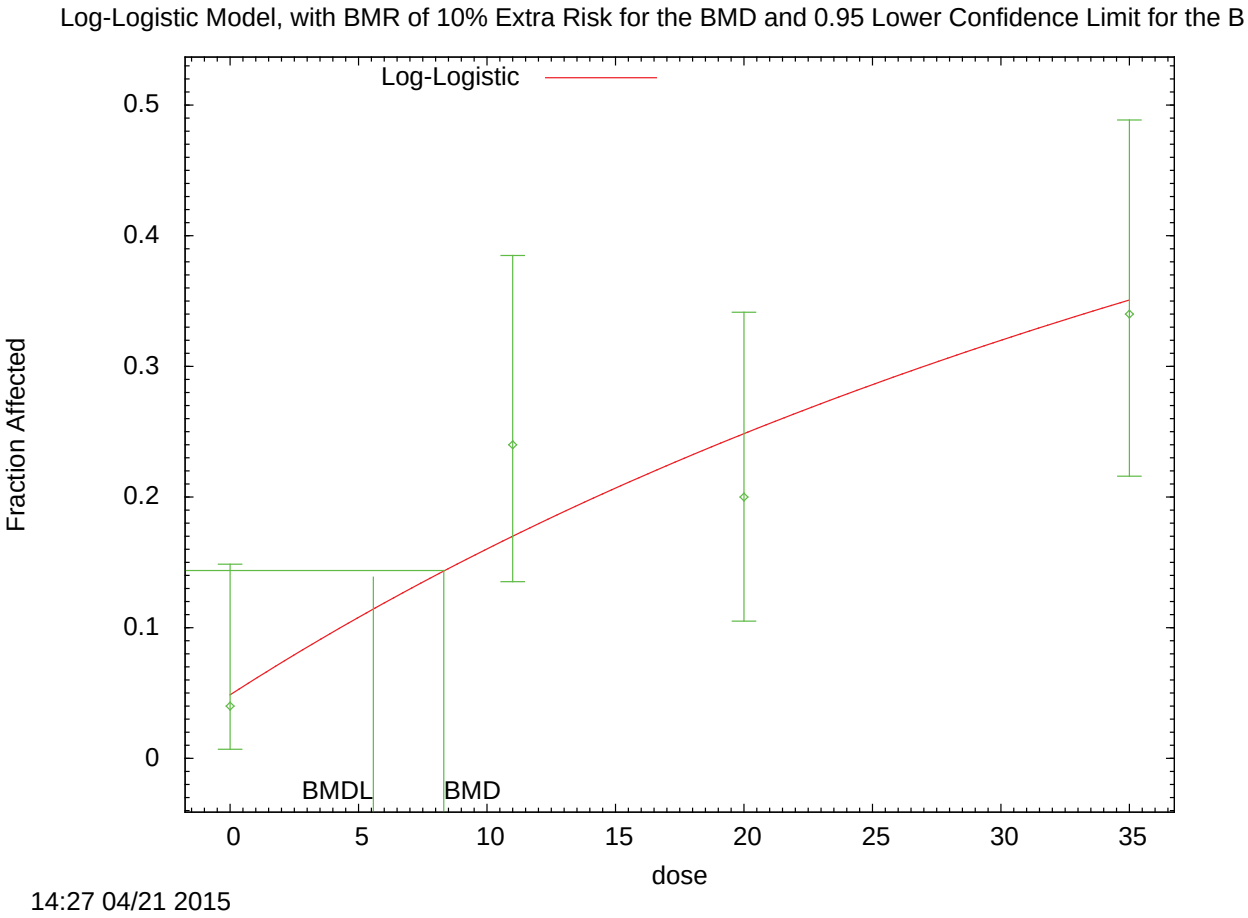

Figure C-2. Fit of Log-logistic Model to Incidence of Renal Pelvis Urothelial Hyperplasia in Female Rats (Matsumoto et al., 2012)

Text Output for Log-Logistic Model to Incidence of Renal Pelvis Urothelial Hyperplasia in Female Rats (Matsumoto et al., 2012)

```
================================================================
        Logistic Model. (Version: 2.14; Date: 2/28/2013)

 ================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = Effect
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 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

User has chosen the log transformed model

Default Initial Parameter Values
background = 0.04
intercept = -4.16226
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

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

	background	intercept
background	1	-0.6
intercept	-0.6	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0.0486009	*	*	*
intercept	-4.31559	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-93.0231	4			
Fitted model	-94.198	2	2.34973	2	0.3089
Reduced model	-101.451	1	16.8563	3	0.0007565

AIC: 192.396

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0486	2.430	2.000	50	-0.283
11.0000	0.1705	8.525	12.000	50	1.307
20.0000	0.2492	12.460	10.000	50	-0.804
35.0000	0.3517	17.586	17.000	50	-0.173

Chi^2 = 2.46 d.f. = 2 P-value = 0.2916

```
   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        8.31753

            BMDL =        5.57528
```

Model Predictions for Incidence of Renal Hydronephrosis in Female Mice

The procedure outlined above was applied to the data (see Table 7) on renal hydronephrosis in female mice exposed chronically to *o*-phenylenediamine dihydrochloride via drinking water for 2 years (Matsumoto et al., 2012). No models provided adequate fit to the data when assessed by the overall goodness-of-fit ($p < 0.1$) (see Table C-3). The data were then modeled without the high-dose group; using the reduced data set, the Gamma, Log-Logistic, Multistage (2-degree), Weibull and Quantal-Linear models provided adequate fit to the data ($p > 0.1$ and scaled residuals <2.0; see Table C-3). The $BMDL_{10}$ from these models providing adequate fit were sufficiently close; therefore, the Log-Logistic model providing the lowest AIC was selected as the best fitting. The BMD_{10} and $BMDL_{10}$ values for incidence of renal hydronephrosis in female mice from this model were 36.41 and 23.08 mg/kg-day, respectively. Figure C-3 shows the Log-Logistic model fit to the data.

Table C-3. Modeling Results for Renal Hydronephrosis in Female Mice[a]

Model Name	AIC	χ^2 Goodness-of-Fit *p*-Value[b]	BMD_{10} mg/kg-d	$BMDL_{10}$ mg/kg-d	Scaled Residual
Gamma[c]	192.94	0.03	89.46	52.77	1.71
Logistic	194.80	0.02	155.31	102.58	1.19
Log-Logistic[d]	192.21	0.04	73.05	42.26	1.67
Log-Probit[d]	197.01	0.01	191.61	102.21	−0.80
Multistage (2-degree)[e]	192.94	0.03	89.46	52.77	1.71
Multistage (3-degree)[e]	192.94	0.03	89.46	52.77	1.71
Probit	194.62	0.02	147.42	96.46	1.17
Weibull[c]	192.94	0.03	89.46	52.77	1.71
Quantal-Linear	192.94	0.03	89.46	52.77	1.71
High Dose Dropped					
Gamma[c]	134.40	0.27	40.22	27.05	0.89
Logistic	136.56	0.07	64.94	50.77	1.44
Log-Logistic[d,f]	**134.12**	**0.34**	**36.41**	**23.08**	**0.75**
Log-Probit[d]	136.37	0.07	57.13	42.45	1.43
Multistage (2-degree)[e]	134.40	0.27	40.22	27.05	0.89
Probit	136.22	0.08	61.31	47.58	1.40
Weibull[c]	134.40	0.27	40.22	27.05	0.89
Quantal-Linear	134.40	0.27	40.22	27.05	0.89

[a]Matsumoto et al. (2012).
[b]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[c]Power restricted to ≥1.
[d]Slope restricted to ≥1.
[e]Betas restricted to ≥0.
[f]Selected model. No models fit the full data set. After dropping the high dose, the Gamma, Log-Logistic, Multistage (2-degree), Weibull and Quantal-Linear 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 (Log-Logistic).

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]).

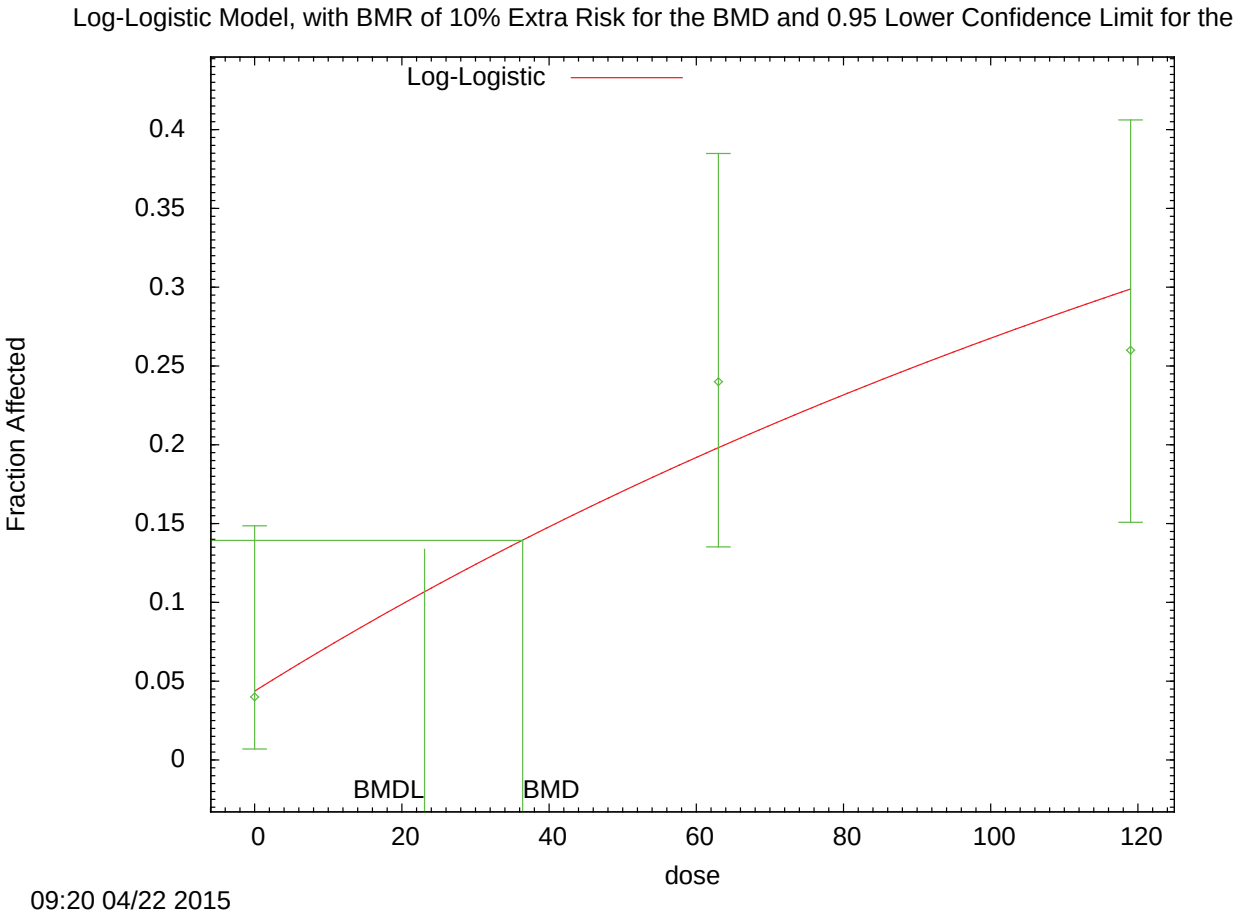

Figure C-3. Fit of Log-Logistic Model to Incidence of Renal Hydronephrosis in Female Mice (Matsumoto et al., 2012)

Text Output for Log-Logistic Model to Incidence of Renal Hydronephrosis in Female Mice (Matsumoto et al., 2012)

```
================================================================
        Logistic Model. (Version: 2.14; Date: 2/28/2013)
 ================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = Effect
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 3
   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

User has chosen the log transformed model

Default Initial Parameter Values
background = 0.04
intercept = -5.43194
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

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

	background	intercept
background	1	-0.5
intercept	-0.5	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0.0436682	*	*	*
intercept	-5.79203	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-64.604	3			
Fitted model	-65.0595	2	0.910965	1	0.3399
Reduced model	-70.709	1	12.2099	2	0.002232

AIC: 134.119

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0437	2.183	2.000	50	-0.127
63.0000	0.1979	9.894	12.000	50	0.747
119.0000	0.2984	14.922	13.000	50	-0.594

Chi^2 = 0.93 d.f. = 1 P-value = 0.3354

Benchmark Dose Computation

```
Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        36.4085

            BMDL =        23.0781
```

MODELING OF CANCER ENDPOINTS

As discussed in the body of the report in the "Derivation of a Provisional Oral Slope Factor" section the tumor types selected for BMD modeling were hepatocellular adenomas and carcinomas in male and female rats and mice, urinary bladder transitional cell papillomas and carcinomas and thyroid follicular adenomas in male rats, and papillary adenomas of the gall bladder in male and female mice exposed to *o*-phenylenediamine via drinking water for 2 years (Matsumoto et al., 2012). The tumor incidences and associated human equivalent doses (HEDs) used in the modeling are shown in Tables 12, B-5, and B-9.

Modeling Procedure for Cancer Incidence Data

BMD modeling of dichotomous cancer data was conducted with the EPA's BMDS (Version 2.6). The Multistage-Cancer model was fit to the incidence data using the extra risk option and a BMR of 10% extra risk. The Multistage-Cancer model was run for all polynomial degrees up to $n - 1$ (where n is the number of dose groups including control). Adequacy of model fit was judged based on the χ^2 goodness-of-fit p-value ($p > 0.1$), magnitude of scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. Among all of the models providing adequate fit, the BMDL from the model with the lowest AIC was selected as a potential POD when BMDL values were within a factor of 2−3. When BMDL values from models providing adequate fit varied more than 2- or 3-fold, the lowest BMDL was selected as a potential POD.

Model Predictions for Hepatocellular Adenomas and/or Carcinomas in Male Mice

The procedure outlined above was applied to the data (see Table 12) on hepatocellular adenomas and/or carcinomas in male mice exposed chronically to *o*-phenylenediamine dihydrochloride via drinking water for 2 years (Matsumoto et al., 2012). The software converged on the 1-degree model, which provided adequate fit ($p > 0.05$); thus, it was selected as the best-fitting model (see Table C-4). The BMD_{10} (HED) and $BMDL_{10}$ (HED) values from this model were 1.26 and 0.90 mg/kg-day, respectively. Figure C-4 shows the model fit to the data.

Table C-4. Modeling Results for Hepatocellular Adenoma and/or Carcinoma in Male Mice[a]

Model	DF	χ^2	χ^2 Goodness-of-Fit *p*-Value[b]	Scaled Residual	AIC	BMD_{10} (HED) mg/kg-d	$BMDL_{10}$ (HED) mg/kg-d
Multistage Cancer (1-degree)[c,d]	2	4.14	0.13	−0.54	249.3	1.26	0.90
Multistage Cancer (2-degree)[c]	2	4.14	0.13	−0.54	249.3	1.26	0.90
Multistage Cancer (3-degree)[c]	2	4.14	0.13	−0.54	249.3	1.26	0.90

[a]Matsumoto et al. (2012).
[b]Values <0.05 fail to meet conventional goodness-of-fit criteria.
[c]Betas restricted to ≥0.
[d]Selected model. All models provided adequate fit to the data.

AIC = Akaike's information criterion; BMD_{10} (HED) = 10% benchmark dose human equivalent dose; $BMDL_{10}$ (HED) = 10% benchmark dose lower confidence limit human equivalent dose; DF = degree(s) of freedom.

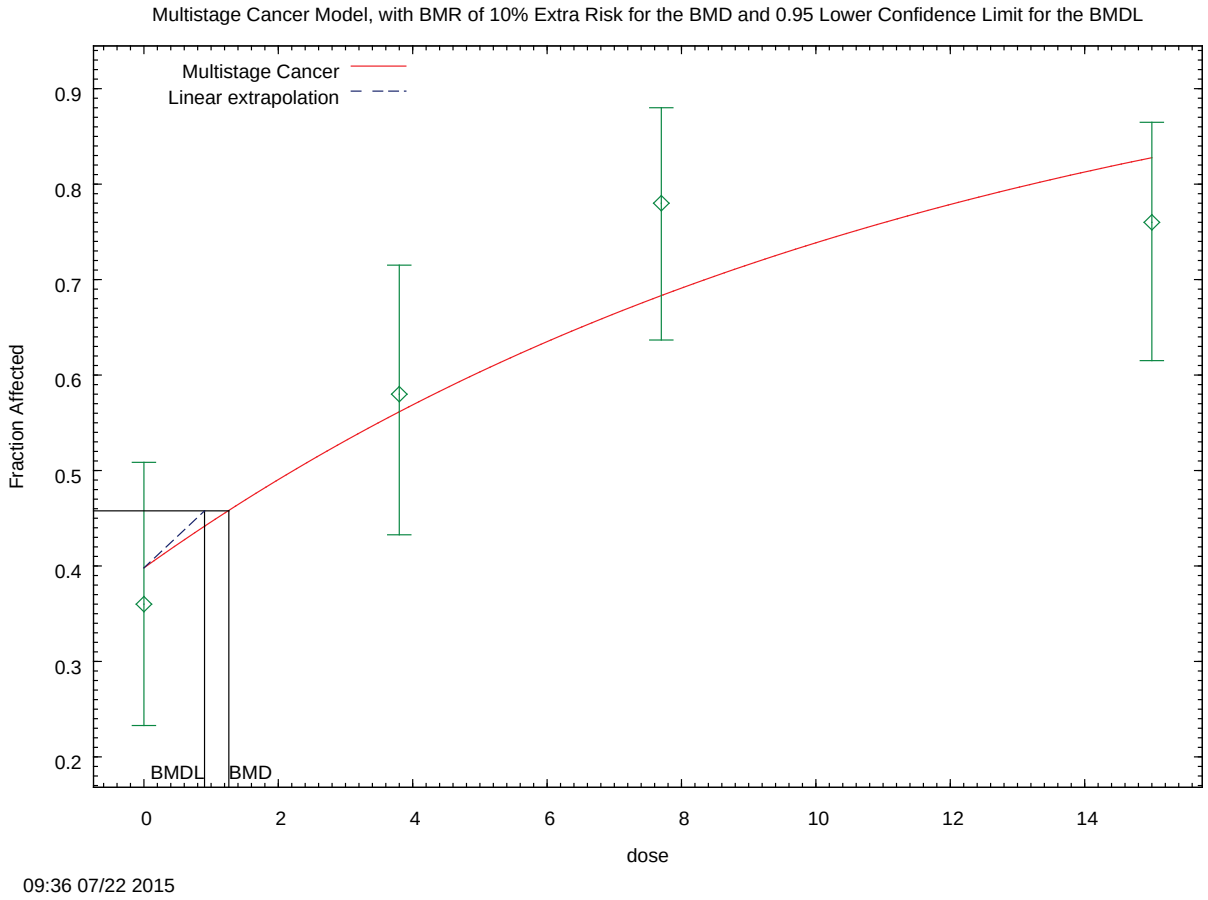

Figure C-4. Fit of Multistage-Cancer (1-Degree) Model to Incidence of Hepatocellular Adenoma and/or Carcinoma in Male Mice (Matsumoto et al., 2012)

Text Output for Multistage-Cancer (1-Degree) Model for Incidence of Hepatocellular Adenoma and/or Carcinoma in Male Mice (Matsumoto et al., 2012)

```
=================================================================
        Multistage Model. (Version: 3.4;  Date: 05/02/2014)
        Input Data File: C:/Users/bowens/BMDS260/Data/msc_livercancer_Opt.(d)
        Gnuplot Plotting File:  C:/Users/bowens/BMDS260/Data/msc_livercancer_Opt.plt
                                          Wed Jul 22 09:36:30 2015
 =================================================================

 BMDS_Model_Run
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   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = Effect
   Independent variable = Dose
```

```
 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 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
                     Background =     0.464296
                        Beta(1) =    0.0663523


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)

Background            1        -0.63

   Beta(1)        -0.63            1



                              Parameter Estimates

                                                      95.0% Wald Confidence Interval
Variable         Estimate        Std. Err.      Lower Conf. Limit   Upper Conf. Limit
Background      0.397509         0.0646602           0.270777             0.52424
Beta(1)         0.0833613        0.0196939           0.0447619            0.121961



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -120.585         4
   Fitted model        -122.662         2       4.15331      2          0.1253
  Reduced model        -132.813         1       24.4558      3         <.0001

           AIC:         249.323


                                 Goodness  of  Fit
                                                                      Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000     0.3975        19.875    18.000      50.000       -0.542
    3.8000     0.5611        28.054    29.000      50.000        0.269
    7.7000     0.6829        34.145    39.000      50.000        1.475
   15.0000     0.8275        41.373    38.000      50.000       -1.262

 Chi^2 = 4.14      d.f. = 2        P-value = 0.1264


   Benchmark Dose Computation

Specified effect =           0.1
```

```
Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =         1.2639

            BMDL =       0.902339

            BMDU =        2.03423

Taken together, (0.902339, 2.03423) is a 90     % two-sided confidence
interval for the BMD

Cancer Slope Factor =      0.110823
```

Model Predictions for Papillary Adenomas of the Gall Bladder in Male Mice

The procedure outlined above was applied to the data (see Table 12) on papillary adenomas of the gall bladder in male mice exposed chronically to *o*-phenylenediamine dihydrochloride via drinking water for 2 years (Matsumoto et al., 2012). The software converged on the 1-degree Multistage-Cancer model, which provided adequate fit ($p > 0.05$) and scaled residuals <2.0; thus, it was selected as the best-fitting model (see Table C-5). The BMD_{10} (HED) and $BMDL_{10}$ (HED) values from this model were 11.6 and 7.4 mg/kg-day, respectively. Figure C-5 shows the model fit to the data.

Table C-5. Modeling Results for Papillary Adenoma of the Gall Bladder in Male Mice[a]

Model	DF	χ^2	χ^2 Goodness-of-Fit *p*-Value[b]	Scaled Residuals	AIC	BMD_{10} (HED) mg/kg-d	$BMDL_{10}$ (HED) mg/kg-d
Multistage Cancer (1-degree)[c,d]	3	0.40	0.94	−0.43	78.8	11.6	7.4
Multistage Cancer (2-degree)[c]	3	0.40	0.94	−0.43	78.8	11.6	7.4
Multistage Cancer (3-degree)[c]	3	0.40	0.94	−0.43	78.8	11.6	7.4

[a]Matsumoto et al. (2012).
[b]Values <0.05 fail to meet conventional goodness-of-fit criteria.
[c]Betas restricted to ≥0.
[d]Selected model. All models provided adequate fit to the data.

AIC = Akaike's information criterion; BMD_{10} (HED) = 10% benchmark dose human equivalent dose; $BMDL_{10}$ (HED) = 10% benchmark dose lower confidence limit human equivalent dose; DF = degree(s) of freedom.

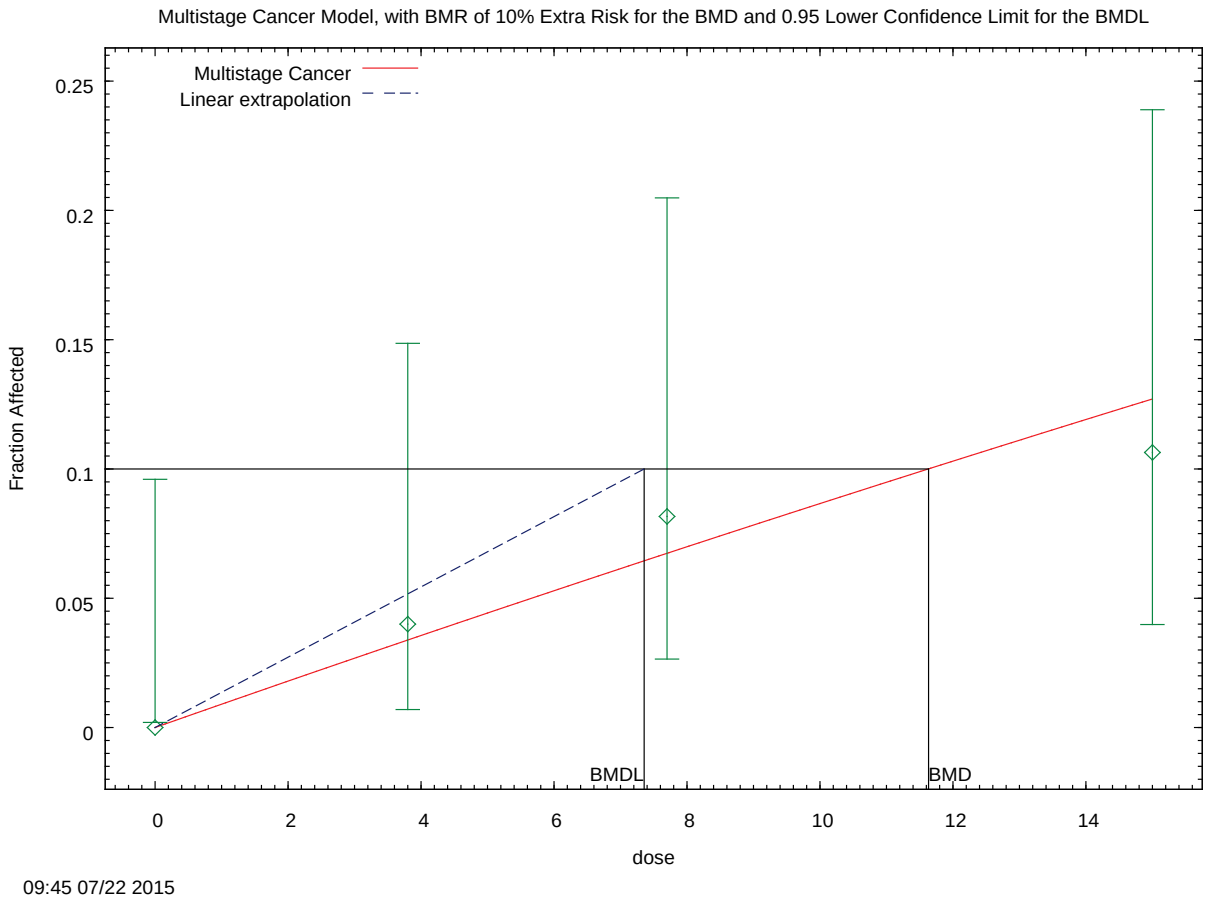

Figure C-5. Fit of Multistage-Cancer (1-Degree) Model to Incidence of Papillary Adenoma of the Gall Bladder in Male Mice (Matsumoto et al., 2012)

Text Output for Multistage-Cancer (1-Degree) Model for Incidence of Papillary Adenoma of the Gall Bladder in Male Mice (Matsumoto et al., 2012)

```
 ======================================================================
        Multistage Model. (Version: 3.4;  Date: 05/02/2014)
        Input Data File: C:/Users/bowens/BMDS260/Data/msc_gallbladder cancer_Opt.(d)
        Gnuplot Plotting File:  C:/Users/bowens/BMDS260/Data/msc_gallbladder
cancer_Opt.plt
                                                Wed Jul 22 09:45:00 2015
 ======================================================================

 BMDS_Model_Run
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   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = Effect
```

```
  Independent variable = Dose

Total number of observations = 4
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1


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
                    Background =    0.0101723
                       Beta(1) =    0.0074551


          Asymptotic Correlation Matrix of Parameter Estimates

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

               Beta(1)

   Beta(1)            1



                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
Background          0               NA
Beta(1)       0.00905685       0.00273192          0.00370238          0.0144113

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



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -38.179         4
   Fitted model        -38.3762         1      0.394311      3          0.9414
  Reduced model        -42.1343         1       7.91053      3          0.0479

          AIC:         78.7524


                                Goodness  of  Fit
                                                                      Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000      46.000        0.000
    3.8000     0.0338         1.692     2.000      50.000        0.241
    7.7000     0.0674         3.301     4.000      49.000        0.399
   15.0000     0.1270         5.970     5.000      47.000       -0.425
```

```
 Chi^2 = 0.40      d.f. = 3        P-value = 0.9407


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

              BMD =        11.6332

             BMDL =        7.35584

             BMDU =        25.1514

Taken together, (7.35584, 25.1514) is a 90     % two-sided confidence
interval for the BMD

Cancer Slope Factor =     0.0135946
```

Model Predictions for MS_Combo-Multiple Tumor Model for All Tumor Types in Male Mice

MS_Combo-multiple tumor BMD modeling was used to combine tumor incidence data for combined hepatocellular adenomas and/or carcinomas and papillary adenomas of the gall bladder in male mice. For each tumor type, the best-fitting Multistage model (i.e., the degree of Poly setting) was maintained in the MS_Combo model run. The calculated combined tumor $BMDL_{10}$ (HED) based on the MS_Combo model is 0.84 mg/kg-day. This $BMDL_{10}$ (HED) is used as the POD to derive the provisional oral slope factor (p-OSF).

Text Output for MS_COMBO Multiple Tumor Model for Combined Tumors in Male Mice

```
================================================================
         MS_COMBO. (Version: 1.9;  Date: 05/20/2014)
         Input Data File: C:\Users\bowens\BMDS260\Data\multi_test.(d)
         Gnuplot Plotting File:  C:\Users\bowens\BMDS260\Data\multi_test.plt
                                              Wed Jul 22 09:13:52 2015
 ================================================================

 BMDS_Model_Run
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   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = Effect
   Independent variable = Dose
   Data file name = livercancer.dax

 Total number of observations = 4
```

Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

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
Background = 0.464296
Beta(1) = 0.0663523

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.7
Beta(1)	-0.7	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.397509	*	*	*
Beta(1)	0.0833613	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-120.585	4			
Fitted model	-122.662	2	4.15331	2	0.1253
Reduced model	-132.813	1	24.4558	3	<.0001

AIC: 249.323

Log-likelihood Constant 112.23100406060614

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.3975	19.875	18.000	50.000	-0.542
3.8000	0.5611	28.054	29.000	50.000	0.269
7.7000	0.6829	34.145	39.000	50.000	1.475
15.0000	0.8275	41.373	38.000	50.000	-1.262

Chi^2 = 4.14 d.f. = 2 P-value = 0.1264

```
   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =         1.2639

            BMDL =       0.902339

            BMDU =        2.03423

Taken together, (0.902339, 2.03423) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =      0.110823



 ======================================================================
        MS_COMBO. (Version: 1.9;  Date: 05/20/2014)
        Input Data File: C:\Users\bowens\BMDS260\Data\multi_test.(d)
        Gnuplot Plotting File:  C:\Users\bowens\BMDS260\Data\multi_test.plt
                                              Wed Jul 22 09:13:52 2015
 ======================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = Effect
   Independent variable = Dose
   Data file name = gallbladdercancer.dax

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 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
                     Background =    0.0101723
                        Beta(1) =    0.0074551


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background
```

have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

Beta(1)

Beta(1) 1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0.00905685	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-38.179	4			
Fitted model	-38.3762	1	0.394311	3	0.9414
Reduced model	-42.1343	1	7.91053	3	0.0479

AIC: 78.7524

Log-likelihood Constant 33.617802094648134

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	46.000	0.000
3.8000	0.0338	1.692	2.000	50.000	0.241
7.7000	0.0674	3.301	4.000	49.000	0.399
15.0000	0.1270	5.970	5.000	47.000	-0.425

Chi^2 = 0.40 d.f. = 3 P-value = 0.9407

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 11.6332

BMDL = 7.35584

BMDU = 25.1514

Taken together, (7.35584, 25.1514) is a 90 % two-sided confidence interval for the BMD

```
Multistage Cancer Slope Factor =     0.0135946


**** Start of combined BMD and BMDL Calculations.****
  Combined Log-Likelihood                    -161.03774986868211
  Combined Log-likelihood Constant            145.84880615525427

   Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =        1.14004
            BMDL =       0.835366
Multistage Cancer Slope Factor =      0.119708
```

APPENDIX D. REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). (2015). 2015 TLVs and BEIs. Based on the documentation of the threshold limit values for chemical substances and physical agents and biological exposure indices. Cincinnati, OH. http://www.acgih.org/forms/store/ProductFormPublic/2015-tlvs-and-beis

Ames, BN; Kammen, HO; Yamasaki, E. (1975). Hair dyes are mutagenic: Identification of a variety of mutagenic ingredients. Proc Natl Acad Sci USA 72: 2423-2427.

Asgård, R; Haghdoost, S; Golkar, SO; Hellman, B; Czene, S. (2013). Evidence for different mechanisms of action behind the mutagenic effects of 4-NOPD and OPD: the role of DNA damage, oxidative stress and an imbalanced nucleotide pool. Mutagenesis 28: 637-644. http://dx.doi.org/10.1093/mutage/get041

Assmann, N; Emmrich, M; Kampf, G; Kaiser, M. (1997). Genotoxic activity of important nitrobenzenes and nitroanilines in the Ames test and their structure-activity relationship. Mutat Res 395: 139-144.

ATSDR (Agency for Toxic Substances and Disease Registry). (2016). Minimal risk levels (MRLs). March 2016. Atlanta, GA: Agency for Toxic Substances and Disease Registry (ATSDR). Retrieved from http://www.atsdr.cdc.gov/mrls/index.asp

Bronaugh, RL; Congdon, ER. (1984). Percutaneous absorption of hair dyes: Correlation with partition coefficients. J Invest Dermatol 83: 124-127. http://dx.doi.org/10.1111/1523-1747.ep12263302

Burnett, C; Loehr, R; Corbett, J. (1977). Dominant lethal mutagenicity study on hair dyes. J Toxicol Environ Health 2: 657-662. http://dx.doi.org/10.1080/15287397709529467

Cal/EPA (California Environmental Protection Agency). (2002). Expedited cancer potency values and no significant risk levels (NSRLs) for four proposition 65 carcinogens: Isobutyl nitrite, nalidixic acid, o-phenylenediamine, o-phenylenediamine hydrochloride. Sacramento, CA: Reproductive and Cancer Hazard Assessment Section, Office of Environmental Health Hazard Assessment. http://www.oehha.ca.gov/prop65/CRNR_notices/pdf_zip/Expedit1Aug2002.pdf

Cal/EPA (California Environmental Protection Agency). (2014). All OEHHA acute, 8-hour and chronic reference exposure levels (chRELs) as of June 2014. Sacramento, CA: Office of Health Hazard Assessment. http://www.oehha.ca.gov/air/allrels.html

Cal/EPA (California Environmental Protection Agency). (2016a). Chemicals known to the state to cause cancer or reproductive toxicity July 15, 2016. (Proposition 65 list). Sacramento, CA: California Environmental Protection Agency, Office of Environmental Health Hazard Assessment. http://oehha.ca.gov/proposition-65/proposition-65-list

Cal/EPA (California Environmental Protection Agency). (2016b). OEHHA toxicity criteria database [Database]. Sacramento, CA: Office of Environmental Health Hazard Assessment. Retrieved from http://www.oehha.ca.gov/tcdb/index.asp

Cebulska-Wasilewska, A; Nowak, D; Niedzwiedz, W; Anderson, D. (1998). Correlations between DNA and cytogenetic damage induced after chemical treatment and radiation. Mutat Res 421: 83-91.

Chung, KT; Murdock, CA; Zhou, Y; Stevens, SE, Jr; Li, YS; Wei, CI; Fernanda, SY; Chou, MW. (1996). Effects of the nitro-group on the mutagenicity and toxicity of some benzamines. Environ Mol Mutagen 27: 67-74. http://dx.doi.org/10.1002/(SICI)1098-2280(1996)27:1<67::AID-EM9>3.0.CO;2-B

DuPont (E. I. du Pont de Nemours and Company). (1967a). Acute oral test [o-phenylenediamine (technical)]. (TSCATS/020614. OTS215307. Section 8D. Doc #878220623). Wilmington, DE: E. E. du Pont de Nemours and Company. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0215307

DuPont (E. I. du Pont de Nemours and Company). (1967b). Acute oral test [o-phenylenediamine]. In Cancer incidence and mortality of workers exposed to meta-toluendiamine: preliminary findings with cover leter 6/24/1983. (TSCATS/020614. OTS215307. Section 8D. EPA Doc #878220624). Wilmington, DE: E. E. du Pont de Nemours and Company. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0215307

DuPont (E. I. du Pont de Nemours and Company). (1968). Acute skin absorption toxicity. In Cancer incidence and mortality of workers exposed to meta-toluenediamine: Preliminary findings with cover letter 6/24/1983. (TSCATS/020619. OTS0215307. EPA DocumentNo. 878220628). Wilmington, DE: E.I. du Pont de Nemours and Company. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0215307

DuPont (E. I. du Pont de Nemours and Company). (1969). Acute dust inhalation toxicity. In Cancer incidence and mortality of workers exposed to meta-toluenediamine: Preliminary findings with cover letter 6/24/1983. (TSCATS/020612. OTS0215307. Section 8D. 878220621). Wilmington, DE: E.I. du Pont de Nemours and Company. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0215307

DuPont (E. I. du Pont de Nemours and Company). (1984). Methemoglobinemia among the workers manufacturing PDA with enclosed table. (TSCATS/031707. OTS0506100. EPA Doc No. 40-8436155). Wilmington, DE: E.I. du Pont de Nemours and Company. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0506100

DuPont (E. I. du Pont de Nemours and Company). (1990). Acute oral neurotoxicity study of o-PDA in rats [TSCA Submission]. In Acute oral neurotoxicity studies of para, meta and ortho-phenylenediamine in rats with cover letter dated 091790. (TSCATS/413738. OTS0528739. Section 4). Haskell Laboratory. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml;jsessionid=99def5715d0543cde79015ea07b8?searchQuery=OTS0528739

DuPont (E. I. du Pont de Nemours and Company). (1992a). Subchronic oral neurotoxicity study of H-18347 in rats. In Subchronic oral neurotoxicity study of ortho-, meta-, and para-phenylenediamine in rats with attachments and cover letter dated 06/30/92. (TSCATS/452725. OTS0572976. Section 4). Newark, DE: Haskell Laboratory. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0572976

DuPont (E. I. du Pont de Nemours and Company). (1992b). Testing of the carcinogenic activity of para-phenylenediamine by peroral administration to pregnant mice (transplacental experiment) with cover letter dated 010793 [EPA Report]. (EPA/OTS; Doc #86-930000088 TSCATS/432079. OTS0544844. EPA document number:86-930000088). Wilmington, DE: Haskell Laboratory. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0544844

ECHA (European Chemicals Agency). (2015). Registered substances. Helsinki, Finland. http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

Gentile, JM; Gentile, GJ; Plewa, MJ. (1987). Mutagenicity of selected aniline derivatives to Salmonella following plant activation and mammalian hepatic activation. Mutat Res Genet Toxicol 188: 185-196. http://dx.doi.org/10.1016/0165-1218(87)90088-7

Gocke, E; Wild, D; Eckhardt, K; King, MT. (1983). Mutagenicity studies with the mouse spot test. Mutat Res 117: 201-212. http://dx.doi.org/10.1016/0165-1218(83)90168-4

Haskell Laboratories. (1967). Primary skin irritation and sensitization tests (In-2883) [TSCA Submission]. In Cancer incidence and mortality of workers exposed to meta-toluenediamine: Preliminary findings with cover letter. (TSCATS/020624. OTS0215307. EPA Document No. 878220630). Newark, DE: E. I. du Pont de Nemours and Company. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0215307

Haskell Laboratories. (1970a). Initial submission: Skin and eye tests in rabbits and guinea pigs with ortho-phenylenediamine (OPD) with cover letter dated 101592. (TSCATS/450947. OTS0571626. EPA Document No. 88-920009968). Wilmington, DE: E. I. DuPont de Nemours and Company. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0571626

Haskell Laboratories. (1970b). Initial submission: Subacute skin absorption toxicity test of o-phenylenediamine in rabbits with cover letter dated 10/15/92. (TSCATS/440832. OTS0555823. EPA DOcument No. 88-920010554). Wilmington, DE: E. I. du Pont de Nemours and Company. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0555823

Haskell Laboratories. (1980). Four-week palatability study in male rats with o-phenylenediamine (OPD) in drinking water. In Cancer incidence and mortality of workers exposed to meta-toluenediamine: preliminary finding with cover letter 6/24/1983. (TSCATS/020616. OTS0215307. Section 8D. EPA Doc No. 878220625). Wilmington, DE: E. I. du Pont de Nemours and Company. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0215307

Haskell Laboratories. (1981). Department of transportation skin corrosion test on rabbit skin. In Cancer incidence and mortality of workers exposed to meta-toluenediamine: preliminary findings with cover letter 6/24/1983. (TSCATS/020622. OTS215307. EPA Document No. 0878220632). Wilmington, DE: E. I. du Pont de Nemours and Company. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0215307

Haskell Laboratories. (1982). Class B poison test on rabbit skin. In Cancer incidence and mortality of workers exposed to meta-toluenediamine: preliminary findings with cover letter. (TSCATS/020613. OTS215307. EPA Document No. 878220622). Newark, DE: E. I. du Pont de Nemours and Company. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0215307

Holland, JM; Gosslee, DG; Williams, NJ. (1979). Epidermal carcinogenicity of bis(2,3-epoxycyclopentyl)ether, 2,2-bis(para-glycidyloxyphenyl)propane, and meta-phenylenediamine in male and female C3H AND C57BL-6 mice. Cancer Res 39: 1718-1725.

HSDB (Hazardous Substances Data Bank). (2013). O-Phenylenediamine, CASRN 95-54-5 [Fact Sheet]. Bethesda, MD: National Library of Medicine. http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+hsdb:@term+@DOCNO+2893

IARC (International Agency for Research on Cancer). (2015). IARC Monographs on the evaluation of carcinogenic risk to humans. Geneva, Switzerland: International Agency for Research on Cancer, WHO. http://monographs.iarc.fr/ENG/Monographs/PDFs/index.php

IBT Labs (Industrial Bio-Test Laboratories, Inc.). (1975a). Acute dermal toxicity study - Albino rabbits. (TSCATS/020454. OTS0206431. EPA document no. 878213658). Ciba-Geigy. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0206431

IBT Labs (Industrial Bio-Test Laboratories, Inc.). (1975b). Acute dermal toxicity study - albino rabbits [with chemical list]. (TSCATS/031947. OTS0506088. EPA Document no. 40-8336146). Ciba-Geigy. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0506088

IBT Labs (Industrial Bio-Test Laboratories, Inc.). (1975c). Acute vapor inhalation toxicity study with o-phenylene diamine cake in rats. (TSCATS/031945. OTS0506088. EPA40-8336146). Ciba Geigy Corporation. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0506088

IBT Labs (Industrial Bio-Test Laboratories, Inc.). (1983). Acute oral toxicity study-female albino rats. (TSCATS/031948. OTS0506088. EPA Doc no. 40-8336146). Ciba Geiby Corp. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0506088

Imaida, K; Ishihara, Y; Nishio, O; Nakanishi, K; Ito, N. (1983). Carcinogenicity and toxicity tests on p-phenylenediamine in F344 rats. Toxicol Lett 16: 259-269.

IPCS (International Programme on Chemical Safety). (2016). INCHEM: Chemical safety information from intergovernmental organizations [Database]: World Health Organization. Canadian Centre for Occupational Health and Safety. Inter-Organization Programme for the Sound Management of Chemicals. Retrieved from http://www.inchem.org/

Ishidate, M, Jr; Yoshikawa, K. (1980). Chromosome aberration tests with Chinese hamster cells in vitro with and without metabolic activation -- a comparative study on mutagens and carcinogens. In PL Chambers; W Klinger (Eds.), Further studies in the assessment of toxic actions (pp. 41-44). New York, NY: Springer-Verlag. http://dx.doi.org/10.1007/978-3-642-67729-8_8

Matsumoto, M; Suzuki, M; Kano, H; Aiso, S; Yamazaki, K; Fukushima, S. (2012). Carcinogenicity of ortho-phenylenediamine dihydrochloride in rats and mice by two-year drinking water treatment. Arch Toxicol 86: 791-804. http://dx.doi.org/10.1007/s00204-012-0800-z

NCI (National Cancer Institute). (1979). Bioassay of p-phenylenediamine dihydrochloride for possible carcinogenicity. (NCI-CG-TR-174; DHEW Publication No. 79-1730). Bethesda, MD: National Institutes of Health. http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr174.pdf

NIOSH (National Institute for Occupational Safety and Health). (2016). NIOSH pocket guide to chemical hazards. Index of chemical abstracts service registry numbers (CAS No.). Atlanta, GA: Center for Disease Control and Prevention, U.S. Department of Health, Education and Welfare. http://www.cdc.gov/niosh/npg/npgdcas.html

NTP (National Toxicology Program). (2014). Report on carcinogens. Thirteenth edition. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service. http://ntp.niehs.nih.gov/pubhealth/roc/roc13/index.html

OECD (Organisation for Economic Co-operation and Development). (2007). Guidance for determining the quality of data for the sids dossiers: (reliability, relevance and adequacy). In Manual for investigation of HPV chemicals Chapter 3: data evaluation. http://www.oecd.org/dataoecd/13/15/36045203.pdf.

OSHA (Occupational Safety & Health Administration). (2006). Table Z-1: Limits for air contaminants. Occupational safety and health standards, subpart Z, toxic and hazardous substances. (OSHA standard 1910.1000, 29 CFR). Washington, DC: U.S. Department of Labor. http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=9992

OSHA (Occupational Safety & Health Administration). (2011). Air contaminants: Occupational safety and health standards for shipyard employment, subpart Z, toxic and hazardous substances. (OSHA Standard 1915.1000). Washington, DC: U.S. Department of Labor. https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10286

Rhone-Poulenc. (1951). Initial submission: Report on the toxicity, mechanism of action and metabolism of hydroquinone with cover letter dated 10/27/92. (TSCATS/440537. OTS0555537. EPA Document Number: 88-920010058). https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0555537

Seiler, JP. (1977). Inhibition of testicular DNA synthesis by chemical mutagens and carcinogens Preliminary results in the validation of a novel short term test. Mutat Res 46: 305-310. http://dx.doi.org/10.1016/0165-1161(77)90007-3

Sherwin Williams. (1992). Initial submission: letter from Sherwin Williams company to USEPA regarding toxicity studies of o-phenylenediamine and other chemicals with attachments and cover letter dated 09/09/1992. (OTS0571101. EPA Doc no. 88-920009445). https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0571101

Sigma-Aldrich. (2015). 78410 Fluka. o-Phenylenediamine, suitable for fluorescence, 99.0% (NT) [Fact Sheet]. http://www.sigmaaldrich.com/catalog/product/fluka/78410?lang=en&region=US

Tarcha, PJ; Chu, VP; Whittern, D. (1987). 2,3-Diaminophenazine is the product from the horseradish peroxidase-catalyzed oxidation of o-phenylenediamine. Anal Biochem 165: 230-233. http://dx.doi.org/10.1016/0003-2697(87)90224-7

Thompson, CZ; Hill, LE; Epp, JK; Probst, GS. (1983). The induction of bacterial mutation and hepatocyte unscheduled DNA synthesis by monosubstituted anilines. Environ Mutagen 5: 803-811. http://dx.doi.org/10.1002/em.2860050605

U.S. EPA (U.S. Environmental Protection Agency). (1985). Health and environmental effects profile for phenylenediamines (o-, m-, p-) [EPA Report]. (ECAO-CIN-P112). Prepared by the Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response.

U.S. EPA (U.S. Environmental Protection Agency). (1988). Recommendations for and documentation of biological values for use in risk assessment (pp. 1-395). (EPA/600/6-87/008). Cincinnati, OH: U.S. Environmental Protection Agency, Office of Research and Development, Office of Health and Environmental Assessment. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=34855

U.S. EPA (U.S. Environmental Protection Agency). (2002). A review of the reference dose and reference concentration processes (pp. 1-192). (EPA/630/P-02/002F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/osa/review-reference-dose-and-reference-concentration-processes

U.S. EPA (U.S. Environmental Protection Agency). (2005a). Guidelines for carcinogen risk assessment [EPA Report] (pp. 1-166). (EPA/630/P-03/001F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www2.epa.gov/osa/guidelines-carcinogen-risk-assessment

U.S. EPA (U.S. Environmental Protection Agency). (2005b). Supplemental guidance for assessing susceptibility from early-life exposure to carcinogens (pp. 1-125). (EPA/630/R-03/003F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum.

U.S. EPA (U.S. Environmental Protection Agency). (2011a). Health effects assessment summary tables (HEAST). Washington, DC: U.S. Environmental Protection Agency, Office of Emergency and Remedial Response. http://epa-heast.ornl.gov/heast.php

U.S. EPA (U.S. Environmental Protection Agency). (2011b). Phenylenediamine, o- (CASRN 95-54-5). Health Effects Assessment Summary Tables for Superfund (HEAST). Washington, DC: Prepared by the Office of Research and Development, National Center for Environmental Assessment, Cincinnati OH for the Office of Emergency and Remedial Response. http://epa-heast.ornl.gov/heast.php?chemical=Phenylenediamine%2C+o-

U.S. EPA (U.S. Environmental Protection Agency). (2011c). Recommended use of body weight 3/4 as the default method in derivation of the oral reference dose (pp. 1-50). (EPA/100/R11/0001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum, Office of the Science Advisor. https://www.epa.gov/risk/recommended-use-body-weight-34-default-method-derivation-oral-reference-dose

U.S. EPA (U.S. Environmental Protection Agency). (2012a). 2012 Edition of the drinking water standards and health advisories [EPA Report]. (EPA/822/S-12/001). Washington, DC: Office of Water. http://www.epa.gov/sites/production/files/2015-09/documents/dwstandards2012.pdf

U.S. EPA (U.S. Environmental Protection Agency). (2012b). o-Phenylenediamine. Exposure assessment tools and models: estimation program interface (EPI) suite. Version 4.11 [Fact Sheet]. US Environmental Protection Agency. http://www.epa.gov/oppt/exposure/pubs/episuite.htm

U.S. EPA (U.S. Environmental Protection Agency). (2016). Integrated risk information system. IRIS assessments [Database]. Washington, DC: U.S. Environmental Protection Agency, Integrated Risk Information System. Retrieved from https://www.epa.gov/iris

Voogd, CE; Van der Stel, JJ; Jacobs, JJ. (1980). On the mutagenic action of some enzyme immunoassay substrates. J Immunol Methods 36: 55-62. http://dx.doi.org/10.1016/0022-1759(80)90093-9

Watanabe, T; Hirayama, T; Fukui, S. (1989). Phenazine derivatives as the mutagenic reaction product from o- or m-phenylenediamine derivatives with hydrogen peroxide. Mutat Res 227: 135-146. http://dx.doi.org/10.1016/0165-7992(89)90037-7

Watanabe, T; Ishihara, N; Ikeda, M. (1976). Toxicity of and biological monitoring for 1,3-diamino-2,4,6-trinitrobenzene and other nitro-amino derivatives of benzene and chlorobenzene. Int Arch Occup Environ Health 37: 157-168. http://dx.doi.org/10.1007/BF00378415

Weisburger, EK; Russfield, AB; Homburger, F; Weisburger, JH; Boger, E; Van Dongen, CG; Chu, KC. (1978). Testing of twenty-one environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity. J Environ Pathol Toxicol 2: 325-356.

WHO (World Health Organization). (2016). Online catalog for the Environmental Health Criteria (EHC) monographs. Available online at http://www.who.int/ipcs/publications/ehc/en/

Wild, D; King, MT; Eckhardt, K. (1980). Cytogenetic effect of ortho-phenylenediamine in the mouse, Chinese hamster, and guinea pig and of derivatives, evaluated by the micronucleus test. Arch Toxicol 43: 249-255. http://dx.doi.org/10.1007/BF00366180

Woodard, GDL. (1951). The toxicity, mechanism of action, and metabolism of hydroquinone with attachment. (OTS0517910. EPA Doc No. 405169110. Section 4). Washington, DC: George Washington University.

Yamada, K; Shirahata, S; Murakami, H; Nishiyama, K; Shinohara, K; Omura, H. (1985). DNA breakage by phenyl compounds. Agric Biol Chem 49: 1423-1428. http://dx.doi.org/10.1271/bbb1961.49.1423

Zeiger, E; Anderson, B; Haworth, S; Lawlor, T; Mortelmans, K. (1988). Salmonella mutagenicity tests: IV: Results from the testing of 300 chemicals. Environ Mol Mutagen 11: 1-158. http://dx.doi.org/10.1002/em.2850110602