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Provisional Peer-Reviewed Toxicity Values for

Nitrofen
(CASRN 1836-75-5)

Superfund Health Risk Technical Support Center
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COMMONLY USED ABBREVIATIONS

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

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR NITROFEN (CASRN 1836-75-5)

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 Environment Assessment (NCEA) scientists and an independent external peer review by three scientific experts.

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

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

QUESTIONS REGARDING PPRTVs

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

INTRODUCTION

Nitrofen is a selective pre- and postemergent herbicide that is currently banned in the United States. All products were voluntarily cancelled in the United States by the manufacturer on September 15, 1983 (U.S. EPA, 1998). The empirical formula for nitrofen is $C_{12}H_7Cl_2NO_3$ (see Figure 1). A table of physicochemical properties is provided below (see Table 1). In this document, "statistically significant" denotes a *p*-value of <0.05.

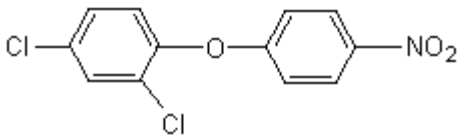

Figure 1. Nitrofen Structure

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

Property (Unit)	Value
Boiling point (ºC at 0.25 mm Hg)[b]	180−190
Melting point (ºC)[b]	70−71
Density (g/cm^3 at 83°C)	1.80
Vapor pressure (mPa at 40ºC)[b]	1.06
pH (unitless)	Not available
Solubility in water (mg/L at 22ºC)	0.7−1.2
Relative vapor density (air = 1)	Not available
Molecular weight (g/mol)	284.1
Flash point (°C)[c]	>200
Octanol/water partition coefficient (log K_{ow} unitless)[b]	5.534

[a]WHO, 1996 (unless otherwise noted).
[b]HSDB, 2011.
[c]IPCS, 1999.

No Reference Dose (RfD), Reference Concentration (RfC), or cancer assessment for nitrofen is included in the IRIS database (U.S. EPA, 2012) or on the Drinking Water Standards and Health Advisories List (U.S. EPA, 2006). No RfD or RfC values were reported in the HEAST (U.S. EPA, 1997). The CARA list (U.S. EPA, 1991a, 1994a) does not include a Health and Environmental Effects Profile (HEEP) for nitrofen. The toxicity of nitrofen has not been reviewed by ATSDR (2009) or the World Health Organization (WHO, 2011). CalEPA (2007) has derived a no-significant-risk level of 9 µg/day for exposure to nitrofen. No occupational exposure limits for nitrofen have been derived by the American Conference of Governmental Industrial Hygienists (ACGIH, 2011), the National Institute of Occupational Safety and Health (NIOSH, 2010), or the Occupational Safety and Health Administration (OSHA, 2006).

The HEAST (U.S. EPA, 1997) did not report a cancer weight-of-evidence classification or an oral slope factor for nitrofen. The International Agency for Research on Cancer (IARC; 2011) states that there is "sufficient evidence" that technical grade nitrofen is carcinogenic to animals and classifies nitrofen in Group 2B (*Possibly Carcinogenic to Humans*). The *12th Report on Carcinogens* (NTP, 2011) states that nitrofen is "*Reasonably Anticipated to be a Human Carcinogen*" based on "*Sufficient Evidence of Carcinogenicity in Experimental Animals*." CalEPA (2008) has derived an inhalation unit risk of 0.000023 per $\mu g/m^3$, an inhalation slope factor of 0.082 per mg/kg-day, and an oral slope factor of 0.082 per mg/kg-day based on the carcinogenic potential for nitrofen.

Literature searches were conducted on sources published from 1900 through November 2011, for studies relevant to the derivation of provisional toxicity values for nitrofen, CAS No. 1836-75-5. Searches were conducted using EPA's Health and Environmental Research Online (HERO) database of scientific literature. HERO searches the following databases: AGRICOLA; American Chemical Society; BioOne; Cochrane Library; DOE: Energy Information Administration, Information Bridge, and Energy Citations Database; EBSCO: Academic Search Complete; GeoRef Preview; GPO: Government Printing Office; Informaworld; IngentaConnect; J-STAGE: Japan Science & Technology; JSTOR: Mathematics & Statistics and Life Sciences; NSCEP/NEPIS (EPA publications available through the National Service Center for Environmental Publications [NSCEP] and National Environmental Publications Internet Site [NEPIS] database); PubMed: MEDLINE and CANCERLIT databases; SAGE; Science Direct; Scirus; Scitopia; SpringerLink; TOXNET (Toxicology Data Network): ANEUPL, CCRIS, ChemIDplus, CIS, CRISP, DART, EMIC, EPIDEM, ETICBACK, FEDRIP, GENE-TOX, HAPAB, HEEP, HMTC, HSDB, IRIS, ITER, LactMed, Multi-Database Search, NIOSH, NTIS, PESTAB, PPBIB, RISKLINE, TRI; and TSCATS; Virtual Health Library; Web of Science (searches Current Content database among others); World Health Organization; and Worldwide Science. The following databases outside of HERO were searched for health-related values: ACGIH, ATSDR, CalEPA, EPA IRIS, EPA HEAST, EPA HEEP, EPA OW, EPA TSCATS/TSCATS2, NIOSH, NTP, OSHA, and RTECS.

REVIEW OF POTENTIALLY RELEVANT DATA (CANCER AND NONCANCER)

Table 2 provides an overview of the relevant database for nitrofen and includes all potentially relevant repeated short-term-, subchronic-, and chronic-duration studies. Principal studies are identified. The phrase "statistical significance" used throughout the document indicates a *p*-value of <0.05.

Table 2. Summary of Potentially Relevant Data for Nitrofen (CASRN 1836-75-5)

Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects[b]	NOAEL[a,c]	BMDL/ BMCL[a]	LOAEL[a,c]	Reference (Comments)	Notes[d]
Human								
1. Oral (mg/kg-day)								
None								
2. Inhalation (mg/m^3)								
None								
Animal								
1. Oral (mg/kg-day)[a]								
Subchronic	25/0 per dose, Sprague-Dawley rat, diet, 7 days/week, 13 weeks	0, 7, 37, 186 (Adjusted)	Decreased body-weight gain in males at 186 mg/kg-day; clinical chemistry changes (decreased glucose and increased total protein, albumin, globulin, and cholesterol) at 186 mg/kg-day in males; Increased relative weights of the male liver, kidneys, and testes at ≥37 mg/kg-day; histopathological effects in male liver at ≥37 mg/kg-day (number of animals affected not reported)	7	Not run	37	O'Hara et al. (1983)	
	10/10 per dose, Wistar-derived rat, diet, 7 days/week, 13 weeks	Male: 0, 9, 46, 230, 1152, 4608 (Adjusted) Female: 0, 10, 51, 256, 1282, 5128 (Adjusted)	Increased mortality in males at ≥1152 mg/kg-day and in females at ≥1282 mg/kg-day; decreased body weight in males at ≥1152 mg/kg-day and in females at 1282 mg/kg-day; increased relative liver weight in males at ≥46 mg/kg-day and in females at all doses; increased relative kidney weight in males at ≥230 mg/kg-day and in females at 1282 mg/kg-day; increased relative heart and spleen weight in males at 1152 mg/kg-day and in females (heart only) at 1282 mg/kg-day; increased relative testes weight at ≥230 mg/kg-day; histopathological liver effects in males at ≥1152 mg/kg-day and females at ≥1282 mg/kg-day	None	Not run	10	Ambrose et al. (1971a)	

Table 2. Summary of Potentially Relevant Data for Nitrofen (CASRN 1836-75-5)

Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects[b]	NOAEL[a,c]	BMDL/ BMCL[a]	LOAEL[a,c]	Reference (Comments)	Notes[d]
	5/5 per dose, Osborne-Mendel rat, diet, 7 days/week, 6 weeks followed by 2 weeks observation	Male: 0, 66, 117, 207, 369, 656 (Adjusted) Female: 0, 71, 126, 224, 398, 709 (Adjusted)	Decreased body-weight gain in males at ≥207 mg/kg-day and in females at ≥126 mg/kg-day	71	Not run	126	NCI (1978a) noncancer results	
	5/5 per dose, Fischer F344 rat, diet, 7 days/week, 4 weeks followed by 2 weeks observation	Male: 0, 453, 667, 978, 1437, 2102 (Adjusted) Female: 0, 512, 753, 1104, 1623, 2373 (Adjusted)	Decreased body-weight gain in both males and females at all doses; increased observation of arched backs and decreased survival in high-dose males and females	None	Not run	453	NCI (1979a) noncancer results	
	5/5 per dose, $B6C3F_1$ mouse, diet, 7 days/week, 6 weeks followed by 2 weeks observation	Male: 0, 241, 428, 760, 1353, 2408 (Adjusted) Female: 0, 260, 462, 822, 1463, 2605 (Adjusted)	Decreased body-weight gain in males at 428 and 760 mg/kg-day and in females at 462 and 822 mg/kg-day; dose-dependent increase in mortality (data not reported)	Not derived	Not run	Not derived	NCI (1978b) noncancer results	
	5/5 per dose, $B6C3F_1$ mouse, diet, 7 days/week, 4 weeks followed by 2 weeks observation	Male: 0, 142, 307, 661, 1672, 3069 (Adjusted) Female: 0, 153, 332, 715, 1808, 3320 (Adjusted)	Mortality at highest dose for both males and females; rough hair and arched backs in males at ≥1672 mg/kg-day and in females at ≥1808 mg/kg-day; mottled kidneys in high-dose females	661	Not run	1672	NCI (1979b) noncancer results	
	1/1 per dose, mongrel dog, diet, 7 days/week, 4 weeks	Male: 0, 169, 421, 1053 (Adjusted) Female: 0, 123, 308, 771 (Adjusted)	Decreased food consumption and body weight in all dogs (severity of decrease not known, as data not reported)	Not derived	Not run	Not derived	Ambrose et al. (1971b)	

Table 2. Summary of Potentially Relevant Data for Nitrofen (CASRN 1836-75-5)

Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects[b]	NOAEL[a,c]	BMDL/ BMCL[a]	LOAEL[a,c]	Reference (Comments)	Notes[d]
Chronic	25/25 per dose, Wistar-derived albino rat, diet, 7 days/week, 97 weeks	Male: 0, 1.09, 11.5, 116 (Adjusted) Female: 0, 1.17, 12.2, 127 (Adjusted)	Poor survival in males and females at all doses (including controls); increased relative kidney and liver weight in males at 116 mg/kg-day; decreased relative splenic weight in females at 127 mg/kg-day	Not derived	Not run	Not derived	Ambrose et al. (1971c)	
	2/2 per dose, purebred beagle dog, diet, 7 days/week, 2 years	0, 0.36, 3.9, 38 (Adjusted)	Increased relative liver weights	3.9	Not run	38	Ambrose et al. (1971d)	
	50/50 per dose (20/20 in controls), Fischer F344 rat, diet, 7 days/week, 78 weeks with an additional 26 weeks of untreated observation	Male: 0, 51.75, 109.23 (HED) Female: 0, 52.03, 108.85 (HED)	None	105.85	Not run	None	NCI (1979c) Noncancer results	
	50/50 per dose (20/20 in controls), Osborne-Mendel rat, diet, 7 days/week, 78 weeks with 32 additional weeks of untreated observation (4 weeks in high-dose males)	Male: 0, 31.38, 70.35 (HED) Female: 0, 21.88, 46.97 (HED)	Decreased survival in males and females at all doses	None	Not run	21.88	NCI (1978c) Noncancer results	
Reproductive and Developmental[e]	**0/13 (8 at highest dose), Sprague-Dawley CD rat, gavage, GDs 8−16, offspring observed through PNDs 133−161**	**0, 0.46, 1.39, 4.17, 12.5**	**Diaphragmatic hernias in pups at ≥1.39 mg/kg-day; hyperactivity (transient) on PNDs 17 and 24 at ≥1.39 mg/kg-day (returned to normal by PND 45); decreased Harderian gland weight at ≥4.17 mg/kg-day; hydronephrosis at ≥4.17 mg/kg-day; decreased pup survival at ≥4.17 mg/kg-day on PNDs 1, 2, and 6**	**0.46**	**0.29**	**1.39**	**Ostby et al. (1985)**	**PS, PR**

Table 2. Summary of Potentially Relevant Data for Nitrofen (CASRN 1836-75-5)

Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects[b]	NOAEL[a,c]	BMDL/ BMCL[a]	LOAEL[a,c]	Reference (Comments)	Notes[d]
Carcinogenic	50/50 per dose (20/20 in controls), Osborne-Mendel rat, diet, 7 days/week, 78 weeks with 32 additional weeks of untreated observation (4 weeks in high-dose males)	Male: 0, 31.38, 70.35 (HED) Female: 0, 21.88, 46.97 (HED)	Pancreatic carcinoma, lymphoma, ovarian granulose cell tumor in females at 46.97 mg/kg-day	NA	24.1	NA	NCI (1978c)	
	50/50 per dose (20/20 in controls), Fischer F344 rat, diet, 7 days/week, 78 weeks with an additional 26 weeks of untreated observation	Male: 0, 51.75, 109.23 (HED) Female: 0, 52.03, 108.85 (HED)	None	NA	Not run	NA	NCI (1979c)	
	50/50 per dose (20/20 in controls), B6C3F$_1$ mouse, diet, 7 days/week, 78 weeks with an additional 12 weeks of untreated observation	**Male: 0, 60.70, 128.26 (HED) Female: 0, 65.38, 137.68 (HED)**	**Hepatocellular carcinomas in male and female mice at all doses; hemangiosarcomas in males at 128.26 mg/kg-day**	**NA**	**2.6**	**NA**	**NCI (1978d)**	**PS**
	50/50 per dose (20/20 in controls), B6C3F$_1$ mouse, diet, 7 days/week, 78 weeks with an additional 13 weeks of untreated observation	Male: 0, 70.66, 147.03 (HED) Female: 0, 76.47, 160.09 (HED)	Hepatocellular adenomas and carcinomas (combined) in males at ≥70.66 mg/kg-day; hepatocellular carcinomas in males at ≥70.66 mg/kg-day; hepatocellular adenomas and carcinomas (combined) in females at ≥76.47 mg/kg-day; hepatocellular carcinomas in females at 160.09 mg/kg-day	NA	6.7	NA	NCI (1979d)	

Table 2. Summary of Potentially Relevant Data for Nitrofen (CASRN 1836-75-5)

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

[a]Dosimetry: NOAEL, BMDL/BMCL, and LOAEL values are converted to an adjusted daily dose (ADD in mg/kg-day) for oral noncancer effects. Values for oral carcinogenic effects are converted to a human equivalent dose (HED in mg/kg-day). All long-term exposure values (4 wk and longer) are converted from a discontinuous to a continuous (weekly) exposure. Values from animal developmental studies are not adjusted to a continuous exposure. Adjusted daily dose (ADD) = dose (ppm) × food consumption per day × (1 ÷ body weight) × (days dosed ÷ total days) (Ambrose et al., 1971a,b,c,d)
Human equivalent dose (HED) = dose (ppm) × food consumption per day × (1 ÷ body weight) × (days dosed ÷ total days) × (body weight animal ÷ body weight human)$^{0.25}$ (NCI, 1978c,d, 1979c,d).

[b]For studies with a BMDL listed, the critical effect used as the POD is listed first.

[c]NOAEL and LOAEL values are determined from the data by the PPRTV authors.

[d]Notes: IRIS = utilized by IRIS, date of last update; PR =peer reviewed; PS = principal study; NPR = not peer reviewed; NA = not applicable.

[e]Additional reproductive and developmental studies are summarized in Table 3.

HUMAN STUDIES

Oral Exposures

No studies were identified.

Inhalation Exposures

No studies were identified.

Other Exposures

Studies evaluating the effects of nitrofen following dermal exposure are reported in the literature in a foreign language and are summarized in the review provided by Burke Hurt et al. (1983). According to these studies, skin and eye irritation were the only effects related to dermal exposure by humans to nitrofen.

ANIMAL STUDIES

Oral Exposures

The effects of oral exposure of animals to nitrofen have been evaluated in 7 subchronic-duration (Ambrose et al., 1971a,b; NCI, 1978a,b, 1979a,b; O'Hara et al., 1983; see Table 2), 2 chronic-duration (Ambrose et al., 1971c,d; see Table 2), 44 reproductive and developmental (see Table 3), and 4 carcinogenic (NCI, 1978c,d, 1979c,d) studies.

Subchronic-duration Studies

O'Hara et al. (1983) conducted a published, peer-reviewed subchronic-duration oral study in which male Sprague-Dawley (COBS-CD(S-D)BR) rats were administered nitrofen (95.7% purity, impurities unknown) for 95 days before mating with untreated females. The study also evaluated male reproductive effects. Compliance with good laboratory practice (GLP) is unknown. Males received 0-, 100-, 500-, or 2500-ppm nitrofen in their food for 95 days prior to cohabitation and then 0-, 6-, 30-, or 155-mg/kg nitrofen in corn oil in 10-mL/kg volumes via gavage during cohabitation with females, up to 10 days until mating. Control rats were fed normal diet or a gavage of corn oil only. The study authors reported the averaged daily doses of nitrofen were 0, 7, 37, and 186 mg/kg-day before mating and during cohabitation. Both sexes were of equal age and were obtained from Charles River Breeding Farms (Kingston, NY). Unmated male and female rats were housed in stainless steel cages with wire mesh floors and fronts. After mating, suspected pregnant female rats were placed in larger cages (17"W × 10"D × 7.5"H) lined with bedding. Cages were inspected daily for births after 20 days of gestation. Environmental conditions were described by a temperature of 23 ± 2°C, relative humidity of 50 ± 15%, and a 12-hour light/12-hour dark cycle. Animals were fed Purina Rodent Laboratory Chow (No. 5001-Meal) and water ad libitum.

Researchers observed male rats twice daily to monitor for any ill health, morbidity, mortality, or reactions to nitrofen treatment (O'Hara et al., 1983). Male rats were weighed 1 week before administering the first dietary nitrofen dose and then weighed every week for the first 13 weeks (91 days) of the 95-day premating period. Mean body weights and food consumption were averaged weekly within dosed male study groups. After a maximum of 10 days of cohabitation with females, male rats were fasted overnight, and blood was removed from the orbital sinus for hematological and chemical analyses. Male rats were then sacrificed by exsanguination, necropsied, and examined thoroughly for gross abnormalities. Male kidneys, liver, and testes were weighed and then reported as percentage of total body weight. These organs were then fixed in 10%-buffered formalin, sectioned in 5-µm slices, and stained with

hematoxylin and eosin for histopathological examination. Pregnant females were observed at different days of gestation, and offspring were observed twice daily for 13 weeks after birth. Pregnant dams were weighed on gestation days (GDs) 0, 6, 10, 16, and 21 and on lactation days (LDs) 0, 4, 10, 15, and 21. Three days after their expected delivery date, females were sacrificed and then grossly examined for lesions in their abdominal region and for the number of implantation sites present in their uterine horns. Subchronic data were analyzed using Sheffe's Multiple Range Test.

No effect on food consumption was observed in males from any dose group (O'Hara et al., 1983). At the highest dose, a decrease in body-weight gain in males was seen throughout the first 13 weeks of exposure to nitrofen. Males exposed to 2500-ppm (186-mg/kg-day) nitrofen for 15 weeks exhibited statistically significant differences in blood glucose (86% of control value), total protein (113% of control value), albumin (111% of control value), globulin (116% of control value), and cholesterol levels (215% of control value) (see Table B.1). Male organ weights were increased in the liver (absolute—2500 ppm [186 mg/kg-day]; relative—≥500 ppm [37 mg/kg-day]), kidneys (relative—≥500 ppm [37 mg/kg-day]), and the testes (absolute and relative—≥500 ppm [37 mg/kg-day]) (see Table B.2). Gross necropsy revealed enlarged (6/25) and darkened (3/25) livers at 2500 ppm (186 mg/kg-day). The same dose resulted in slight-to-marked hydrotrophy of the liver and increased cytoplasmic basophilia of the male centribular hepatocytes (no further details reported). At 500 ppm (37 mg/kg-day), slight-to-very slight effects were seen in the hepatocytes of males (no further details reported). While histopathological data are discussed and pictures provided to illustrate the effects, no effort was made by the study authors to quantify the histopathological effects of nitrofen to male livers. No effects on gestation, fertility, litter size, weight, or sex ratio were seen in any dose group. Based on increased relative weights of the male liver, kidney, and testes, a LOAEL of 500 ppm (37 mg/kg-day) and a NOAEL of 100 ppm (7 mg/kg-day) are assigned for subchronic-duration oral exposure to nitrofen.

In a published subchronic-duration study, Ambrose et al. (1971a) investigated the oral toxicity of nitrofen (95% purity) in Wistar-derived albino rats. Impurities consisted of 3% *p*-chloronitrobenzene, 1% dichlorophenol, and 1% unknown. It is unclear whether the study was conducted in compliance with GLP. The study authors administered 0-, 100-, 500-, 2500-, 12,500-, or 50,000-ppm nitrofen in commercial Purina Laboratory Chow to groups of 10 male and 10 female rats ad libitum for 13 weeks. Adjusted daily doses are 0, 9, 46, 230, 1152, and 4608 mg/kg-day for males and 0, 10, 51, 256, 1282, and 5128 mg/kg-day for females. Animals were obtained from Albino Farms in Red Bank, NJ. The study authors measured animal weights weekly and food consumption over 3 days at the end of the first month of exposure and at study termination. At study termination, the study authors performed hematological examinations and urinary analyses on five rats/sex/dose. Additionally, at study termination, the study authors weighed the heart, spleen, kidneys, liver, and testes of surviving rats and performed histopathological examinations on the bladder, lung, small and large intestines, stomach, pancreas, adrenals, brain, pituitary, thyroid, bone marrow, skeletal muscle, skin, heart, spleen, kidneys, liver, and testes. The statistical methods were not reported.

All rats administered 50,000-ppm (4608-mg/kg-day males; 5128-mg/kg-day females) nitrofen experienced mortality in the first week (Ambrose et al., 1971a). For rats administered 12,500-ppm nitrofen (1152-mg/kg-day males; 1282-mg/kg-day females), 4/10 males and 4/10 females experienced mortality before study termination. No significant mortality or

changes in food consumption or growth were observed in rats administered 0-, 100- (9-mg/kg-day males; 10-mg/kg-day females), or 500-ppm nitrofen (46-mg/kg-day males; 51-mg/kg-day females). Significantly reduced body weight was observed in male and female rats administered 12,500-ppm (1152-mg/kg-day males; 1282-mg/kg-day females) nitrofen and male rats administered 2500-ppm nitrofen (230-mg/kg-day). Values from hematological examinations and urinary analyses were not significantly different from the control. Organ weights following exposure to nitrofen are reported (see Table B.3). A dose-dependent increase in relative liver weight was observed in both sexes at all dose groups, except for male rats administered 100-ppm nitrofen (9-mg/kg-day males; 10-mg/kg-day females). Female rats administered 12,500-ppm nitrofen (1282-mg/kg-day) had increased relative organ weights for the heart and kidney. Males administered 12,500-ppm nitrofen (1152-mg/kg-day) had significantly increased relative organ weights for the heart, spleen, kidney, liver, and testes. Males from the 2500-ppm dose group (230-mg/kg-day) had significantly increased relative organ weights for the liver, kidney, and testes. Edema, glycogen granules located on cell peripheries, cytoplasmic swelling, and liver nuclei possessing prominent nucleoli were observed in male and female rats administered ≥12,500-ppm (1152-mg/kg-day males; 1282-mg/kg-day females) nitrofen. Based on increased relative liver weights in females, a LOAEL of 100 ppm (10 mg/kg-day) is determined; due to effects being seen at all doses in females, a NOAEL is precluded.

The National Cancer Institute (NCI) (1978a) performed a peer-reviewed subchronic-duration study to investigate the effects of nitrofen in rats. It is unclear whether the studies are GLP compliant. Technical-grade nitrofen (with a manufacturer's estimated purity of 87% and gas-liquid chromatography-estimated purity of greater than 80%) was administered in the feed to Osborne-Mendel rats. A total of at least five impurities were detected and consisted of xylene, dichlorphenol, *p*-chloronitrobenzene, and multiple chloronitrodiphenyl ethers. The percentages of the impurities were not reported. The rats were obtained from the Battelle Memorial Institute (Columbus, OH). The basal laboratory diet for all animals consisted of 2% Duke's® corn oil added to Wayne Lab-Blox® meal (Allied Mills, Inc.). Food and water were supplied ad libitum. For the duration of the experiment, rats were individually housed in steel and wire-mesh cages. Temperature was maintained at 20−24°C, and relative humidity was maintained at 45−55%. Twelve-hour fluorescent light/dark cycles were provided. All animals were weighed immediately prior to the start of the experiment.

The subchronic toxicity test was initially performed in rats to help determine the maximum tolerated dose of nitrofen that would be administered in the chronic-duration study (NCI, 1978a). Nitrofen mixed with a small amount of corn oil was administered in the feed to Osborne-Mendel rats (5/sex/dose group) at concentrations of 0, 1000, 1780, 3160, 5620, or 10,000 ppm. Adjusted daily doses are calculated using default data for body weight (U.S. EPA, 1994b) and food consumption (U.S. EPA, 1988) and are 0, 66, 117, 207, 369, and 656 mg/kg-day for males and 0, 71, 126, 224, 398, and 709 mg/kg-day for females. Test diets were administered for 6 weeks; after dosing, there was a 2-week observation period in which all test animals were fed the basal diet. To select the initial high doses for the chronic-duration study, the study authors examined two criteria—mortality and retardation in body-weight gain (expressed as a percentage of the weight gain of the control animals); no other endpoints were evaluated. No mortality was observed in any test group. Body-weight gain retardation was 10% and 25% in the 3160-ppm (207 mg/kg-day) and 5620-ppm (369 mg/kg-day) groups, respectively, for males, and 17% and 26% in the 1780-ppm (126 mg/kg-day) and 3160-ppm groups

(224 mg/kg-day), respectively, for females. Effects on body-weight gain at 10,000 ppm (656 mg/kg-day) in males and at ≥5620 ppm (398 mg/kg-day) in females were not reported. However, it is assumed that nitrofen had an effect on body-weight gain at these levels as well as the concentrations estimated to induce 20% body-weight gain retardation were selected as the initial high doses for the chronic-duration study—4600 ppm for males and 2600 ppm for females. Based on decreased body-weight gain in female rats, a LOAEL of 1780 ppm (126 mg/kg-day) and a NOAEL of 1000 ppm (71 mg/kg-day) are determined.

NCI (1979a) performed an additional peer-reviewed subchronic-duration study to investigate the effects of nitrofen in a different strain of rats. It is unclear whether the study is GLP compliant. Technical-grade nitrofen (purity not specified) was administered in feed to Fischer F344 rats. The rats (4 weeks old) were obtained from A. R. Schmidt (Madison, WI) and Laboratory Supply Company, Inc. (Indianapolis, IN). The basal laboratory diet for all animals consisted of Wayne Lab-Blox® meal (Allied Mills, Inc.). Food and acidulated water (pH 2.5) were provided ad libitum. Animals were grouped and distributed among cages, where the average body weight per cage was approximately equal for the particular species and sex. Animals were housed by sex in groups of four in polycarbonate cages suspended from aluminum racks. Temperature was maintained at 22−26°C, and relative humidity was maintained at 45−55%. Fluorescent light was provided for 8 hours per day. All animals were weighed immediately prior to the start of the experiment.

Subchronic toxicity tests were initially performed in rats to help determine concentrations of nitrofen that would be administered in the chronic-duration studies (NCI, 1979a). Nitrofen was administered in the feed to Fischer F344 rats (5/sex/dose group) at concentrations of 0, 6800, 10,000, 14,670, 21,560, or 31,530 ppm. Adjusted daily doses are calculated using default data for body weight (U.S. EPA, 1994b) and food consumption (U.S. EPA, 1988) and are 0, 453, 667, 978, 1437, and 2102 mg/kg-day in males and 0, 512, 753, 1104, 1623, and 2373 mg/kg-day in females. Test diets were administered for a total of 4 weeks; after dosing, there was a 2-week observation period in which all test animals were fed the basal diet. Two times per week, individual body weights and food consumption data were reported. At the end of the study, animals were euthanized and necropsied. The study gives no indication that a histopathological examination was performed. No statistical tests were reported.

Table B.4 shows survival, mean body-weight change, and incidence of arched back in rats following exposure to nitrofen (NCI, 1979a). The results indicated decreased body-weight gain in both sexes at all doses. An increase in arched backs and a decrease in survival in males and females of the highest dose group were observed compared to controls. No other endpoints were reported. For this study, a LOAEL of 6800 ppm (453 mg/kg-day) is determined based on decreased body-weight in male rats. Because treatment-related effects were seen at the lowest dose, derivation of a NOAEL is not feasible.

NCI (1978b) evaluated the effects of subchronic-duration exposure to nitrofen in mice. Purity and GLP compliance are the same as reported in NCI (1978a). Experimental design was identical to that reported for NCI (1978a) with a few exceptions. $B6C3F_1$ mice were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA). Mice were housed by sex in groups of 10 in polypropylene cages with solid bottoms and filter tops. Nitrofen was mixed with a small amount of corn oil and administered in the feed to mice (5/sex/dose group) at concentrations of 0, 1780, 3160, 5620, 10,000, or 17,800 ppm. Adjusted daily doses are

calculated using default data for body weight (U.S. EPA, 1994b) and food consumption (U.S. EPA, 1988) and are 0, 241, 428, 760, 1353, and 2408 mg/kg-day for males and 0, 260, 462, 822, 1463, and 2605 mg/kg-day for females.

The study authors reported retardation in body-weight gain at concentrations of 3160 and above (428 mg/kg-day males; 462 mg/kg-day females) but noted that observations were not clearly dose related (NCI, 1978b). In the 3160 ppm group (428 mg/kg-day males; 462 mg/kg-day females), males experienced a body-weight-gain reduction of 12%, while females showed an 8%-reduction. At 5620 ppm (760 mg/kg-day males; 822 mg/kg-day females), body-weight-gain reduction increased to 37% in males and 40% in females. No further results for body weight were reported. In both sexes, mortality increased with concentration (data not reported). No other endpoints were reported. For males and females, the study authors selected the initial high dose for the chronic-duration study as 3550 ppm. Because complete data for body weight and mortality were not available and the study authors noted that effects on body weight were not clearly dose related, it is not feasible to develop a LOAEL or NOAEL for this study.

NCI (1979b) conducted an additional study evaluating the effects of subchronic-duration exposure to nitrofen in mice. GLP compliance and compound purity are unknown. Experimental design was identical to that reported for NCI (1979a) with some exceptions. B6C3F_1 mice (4 weeks old) were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA). Animals were housed by sex in groups of five in polycarbonate cages suspended from aluminum racks. Mice were exposed to 0-, 1180-, 2550-, 5500-, 13,900-, or 25,520-ppm nitrofen in the feed. Adjusted daily doses are calculated using default data for body weight (U.S. EPA, 1994b) and food consumption (U.S. EPA, 1988) and are 0, 142, 307, 661, 1672, and 3069 mg/kg-day for males and 0, 153, 332, 715, 1808, and 3320 mg/kg-day for females.

Table B.5 provides survival, mean body-weight change, and observations of abnormal clinical signs in mice following exposure to nitrofen (NCI, 1979b). Survival was decreased in the highest dose group for both sexes. Abnormal clinical signs were observed in all animals of both sexes at the two highest doses (13,900 [1672 mg/kg-day males; 1808 mg/kg-day females] and 25,520 ppm [3069 mg/kg-day males; 3320 mg/kg-day females]). Males and females in these groups had arched backs and rough hair. Females in the 25,520 ppm group (3320 mg/kg-day) also experienced mottled kidneys. Mean body-weight change results were comparable to controls for both sexes at all doses. No other effects were reported. For this study, the LOAEL and NOAEL are 13,900 ppm (1672 mg/kg-day) and 5500 ppm (661 mg/kg-day), respectively, for observations of abnormal clinical signs in male mice.

Ambrose et al. (1971b), in a published subchronic-duration study, investigated the oral toxicity of nitrofen (95% purity) in mongrel dogs. Impurities consisted of *p*-chloronitrobenzene (3%), dichlorophenol (1%), and unknowns (1%). Dogs were obtained from Medical College of Virginia, Central Animals Facilities. It is unknown whether the study was conducted in compliance with GLP. The study authors administered 0-, 4000-, 10,000-, or 25,000 ppm nitrofen in basal diet to 1 male and 1 female dog per dose for 4 weeks. Adjusted daily doses are calculated using default data for body weight (U.S. EPA, 1994b) and food consumption (U.S. EPA, 1988) and are 0, 169, 421, and 1053 mg/kg-day for males and 0, 123, 308, and 771 mg/kg-day for females. The basic diet consisted of 87% ground basal diet, 12% corn oil,

and 1% USP cod liver oil. The study authors measured animal weights weekly and food consumption daily. Decreases in food consumption and body weight were observed in all dogs administered nitrofen in basal diet (data not reported). Due to small sample sizes and the absence of data for critical effects, neither a LOAEL nor a NOAEL can be determined for this study.

Chronic-duration Studies

Parallel to the subchronic-duration study in rats, Ambrose et al. (1971c) published a chronic-duration study investigating the oral toxicity of nitrofen (95% purity) in Wistar-derived albino rats. Impurities consisted of *p*-chloronitrobenzene (3%), dichlorophenol (1%), and unknown (1%). It is unknown whether the study was conducted in compliance with GLP. The study authors administered 0-, 10-, 100-, or 1000 ppm nitrofen in commercial Purina Laboratory Chow to groups of 25 male and 25 female rats ad libitum for 97 weeks. Adjusted daily doses are calculated by averaging the body-weight data measured at intervals (1, 3, 6, 13, 26, 52, 78, and 96 weeks) for each dose level in the study and using default data for food consumption (U.S. EPA, 1988). These values are 0, 1.09, 11.5, and 116 mg/kg-day for males and 0, 1.17, 12.2, and 127 mg/kg-day for females. Animals were obtained from Albino Farms in Red Bank, NJ. The study authors measured animal weights weekly and food consumption over 3 days at the end of Months 1, 3, 6, and 12. The study authors performed hematological examinations and urinary analyses on 5 rats/sex/dose every 3 months. Additionally, at study termination, the study authors performed histological examinations; weighed the heart, spleen, kidneys, liver, and testes of surviving rats; and calculated organ-to-body weight ratios. The statistical methods were not reported.

Poor survival was observed in male and female rats in all dose groups, including controls, after 65 weeks, forcing the study to end at 97 weeks (see Table B.6). No explanation was given for the decreased rate of survival. Significantly reduced growth was observed irregularly in male and female rats administered 100- (11.5-mg/kg-day males; 12.2-mg/kg-day females) and 1000-ppm (116-mg/kg-day males; 127-mg/kg-day females) nitrofen at weekly intervals throughout the study (see Table B.6). No significant changes in food consumption were observed. The study authors noted that values from hematological examinations and urinary analyses were not significantly different from the control (data not reported). Male rats administered 1000-ppm (116-mg/kg-day) nitrofen had increased organ-to-body-weight ratios in the kidney and liver (see Table B.7). Decreased relative splenic weight was seen in females at 1000 ppm (127 mg/kg-day) (see Table B.7). No histopathological findings in any of the dose groups differed from those found in the control groups (data not reported). Given the low rate of survival seen at all doses, derivation of a NOAEL or LOAEL is not feasible.

NCI (1978c) conducted a study to evaluate the effects of nitrofen in the Osborne-Mendel rat. GLP compliance is unknown. Technical-grade nitrofen (with a manufacturer's estimated purity of 87% and gas-liquid chromatography estimated purity of greater than 80%) was administered in the feed to Osborne-Mendel rats. A total of at least five impurities were detected and consisted of xylene, dichlorphenol, *p*-chloronitrobenzene, and multiple chloronitrodiphenyl ethers. The percentages of the impurities were not reported. The rats were obtained from the Battelle Memorial Institute (Columbus, OH). The basal laboratory diet for all animals consisted of 2% Duke's® corn oil added to Wayne Lab-Blox® meal (Allied Mills, Inc.). Food and water were supplied ad libitum. For the duration of the experiment, rats were individually housed in

steel and wire-mesh cages. Temperature was maintained at 20−24°C, and relative humidity was maintained at 45−55%. Twelve-hour fluorescent light/dark cycles were provided.

Nitrofen was administered at concentrations of 0 (20/sex); initial low and high concentrations of 2300 ppm and 4600 ppm, respectively, for males (50/dose); and initial low and high concentrations of 1300 ppm and 2600 ppm, respectively, for females (50/dose) (NCI, 1978c). Rats were treated for 78 weeks. At Week 46, study authors determined that the male high-dose rats were not tolerating 4600 ppm, so the dose was decreased to 2300 ppm (resulting in a time-weighted average of 3627 ppm over the 78 weeks). Adjusted daily doses (HEDs) are calculated using body-weight data as reported by the study authors and default data for food consumption (U.S. EPA, 1988) and are 0, 105 (31.38), and 241 (70.35) mg/kg-day for males and 0, 83 (21.88), and 183 (46.97) mg/kg-day for females. Control and low-dose males and all female rats were observed (untreated; fed basal diet and corn oil mixture) for an additional 32 weeks. Male high-dose rats were observed (untreated) for 4 weeks after treatment before being sacrificed at Week 83 of the study.

All animals were observed daily for mortality (NCI, 1978c). Study authors recorded body weights, food consumption, and data regarding appearance, behavior, signs of toxic effects, and incidence, size, and location of tissue masses on a weekly basis for the first 10 weeks, then once per month for the remainder of the study. This study focused primarily on the possible carcinogenic potential of nitrofen in rats and did not report organ weights or results for serum or clinical chemistry. Observation and palpation were used to find tissue masses. All animals in the study were necropsied. Study authors performed gross and microscopic examination of major tissues, organs, and gross lesions. Microscopic examination was completed for the following: skin, subcutaneous tissue, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, bile duct, pancreas, esophagus, stomach, small and large intestines, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, pancreatic islets, testis, prostate, brain, muscle, uterus, mammary gland, and ovary. Time-adjusted analysis was used on animals that survived at least 52 weeks, or earlier, if the first tumor was found before this time (NCI, 1978c). Animals with early mortality and no tumors were excluded. Life-table methods were used when necessary to examine tumor incidence. Using the exact interval on the odds ratio, study authors calculated the 95% confidence interval for the relative risk of each dose group compared to the control.

In male and female rats, the study authors reported a dose-related depression of body-weight gain throughout the treatment period (NCI, 1978c). In general, through the first 10 weeks of treatment, animal appearance and behavior were normal; however, hunched appearance, abdominal urine stains, and labored respiration did occur intermittently. From Week 14 onward, the number of animals with a hunched appearance increased with time. At the end of treatment at 78 weeks, 75% of low-dose and 95% of high-dose animals exhibited a hunched appearance. Other clinical observations included urine stains and bloody vaginal discharge in females, which was noted most consistently in the last 3 months of the study. Labored respiration and other respiratory signs (at low-to-moderate frequency) occurred in all groups, particularly during the second year of the study. Decreased survival was seen in both males and females at all doses. In male rats, 45% of controls, 60% of low-dose, and 30% of high-dose animals survived until the end of the study. By Week 45, about 50% of high-dose males were dead. The study authors concluded that this precluded meaningful statistical analysis to evaluate the occurrence of late-developing tumors in this group. In females, 80% of controls,

74% of low-dose, and 56% of high-dose animals survived until the end of the study. The study authors noted that adequate survival was seen in all female groups to evaluate tumor development.

The incidence of primary neoplasms in female rats treated with nitrofen is summarized in Table B.8 (NCI, 1978c). In females, long-term dietary exposure to nitrofen was associated with highly invasive neoplasms of the pancreas (2/50 rats or 4%, in the low-dose group, and 7/50 or 14%, in the high-dose group). Most of the pancreatic tumors appeared to be ductal carcinomas that were highly anaplastic and poorly differentiated and were also associated with marked desmoplasia, ischemic necrosis, inflammation, and hemorrhage. Invasion of the abdominal cavity was also observed, and all metastasized to the lung (data not shown). Although few animals suffered from neoplasms in the pancreas, study authors noted that these types of neoplasms are rare for this strain of rat, so their occurrence is likely related to nitrofen administration. Various tumors affecting the reproductive system, including the vagina, uterus, and ovary, were also observed in female rats. The study authors reported that vaginal and uterine carcinomas are unusual neoplasms in Osborne-Mendel rats. High-dose female rats also developed an increase in lymphomas (see Table B.8). No other treatment-related neoplasms were observed.

In male rats, nitrofen intake was related to a life-shortening effect, especially in high-dose males (NCI, 1978c). The primary effect of treatment in high-dose males was massive hemorrhage of genitalia and the pelvic cavity. In addition, males of the high-dose group experienced massive centrilobular necrosis in the liver. There was also a high incidence of pneumonia in the low-dose males, which may have been increased by stress. Although carcinogenicity was not observed in males, the study authors noted that the high rate of early mortality may have precluded males from displaying carcinogenic responses. Study authors concluded that under the study conditions, dietary administration of nitrofen was carcinogenic to the female rat pancreas and reproductive system and that carcinogenicity in male rats could not be adequately determined.

For the chronic-duration study in Fischer F344 rats (6 weeks old), NCI (1979c) administered nitrofen in the diet for 78 weeks. GLP compliance is unknown. Technical-grade nitrofen (purity not specified) was administered in feed to Fischer F344 rats. The rats (4 weeks old) were obtained from A. R. Schmidt (Madison, Wisconsin) and Laboratory Supply Company, Inc. (Indianapolis, IN). The basal laboratory diet for all animals consisted of Wayne Lab-Blox® meal (Allied Mills, Inc.). Food and acidulated water (pH 2.5) were provided ad libitum. Animals were grouped and distributed among cages where the average body weight per cage was approximately equal for the particular species and sex. Animals were housed by sex in groups of four in polycarbonate cages suspended from aluminum racks. Temperature was maintained at 22−26°C, and relative humidity was maintained at 45−55%. Fluorescent light was provided for 8 hours per day. All animals were weighed immediately prior to the start of the experiment.

Nitrofen was administered in the feed at a concentration of 0 (control; 20/sex) and low and high concentrations of 3000 ppm and 6000 ppm, respectively, for males and females (50/sex/dose) (NCI, 1979c). Adjusted daily doses (HEDs) are calculated using body-weight data reported by the study authors and default food consumption data (U.S. EPA, 1988) and are 0, 195 (51.75), and 419 (109.23) mg/kg-day for males and 0, 221 (52.03), and 470 (108.85) mg/kg-day for females. All treated animals were dosed for 78 weeks. Following the treatment

period, rats were observed (untreated) for an additional 26 weeks. Control animals received the basal laboratory diet for the total 104 weeks.

All animals were weighed before the study and at monthly intervals throughout the duration (NCI, 1979c). They were examined two times a day, and food consumption was monitored in 20% of each dose group on a monthly basis. This study was designed to evaluate nitrofen for possible carcinogenicity and did not evaluate organ weights or serum or clinical chemistry endpoints. At the end of the treatment period, animals were euthanized and necropsied. Gross and microscopic examinations were performed on all major tissues, organs, and gross lesions. The following tissues were also microscopically examined: skin, subcutaneous tissue, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, pancreas, esophagus, stomach, small and large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, brain, uterus, mammary gland, and ovary. Some animals, due to early death or cannibalization, did not have all organs examined. Time-adjusted analysis was used on animals that survived at least 52 weeks, or earlier, if the first tumor was found before this time. Animals with early mortality and no tumors were excluded. Life-table methods were used when necessary to examine tumor incidence. Using the exact interval on the odds ratio, study authors calculated the 95% confidence interval for the relative risk of each dose group compared to the control.

For the chronic testing results in Fischer F344 rats, study authors reported dose-related mean body-weight depression in male rats until Week 75 and in female rats throughout the study (NCI, 1979c). However, the data were only reported as line graphs with no data points. Furthermore, the appearance of the female body-weight graphic suggests that values were comparable to controls at the end of the study. No clinical observations were reported. For survival, results of Tarone's test for dose-related mortality were not significant for males or females. There was no significant excess in neoplastic or nonneoplastic lesions in treated rats when compared to control animals. Therefore, the NOAEL for noncancer effects in this study is 6000 ppm ($108.85_{ADJ,HED}$ mg/kg-day; derivation of a LOAEL is precluded.

NCI (1978d) is selected as the principal study for deriving the oral slope factor (p-OSF). NCI (1978d) conducted a chronic-duration study in $B6C3F_1$ mice in which nitrofen was administered in the feed. Compound purity and GLP compliance are as reported in NCI (1978c). Experimental design was identical to that reported for NCI (1978c) with some exceptions. $B6C3F_1$ mice were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA). Mice were housed by sex in groups of 10 in polypropylene cages with solid bottoms and filter tops. As with the rat bioassay (NCI, 1978c), this study focused on the ability of nitrofen to form neoplastic lesions in mice. Nitrofen was administered at a concentration of 0 (control; 20/sex) and initial low and high concentrations of 1775 ppm and 3550 ppm, respectively, for males and females (50/sex/dose). Mice were treated for 78 weeks. Following the treatment period, mice were observed (untreated; fed basal diet and corn oil mixture) for an additional 12 weeks. During Week 7, after appearing to tolerate the initial low and high doses, the low dose was increased from 1775 ppm to 2000 ppm, and the high dose was increased from 3550 ppm to 4000 ppm. During Week 22, the low dose was increased from 2000 ppm to 2500 ppm, and the high dose was increased from 4000 ppm to 5000 ppm. These final concentrations were maintained for the remainder of the 78-week treatment period. The resulting time-weighted average concentrations for the low- and high-dose groups over the 78-week treatment period were 2348 ppm and 4696 ppm, respectively. Human equivalent daily

doses (HEDs) are calculated using body-weight data provided by the study authors and default data for food consumption (U.S. EPA, 1988) and are 0, 418 (60.70), and 899 (128.26) mg/kg-day for males and 0, 469 (65.38), and 1004 (137.68) mg/kg-day for females.

In male mice, no effects on weight gain were seen compared to controls (NCI, 1978d). Female mice displayed a dose-related depression in body-weight gain (data provided in the form of a line graph). In general, throughout the first year of the study, animal appearance and behavior were comparable among treated and control groups; however, beginning in Week 54 and through the end of the study, treated mice, particularly the females, showed pronounced bloating/abdominal distension (data not reported). Necropsy of these animals later revealed liver tumors determined to be hepatocellular carcinomas. In female mice, nitrofen did not have any effects on survival. In male mice, survivability was unusually low in male controls. The study authors noted that no common cause for the decrease in the survival rate was available. Only 10% of the control group males survived until the end of the study, compared to 54% and 34% in the low- and high-dose groups, respectively. In females, 85% of controls, 54% of low-dose, and 62% of high-dose animals survived. The study authors concluded that survival was considered adequate to evaluate the carcinogenicity of nitrofen in the female mice.

The incidence of neoplasms in male and female mice treated with nitrofen is summarized in Table B.9 (NCI, 1978d). In both males and females, long-term dietary exposure to nitrofen was associated with a high occurrence of hepatocellular carcinomas at all treatment levels, and results were statistically significant for low- (36/49, 73%) and high-dose (46/48, 96%) groups. Tumors were generally well differentiated, and most were confined to the liver, but a few metastasized. The occurrence of fibromas or fibrosarcomas in male mice was significant for low-dose males; however, given that these tumors were not observed in the high-dose males, the study authors were uncertain whether the occurrence of these tumors was treatment related.

NCI (1979d) further explored the chronic-duration/carcinogenic effects of nitrofen in $B6C3F_1$ mice. GLP compliance is unknown, and compound purity was not reported. Study methods are the same as reported in NCI (1979c) with some exceptions. Mice (4 weeks old) were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA). Animals were housed by sex in groups of five in polycarbonate cages suspended from aluminum racks. Similar to the study conducted in rats (NCI, 1979d), this study focused on the possible carcinogenicity of nitrofen and did not report organ weights or serum or clinical chemistry. For the chronic-duration study in $B6C3F_1$ mice (6 weeks old), nitrofen was administered in the feed at a concentration of 0 (control; 20/sex) and low and high concentrations of 3000 ppm and 6000 ppm, respectively, for males and females (50/sex/dose). Adjusted daily doses (HEDs) are calculated using body-weight data provided by the study authors and default data for food consumption (U.S. EPA, 1988) and are 0, 473 (70.66), and 998 (147.03) mg/kg-day for males and 0, 534 (76.47), and 1136 (160.09) mg/kg-day for females. All treated animals were dosed for 78 weeks. Following the treatment period, rats were observed (untreated) for an additional 13 weeks. Control animals received the basal laboratory diet for the total 91 weeks.

NCI (1979d) reported dose-related mean body-weight depression in both male and female mice throughout the study (data were provided in graphical form only and did not include data points). No clinical observations were reported. For survival, results of Tarone's test for dose-related mortality were not significant for males or females. In males, 95% (19/20) of controls, 96% (48/50) of low-dose animals, and 80% (40/50) of high-dose animals survived until

the end of the study. In females, 60% (12/20) of controls, 86% (43/50) of low-dose animals, and 96% (48/50) of high-dose animals survived until the end of the study. The study authors reported treatment-related incidences of liver neoplasms and hyperplasias (see Table B.10). There were significant positive dose-related trends in incidences of hepatocellular carcinomas and adenomas in both sexes of mice. These carcinomas had areas of prominent trabecular formations and hepatocytes that formed in cords that were several cells thick. Hepatocellular adenomas were expansile lesions. Livers with hyperplasia consisted of single or multiple foci in which hepatocytes (and their nuclei) were enlarged. The cytoplasm was described as abundant and frequently vacuolated. Bile duct carcinomas were characterized by small, elongated cells consisting of scanty cytoplasm and dark nuclei. Treatment-related neoplasms were not seen in any other tissues. Under the conditions of the study, the study authors concluded that dietary administration of nitrofen is carcinogenic to the liver of $B6C3F_1$ mice, causing hepatocellular carcinomas in males and females.

Ambrose et al. (1971d), in a published chronic-duration study, investigated the oral toxicity of nitrofen (95% purity) in 6-month-old purebred beagle dogs. Impurities consisted of 3% *p*-chloronitrobenzene, 1% dichlorophenol, and 1% unknown. It is unknown whether the study was conducted in compliance with GLP. The study authors administered 0-, 20-, 200-, or 2000-ppm nitrofen in basal diet to two dogs/sex/dose for 2 years. Adjusted daily doses are calculated using terminal body-weight data provided in the study (Ambrose et al., 1971d) and default food consumption data (U.S. EPA, 1988) and are 0, 0.36, 3.9, and 38 mg/kg-day. The basic diet consisted of 87% ground basal diet, 12% corn oil, and 1% USP cod liver oil. Dogs were immunized against distemper, infectious hepatitis, and leptospirosis, and were treated for intestinal parasites. The study authors measured animal weights weekly and food consumption daily. The study authors conducted hematological examinations and urinary analyses at the beginning of the study and every 3 months thereafter. At study termination, the study authors tested for bromosulfalein (BSP) retention, serum glutamic-oxaloacetic acid transaminase (SGOT), serum alkaline phosphatase (SAP), and blood urea nitrogen (BUN). Additionally, at study termination, the study authors performed histological examinations; weighed the heart, spleen, kidneys, liver, and testes of surviving dogs; and calculated organ-to-body weight ratios. The statistical methods were not reported.

No changes in mortality, food consumption, or growth were observed in any of the test animals (Ambrose et al., 1971d). Values from hematological examinations and urinary analyses were not significantly different from the control. BSP, SGOT, SAP, and BUN values indicated no adverse effects. Dogs administered 2000-ppm nitrofen had significantly increased liver-to-body weight ratios (see Table B.11). No histopathological findings in any of the dose groups differed from those found in the control groups. A LOAEL of 2000 ppm (38 mg/kg-day) and a NOAEL of 200 ppm (3.9 mg/kg-day) are determined based on increased relative liver weights in combined males and females.

Reproductive and Developmental Studies

A total of 44 assays covering four species of animals (rats, mice, rabbits, and hamsters) have been performed to evaluate the reproductive and developmental effects of nitrofen. No reproductive effects were seen following exposure to the nitrofen. Administration of nitrofen to pregnant animals resulted in a number of developmental effects, with the most prevalent being neonatal lethality, diaphragmatic hernias, renal defects, and malformation of the Harderian gland. The rat was determined to be most sensitive species. Table 3 provides a summary of the

available literature concerning the reproductive and developmental effects of nitrofen. The selected principal study is included in Tables 2 and 3 and summarized below.

The study by Ostby et al. (1985) is selected as the principal study for deriving the subchronic and chronic p-RfDs. Ostby et al. (1985) published a study examining the developmental effects of prenatal exposure to nitrofen in the Sprague-Dawley CD rat. Rats were obtained from Charles River Breeding Laboratory in Wilmington, MA, and were provided Purina Rodent Laboratory Chow and water ad libitum. Study authors did not report whether the study was conducted in compliance with GLP guidelines. Nitrofen was recrystallized from technical grade to a purity of 99.59% and administered in 0.2-mL corn oil by gavage to female 90-day-old pregnant Sprague-Dawley CD rats at doses of 0, 0.46, 1.39, 4.17, or 12.5 mg/kg-day on GDs 8−16. Dosing solutions were prepared based on the average weight of all rats on GD 7. Dams were weighed on GDs 7 and 17. Any dams that had not delivered by GD 24 were necropsied. Dead pups found on postnatal day (PND) 0 (the day of birth) were necropsied for cause of death. Numbers of pups per litter and litter weights were recorded on PNDs 0, 1, 2, and 6. Percentages of eyes open on PND 16 were calculated.

Two blocks of behavioral tests were performed in which the offspring were run through activity mazes for 1 hour, and the number of photocell beam interruptions per individual was recorded (Ostby et al., 1985). In the first block, 86 pups (20 control, ~16 per dose group) were tested on PNDs 17 and 24; 64 pups (22 control, ~10 per dose group) were tested on PNDs 45 and 49; and 32 pups (8 control, ~7 per dose group) were tested on PND 90. Each dose group contained an equal number of males and females for the first two tests; only male pups were tested in the last test. Block 2 differed from the first block in that it only tested 24 controls and 16 rats from the 12.5-mg/kg-day dose group on PNDs 17 and 24.

Pups were weaned on PND 29 (Ostby et al., 1985). Females were checked daily from PNDs 32−44 for age at vaginal opening and first estrus. On PND 54, offspring were placed into nonlittermate pairs and mated. Females were allowed to deliver three litters, and number of pups per litter was recorded. Males were sacrificed by asphyxiation and necropsied on PNDs 133−161. Weights of the body, seminal vesicles, testes, liver, right kidney, lungs, and Harderian glands were recorded. Kidneys were examined for hydronephrosis and kidney cortex diameter. Eyes were examined for the presence of porphyrin rings. Data were evaluated using analysis of variance in SAS. Significant effects were then tested using *t*-tests and analyzed using linear regression. The unit of analysis for all preweanling data was the litter mean, and the unit of analysis for all postweanling data was the individual animal.

No effects were observed on maternal viability or weight change from GDs 7−17 at any dose level (Ostby et al., 1985). Examination of dams that had not delivered by GD 24 revealed one case of resorbed pups at the 4.17-mg/kg-day dose level. One female delivered only dead pups at the 4.17-mg/kg-day dose level. The number of pups per litter on PND 0 was significantly reduced at the 0.46- and 4.17-mg/kg-day dose levels, and the number of live pups on PNDs 1, 2, and 6 was statistically significantly reduced at the 0.46-, 4.17-, and 12.5 mg/kg day dose levels. Necropsy of pups found dead shortly after birth revealed that most of the dead pups at 1.39 mg/kg-day (3/4, 75%), 4.17 mg/kg-day (2/3, 67%), and 12.5 mg/kg-day (5/5, 100%) had diaphragmatic hernias (see Table B.12). The percentages of litters containing pups with diaphragmatic hernias were increased at 1.39 (3/11, 27%), 4.17 (2/10, 20%), and 12.5 mg/kg-day (3/7, 43%) (see Table B.12). This increase did not reach statistical significance

but is deemed biologically relevant as a number of other studies have reported statistically increased numbers of diaphragmatic hernias in rat and mouse litters at higher dose levels following gestational exposure to nitrofen (see Table 3). Furthermore, given that most of the pups found dead on Day 0 had diaphragmatic hernias, it appears that this malformation may be the primary cause of early neonatal mortality following exposure to low levels of nitrofen. No effects were seen on average pup weight on PND 1 or 2 (see Table B.12). No delay in eye opening was observed in pups on PND 16. Results of the behavioral locomotor tests are shown in Table B.13. Pups in the 1.39-, 4.17-, and 12.5-mg/kg-day dose groups were hyperactive compared to controls on PNDs 17 and 24. However, these effects were transient because no difference in locomotor activity was observed in tests performed on PNDs 45, 49, or 90. No significant difference in the age at vaginal opening and first estrus between treated females and controls was observed, and no effects were seen in the difference of litter size over three cycles between treated and control groups. Results of the necropsy performed on adult offspring on PNDs 133−161 are presented in Table B.14. Weight of Harderian glands was significantly reduced at the 4.17- and 12.5-mg/kg-day dose levels, and porphyrin rings (indicative of chromodacryorrhea) were observed in 13 individuals in the 12.5-mg/kg-day dose group. Severe cases of hydronephrotic kidneys were observed in animals treated with 4.17 (3 cases) and 12.5 mg/kg-day (6 cases), while no instances were observed in controls or lower dose groups. No effects were observed in body weight, weight of testes, seminal vesicle weight, liver weight, lung weight, right kidney weight, or kidney cortex diameter (data not shown). Based on data for number of litters with pups having diaphragmatic hernias, the NOAEL and LOAEL are 0.46 and 1.39 mg/kg-day, respectively.

Table 3. Summary of Oral Reproductive and Developmental Studies for Nitrofen (CASRN 1836-75-5)

Study Type, Number of Male/Female, Strain Species, Route of Administration, Study Duration	Dosimetry, Purity of Nitrofen[a]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference
Rat					
Developmental, 0/13 (8 at highest dose), Sprague-Dawley CD rat, gavage, dosed on GDs 8−16, offspring observed until PND 133-161, then necropsied	0, 0.46, 1.39, 4.17, or 12.5 mg/kg-day, purity 99.59%	Decreased live pups/litter at birth at PNDs 1, 2, and 6 at ≥0.46 mg/kg-day; diaphragmatic hernias found in pups that died immediately after birth at 1.39- (3/4), 4.17- (2/3), and 12.5- (5/5) mg/kg-day dose levels; increased percentage of litters containing pups with diaphragmatic hernias at 1.39 (3/11, 27%), 4.17 (2/10, 20%), and 12.5 mg/kg-day (3/7, 43%); decreased Harderian gland weight at ≥4.17 mg/kg-day; transient effect on locomotor activity (hyperactivity) at ≥1.39 mg/kg-day on PNDs 17 and 24 as determined by figure-8 maze activity assay; activity returned to normal by PND 45	0.46 mg/kg-day	1.39 mg/kg-day	Ostby et al. (1985)
Developmental, 0/unreported number, Sprague-Dawley rat, gavage, dosed on GDs 8−16, offspring evaluated for kidney effects on PNDs 3 and 6	0, 4.17, 12.5, or 25 mg/kg-day, purity not reported	Altered physiological responses in kidneys at ≥4.17 mg/kg-day; decreased kidney weight and body weight at ≥12.5 mg/kg-day; renal protein content and glomerular counts decreased at ≥12.5 mg/kg-day; diaphragmatic hernias in dying pups at 4.17 (2/3), 12.5 (35/35), and 25 mg/kg-day (19/32); decreased pup survival at 12.5 mg/kg-day (69%) and 25 mg/kg-day (37%)	None	4.17 mg/kg-day	Kavlock and Gray (1983a)
Developmental, 0/131 (20 in highest dose group, 37 per group at other doses), Long-Evans rat, gavage, dosed on GDs 6−15, dams were weighed on GDs 6, 8, 10, 13, 16, and 20; half of dams were sacrificed on GD 20, and half were allowed to give birth (generally on GD 21) and were kept with pups until weaning on GD 24	0, 6.25, 12.5, or 25 mg/kg-day, technical grade nitrofen, purity 96.6%	Decreased pup weight at birth at the 25-mg/kg-day dose level; increase in incidence of chromodacryorrhea indicative of Harderian gland dysfunction at 6.25 mg/kg-day (10.6%) and 12.5 mg/kg-day (35.5%); increase in renal defects (kidney and ureter dilation, absence of renal papilla) at ≥6.25 mg/kg-day; delayed development of the renal papilla and decreased pup survival (0% survival by PND 2) in 25-mg/kg-day dose group	None	6.25 mg/kg-day	Kavlock et al. (1988)

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Study Type, Number of Male/Female, Strain Species, Route of Administration, Study Duration	Dosimetry, Purity of Nitrofen[a]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference
2-Generation reproductive study, 10/20 per dose, Sherman rat, diet, F1a generation bred on Day 68, F1b generation bred on Day 200, F2a generation bred at time of weaning of F1b generation, exact duration not reported	0, 1.5, 7.6, or 38 mg/kg-day, technical grade nitrofen, purity 89%	Decreased percent survival at ≥7.6 mg/kg-day in the F1a, F1b, and F2a pups; no effects regarding food consumption or body-weight gain	1.5 mg/kg-day	7.6 mg/kg-day	Kimbrough et al. (1974)
3-Generation reproductive study, 25/25 per dose, Wistar albino rat, diet, dosed for 11 weeks, then 20 females (F0) from each dose level were mated to produce F1a and F1b generations; females from F1b were mated to produce F2a and F2b generations; females from F2b were mated to form F3a and F3b generations	0, 0.97, 9.7, or 97 mg/kg-day, purity not reported	Decreased pup viability at birth, and 0% pup survival to PND 5 at 97 mg/kg-day; increased number of stillborn pups at 9.7 mg/kg-day (effect most prominent in the F1a and F1b offspring)	0.97 mg/kg-day	9.7 mg/kg-day	Ambrose et al. (1971e)
Developmental, 0/exact number not reported (5 or 6 per dose group, 24 [control]), CD rat, administration route not specified, dosed on GDs 8−16; treated dams sacrificed and fetuses examined on GD 21; 6 control dams sacrificed and fetuses examined on GDs 19, 20, 21, and 22	0, 12.5, or 25 mg/kg-day, purity not reported	Dose-related decrease in fetal body weight and decrease in fetal body-weight index at both dose levels; dose-related decrease in absolute brain, lung, liver, and kidney weight and development; average fetal survival decreased with dose (no statistics reported)	None	12.5 mg/kg-day	Kavlock et al. (1982)
Developmental, 0/unspecified number, 10−16 pups per litter, Sprague-Dawley rat, gavage, dosed on GDs 7−21	0, 12.5 mg/kg-day, purity not reported	Significantly reduced absolute and relative kidney weights at birth and significantly reduced absolute kidney weight at PND 10; decreased renal concentrating ability; postnatal survival through PND 10 was significantly decreased by treatment to 22%	None	12.5 mg/kg-day	Chase-Deesing et al. (1986)

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Study Type, Number of Male/Female, Strain Species, Route of Administration, Study Duration	Dosimetry, Purity of Nitrofen[a]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference
Developmental, 0/unspecified number, Sprague-Dawley CD rat, gavage, dosed on GDs 8−16, male offspring sacrificed and necropsied on PND 70	0, 12.5 mg/kg-day, purity not reported	Decrease in litter size on PND 3; defects of Harderian gland in 5 males necropsied on PND 70 (1 missing both Harderian glands, 2 missing 1 Harderian gland, 2 with abnormal secretions of Harderian gland)	None	12.5 mg/kg-day	Gray et al. (1982a)
Developmental, 0/unreported number, Fischer-344 rat, gavage, dosed on GDs 10−13	0, 15 mg/kg-day, purity not reported	Decreased average pup weight at birth	None	15 mg/kg-day	Raub et al. (1983)
Developmental, 0/number not reported, Sprague-Dawley rat, gavage, dosed on GDs 8−18	0, 20, 31.2, or 50 mg/kg-day, purity >98%	Decreased birth weights in 31.2- and 50-mg/kg-day groups; labored breathing and cyanosis in all treated pups; decreased pup survival on PNDs 1 and 25 at all doses	None	20 mg/kg-day	Stone and Manson (1981)
Developmental, 0/exact number not reported, Fischer-344 rat, gavage, dosed on GDs 10−13, some dams (numbers not reported) sacrificed and offspring recovered on GD 21, the rest carried to term and were examined until 70 days after birth	0, 20, or 40 mg/kg-day, purity ~99%	Labored breathing and cyanosis shortly after birth in both dose groups; increased incidence of diaphragmatic hernia in pups at both doses; decreased heart rates in pups (doses not reported); decreased body weights at birth in both dose groups; increased percentage of offspring stillborn at 20 mg/kg-day (11%) and 40 mg/kg-day (25%); 0% survival to PND 2 in 40-mg/kg-day dose group	None	20 mg/kg-day	Lau et al. (1986)
Developmental, 0/exact number not reported, Sprague-Dawley rat, gavage, dosed on GDs 10−13; some dams (numbers not reported) sacrificed and offspring recovered on GD 21, the rest carried to term and were examined until 70 days after birth	0, 20, or 40 mg/kg-day, purity ~99%	Labored breathing and cyanosis shortly after birth in both dose groups; decreased heart rates in pups (doses not reported); increased incidence of diaphragmatic hernia in pups at ≥20 mg/kg-day; decreased body weights at birth in both dose groups; increased percentage of offspring stillborn at 20 and 40 mg/kg-day (data not provided); 0% survival to PND 2 in 40-mg/kg-day dose group	None	20 mg/kg-day	Lau et al. (1986)

Table 3. Summary of Oral Reproductive and Developmental Studies for Nitrofen (CASRN 1836-75-5)

Study Type, Number of Male/Female, Strain Species, Route of Administration, Study Duration	Dosimetry, Purity of Nitrofen[a]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference
Developmental, 0/9−11 per dose group, Sherman rat, gavage, dosed on GDs 7−15, pups observed until weaning	0, 10, 20, or 50 mg/kg-day, technical grade nitrofen, purity 89%, or 0, 20, or 50 mg/kg-day, purity 99%	Decreased number of live offspring per litter at birth (7.1 for technical and 7.5 for pure nitrofen compared to 12.4 in control) and at weaning (3.6 for technical and 3.9 for pure nitrofen compared to 12.4 in control) at 20 mg/kg-day; low number of live offspring per litter at birth (2.7 for technical and 2.2 for pure nitrofen compared to 12.7 in control) and no live offspring at weaning at 50-mg/kg-day dose level	10 mg/kg-day	20 mg/kg-day	Kimbrough et al. (1974)
Developmental, 0/number not reported, Sprague-Dawley rat, dosed on GDs 7−21, fetal rats recovered by cesarean section on GD 21	0, 25 mg/kg-day, purity 99%	Absolute fetal body, liver, kidney, intestine, heart, and lung weights, as well as organ DNA, RNA, and protein levels decreased in treated pups	None	25 mg/kg-day	Zeman et al. (1986)
Developmental, 0/12 per dose group, Sherman rat, gavage, dosed on GDs 7−18, cesarean sections performed on GD 21	0, 50 mg/kg-day, technical grade nitrofen, purity 89%	Fetal cyanosis at birth in pups of treated dams; high fetal mortality in pups of treated dams (data not provided)	None	50 mg/kg-day	Kimbrough et al. (1974)
Developmental, 0/18 (10 treated, 8 control), Sprague-Dawley rat, dosed on GDs 8−18, dams sacrificed and fetuses recovered by cesarean section on GDs 20 or 21	0, 50 mg/kg-day, purity >98%	Decreased fetal body and lung weights (absolute and relative) in treated pups apparent at GD 20; histological analysis revealed no effects	None	50 mg/kg-day	Stone and Manson (1981)
Developmental, 0/unspecified number, Sprague-Dawley rat, gavage, dosed on GD 11	0, 50, or 100 mg/kg-bw, purity not reported	Hydronephrosis combined with decreased maximal urine osmotic concentration at 50 and 100 mg/kg-bw; decreased fetal percentage survival to PND 2 (63%) and PND 29 (21%) at 100 mg/kg-bw	None	50 mg/kg-bw	Daston et al. (1988)

Table 3. Summary of Oral Reproductive and Developmental Studies for Nitrofen (CASRN 1836-75-5)

Study Type, Number of Male/Female, Strain Species, Route of Administration, Study Duration	Dosimetry, Purity of Nitrofen[a]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference
Developmental, 0/unspecified number, Long-Evans rat, gavage, dosed on GD 11, fetuses recovered on GD 22	0, 70, 115, 265, or 400 mg/kg-bw, trace contaminant identified as reduction product, 2,4-dichloro-4'-amino diphenyl ether present in less than ppm amounts	Significant increase in incidence of hydronephrosis and diaphragmatic hernias at all dose levels; dose-related increase in neonatal lethality	None	70 mg/kg-day	Costlow and Manson (1981)
Developmental, 0/unreported number, Long-Evans rat, gavage, dosed on GD 11, offspring observed until sacrificed and necropsied on PND 35	75 mg/kg-bw, purity > 99.9%	Decreased number of pups with eyes open on PND 16 (58% compared to 76% in control); decrease in Harderian gland weights compared to controls; no effects on postnatal viability or growth through weaning	None	75 mg/kg-day	Kavlock and Gray (1983b)
Developmental, 0/unspecified number, Long-Evans rat, gavage, dosed on GD 11, pups observed until maturity	0, 75, 115, 150, 200, or 250 mg/kg-bw, trace contaminant identified as reduction product, 2,4-dichloro-4'-amino diphenyl ether present in less than ppm amounts	Decrease in body weight at ≥ 115 mg/kg-bw; increased incidence of hydronephrosis at all doses except 150 mg/kg-bw; increased incidence of cardiac malformation at 150 and 250 mg/kg-bw	75 mg/kg-day	115 mg/kg-day	Costlow and Manson (1981)
Developmental, 0/unspecified number, Long-Evans rat, gavage, dosed on GDs 7−9, 9−11, or 12−14; pups observed until 48 hours after birth	0, 150 mg/kg-day, trace contaminant identified as reduction product, 2,4-dichloro-4'-amino diphenyl ether present in less than ppm amounts	Decreased birth weight (86−87% of controls) and survival rate (0−26% of controls) to PND 2 in groups of treated pups for each dosing period	None	150 mg/kg-day	Costlow and Manson (1981)

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Study Type, Number of Male/Female, Strain Species, Route of Administration, Study Duration	Dosimetry, Purity of Nitrofen[a]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference
Developmental, 0/unspecified number, Long-Evans rat, gavage, dosed on GDs 9, 10, 11, or 12; pups observed after birth and sacrificed and autopsied on PND 35	0, 150 mg/kg-bw, trace contaminant identified as reduction product, 2,4-dichloro-4'-amino diphenyl ether present in less than ppm amounts	Decreased birth weight (84−92% compared to controls) and decreased survival rate (44−96% compared to controls) at PND 2 in groups of treated pups for each dosing period	None	150 mg/kg-day	Costlow and Manson (1981)
Developmental, 0/unreported number, Sprague-Dawley rat, gavage, dosed on GD 11, single administration, dams sacrificed and fetuses recovered on GD 21	0, 200, 250, 300, 350, or 400 mg/kg-bw, purity not reported	Decreased fetal average body weight at all doses; increased number of cardiovascular anomalies (study authors noted ventricular septal defects and anomalous right subclavian arteries, but no further detail was provided) at all dose levels	None	200 mg/kg-day	Kim et al. (1999)
Developmental, 0/17 (10 dosed, 7 control), Sprague-Dawley rat, single administration, administration route not reported, dosed on GD 10	0, 400 mg/kg-bw[c], purity not reported	Decreased birth weight (87% of control) and lung-to-body-weight ratio (60% of control) in nitrofen-treated pups	None	400 mg/kg-bw	Ijsselstijn et al. (1997)
Developmental, 0/unspecified number, Sprague-Dawley rat, gavage, dosed on GD 9.5, fetuses recovered on GD 13.5	0, 490 mg/kg-bw[d], purity not reported	Defects in propagation of calcium across airway smooth muscle (ASM)	None	490 mg/kg-bw	Featherstone et al. (2006)
Developmental, 0/unspecified number, Sprague-Dawley rat, gavage, single administration, GD of dosing not reported	0, 735 mg/kg-bw[d], purity ~ 98%	Abnormal development of the pleuroperitoneal fold in exposed embryos recovered on GDs 13.5−14	None	735 mg/kg-bw	Clugston et al. (2010)
Developmental, 0/unspecified number, Long-Evans rat, gavage, dosed on GDs 15−20; pups observed until 48 hours after birth	20−100 mg/kg-day, trace contaminant identified as reduction product, 2,4-dichloro-4'-amino diphenyl ether present in less than ppm amounts	No effects observed in newborns (no data provided)	100 mg/kg-day	None	Costlow and Manson (1981)

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Study Type, Number of Male/Female, Strain Species, Route of Administration, Study Duration	Dosimetry, Purity of Nitrofen[a]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference
Developmental, 0/unreported number, rat (strain unreported), administration route not reported, dosed on GDs 7−16	5 dose levels from 0−25 mg/kg-day, purity not reported	Diaphragmatic hernias at 1.39 mg/kg-day and above; Harderian gland effects (not specified) at 12.5 mg/kg-day and above	None	None	Gray et al. (1982b)[e]
Developmental, 0/number not reported, rat (strain not reported), route of administration not reported, dosed on GD 10	Not reported	Diaphragmatic hernia; decreased growth of lung primordium	None	None	Piersma et al. (1993)[e]
Developmental, 0/unreported number, Fischer F344 rat, administration route not reported, dosed on GDs 10−13; ECGs recorded on GD 21, immediately following birth, and on PNDs 2 and 70	0, 20, 40 mg/kg-day, purity not reported	Diaphragmatic hernias (doses unreported); irregular and gasping respiratory movements and cyanosis of all treated litters; 0% pup survival to PND 1, and distension of abdomen in high-dose group; low body weights in low-dose group until PND 70; dose-related decrease in heart rate	None	None	Robinson and Cameron (1984)[e]
Developmental, 0/unreported number, LEH rat, administration route not reported, dosed on GDs 10−12	200 mg/kg-day (no control reported), purity not reported	Effects not reported	None	None	Kang and Manson (1987)[e]
Developmental, 0/unreported number, rat (strain unreported), gavage, dosed on GDs 7−21	0, 25 mg/kg-day, purity not reported	Reduced body weight, intestinal weight, and intestinal length; elevated enzyme activity	None	None	Mahboob et al. (1985)[e]
Mouse					
Developmental, 0/unspecified number, CD-1 mouse, gavage, dosed on GDs 7−17, dams carried to term, and pups were weaned on PND 30, males sacrificed and necropsied on PND 110, females sacrificed and necropsied on PND 130	0, 6.25, 12.5, 25, 50, 100, 150, or 200 mg/kg-day, purity 99.6%	Decreased lung and liver weights at all dose levels on PND 110; decreased absolute Harderian gland weight at all dose levels, and absence of glands at 25 (4%), 50 (65%), and 100 (97%) mg/kg-day; retardation of growth rates at ≥12.5 mg/kg-day; reduced body weights at birth in the 150- and 200-mg/kg-day dose groups, and at PND 3 in the 100-mg/kg-day group; incidence of diaphragmatic hernia (6%), cleft palate (15%), and distended abdomen (22%) at 200 mg/kg-day; 100% pup mortality by PND 3 at 150 and 200 mg/kg-day	None	6.25 mg/kg-day	Gray et al. (1983a)

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Study Type, Number of Male/Female, Strain Species, Route of Administration, Study Duration	Dosimetry, Purity of Nitrofen[a]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference
Developmental, 0/12−14, CD-1 mouse, gavage, dosed on GDs 7−17, litters from prenatal untreated control dams (CON) were cross-fostered with prenatal nitrofen treated (N) dams or untreated (CON) dams. Litters from treated (N) dams were cross-fostered with CON dams. Final groups consisted of N/CON, N/N, CON/N and CON/CON. Growth and viability of pups evaluated on PNDs 1,3,10 and 20. Pups were necropsied on PND 110.	0 (corn oil control) or 100 mg/kg-day, purity 99.6%	Decreased lung and liver weights on PND 110; decreased absolute Harderian gland weights, and absence of glands (97%); retardation of growth rates; reduced body weights on PND 3 among groups (N/CON and N/N) treated prenatally. Developmental effects not seen in groups (CON/N and CON/CON) without prenatal nitrofen treatment.	NA	NA	Gray et al. (1983b)
Developmental, 0/unreported number, Swiss-Webster CD-1 mouse, gavage, dosed on GDs 6−15, females carried litters to term, offspring sacrificed at weaning on PNDs 27−33	0, 10, 50, 100, 250, or 500 mg/kg-day, purity ≥99%	Decreased pup body weight in males (80% of control) and females (83% of control), and in Harderian gland size in males (69% of control) and females (76% of control) at 10 mg/kg-day; decrease in the percentage prenatal litter survival at 100 (87%), 250 (63%), and 500 (0%) mg/kg-day (compared to 94% in control); decreased survival per litter to PND 15 at 50 (81% of control), 100 (48% of control), and 250 (2% of control) mg/kg-day Decreased maternal weight gain per female at 10 (71% of control), 50 (67% of control), and 100 (88% of control) mg/kg-day	Developmental: None Maternal: None	Developmental: 10 mg/kg-day Maternal: 10 mg/kg-day	Francis et al. (1999)
Developmental, 0/unspecified, CD-1 mouse, gavage, dosed on GDs 7−17, pups sacrificed and examined on PNDs 3, 8, 13, and 110	0, 100 mg/kg-day, purity not reported	Delayed eye opening and decreased weight/absence of Harderian glands in treated pups	None	100 mg/kg-day	Gray et al. (1982a)

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Study Type, Number of Male/Female, Strain Species, Route of Administration, Study Duration	Dosimetry, Purity of Nitrofen[a]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference
Developmental, 0/169 (76 treated, 93 control), Swiss Webster mouse, gavage, dosed on GD 8, embryos recovered by cesarean section and examined on GDs 12, 14, 16, or 17	0, 1111 mg/kg-bw[d], purity not reported	Severe fetal craniofacial defects (no further detail provided) in groups recovered on GDs 12, 14, 16, and 17	Developmental: None	Developmental: 1111 mg/kg-day	Acosta et al. (2001)
Developmental, 0/unreported number, mouse (strain unreported), administration route not reported, dosed on GDs 8−12	0, 200 mg/kg-day, purity not reported	0% pup viability in treatment group; eyeless offspring; reduction in size of Harderian gland	None	None	Gray et al. (1982b)[e]
Developmental, 0/unreported number, mouse (strain unreported), administration route not reported, dosed on GDs 7−17	0−200 mg/kg-day (exact dose levels not reported), purity not reported	Reduced size of Harderian gland at 6.25 mg/kg-day and above, and destruction of Harderian gland in 4% of pups at 50 mg/kg-day and 35% at 100 mg/kg-day; 50% pup viability at 100 mg/kg-day and 0% viability at 150 mg/kg-day and above; eyeless offspring at 200 mg/kg-day	None	None	Gray et al. (1982b)[e]
Developmental, 0/unreported number, mouse (strain unreported), administration route not reported, dosed on GDs 7−17	0, 50−200 mg/kg-day (exact dose levels not reported, control not specified), purity not reported	Reproductive problems at 50 and 100 mg/kg-day; stunted growth in all treated groups	None	None	Gray et al. (1982b)[e]
Rabbit					
Developmental, 0/15 per dose group, New Zealand White Rabbit, capsule, dosed on GDs 6−18, 10 rabbits per group sacrificed on GD 28 and examined for developmental effects, remaining 5 rabbits per group were sacrificed along with offspring on PND 2	0, 5, 20, or 80 mg/kg-day, purity not reported	Decreased live fetuses per litter at 80 mg/kg-day	20 mg/kg-day	80 mg/kg-day	Siou (1979) as reported in Burke Hurt et al. (1983)
Hamster					
Developmental, 0/unreported number, hamster (strain unreported), administration route not reported, dosed on GDs 7−11	0, 25, 50, 100, 200, or 400 mg/kg-day, purity not reported	Harderian glands, lungs, adrenals, seminal vesicles, epididymides, testes, sperm counts, and flank gland development were reduced (doses not reported)	None	None	Gray (1984)[e]

Table 3. Summary of Oral Reproductive and Developmental Studies for Nitrofen (CASRN 1836-75-5)

Study Type, Number of Male/Female, Strain Species, Route of Administration, Study Duration	Dosimetry, Purity of Nitrofen[a]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference
Developmental, 0/unreported number, hamster (strain unreported), administration route not reported, dosed on GDs 8−9, 11−12, or 14−15	0, 400 mg/kg-day, purity unreported	Uterus unicornis and ipsilateral renal agenesis in females and unilateral agenesis of vas deferens and/or epididymis and seminal vesicle in male pups following treatment on GDs 8−9; spermatic granulomas in male pups treated later in gestation; serum thyroxin levels reduced in males at PND 25	None	None	Gray (1984)[e]
Developmental, 0/unreported number, hamster (strain unreported), administration route not reported, dosed on GDs 7−11	0−400 mg/kg-day (exact dose levels not reported), purity not reported	Reduced size of Harderian gland at 100 mg/kg-day and above; severe reproductive effects (further details not provided)	None	None	Gray et al. (1982b)[e]

[a]Units are reported in mg/kg-day or mg/kg-bw (for single-administration studies).
[b]Not reported by the study author(s) but determined from data for this review.
[c]Dose reported in study as mg; converted using the following equation: Dose (mg/kg-day) = provided dose (mg) ÷ average body weight (kg), where average body weight is the body weight provided by the study authors.
[d]Dose reported in study as mg; converted using the following equation: Dose (mg/kg-day) = provided dose (mg) ÷ average body weight (kg), where average body weight is the average subchronic body weight for females of the species and strain provided by the study authors.
[e]Only abstract available; no NOAELs or LOAELs were derived due to lack of information.

NA = Not applicable because the study (Gray et al., 1983b) was not designed to identify a LOAEL.

Inhalation Exposures

Subchronic-duration Studies

No studies were identified.

Chronic-duration Studies

No studies were identified.

Developmental and Reproductive Studies

No studies were identified.

OTHER DATA (SHORT-TERM TESTS, OTHER EXAMINATIONS)

Studies investigating the kinetics of nitrofen in rats, sheep, and cows (Brown and Mason, 1986; Costlow and Manson, 1983; Hunt et al., 1977; Gutenmann and Lisk, 1967) and the potential mode of action for nitrofen (Noble et al., 2007; Brandsma et al., 1994; Manson et al., 1984, as cited by Manson, 1986) are summarized in Table 4. The data evaluating the genotoxicity/mutagenicity of nitrofen are also included in Table 4. Ames assays testing the genotoxicity of nitrofen found equivocal evidence of genotoxic effects in various strains of *Salmonella typhimurium* (Dunkel et al., 1985; Moriya et al., 1983; Tanaka et al., 1996). Data reported in other Ames assays were also available (Byeon et al., 1976; Jeang and Li, 1980; Shirasu et al., 1982, as cited in Burke Hurt et al., 1983). The TA98 and TA100 *S. typhimurium* strains exhibited positive and negative results, both with or without rat liver S9 activation. The YG1026 and YG1029 strains exhibited a positive result with S9 activation in one study (Tanaka et al., 1996). A possible explanation of these positive genotoxicity results may be that a low-level impurity in technical grade nitrofen preparations—4,4'-dichlorobenzene—could have caused genotoxic effects to strains TA98 and TA100 (Paik and Lee, 1977; Burke Hurt et al., 1983).

Cultured mouse lymphoma cells and human lymphocytes were also used to assess the genotoxicity of nitrofen (Paik and Lee, 1977; McGregor et al., 1996). These studies did not indicate any increase in forward mutation or unscheduled DNA repair synthesis due to nitrofen. Several chromosomal aberration studies were conducted with nitrofen in rats (McLeod and McCarthy., 1981; Reustle and Scribner, 1980; Kiryushin, 1975) and in freshly germinated barley roots (Oku, 1976, as cited by Burke Hurt et al., 1983). No chromosomal aberrations were observed in these studies. Furthermore, mice exposed to nitrofen in doses up to 1.69 mg/kg did not exhibit any increases in the number of micronuclei present in polychromatic erythrocytes (Siou, 1978, as cited by Burke Hurt et al., 1983). These findings suggest that nitrofen is not genotoxic.

Noble et al. (2007) conducted in vitro cell assays as well as whole animal rodent studies in order to test hypotheses concerning the mechanisms by which nitrofen induces diaphragmatic hernias in rodents. Study authors investigated the interactions of nitrofen with various stages of the retinoid signaling pathway; with vitamins A, C, and E; and with the thyroid signaling pathway. A luciferase assay conducted with P19 cells revealed that nitrofen application did not affect the mRNA expression of any elements in the retinoid signaling cascade. A yeast-HRE assay revealed significant inhibition of receptor binding at >100μM nitrofen, but no significant effect at concentrations that induce diaphragmatic hernia without being lethal. A clear dose-dependent decrease in retinoic acid levels was observed with increasing nitrofen concentration in a dual assay system with luciferase and Retinoic Acid Receptor Response

Element (RARE)-luciferase, and significantly lower levels of retinoic acid were observed in embryos exposed to nitrofen on GD 9. Study authors observed a rescue effect when vitamin A was administered with nitrofen, but no significant effects when nitrofen was administered with vitamins C or E. Study authors attributed this effect to an increase in the substrate for RALDH in the retinoid signaling cascade, not to the oxidizing properties of the vitamin. Tests conducted to determine the thyromimetic effects of nitrofen returned negative results; nitrofen does not exert teratogenic effects through interactions with the thyroid signaling pathway. Study authors concluded that the perturbation of retinoic acid is the primary effect of nitrofen on the retinoid signaling pathway, and that this may be an underlying cause of nitrofen-induced diaphragmatic hernia in rodent models.

Table 4. Other Studies

Tests	Methods	Dosimetry	Results	References
Ames assay	*Salmonella typhimurium*, TA98, TA100, TA1535, TA1537, TA1538, *Escherichia coli* WP2 *urvA*, ± S9 mix	Unknown (partial PDF missing doses)	+ without S9 in TA98, TA100, and TA1538; − without S9 at TA1535, TA1537, WP2 *urvA* + with S9 in TA98, TA100, and TA1538; − with S9 at TA1535, TA1537, WP2 *urvA*	Dunkel et al. (1985)
Ames assay	*S. typhimurium*, TA98, TA100, TA1535, TA1537, TA1538, WP2 *hcr*, ± S9 mix	0−500 µg/plate	+ without S9 in TA100; − without S9 at TA1535, TA1537, TA1538, WP2 *hcr* + with S9 in TA98; − with S9 at TA1535, TA1537, TA1538, WP2 *hcr*	Moriya et al. (1983)
Ames assay	*S. typhimurium*, TA98, TA100, YG1021, YG1024, YG1026, YG1029, + S9 mix	Not reported	+ with S9 mix in YG1026 and YG1029; − with S9 at TA98, TA100, YG1021	Tanaka et al. (1996)
Ames assay	Unspecified, Ames system	Not reported	Positive	Byeon et al. (1976), Jeang and Li (1980), Shirasu et al. (1982), as cited by Burke Hurt et al. (1983)
Lymphoma assay (mouse and human lymphocytes)	L5178Y mouse lymphoma cells, human lymphocytes grown in culture	0, 10, and 20 mM	negative for methotrexate-resistant mutants in mouse lymphoma cells; did not induce unscheduled DNA repair synthesis in human lymphocytes	Paik and Lee (1977)
Mouse lymphoma assay	L5178Y mouse lymphoma cells	0, 30, and 60 µg/mL	Borderline response at *tk* locus in L5178Y mouse cells	McGregor et al. (1996)
Chromosomal aberrations	8 male Charles River CD-1 rat	0, 0.05, 0.125, and 0.5 g/kg-day for 5 days	No increase in chromosomal aberrations from bone marrow	McLeod and McCarthy (1981)
Chromosomal aberrations	8 male Charles River CD-1 rat	0, 0.39, 0.79, and 1.59 g/kg-day for 5 days	No increase in chromosomal aberrations from bone marrow	Reustle and Scribner (1980)
Chromosomal aberrations	Rat, strain not specified	$1/15^{th}$ or $1/200^{th}$ LD_{50} to rats for 6 days	No increase in chromosomal aberrations	Kiryushin (1975)
Chromosomal aberrations	Freshly germinated barley roots	800- and 8000-ppm nitrofen emulsion	No increase in chromosomal aberrations	Oku (1976), as cited by Burke Hurt et al. (1983)

Table 4. Other Studies

Tests	Methods	Dosimetry	Results	References
In vivo micronuclei assay	5−10 male Swiss mouse, gavage, examined for the presence of micronuclei in polychromatic erythrocytes in the bone marrow	0, 0.34, 1.35, and 1.69 mg/kg	No increase in micronuclei	Siou (1978), as cited by Burke Hurt et al. (1983)
Toxicokinetics study	Long-Evans Hooded rat; single oral maternal dose of 240 mg/kg ^{14}C-labeled nitrofen on GD 10; maternal and embryonic tissues collected after 1.5−72 hours; HPLC of maternal fat, plasma, liver, heart, and embryo-placental complex	240 mg/kg ^{14}C-labeled nitrofen on GD 10	After 3−12 hours: uptake, peak maternal tissue concentrations (highest in liver); after 3 hours: radioactivity detected in embryo-placental complex; after 12 hours: accumulation in maternal fat underway; after 24 hours: 100-fold higher accumulation in maternal fat compared to blood; after 48 hours: redistribution of nitrofen to maternal heart, liver, and the embryonic compartment; half-life of measured radioactivity in blood estimated to be 42 hours; half-life of detectable nitrofen (parent compound) in blood calculated to be 68 hours; embryonic compartment contained only nitrofen parent compound and was a deep compartment for the compound; 4'-amino, 4'-acetylamine, 5-hydroxy were the dominant metabolites detected in maternal tissues	Brown and Manson (1986)
Toxicokinetics study	8 Long-Evans rat; single oral maternal dose of 240 mg/kg ^{14}C-labeled nitrofen on GD 10; toxicokinetic analysis of dam tissues; UV profile HPLC analysis of metabolites in embryo-placental complex extracts; analysis of 4'-amino, acetylamine and hydroxyl metabolites	120 mg/kg ^{14}C-labeled nitrofen on GD 11	8-hour absorption phase in dams, maximal maternal blood concentration of 10 μg/mL; half-life in maternal blood of 8 days; 100-fold higher accumulation in maternal fat compared to blood; volume of distribution (V) of 41.3 liters (0.413 if adjusted to the 1% absorption reported elsewhere based on concentration in excreta); no detectable radiation from ^{14}C-labeled nitrofen in embryo-placental complex extracts; acetylamine metabolite only HPLC-detectable nitrofen compound in embryo-placental complex extracts	Costlow and Manson (1983)

Table 4. Other Studies

Tests	Methods	Dosimetry	Results	References
Toxicokinetics study	Delaine ewe; gelatin capsule of ^{14}C-labeled nitrofen and grain; blood, urine, and feces collected every 0.5 hour (0−4 hours), hourly (4−9 hours), and thereafter every 4 hours (until 100 hours); combustion analysis, TLC analysis, audioradiography, liquid scintillation counting of extracts	40 mg/kg	Radioactivity detected at highest levels in blood after 19 hours; aminonitrofen and nitrofen the most recovered radioactive compounds; 11% of applied dose excreted as nitrofen in feces; 39% of the applied dose was recovered in blood, urine, and feces (37.2% of applied dose) after 99 hours; urine contained conjugated nitrofen that was 25% or 70% liberated and extractable by β10-glucoronidase or sulfatase, respectively	Hunt et al. (1977)
Toxicokinetics study	Holstein cow; 5-ppm pure recrystallized nitrofen in feed (based on a daily ration of 26.2 kg); urine feces, milk collected in morning and evening; deconjugation via orthophosphoric acid digestion; samples extracted in acetone; methylation with diazomethane; affinity gas chromatography; also examined stability of nitrofen in rumen fluid	5 ppm in feed for 4 days	No detection of nitrofen in milk, urine, or feces; recovery of nitrofen spiked into milk, urine, or feces samples was possible to levels as low as 0.2 ppm; 2,4-dichloro-4'-aminodiphenyl ether detected in rumen fluid	Gutenmann and Lisk (1967)
Mode-of-action study	Bacterial recombinant (α_1 and β_1 forms of thyroid hormone receptor) binding assay; chicken α type rat β type thyroxine hormone receptor as recombinant in an *E. coli* pop 2136 strain vector; measured [^{125}I] labeled triiodothyronine ([^{125}I]T3) specific binding to α_1 chicken and β_1 rat thyroid hormone receptor protein in the presence of excess nonradioactive T3 and nitrofen at various concentrations	10, 100, or 1000 μM nitrofen	Decreased maximal binding capacity of T3 to the α_1 chicken and β_1 rat forms of thyroid hormone receptor in a noncompetitive (allosteric) way; these effects of nitrofen were dose dependent; this result indicates that nitrofen inhibits the binding of T3 to the T3 receptor	Brandsma et al. (1994)

Table 4. Other Studies

Tests	Methods	Dosimetry	Results	References
Mode-of-action study	Euthyroid and thyroparathyroidectomized (TPTX) adult female rats, strain not specified; coadministered thyroxine (T4) to test recovery of hypothalamic-pituitary-thyroid function in nonpregnant, pregnant, and fetal rats	15 and 30 mg/kg-day for 2 weeks	Euthyroid rats: significant decrease in thyroid stimulating hormone and T4; TPTX rats: coadministration of T4 with nitrofen resulted in a 70% reduction in the frequency of malformed fetuses compared to nitrofen alone	Manson et al. (1984), as cited by Manson (1986)
Mode-of-action study	Retinoid and thyroid hormone signaling pathways examined in vitro in conjunction with rat whole-animal in vivo studies; thyroid hormone, thyroid hormone receptor function assays; yeast hormone response element (HRE) assay: binding to nuclear receptors including thyroid receptor and retinoic acid receptors (transgenic yeast); dual luciferase assay: retinoic acid production measured by retinoic acid receptor RARE-luciferase enzymes	10nM, 100nM, 1μM, 10μM, and 100μM	Nitrofen interacts with various elements of the retinoid signaling cascade to ultimately disturb the levels of retinoic acid; no significant effects were observed on the thyroid signaling pathway; no effect was observed when P19 cells were treated with vitamins C or E along with nitrofen, but vitamin A produced a rescue effect when administered with nitrofen	Noble et al. (2007)

DERIVATION OF PROVISIONAL VALUES

Table 5 presents a summary of noncancer reference values. Table 6 presents a summary of cancer values. For cancer, the toxicity value was converted to HED units, and the conversion process is presented in the section on derivation of provisional cancer potency values.

Table 5. Summary of Noncancer Reference Values for Nitrofen (CASRN 1836-75-5)

Toxicity Type (Units)	Species/Sex	Critical Effect	p-Reference Value	POD Method	POD	UF_C	Principal Study
Subchronic p-RfD (mg/kg-day)	Rat/litter data	Diaphragmatic hernias	3×10^{-3}	$BMDL_{05}$	0.29	100	Ostby et al. (1985)
Chronic p-RfD (mg/kg-day)	Rat/litter data	Diaphragmatic hernias	3×10^{-3}	$BMDL_{05}$	0.29	100	Ostby et al. (1985)
Subchronic p-RfC (mg/m^3)	None	None	None	None	None	None	None
Chronic p-RfC (mg/m^3)	None	None	None	None	None	None	None

Table 6. Summary of Cancer Reference Values for Nitrofen (CASRN 1836-75-5)

Toxicity Type	Species/ Sex	Tumor Type	Cancer Value	Principal Study
p-OSF	Rat/F	Hepatocellular carcinomas	3.8×10^{-2} $(mg/kg\text{-}day)^{-1}$	NCI (1978d)
p-IUR	None	None	None	None

DERIVATION OF ORAL REFERENCE DOSES

Derivation of Subchronic Provisional RfD (Subchronic p-RfD)

Based on the available literature, there were seven subchronic-duration, four chronic-duration, several developmental and reproductive studies (see Table 3), and four carcinogenic studies involving oral exposures to nitrofen. When compared to adult animals exposed for any duration, the effects in fetal animals are seen at much lower relative doses, indicating that the critical effect for nitrofen is developmental in nature. **The developmental study by Ostby et al. (1985) is selected as the principal study for derivation of the subchronic p-RfD.** Details of this study are provided in the "Review of Potentially Relevant Data" section. Table 3 summarizes the available studies reporting developmental effects in animals following oral exposure to nitrofen. The developmental effect of diaphragmatic hernias is the most consistent, with other reported effects (i.e., renal, Harderian gland, heart, and neurobehavioral) not consistently reported. Diaphragmatic hernias showed a strong biological gradient as this effect was related to dose over several independent studies. Diaphragmatic hernias were also reported across species (rat and mouse) with effects in the rat being more sensitive, as indicated by Ostby et al. (1985). Furthermore, at the higher dose levels, most of the pups found dead on PND 0 had diaphragmatic hernias (75% at 1.39 mg/kg-day, 67% at 4.17 mg/kg-day, and 100% at 12.5 mg/kg-day), suggesting that this malformation may be a primary cause of neonatal mortality following maternal exposure to nitrofen (see Table B.12). Thus, diaphragmatic hernias in rat pups is chosen as the critical effect.

The Ostby et al. (1985) rat data for the number of litters with pups having diaphragmatic hernias gives a $BMDL_{05}$ of 0.292 mg/kg-day. Litter-specific data for this study were not available, and the results were reported as mean data. Therefore, use of nested models provided by BMD software was precluded, and regular dichotomous BMD models were used to determine the point of departure (POD) (U.S. EPA, 2012). The dichotomous data models in the EPA BMDS (version 2.1.2) were fit to the data for the number of litters with pups having diaphragmatic hernias following exposure of maternal rats to nitrofen by gavage on GDs 8−16 (see Table B.12). Table C.1 lists the BMD output models considered for derivation of the chronic and subchronic p-RfD with curve and BMD output text provided for the selected model in the BMD supplement to this document provided in Appendix C (see Figure C.1 and the subsequent text output). All models provided adequate fit to the diaphragmatic hernia litter data. The LogLogistic model is considered the best model to fit the data and was used as the POD as it provided the lowest AIC (37.935) and a $BMDL_{05}$ of 0.292 mg/kg-day (see Table C.1).

The subchronic p-RfD for nitrofen, based on the $BMDL_{05}$ of 0.292 mg/kg-day in nested rat data (Ostby et al., 1985), is derived as follows:

$$\textbf{Subchronic p-RfD} = \textbf{BMDL}_{\textbf{05}} \div \textbf{UF}_{\textbf{C}}$$
$$= 0.292 \text{ mg/kg-day} \div 100$$
$$= \textbf{3} \times \textbf{10}^{\textbf{-3}} \textbf{ mg/kg-day}$$

Table 7 summarizes the uncertainty factors for the subchronic p-RfD for nitrofen.

Table 7. Uncertainty Factors Used to Derive a Subchronic p-RfD for Nitrofen

UF	Value	Justification
UF_A	10	A UF_A of 10 is applied for interspecies extrapolation to account for potential toxicokinetic and toxicodynamic differences between rats and humans. There are no data to determine whether humans are more or less sensitive than rats to the developmental effects of nitrofen.
UF_D	1	A UF_D of 1 is applied because the database includes 1 acceptable 2-generation reproduction study in rats (Kimbrough et al., 1974), 1 acceptable 3-generation reproduction studies in rats (Ambrose et al., 1971e), and multiple developmental studies across 4 species (rat, mouse, rabbit, hamster; see Table 3).
UF_H	10	A UF_H of 10 is applied for intraspecies differences to account for potentially susceptible individuals in the absence of information on the variability of response in humans.
UF_L	1	A UF_L of 1 is applied because the POD is a BMDL.
UF_S	1	A UF_S of 1 is applied because a developmental study (Ostby et al., 1985) was utilized as the principal study to derive the subchronic p-RfD.
UF_C	100	

The confidence of the subchronic p-RfD for nitrofen is high as explained in Table 8.

Table 8. Confidence Descriptors for Subchronic p-RfD for Nitrofen

Confidence Categories	Designation[a]	Discussion
Confidence in study	H	Confidence in the key study is high. Ostby et al. (1985) examined appropriate developmental toxicity endpoints, although only 8−13 maternal rats per dose group were used, resulting in a small number of litters per dose (7−11). The study was peer reviewed. GLP compliance is unknown. The study included multiple effect levels, and both a NOAEL and LOAEL are identified. The data used as the critical effect were modeled using BMD software and provided a good fit. The key endpoint of diaphragmatic hernias in the litters of pups is seen in multiple independent studies and in two species—rat and mouse.
Confidence in database	H	The database includes subchronic-duration toxicity studies in 2 species (rat and mouse), chronic-duration toxicity studies in 2 species (rat and mouse), developmental toxicity studies in 4 species (rat, mouse, rabbit, and hamster), and one 2-generation reproductive study and one 3-generation reproductive study in rats.
Confidence in subchronic p-RfD[b]	H	The overall confidence in the subchronic p-RfD is high.

[a]L = Low, M = Medium, H = High.
[b]The overall confidence cannot be greater than lowest entry in table.

Derivation of Chronic Provisional RfD (Chronic p-RfD)

Although chronic toxicity testing of nitrofen has been conducted, effects in fetal animals occurred at much lower relative doses indicating that the critical effect is developmental. Therefore, the critical endpoint is diaphragmatic hernias as indicated by Ostby et al. (1985). This is the same critical effect used to derive the subchronic p-RfD. A full description concerning the selection of this endpoint as the critical effect and calculation of the appropriate $BMDL_{05}$ are provided in the section on the derivation of the subchronic p-RfD. Consistent with the practice of the EPA, the developmental period is recognized as a susceptible lifestage where exposure during certain time windows is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991b). Therefore, a UF for extrapolation from less-than-chronic results is not used, and the chronic p-RfD is derived as follows:

$$\textbf{Chronic p-RfD} = \textbf{BMDL}_{\textbf{05}} \div \textbf{UF}_{\textbf{C}}$$
$$= 0.292 \text{ mg/kg-day} \div 100$$
$$= \mathbf{3 \times 10^{-3} \text{ mg/kg-day}}$$

Table 9 summarizes the uncertainty factors for the chronic p-RfD for nitrofen.

Table 9. Uncertainty Factors Used to Derive a Chronic p-RfD for Nitrofen

UF	Value	Justification
UF_A	10	A UF_A of 10 is applied for interspecies extrapolation to account for potential toxicokinetic and toxicodynamic differences between rats and humans. There are no data to determine whether humans are more or less sensitive than rats to the developmental effects of nitrofen.
UF_D	1	A UF_D of 1 is applied because the database includes 1 acceptable 2-generation reproduction study in rats (Kimbrough et al., 1974), 1 acceptable 3-generation reproduction study in rats (Ambrose et al., 1971e), and multiple developmental studies across 4 species (rat, mouse, rabbit, hamster; see Table 3).
UF_H	10	A UF_H of 10 is applied for intraspecies differences to account for potentially susceptible individuals in the absence of information on the variability of response in humans.
UF_L	1	A UF_L of 1 is applied because the POD is a BMDL.
UF_S	1	A UF_S of 1 is applied because a developmental study (Ostby et al., 1985) was utilized as the principal study to derive the chronic p-RfD. Because the developmental period is identified by the EPA as a susceptible lifestage where exposure during times of development may be more relevant than exposure over a lifetime, a UF was not used to account for extrapolation from less than chronic results.
UF_C	100	

The confidence of the chronic p-RfD for nitrofen is high as explained in Table 10.

Table 10. Confidence Descriptors for Chronic p-RfD for Nitrofen

Confidence Categories	Designation[a]	Discussion
Confidence in study	H	Confidence in the key study is high. Ostby et al. (1985) examined appropriate developmental toxicity endpoints, although only 8−13 maternal rats per dose group were used, resulting in a small number of litters per dose (7−11). The study was peer reviewed. GLP compliance is unknown. The study included multiple effect levels, and both a NOAEL and LOAEL are identified. The data used as the critical effect were modeled using BMD software and provided a good fit. The key endpoint of diaphragmatic hernias in the litters of pups is seen in multiple independent studies and in two species—rat and mouse.
Confidence in database	H	The database includes subchronic-duration toxicity studies in 2 species (rat and mouse), chronic-duration toxicity studies in 2 species (rat and mouse), developmental toxicity studies in 4 species (rat, mouse, rabbit, and hamster), and one 2-generation reproductive study and one 3-generation reproductive study in rats.
Confidence in chronic p-RfD[b]	H	The overall confidence in the chronic p-RfD is high.

[a]L = Low, M = Medium, H = High.
[b]The overall confidence cannot be greater than lowest entry in table.

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

Derivation of a provisional subchronic or chronic RfC for nitrofen is precluded because no quantitative human or animal studies examining the effects of subchronic or chronic inhalation exposure to nitrofen have been identified. Derivation of a screening value is precluded for the same reason.

CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR

Table 11 identifies the cancer weight-of-evidence descriptor for nitrofen as "*Likely to be Carcinogenic to Humans*." NCI (1978c) reported treatment-related tumor increases in the hematopoietic system (lymphoma), pancreas (carcinoma), and ovaries (granulosa cell tumor) of female Osborn-Mendel rats at the highest dose (see Table B.8) following 78 weeks of exposure to nitrofen in feed and an additional 32 weeks of untreated observation. No treatment-related tumors were found in the male rat. A study conducted in F344 rats reported no treatment-related tumors in males or females following exposure to nitrofen in feed for 78 weeks, with 26 weeks of untreated observation (NCI, 1979c). NCI (1978d) found an increased incidence of hepatocellular carcinomas in both male and female $B6C3F_1$ mice (see Table B.9) following 78 weeks of exposure to nitrofen in feed and an additional 12 weeks of untreated observation. The liver tumors were increased in a dose-dependent manner. NCI (1979d) conducted an additional study in $B6C3F_1$ mice, which also reported an increased incidence in hepatocellular carcinomas and hepatocellular carcinomas and adenomas (combined) in both males and females (see Table B.10). These liver tumors were increased at all doses in both sexes except for female hepatocellular carcinomas, which were only increased at the high dose.

Table 11. Cancer WOE Descriptor for Nitrofen

Possible WOE Descriptor	Designation	Route of Entry (Oral, Inhalation, or Both)	Comments
"Carcinogenic to Humans"	N/A	N/A	Convincing epidemiologic evidence of a causal association between human exposure to nitrofen and cancer does not exist.
"Likely to Be Carcinogenic to Humans"	**Selected**	**Oral feed**	**The available evidence of carcinogenicity in Osborn-Mendel rats (lymphoma, pancreatic carcinomas, and ovary granulose cell tumors in females) and $B6C3F_1$ mice (hepatocellular carcinomas and adenomas in males and females) exposed orally (in feed) to nitrofen indicates that nitrofen is likely to be carcinogenic to humans.**
"Suggestive Evidence of Carcinogenic Potential"	N/A	N/A	The evidence from human and animal data is more than suggestive of carcinogenicity, which raises a concern for carcinogenic effects but is judged sufficient for a stronger conclusion.
"Inadequate Information to Assess Carcinogenic Potential"	N/A	N/A	Available adequate information exists to assess carcinogenic potential.
"Not Likely to Be Carcinogenic to Humans"	N/A	N/A	No strong evidence of noncarcinogenicity in humans is available.

DERIVATION OF PROVISIONAL CANCER POTENCY VALUES

Derivation of Provisional Oral Slope Factor (p-OSF)

NCI (1978d) is selected as the principal study for derivation of the p-OSF. The cancer endpoint is the incidence of hepatocellular carcinomas in female mice. Details of this study are provided in the "Review of Potentially Relevant Data" section. It should be noted that while pancreatic tumors in the rat reported by NCI (1978c) were observed at a lower $DOSE_{ADJ,HED}$ (46.97 mg/kg-day) than the lowest $DOSE_{ADJ,HED}$ for liver tumors in the mouse (65.38 mg/kg-day) (NCI, 1978d), the mouse liver tumors reported by NCI (1978d, 1979d) provided the clearest evidence for carcinogenic potential and the greatest statistical power for analysis. For example, NCI (1978c) reported pancreatic carcinomas in 14% (7/50) of female rats at the adjusted human equivalency dose of 46.97 mg/kg-day. In comparison, hepatocellular carcinomas were seen in 88% (36/41) of female mice at the slightly higher adjusted human equivalency dose of 65.38 mg/kg-day (NCI, 1978d). For further comparison, BMD modeling was conducted using data for liver tumor formation reported by the two NCI mouse studies and pancreatic carcinomas seen in rats (NCI, 1978c). The lowest $BMDL_{10ADJ,HED}$ of 2.6 for hepatocellular carcinomas in female mice (see Figure C.2.) was over 9 times lower than the $BMDL_{10ADJ,HED}$ of 24.1 for rat pancreatic carcinomas.

Dosimetric adjustments were made for oral dietary administration of nitrofen by adjusting doses for oral cancer analysis (p-OSF). A sample calculation is shown below for the female low-dose group in NCI (1978d).

$$\mathbf{DOSE_{ADJ,HED}} = \text{dose} \times \text{food consumption per day} \times (1 \div \text{body weight}) \times (\text{days dosed} \div \text{total days}) \times (\text{body weight animal} \div \text{body weight human})^{0.25}$$

$$= 2348 \text{ ppm} \times 0.0061 \text{ kg/day} \times (1 \div 0.026475258 \text{ kg}) \times (546 \div 630) \times (0.026475258 \text{ kg} \div 70 \text{ kg})^{0.25}$$

$$= \mathbf{65.38 \text{ mg/kg-day}}$$

Table B.9 presents BMD input data for incidence of hepatocellular carcinomas in female mice exposed to nitrofen in feed for 78 weeks. Hoover et al. (1980) compared the histological characteristics of the liver tumors induced by nitrofen to the spontaneous neoplasms seen in the control animals. The nitrofen-induced tumors generally consisted of solid sheets or nodules composed of large eosinophilic hepatocytes. A total of three basophilic tumors were seen in the low-dose males. However, all of the tumors examined in treated females were eosinophilic in nature. Spontaneously formed tumors seen in the controls consisted of small basophilic cells arranged in solid or trabecular fashion. The differences in histological characteristics between the liver tumors from mice exposed to nitrofen and those occurring in control mice suggest that nitrofen induces unique liver tumors and does not act as a promoter of neoplasms formed spontaneously. Given the large increase in incidence of the mouse liver tumors compared to controls along with the observed morphological differences, it is concluded that the mouse liver tumors are relevant for deriving the p-OSF. As shown in Table C.2, hepatocellular carcinomas in female mice from the NCI (1978d) study provide the lowest credible POD and are, therefore, used for derivation of the p-OSF. Adequate model fit is obtained for the hepatocellular carcinomas using the multistage-cancer model. The modeling results yield a $BMDL_{10ADJ,HED}$ of 2.6 mg/kg-day. The curve and BMD output text are provided for the selected model in the BMD supplement to this document found in Appendix C (see Figure C.2 and the text output that follows the figure).

p-OSF = $0.1 \div BMDL_{10ADJ,HED}$
= $0.1 \div 2.6$ mg/kg-day
= **3.8×10^{-2} (mg/kg-day)$^{-1}$**

Derivation of Provisional Inhalation Unit Risk (p-IUR)

No human or animal studies examining the carcinogenicity of nitrofen following inhalation exposure have been identified. Therefore, derivation of a p-IUR is precluded.

APPENDIX A. PROVISIONAL SCREENING VALUES

No screening values are presented.

APPENDIX B. DATA TABLES

Table B.1. Clinical Chemistry Values of Male Sprague-Dawley Rats Orally Exposed to Nitrofen for 15 Weeks[a]

Parameter	Exposure Group, ppm (Adjusted Daily Dose, mg/kg-day)			
	0	100 (7)	500 (37)	2500 (186)
Sample size	25	25	25	24
Glucose (mg/dL)	102.0 ± 11.1	98.6 ± 13.7 (97)	99.3 ± 10.4 (97)	88.0 ± 12.0[b] (86)
Total protein (g/dL)	6.1 ± 0.2	6.0 ± 0.3 (98)	6.1 ± 0.3 (100)	6.9 ± 0.3[b] (113)
Albumin (g/dL)	3.5 ± 0.2	3.5 ± 0.2 (100)	3.5 ± 0.2 (100)	3.9 ± 0.3[b] (111)
Globulin (g/dL)	2.5 ± 0.3	2.5 ± 0.2 (100)	2.5 ± 0.2 (100)	2.9 ± 0.3[b] (116)
Cholesterol (mg/dL)	55.8 ± 12.5	52.9 ± 15.4 (95)	64.8 ± 17.4 (116)	119.9 ± 32.5[b] (215)

[a]Values are mean ± SD (% of control)
[b]Significantly different from control ($p < 0.05$) as reported by study authors.

Source: O'Hara et al. (1983).

Table B.2. Body and Organ Weights of Male Sprague-Dawley Rats Orally Exposed to Nitrofen for 15 Weeks[a]

Parameter		Exposure Group, ppm (Adjusted Daily Dose, mg/kg-day)			
		0	100 (7)	500 (37)	2500 (186)
Sample size		25	25	25	24
Terminal body weight	Absolute (g)	479 ± 43	460 ± 42 (96)	462 ± 39 (96)	420 ± 39[b] (88)
Kidney weight	Absolute (g)	3.33 ± 0.47	3.31 ± 0.41 (99)	3.48 ± 0.37 (105)	3.44 ± 0.32 (103)
	Relative	0.70 ± 8.1	0.72 ± 6.2 (104)	0.75 ± 4.7[b] (108)	0.82 ± 5.7[b] (118)
Liver weight	Absolute (g)	12.5 ± 2.2	12.0 ± 1.6 (96)	13.7 ± 1.7 (110)	19.5 ± 2.4[b] (156)
	Relative	2.61 ± 36	2.61 ± 19 (100)	2.96 ± 17[b] (113)	4.66 ± 42[b] (179)
Testes weight	Absolute (g)	3.15 ± 0.41	3.14 ± 0.32 (100)	3.48 ± 0.33[b] (110)	3.52 ± 0.23[b] (112)
	Relative	0.67 ± 10.9	0.69 ± 8.9 (103)	0.76 ± 8.3[b] (114)	0.85 ± 10.5[b] (127)

[a]Values are mean ± SD (% of control); relative organ weights reported as percentage of body weight × 100.
[b]Significantly different from control ($p < 0.05$) as reported by study authors.

Source: O'Hara et al. (1983).

Table B.3. Relative Organ Weights of Male and Female Wistar Rats Orally Exposed to Nitrofen for 13 Weeks[a]

Parameter	Exposure Group, ppm (Adjusted Daily Dose, mg/kg-day)				
Male	**0**	**100 (9)**	**500 (46)**	**2500 (230)**	**12,500 (1152)**
Sample size	9	10	9	10	6
Heart (g/kg)	2.7 ± 0.3	2.7 ± 0.3 (100)	2.8 ± 0.2 (104)	3.0 ± 0.6 (111)	3.5 ± 0.3[b] (130)
Spleen (g/kg)	1.6 ± 0.2	1.6 ± 0.1 (100)	1.5 ± 0.1 (94)	1.9 ± 0.7 (119)	2.5 ±0.6[b] (156)
Kidney (g/kg)	7.2 ± 0.7	7.1 ± 0.7 (99)	7.6 ± 0.6 (106)	8.5 ± 0.6[b] (118)	10.5 ± 0.7[b] (146)
Liver (g/kg)	33.7 ± 5.0	34.9 ± 5.1 (104)	40.9 ± 6.2[b] (121)	56.7 ± 5.9[b] (168)	121.7 ± 18.1[b] (361)
Testes (g/kg)	8.7 ± 1.3	9.1 ± 0.6 (105)	9.5 ± 0.9 (109)	10.3 ± 1.0[b] (118)	10.9 ± 3.9[b] (125)
Female	**0**	**100 (10)**	**500 (51)**	**2500 (256)**	**12,500 (1282)**
Sample size	9	10	9	10	6
Heart (g/kg)	3.0 ± 0.1	3.3 ± 0.4 (111)	3.0 ± 0.2 (100)	3.2 ± 0.5 (111)	4.3 ±0.7[b] (143)
Spleen (g/kg)	2.5 ± 1.0	2.9 ± 1.2 (113)	2.7 ± 0.9 (108)	2.6 ± 1.0 (107)	2.8 ± 1.1 (112)
Kidney (g/kg)	7.1 ± 0.5	7.6 ± 0.6 (116)	7.8 ± 0.8 (110)	8.2 ± 0.5 (104)	11.1 ± 1.3[b] (156)
Liver (g/kg)	33.5 ± 2.4	37.3 ± 3.9[b] (107)	39.5 ± 2.9[b] (118)	52.1 ± 4.5[b] (115)	101.0 ± 5.4[b] (301)

[a]Values are mean ± SD (% of control).
[b]Significantly different from control ($p < 0.05$) as reported by study authors.

Source: Ambrose et al. (1971a).

Table B.4. Survival, Clinical Effects, and Mean Weight Loss of Male and Female Fischer F344 Rats Orally Exposed to Nitrofen for 4 Weeks

Parameter	Exposure Group, ppm (Adjusted Daily Dose, mg/kg-day)					
Male	**0**	**6800 (453)**	**10,000 (667)**	**14,670 (978)**	**21,560 (1437)**	**31,530 (2102)**
Sample size	5	5	5	5	5	5
No. survived (%)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	1 (20)
Mean weight gain[a]	--	-13	-17	-36	-52	-58
No. with arched backs (%)	0	0	0	0	0	4 (80)
Female	**0**	**6800 (512)**	**10,000 (753)**	**14,670 (1104)**	**21,560 (1623)**	**31,530 (2373)**
Sample size	5	5	5	5	5	5
No. survived (%)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	1 (20)
Mean weight gain[a]	--	-24	-16	-34	-43	-47
No. with arched backs (%)	0	0	0	0	0	4 (80)

[a]Mean body-weight gain less than that of controls indicated by "-"; values are percentages as reported by study authors.

Source: NCI (1979a).

Table B.5. Survival, Clinical Observations, and Mean Weight Loss of Male and Female B6C3F$_1$ Mice Orally Exposed to Nitrofen for 4 Weeks

Parameter	Exposure Group, ppm (Adjusted Daily Dose, mg/kg-day)					
Male	**0**	**1180 (142)**	**2550 (307)**	**5500 (661)**	**13,900 (1672)**	**25,520 (3069)**
Sample size	5	5	5	5	5	5
No. survived (%)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	2 (40)
Mean weight gain[a]	--	5	-5	-4	-4	1
Clinical signs (%)	0	0	0	0	5[b] (100)	5[b] (100)
Female	**0**	**1180 (153)**	**2550 (332)**	**5500 (715)**	**13,900 (1808)**	**25,520 (3320)**
Sample size	5	5	5	5	5	5
No. survived (%)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	3 (60)
Mean weight gain[a]	--	7	-1	8	-2	3
Clinical signs (%)	0	0	0	0	5[b] (100)	5[b,c] (100)

[a]Mean body-weight gain relative to controls (±%).
[b]Rough hair and arched backs.
[c]Mottled kidneys.

Source: NCI (1979b).

Table B.6. Mean Body Weights and Mortalities of Male and Female Wistar-Derived Albino Rats Orally Exposed to Nitrofen for 97 Weeks[a]

Parameter	Exposure Group, ppm (Adjusted Daily Dose, mg/kg-day)			
Male	**0**	**10 (1.09)**	**100 (11.5)**	**1000 (116)**
Start	58 ± 6	58 ± 7 [100]	58 ± 6 [100]	58 ± 6 [100]
Week 1	83 ± 9	80 ±13 [96]	78 ± 11 [94]	75 ± 12 [90]
Week 3	153 ± 26	149 ± 29 [97]	146 ± 23 [95]	139 ± 22 [91]
Week 6	264 ± 33	258 ± 32 (1) [98]	252 ± 30 [95]	231 ± 30[b] [88]
Week 13	375 ± 38	369 ± 45 [98]	362 ± 34 [97]	351 ± 32 (2) [94]
Week 26	458 ± 55	450 ± 45 (2) [98]	435 ± 42 (1) [95]	432 ± 30 [94]
Week 52	504 ± 65 (3)	486 ± 46 (7) [98]	465 ± 50[b] (5) [92]	458 ± 37[b] (5) [91]
Week 78	513 ± 69 (7)	506 ± 97 (14) [99]	452 ± 55[b] (10) [88]	457 ± 45[b] (10) [89]
Week 97	489 ± 65 (10)	460 ± 58 (20) [94]	423 ± 58[b] (14) [87]	447 ± 41 (16) [91]
Female	**0**	**10 (1.17)**	**100 (12.2)**	**1000 (127)**
Start	54 ± 5	54 ± 7 [100]	54 ± 5 [100]	53 ± 7 [98]
Week 1	73 ± 9	73 ±10 [100]	68 ± 12 [93]	70 ± 9 [96]
Week 3	129 ± 13	127 ± 13 (2) [98]	118 ± 16[b] (1) [91]	120 ± 14[b] [93]
Week 6	182 ± 17	185 ± 12 (3) [102]	174 ± 12 [96]	172 ± 18[b] [95]
Week 13	232 ± 20 (2)	233 ± 16 [100]	221 ± 14 (2) [95]	215 ± 22[b] (3) [93]
Week 26	281 ± 28	275 ± 20 [98]	264 ± 15[b] (3) [94]	259 ± 32[b] [92]
Week 52	321 ± 46 (3)	308 ± 31 (5) [96]	311 ± 31 (5) [97]	300 ± 46 (7) [93]
Week 78	320 ± 53 (15)	333 ± 53 (11) [104]	325 ± 41 (12) [102]	307 ± 46 (13) [96]
Week 97	320 ± 12 (20)	340 ± 31 (17)	313 ± 47 (20)	274 ± 44 (19) [86]

[a]Weights expressed as mean (g) ± SD; (cumulative mortality [% of control]).
[b]Significantly different from control ($p \leq 0.05$) as reported by the study authors.

Source: Ambrose et al. (1971c).

Table B.7. Organ-to-Body-Weight Ratios of Male and Female Wistar-Derived Rats Orally Exposed to Nitrofen for 97 Weeks[a]

Parameter	Exposure Group, ppm (Adjusted Daily Dose, mg/kg-day)			
Male	**0**	**10 (1.09)**	**100 (11.5)**	**1000 (116)**
Sample size[b]	5	8	5	6
Heart (g/kg)	3.5 ± 0.7	3.0 ± 0.4 (86)	3.2 ± 0.5 (91)	3.4 ± 0.4 (97)
Spleen (g/kg)	2.1 ± 0.6	1.8 ± 0.4 (86)	2.0 ± 0.4 (95)	2.1 ± 0.5 (100)
Kidney (g/kg)	8.4 ± 0.9	8.8 ± 2.2 (105)	8.8 ± 1.6 (105)	10.7 ± 2.5[c] (127)
Liver (g/kg)	37.1 ± 3.9	34.9 ± 2.7 (94)	37.0 ± 7.9 (100)	50.4 ± 5.9[c] (136)
Testes (g/kg)	7.3 ± 1.2	6.8 ± 0.8 (93)	8.2 ± 2.0 (112)	7.8 ± 1.1 (107)
Female	**0**	**10 (1.17)**	**100 (12.2)**	**1000 (127)**
Sample size[b]	15	5	11	9
Heart (g/kg)	4.0 ± 0.3	3.6 ± 0.4 (90)	4.3 ± 1.1 (108)	4.0 ± 0.3 (100)
Spleen (g/kg)	3.6 ± 0.8	2.8 ± 0.7 (78)	3.2 ± 0.9 (89)	2.6 ± 0.5[c] (72)
Kidney (g/kg)	10.9 ± 1.4	9.5 ± 1.4 (87)	10.2 ± 1.3 (94)	10.0 ± 2.4 (92)
Liver (g/kg)	42.7 ± 4.9	39.8 ± 3.4 (93)	43.4 ± 6.6 (102)	46.9 ± 9.2 (110)

[a]Values are mean ratios of organ to body weight ± SD (% of control).
[b]Animals that survived to study termination were included.
[c]Significantly different from control ($p \leq 0.05$), as reported by the study authors.

Source: Ambrose et al. (1971c).

Table B.8. Incidence of Neoplasms in Female Osborne-Mendel Rats Orally Exposed to Nitrofen for 78 Weeks

Parameter		Exposure Group, ppm (Human Equivalency Dose, mg/kg-day)		
		0	**1300 (21.88)**	**2600 (46.97)**
Hematopoietic System	Lymphoma	0/20 (0%)	0/50 (0%)	4/50 (6%)*
Pancreas	Carcinoma	0/20 (0%)	2/50 (4%)	7/50 (14%)**
Uterus	Carcinoma	0	0	2/49 (4%)
	Squamous cell carcinoma	0	1/50 (2%)	0
	Adenocarcinoma	0	1/50 (2%)	1/49 (2%)
	Endometrial stromal polyp	2/20 (10%)	3/50 (6%)	1/49 (2%)
	Endometrial stromal sarcoma	0	1/50 (2%)	0
Vagina	Carcinoma	0	0	1/50 (2%)
	Squamous cell carcinoma	0	0	1/50 (2%)
Ovary	Carcinoma	0	1/50 (2%)	2/49 (4%)
	Squamous cell carcinoma	0	1/50 (2%)	0
	Cystadenocarcinoma	0	1/50 (2%)	0
	Granulosa-cell tumor	0	0	4/49 (8%)*
	Granulosa-cell carcinoma	1/20 (5%)	1/50 (2%)	0

*Significantly different from control ($p \leq 0.05$), as reported by the study authors.
**Significantly different from control ($p \leq 0.01$), as reported by the study authors.

Source: NCI (1978c).

Table B.9. Incidence of Neoplasms in Male and Female B6C3F$_1$ Mice Orally Exposed to Nitrofen for 78 Weeks

Parameter		Exposure Group, ppm[a] (Human Equivalency Dose, mg/kg-day)		
Male		**0**	**2348 (60.70)**	**4696 (128.26)**
Subcutaneous tissue	Fibroma	0	2/44 (5%)	0
	Fibrosarcoma	0	8/44 (18%)*	0
	Hemangiopericytoma	0	1/44 (2%)	0
	Neurofibroma	0	1/44 (2%)	0
Liver	Hepatocellular carcinoma	4/20 (20%)	36/49 (73%)**	46/48 (96%)**
	Hemangiosarcoma	0	1/44 (2%)	4/48 (8%)
Urinary bladder	Transitional-cell carcinoma	0	0	2/40 (5%)
Female		**0**	**2348 (65.38)**	**4696 (137.68)**
Liver	Hepatocellular carcinoma	0	36/41 (88%)**	43/44 (98%)**
	Hemangiosarcoma	0	0	4/44 (9%)
Urinary Bladder	Transitional-cell papilloma	0	0	1/41 (2%)

[a]Represents time-weighted average concentrations over the 78-week treatment period as reported by study authors.

*Significantly different from control ($p \leq 0.05$), as reported by the study authors.
**Significantly different from control ($p \leq 0.01$), as reported by the study authors.

Source: NCI (1978d).

Table B.10. Incidence of Neoplasms in the Livers of Male and Female B6C3F$_1$ Mice Orally Exposed to Nitrofen for 78 Weeks

Parameter	Exposure Group, ppm (Human Equivalency Dose, mg/kg-day)		
Male	**0**	**3000 (70.66)**	**6000 (147.03)**
Sample size	20	49	48
Hyperplasia[a]	0	9 (18%)	4 (8%)
Hepatocellular adenoma or carcinoma	1 (5%)	31 (63%)**	40 (83%)**
Hepatocellular carcinoma	0	13 (27%)**	20 (42%)**
Bile duct carcinoma (hepatoblastoma)	0	3 (6%)	4 (8%)
Female	**0**	**3000 (76.47)**	**6000 (160.09)**
Sample size	18	48	50
Hyperplasia[b]	0	11 (23%)	22 (22%)
Hepatocellular adenoma or carcinoma	0	14 (29%)**	30 (60%)**
Hepatocellular carcinoma	0	5 (10%)	13 (26%)*
Bile duct carcinoma (hepatoblastoma)	0	1 (2%)	0

[a]Adjusted daily doses calculated for noncancerous endpoints in males are 0, 515, and 1029 mg/kg-day
[b]Adjusted daily doses calculated for noncancerous endpoints in females are 0, 518, and 1037 mg/kg-day.

*Significantly different from control ($p \leq 0.05$), as reported by the study authors.
**Significantly different from control ($p \leq 0.01$), as reported by the study authors.

Source: NCI (1979d).

Table B.11. Terminal Body Weight and Organ-to-Body-Weight Ratios of Male and Female Beagle Dogs Orally Exposed to Nitrofen for 2 Years[a]

Parameter	Exposure Group, ppm (Adjusted Daily Dose, mg/kg-day)			
	0	**20 (0.36)**	**200 (3.9)**	**2000 (38)**
Sample size[b]	4	4	4	4
Terminal body weight (kg)	10.99 ± 0.36	11.30 ± 1.39 (103)	10.50 ± 1.83 (96)	10.65 ± 2.02 (97)
Heart (g/kg)	8.17 ± 1.53	7.87 ± 0.99 (96)	7.73 ± 1.37 (95)	7.93 ± 1.25 (97)
Spleen (g/kg)	8.85 ± 2.40	6.42 ± 1.04 (73)	6.06 ± 2.67 (68)	7.55 ± 3.09 (85)
Kidney (g/kg)	4.58 ± 0.29	5.23 ± 0.82 (114)	4.63 ± 0.69 (101)	5.67 ± 0.64 (124)
Liver (g/kg)	26.25 ± 2.8	27.13 ± 3.3 (103)	28.63 ± 1.8 (109)	40.45 ± 4.3* (154)
Testes[c] (g/kg)	1.94	2.14 (110)	1.85 (95)	1.97 (102)

[a]Values are mean ± SD (% relative to control).
[b]Two dogs of each sex per diet level.
[c]SD not reported by study authors.
*Significantly different from control ($p \leq 0.05$), as reported by the study authors.

Source: Ambrose et al. (1971d).

Table B.12. Neonatal Effects on Sprague-Dawley Rat Pups Exposed to Nitrofen on GDs 8−16

Parameter	Exposure Group, mg/kg-day				
	0	**0.46**	**1.39**	**4.17**	**12.5**
No. of litters	9	9	11	10	7
No. of pups/litter Day 0 (%)[a]	12.1	8.2[b] (68)	10.5 (87)	9.2[c] (76)	10.1 (83)
No. live pups Day 1 (%)[a]	11.9	8.0[b] (67)	9.9 (83)	8.9[c] (75)	8.4[c] (71)
No. live pups Day 2 (%)[d]	11.8 (98)	8.0[b] (98)	9.8 (93)	8.9[c] (97)	8.4[c] (83)
No. live pups Day 6 (%)[d]	11.6 (96)	7.7[b] (89)	9.3 (88)	8.5[c] (84)	8.1[c] (78)
No. dead pups recovered Day 0	4	2	4	3	5
No. pups with diaphragmatic hernias (%)[e]	0	0	3 (75)	2 (67)	5 (100)
No. litters from which dead pups recovered Day 0 (%)[f]	2 (22)	2 (22)	3 (27)	3 (30)	3 (43)
No. litters with pups having diaphragmatic hernias (%)[f]	0	0	3 (27)	2 (20)	3 (43)
Average pup weight Day 1 (g) (%)[a]	6.9	7.2 (104)	7.0 (101)	6.8 (99)	6.7 (97)
Average pup weight Day 2 (g) (%)[a]	8.1	8.2 (101)	7.8 (96)	8.0 (99)	7.7 (95)

[a]Percent of control; calculated for this review.
[b]Significantly different from control ($p \leq 0.01$) by *t*-test performed by study authors.
[c]Significantly different from control ($p \leq 0.05$) by *t*-test performed by study authors.
[d]Percent survival from Day 1; provided by study authors.
[e]Percent of total no. dead pups Day 0; calculated for this review.
[f]Percent of total no. litters; calculated for this review.

Source: Ostby et al. (1985).

Table B.13. Effects on Locomotor Activity of Sprague-Dawley Rats Exposed to Nitrofen on GDs 8−16[a]

Parameter		Exposure Group, mg/kg-day				
		0	**0.46**	**1.39**	**4.17**	**12.5**
Block 1	Day 17	251 ± 31	305 ± 38 (122)	323 ± 38 (129)	273 ± 34 (109)	345 ± 34[b] (137)
	Day 24	266 ± 38	307 ± 38 (115)	363 ± 38 (136)	421 ± 38[c] (158)	359 ± 44 (135)
	Days 45 & 49	682 ± 66	632 ± 63 (93)	638 ± 61 (94)	660 ± 69 (97)	689 ± 59 (101)
	Day 90	749 ± 53	734 ± 50 (97)	648 ± 63 (87)	719 ± 95 (96)	748 ± 65 (100)
Block 2	Day 17	234 ± 22	-	-	-	306 ± 23[b] (131)
	Day 24	294 ± 25	-	-	-	377 ± 32[b] (128)
Blocks 1 & 2	Days 17 & 24	264 ± 16	304 ± 29 (115)	341 ± 29[b] (129)	345 ± 28[b] (131)	341 ± 20[c] (129)

[a]Values are mean ± SD (% of control, calculated for this review); values represent number of photocell beam interruptions in a figure-8 maze with 8 photocells over a 1-hour period. Blocks refer to sequential experiments.
[b]Significantly different from control ($p \leq 0.05$) as reported by study authors.
[c]Significantly different from control ($p \leq 0.01$) as reported by study authors.

Source: Ostby et al. (1985).

Table B.14. Necropsy Results of 133−161-Day-Old Male Sprague-Dawley Rats Exposed to Nitrofen on GDs 8−16[a]

Parameter	Exposure Group, mg/kg-day				
	0	**0.46**	**1.39**	**4.17**	**12.5**
Sample size	25	25	25	22	17
Body weight (g)	640.9 ± 76[b]	652.9 ± 70 (102)	621.2 ± 53 (97)	644.9 ± 66 (101)	655.3 ± 59 (102)
Seminal vesicle weight (g)	1.96 ± 0.29	1.98 ± 0.33 (101)	1.97 ± 0.38 (101)	1.90 ± 0.31 (97)	1.90 ± 0.35 (97)
Harderian gland weight (g)	0.392 ± 0.06	0.385 ± 0.05 (98)	0.391 ± 0.04 (100)	0.358 ± 0.07[c] (91)	0.303 ± 0.04[d] (77)
No. animals with hydronephrotic kidneys (%)	0	0	0	3 (14)	6 (35)
No. animals with porphyrin rings around one or both eyes (%)	0	0	0	0	13 (76)
Sample size	6	6	6	6	6
Testes weight (g)	3.85 ± 0.29	3.39 ± 0.61 (88)	3.71 ± 0.22 (96)	3.86 ± 0.42 (100)	3.90 ± 0.26 (101)
Liver weight (g)	27.01 ± 4.46	27.78 ± 3.96 (103)	24.83 ± 2.76 (92)	30.79 ± 5.12 (114)	25.72 ± 3.90 (95)
Lung weight (g)	1.80 ± 0.19	1.85 ± 0.11 (103)	1.98 ± 0.42 (110)	2.13 ± 0.23 (118)	1.88 ± 0.13 (104)
Right kidney weight (g)	2.40 ± 0.17	2.47 ± 0.22 (103)	2.31 ± 0.28 (96)	2.70 ± 0.21 (113)	2.39 ± 0.24 (100)

[a]Absolute weights expressed as mean ± SD (% of control).
[b]Sample size = 24.
[c]Significantly different from control ($p \leq 0.05$) as reported by study authors.
[d]Significantly different from control ($p \leq 0.01$) as reported by study authors.

Source: Ostby et al. (1985).

APPENDIX C. BMD OUTPUTS

Subchronic and Chronic Endpoints:

Table C.1. Model Predictions for Rat Litters with Pups Having Diaphragmatic Hernias

Model	Goodness-of-Fit *p*-Value[a]	AIC for Fitted Model	BMD_{05} mg/kg-day	$BMDL_{05}$ mg/kg-day	Conclusions
Gamma	0.16	40.761	0.828	0.453	
Weibull	0.16	40.761	0.828	0.453	
LogProbit	0.14	42.643	2.866	1.191	
LogLogistic	**0.43**	**37.935**	**0.587**	**0.292**	**Lowest AIC Lowest BMDL**
Multistage	0.16	40.761	0.828	0.453	
Logistic	0.20	42.235	2.624	1.547	
Probit	0.20	42.125	2.386	1.427	
Quantal Linear	0.16	40.761	0.828	0.453	

[a]Values < 0.10 fail to meet conventional goodness-of-fit criteria.

AIC = Akaike's Information Criteria; BMD = benchmark dose; BMDL = BMD lower limit

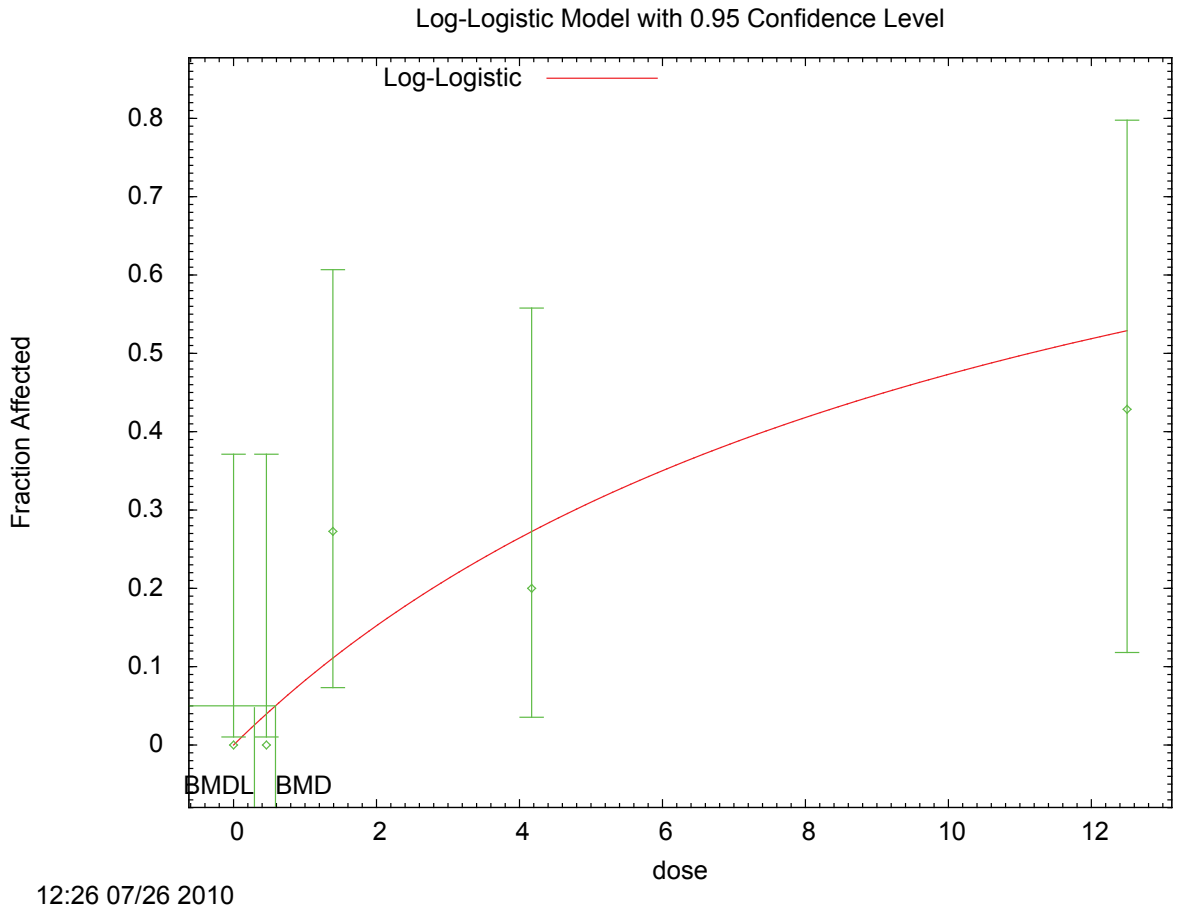

Figure C.1. LogLogistic BMD Model for Rat Litters with Pups Having Diaphragmatic Hernia Data (Ostby et al., 1985)

Text Output for LogLogistic BMD Model for Rat Litters with Pups Having Diaphragmatic Hernia Data (Ostby et al., 1985)

```
================================================================
        Logistic Model. (Version: 2.13; Date: 10/28/2009)
        Input Data File: C:/1/Ostby_1985_LittersWithPupsDiaHern05_LogLogistic_1.(d)
        Gnuplot Plotting File:  C:/1/Ostby_1985_LittersWithPupsDiaHern05_LogLogistic_1.plt
                                                Mon Jul 26 12:26:26 2010
================================================================

[add_notes_here]
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the probability function is:

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


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

Total number of observations = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
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
intercept = -2.57481
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

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

intercept

intercept 1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0	*	*	*
intercept	-2.41098	*	*	*
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	-16.2299	5			
Fitted model	-17.9674	1	3.47512	4	0.4817
Reduced model	-21.2537	1	10.0477	4	0.03963

AIC: 37.9348

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Scaled Size	Residual
0.0000	0.0000	0.000	0.000	9	0.000
0.4600	0.0396	0.357	0.000	9	-0.609
1.3900	0.1109	1.220	3.000	11	1.709
4.1700	0.2723	2.723	2.000	10	-0.514
12.5000	0.5287	3.701	3.000	7	-0.530

Chi^2 = 3.84 d.f. = 4 P-value = 0.4283

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.58657

BMDL = 0.292378

Cancer Endpoints:

Table C.2. Summary of the PODs for the p-OSF for Nitrofen

Study and Year	Endpoint	Gender/ Species	File Name	BMD	BMDL	BMD/ BMDL	*p*-Value Test	AIC	Scaled Residual of Interest	Model Notes
NCI, 1978d	**Hepatocellular Carcinoma**	**Female Mouse**	**NCI_1978_new_Liver_HepaCarc_mouse_F_MultiCanc2_1.out**	**3.5**	**2.6**	**1.3**	**0.8849**	**42.18**	**0.000**	**Lowest BMDL**
NCI, 1978d	Hepatocellular Carcinoma	Male Mouse	NCI_1978_new_Liver_HepaCarc_mouse_M_MultiCanc2_1.out	7.3	4.1	1.8	NA	99.34	0.000	Failed *p*-value test
NCI, 1979d	Hepatocellular Adenoma or Carcinoma	Male Mouse	NCI_1979_new_Liver_HepaAdemCarc_mouse_M_MultiCanc2_1.out	8.4	6.7	1.3	0.6397	119.85	-0.052	
NCI, 1979d	Hepatocellular Adenoma or Carcinoma	Female Mouse	NCI_1979_new_Liver_HepaAdemCarc_mouse_F_MultiCanc2_1.out	27.7	16.1	1.7	1.0000	129.25	0.000	
NCI, 1979d	Hepatocellular Adenoma	Male Mouse	NCI_1979_new_Liver_HepaAdenoma_mouse_M_MultiCanc2_1.out	27.3	19.2	1.4	0.1514	143.61	-0.397	
NCI, 1979d	Hepatocellular Carcinoma	Male Mouse	NCI_1979_new_Liver_HepaCarc_mouse_M_MultiCanc2_1.out	26.9	20.4	1.3	0.8891	124.13	0.000	
NCI, 1978c	Pancreatic Carcinoma	Female Rat	NCI_1978_new_Panc_CarcNOS_rat_F_MultiCanc2_1.out	38.3	24.1	1.6	1.0000	61.29	0.000	
NCI, 1979d	Hepatocellular Adenoma	Female Mouse	NCI_1979_new_Liver_HepaAdem_mouse_F_MultiCanc2_1.out	40.0	29.4	1.4	0.9941	112.44	0.088	
NCI, 1979d	Hepatocellular Carcinoma	Female Mouse	NCI_1979_new_Liver_HepaCarc_mouse_F_MultiCanc2_1.out	73.9	43.0	1.7	1.0000	93.38	0.000	

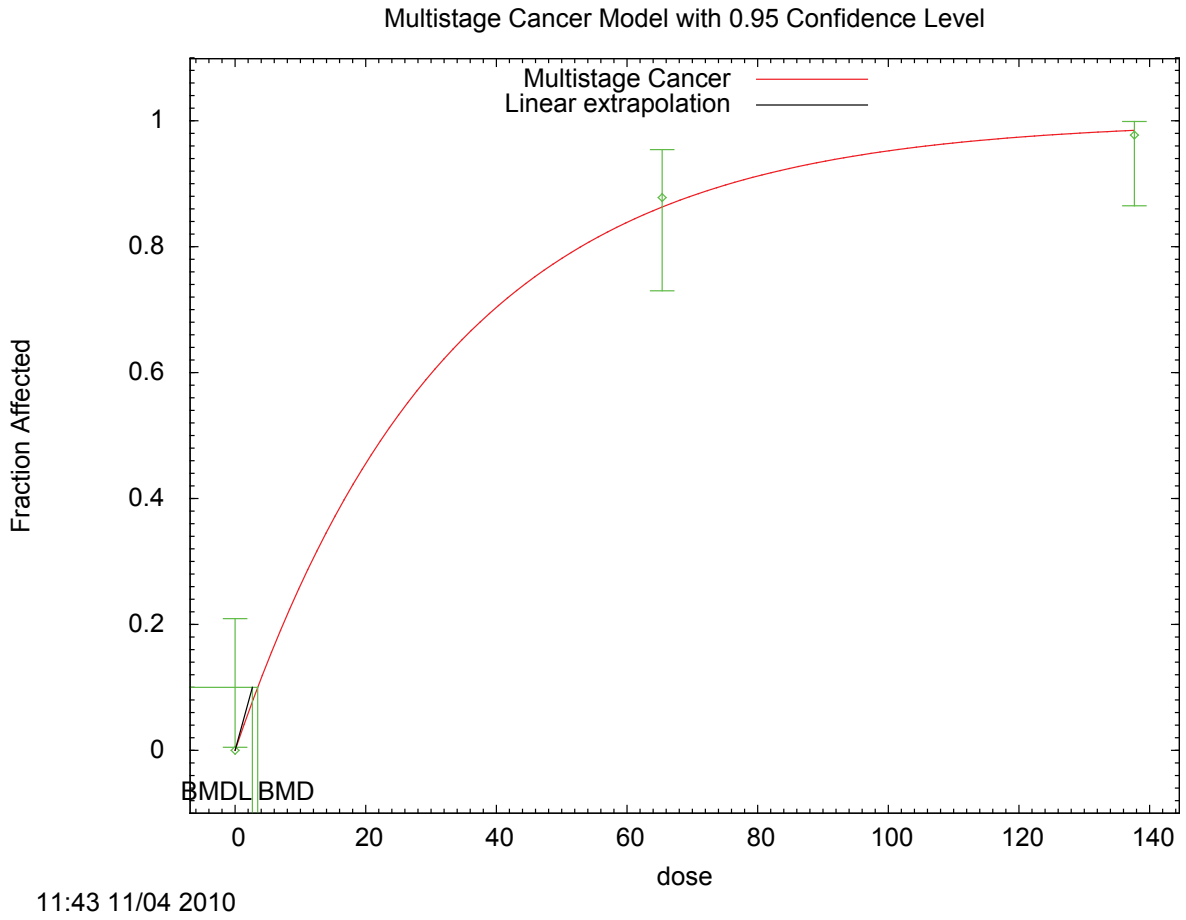

Figure C.2. Multistage-Cancer BMD Model for Hepatocellular Carcinoma in Female Mouse Data (NCI, 1978d)

Text Output for Multistage-Cancer BMD Model for Hepatocellular Carcinoma in Female Mouse Data (NCI, 1978d)

```
=============================================================================
        Multistage Cancer Model. (Version: 1.9;  Date: 05/26/2010)
        Input Data File: C:/36/NCI_1978_new_Liver_HepaCarc_mouse_F_MultiCanc2_1.(d)
        Gnuplot Plotting File:
C:/36/NCI_1978_new_Liver_HepaCarc_mouse_F_MultiCanc2_1.plt
                                                Thu Nov 04 12:43:27 2010
 =============================================================================

 Incidence_of_Hepatocellular_Carcinoma_in_Female_B6C3F1_Mice
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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

   The parameter betas are restricted to be positive
```

```
  Dependent variable = DichEff
  Independent variable = Dose

Total number of observations = 3
Total number of records with missing values = 0
Total number of parameters in model = 3
Total number of specified parameters = 0
Degree of polynomial = 2


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



                 Default Initial Parameter Values
                    Background =     0.101802
                       Beta(1) =    0.0274117
                       Beta(2) =            0


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -Background    -Beta(2)
                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            *               *                   *
        Beta(1)        0.0304142            *               *                   *
        Beta(2)                0            *               *                   *

* - Indicates that this value is not calculated.


                       Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
    Full model        -19.9753         3
  Fitted model        -20.0879         1      0.225146      2          0.8935
 Reduced model        -57.3584         1       74.7661      2         <.0001

          AIC:         42.1758


                                Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size          Residual
  ---------------------------------------------------------------------------
```

```
    0.0000     0.0000         0.000     0.000          19        0.000
   65.3846     0.8631        35.388    36.000          41        0.278
  137.6762     0.9848        43.332    43.000          44       -0.409

 Chi^2 = 0.24      d.f. = 2        P-value = 0.8849


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        3.46419

            BMDL =        2.64773

            BMDU =        7.22885

Taken together, (2.64773, 7.22885) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =     0.0377682
```

APPENDIX D. REFERENCES

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