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Provisional Peer Reviewed Toxicity Values for

p-Nitrotoluene (4-Nitrotoluene)
(CASRN 99-99-0)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
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Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level
MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration
p-RfD	provisional oral reference dose

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

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR *p*-NITROTOLUENE (4-NITROTOLUENE) (CASRN 99-99-0)

Background

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

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

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

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

Disclaimers

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

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

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

The HEAST (U.S. EPA, 1997) listed a subchronic oral RfD of $1x10^{-1}$ mg/kg-day and a chronic oral RfD of $1x10^{-2}$ mg/kg-day for *p*-nitrotoluene. The assessments were derived by analogy from a LOAEL of 200 mg/kg-day for spleen lesions in rats gavaged with *o*-nitrotoluene for six months (Ciss et al., 1980), and included an uncertainty factor (UF) of 1000 (10 for extrapolation from animal data, 10 for sensitive individuals, and 10 for the use of a LOAEL) for the subchronic RfD, and 10,000 (including an additional UF of 10 for the use of a subchronic study) for the chronic RfD. The source document was a Health and Environmental Effects Profile (HEEP) for nitrotoluenes (U.S. EPA, 1986). Aside from the HEEP, no additional relevant documents were included in the Chemical Assessment and Related Activities (CARA) list (U.S. EPA, 1991a, 1994). *p*-Nitrotoluene was not listed on IRIS (U.S. EPA, 2007) or the Drinking Water Standards and Health Advisories list (U.S. EPA, 2006).

The HEAST (U.S. EPA, 1997) did not list an RfC for *p*-nitrotoluene. The 1986 HEEP contained no information on the inhalation toxicity of *p*-nitrotoluene. Relevant occupational exposure values for all isomers of nitrotoluene included an OSHA (2007) PEL-TWA of 30 mg/m^3 (5 ppm), and ACGIH (2001, 2007) TLV-TWA and NIOSH (2005) REL-TWA of 11 mg/m^3 (2 ppm). All three occupational values included a notation for risk of skin absorption.

p-Nitrotoluene was not listed on the HEAST cancer table (U.S. EPA, 1997). The 1986 HEEP categorized *p*-nitrotoluene as a Group D chemical, not classifiable as to human carcinogenicity, because no human data were available and the animal data were inadequate: a 24-week study that showed an equivocal increase in lung tumors in injected mice (Slaga et al., 1985). IARC (1996) considered *p*-nitrotoluene to be not classifiable as to its carcinogenicity in humans (Group 3), because of inadequate evidence in humans and animals, and limited evidence for genotoxicity in mammalian systems. NTP completed chronic (2002) and subchronic (1992)

oral (feeding) assays for *p*-nitrotoluene in rats and mice, which were used in the preparation of this document.

Neither ATSDR (2007) nor WHO (2007) reviewed the toxicity of *p*-nitrotoluene. A toxicity review on aromatic nitro compounds (Weisburger and Hudson, 2001) was consulted for relevant information. Literature searches were conducted for the period from 1985 to 2007 to identify data relevant for the derivation of a provisional RfD and RfC, and a cancer assessment for *p*-nitrotoluene. Databases searched included the following: TOXLINE, MEDLINE, CANCERLIT, TOXLIT/BIOSIS, RTECS, HSDB, GENETOX, CCRIS, TSCATS, EMIC/EMICBACK, and DART/ETICBACK.

REVIEW OF PERTINENT LITERATURE

Human Studies

No data were located regarding effects in humans following chronic or subchronic exposure to *p*-nitrotoluene. Linch (1974) discussed the cyanosis and hemoglobin-depressing effects of occupational exposure to nine chemicals, including p-nitrotoluene, noting that cyanogenic activity primarily resulted from metabolites produced by either oxidation of the amine or reduction of the nitro group. This study further noted that a subset of workers seemed to be more susceptible to cyanosis and proposed a pre-employment methemoglobin reduction screening test to identify these individuals. Finally, Linch (1974) proposed "biological TLVs" based on urinary excretion data during cyanosis episodes and subsequent cyanosis-free periods. These data were not associated with airborne concentrations or other exposure data, so their applicability to derivation of toxicity values was limited.

Animal Studies

Animal studies for *p*-nitrotoluene included subchronic and chronic oral studies in rats and mice. No subchronic or chronic inhalation bioassays for *p*-nitrotoluene were located in the literature search. However, in an unpublished study, Haskell Laboratories (1972) reported eye irritation in male rats exposed for one hour to atmospheres containing 230 mg/m^3 (41 ppm) of *p*-nitrotoluene.

Short Term Animal Studies

In a two-week study, female $B6C3F_1$ mice (8-12 per group) were gavaged daily with 0, 200, 400, or 600 mg/kg-day of *p*-nitrotoluene in corn oil for two weeks (Burns et al., 1994). Standard toxicity endpoints were evaluated, including clinical signs, body weights, hematology, and serum chemistry. At termination on day 15, gross pathology, organ weights, and histopathology of the brain, liver, thymus, spleen, lungs, kidneys, and lymph nodes also were evaluated. In addition, on day 15, a battery of 12 *in vitro* or *in vivo* tests was administered to evaluate immune function; different sets of mice were exposed to provide sufficient subjects for immune testing. Exposure to *p*-nitrotoluene had no adverse effect on mortality, the incidence of clinical signs, or body weight gain. There were statistically significant trends for small dose-

related increases in relative and absolute liver and spleen weights, but only the increases in relative liver weights in the 600 mg/kg-day group were significantly different from the controls. Liver histopathology, evidenced by reversible swelling of hepatocytes near the central veins, was noted in all mice treated with ≥400 mg/kg-day, and was moderate rather than mild in three of the eight high-dose mice. There was a statistically significant trend for increased reticulocyte counts, but no other effect on hematology. The authors attributed increases in serum albumin and serum total protein to treatment-related dehydration. Mice exposed to *p*-nitrotoluene showed dose-related selective decreases in spleen CD^{4+} and CD^{3+} T helper cells, and IgM antibody-forming cells; these reductions in high-dose mice were statistically significant compared to controls. The calculated stimulation index for the mixed leukocyte response was significantly lower in high-dose mice. The delayed hypersensitivity response to keyhole limpet hemocyanin was reduced in low- and high-dose mice. Dose-related increases in phagocytic activity by peritoneal macrophages showed a statistically significant positive trend. Exposure at the high dose reduced the resistance of mice to *Listeria monocytogenes* infection *in vivo*. The authors concluded that most of the immunological effects of *p*-nitrotoluene exposure were related to adverse effects on CD^{4+} T helper cells. The low dose of 200 mg/kg-day was a LOAEL for the delayed hypersensitivity response in mice gavaged for two weeks.

Two-week studies also were conducted by NTP (1992; Dunnick et al., 1994) in rats and mice as range-finding studies for subsequent 13-week studies. Groups of F344/N rats (5 per gender per dose) were fed diets containing 0, 1250, 2500, 5000, 10,000 or 20,000 ppm of *p*-nitrotoluene (purity >96%) for two weeks. The average daily intakes of *p*-nitrotoluene were reported as 0, 106, 211, 446, 723 or 869 mg/kg-day in male rats and 0, 105, 203, 404, 610 or 611 mg/kg-day in female rats. At necropsy, all rats were examined for gross lesions. Representative portions of gross lesions and liver, kidney, spleen, and stomach were examined for histopathology; the thymus of animals in the control and three highest treatment groups also was examined for histopathology. *p*-Nitrotoluene had no effect on survival. Food consumption was markedly reduced in both genders at 10,000 and especially at 20,000 ppm. Body weight gain was reduced at ≥5000 ppm (446 mg/kg-day) in males and ≥10,000 ppm (610 mg/kg-day) in females; animals of both genders actually lost weight at 20,000 ppm. No treatment-related clinical signs and no treatment-related gross lesions were observed. Increased congestion and extramedullary hematopoiesis of the spleen were observed in 1 male at 5000 ppm (446 mg/kg-day) and most rats at ≥10,000 ppm (610 or 723 mg/kg-day). Lymphoid depletion in the thymus and spleen of some rats at ≥10,000 ppm was attributed to the body weight effects.

Similarly, groups of mice (5 per gender per dose) were fed diets containing 0, 675, 1250, 2500, 5000 or 10,000 ppm of *p*-nitrotoluene (purity >96%) for two weeks (NTP, 1992; Dunnick et al., 1994). The average daily intakes of *p*-nitrotoluene were reported as 0, 202, 397, 588, 920 or 1548 mg/kg-day for male mice and 0, 388, 647, 755, 1262 or 2010 mg/kg-day for female mice. At necropsy, all mice were examined for gross lesions and representative portions of gross lesions and the liver were examined for histopathology. *p*-Nitrotoluene had no effect on survival. At the highest dose, food consumption was reduced in both genders, body weight gain was reduced in females, and body weight loss occurred in males. No treatment-related clinical signs were observed. Dose-related increases in relative liver weights were observed in all male and female groups at ≥105 or 106 mg/kg-day. However, no chemically related gross or microscopic lesions were observed. Increased hematopoiesis of the spleen was noted, but was

not dose-related in severity or incidence and was not considered related to treatment by NTP (1992).

Subchronic Animal Studies

In the subchronic rat study, groups of F344/N rats (10 per gender per group) were fed diets containing 0, 625, 1250, 2500, 5000 or 10,000 ppm of *p*-nitrotoluene (purity >96%) for 13 weeks (NTP, 1992; Dunnick et al., 1994). Average daily intakes of *p*-nitrotoluene were reported as 0, 42, 82, 165, 342 or 723 mg/kg-day for male rats and 0, 44, 82, 164, 335 or 680 mg/kg-day for female rats. Animals were observed twice daily for mortality and moribundity, and weekly for feed consumption, body weight, and clinical signs. Clinical chemistry and hematology evaluations were conducted on satellite groups (10/gender/dose) after 1 and 3 weeks of treatment, and on the main groups at termination. Reproductive system evaluations (vaginal cytology, sperm morphology, sperm density, and sperm motility) were conducted on the 0, 2500, 5000, and 10,000 ppm groups at the end of the study. At termination, a complete necropsy was performed on all animals. Organ weights were recorded for heart, liver, lungs, right kidney, thymus, and right testicle. All animals were evaluated for histopathology in gross lesions, tissue masses or suspect tumors and regional lymph nodes, and 40 tissues.

13-week treatment with *p*-nitrotoluene had no effect on rat survival (NTP, 1992; Dunnick et al., 1994). Feed consumption and body weight gain were reduced in both genders at the two highest doses (335-723 mg/kg-day); no clinical signs were related to treatment. Blood analyses in satellite groups after one week of treatment revealed increased erythrocyte counts, hemoglobin concentration, and hematocrit at the two highest doses and reduced reticulocytes at the highest dose in both genders, and reduced platelet counts in females at the three highest doses. After three weeks of treatment, changes at the highest dose included reduced erythrocyte counts in males and hemoglobin concentration in females, and increased methemoglobin in males and nucleated erythrocyte counts in both genders. After 13 weeks of treatment, the blood methemoglobin concentration was increased in both genders at 10,000 ppm (680 or 723 mg/kg-day). Evidence of erythrocyte destruction—reduced erythrocyte count, hemoglobin concentration, and hematocrit in females—also occurred at ≥342 mg/kg-day in males and ≥44 mg/kg-day in females, although in both genders the changes were prominent only at 680 or 723 mg/kg-day. Nucleated erythrocyte counts were elevated in males at 723 mg/kg-day and females at ≥335 mg/kg-day; in females, reticulocyte counts were elevated at ≥723 mg/kg-day.

The only significant clinical chemistry change observed after one week was elevated serum alanine aminotransferase (ALT) in females at the two highest doses (NTP, 1992). After three weeks, bile acids were elevated in high-dose females and serum ALT was elevated at the high dose in both genders. After thirteen weeks, bile acids and serum urea nitrogen were elevated in high-dose males and total protein was decreased in males at ≥82 mg/kg-day and females at ≥335 mg/kg-day.

After 13 weeks of treatment, relative liver and kidney weights were increased in male rats at ≥165 mg/kg-day and females at the highest dose (NTP, 1992; Dunnick et al., 1994). Although average relative testis weights were not significantly changed, small testes in 2/10 males at the highest dose were considered to be potentially related to treatment. Dose-related increases in the

incidence and severity of microscopic lesions were observed in the kidney, spleen, and testis; none of the specific lesions occurred in control animals. Hyaline droplet nephropathy in conjunction with increased α-2u-globulin was observed in all treated males, but no control males. Two other kidney lesions, karyomegaly and Periodic Acid-Schiff staining pigmentation thought to be lipofuscin[1], occurred at ≥165 mg/kg-day and 680 mg/kg-day, respectively, in males and in all treated females. Spleen lesions, including hematopoiesis, hemosiderosis, and congestion were not seen in controls but occurred in all treated groups in both genders. As summarized in Table 1, at the low dose, at least 5/10 females (44 mg/kg-day) and 8/10 males (42 mg/kg-day) exhibited at least one of the spleen lesions; almost all animals at higher doses had these lesions. The severity of the lesions was minimal in the 4 lower dose groups, progressing to mild in the 680 and 723 mg/kg-day groups. Degeneration of the testis, including absence of spermatogenesis, decreased germinal epithelium, and presence of syncytial giant cells representing degenerate spermatids occurred in high-dose males. Epididymal sperm concentration and testicular spermatid head count were reduced in high-dose males. In high-dose females, 9/10 rats had no discernable estrous cycle, although no gross or microscopic histopathology of the ovary was observed. In this 13-week study, the lowest dietary exposure level (42 mg/kg-day in males and 44 mg/kg-day in females), was the LOAEL for high incidence but minimal severity spleen lesions, including hematopoiesis, hemosiderosis, and congestion in male and female rats.

Table 1. Incidence of Treatment-Related Spleen Lesions in Groups of 10 F344/N Rats Following 13-Week Feeding with *p*-Nitrotoluene

	Male rats Dose (mg/kg-day)							Female rats Dose (mg/kg-day)					
Lesion	**0**	**42**	**82**	**165**	**342**	**723**		**0**	**44**	**82**	**164**	**335**	**680**
ppm	0	625	1250	2500	5000	10,000		0	625	1250	2500	5000	10,000
Hematopoiesis	0/10	6/10	9/10	10/10	10/10	10/10		0/10	4/10	4/10	5/10	9/10	10/10
Pigmentation	0/10	6/10	8/10	10/10	9/10	10/10		0/10	5/10	6/10	10/10	10/10	10/10
Congestion	0/10	8/10	10/10	9/10	10/10	10/10		0/10	4/10	6/10	10/10	10/10	10/10

For the subchronic mouse study, NTP (1992; Dunnick et al., 1994) fed groups of $B6C3F_1$ mice (10 per gender per group) diets containing 0, 625, 1250, 2500, 5000 or 10,000 ppm of *p*-nitrotoluene (purity >96%) for 13 weeks. Average daily intakes of *p*-nitrotoluene were reported as 0, 202, 397, 588, 920 or 1548 mg/kg-day for male mice and 0, 388, 647, 755, 1262 or 2010 mg/kg-day for female mice. Mice were analyzed similarly to rats with respect to systemic and reproductive toxicity. However, the subchronic study did not include analyses of hematology or clinical chemistry, and the mouse liver weights included the weight of the gall bladder. Treatment with *p*-nitrotoluene had no effect on mouse survival. Feed consumption and body weight gain were reduced in both genders at the two highest doses, but only the highest dose changes were biologically significant (>10%). No clinical signs were related to treatment. Dose-related increases in relative and/or absolute liver weights occurred in all treated groups. *p*-Nitrotoluene had no adverse effect on the incidence of gross or microscopic lesions in any tissue

[1] Lipofuscin is a brown pigment, a product of oxidation of lipids and lipoproteins.

or on the measured reproductive parameters. Although no liver histopathology was detected in the NTP (1992) subchronic mouse study, evidence from the chronic study discussed below (NTP, 2002) suggested that extended exposure to *p*-nitrotoluene at these dose levels might result in liver histopathology. Thus, the liver weight increases observed in this subchronic study might represent an initial sign of hepatic toxicity. The lowest dietary exposure level in this subchronic study, 625 ppm (202 mg/kg-day in males and 388 mg/kg-day in females), was a minimal LOAEL for increased liver weight in mice of both genders.

Other subchronic studies of *p*-nitrotoluene were limited, but supported the occurrence of hematological, spleen, and testicular effects in exposed rats. Wistar rats (10 per gender per group) were gavaged with 0 or 400 mg/kg-day of *p*-nitrotoluene (99% purity) in 1% methyl cellulose 5 days/week for 6 months (Ciss et al., 1980). After 3 months, groups were subdivided (5 per gender) and combined with treated or untreated animals to evaluate breeding and reproductive effects. This study did not describe randomization or husbandry procedures. Rats were evaluated daily for behavior, clinical signs, mortality, and the number and vitality of offspring. Body weights were recorded weekly. Hematological and clinical chemistry parameters were recorded, although the study did not specify when the blood samples were taken for these tests. Rats surviving at termination and dying prematurely were necropsied, and the major organs were weighed and examined histologically; organ weight changes were not reported quantitatively. F_1 animals also were evaluated histologically at termination. There were no treatment-related effects on mortality or growth. Treatment caused alopecia in female rats, and slight decreases in hemoglobin levels in both genders. In treated males, adverse effects noted in the spleen included increased size and weight; testicular effects included testicular atrophy in nine of ten treated rats and necrosis of the seminiferous tubules in five of nine rats. No histological lesions were detected in females or offspring. Treatment had no effect on reproduction, including numbers of litters or litter size, or in the health of offspring. The single dose level of 400 mg/kg-day in this study was a LOAEL for testicular atrophy and splenomegaly in male rats and for reduced hemoglobin in both genders.

White rats (number and gender not specified) were gavaged with 392 mg/kg-day of *p*-nitrotoluene, daily for 30 days or 3 days per week for 3 months (Kovalenko, 1973; Vasilenko and Kovalenko, 1975). Reduced body weight gain was observed. Treatment had no effect on liver or kidney weights; liver function, measured by bromosulfophthalein retention time; or kidney function as indicated by levels of urea in blood and urine. Rats in both exposure regimes exhibited slight sulfhemoglobinemia and Heinz body formation. In rats treated for 30 days, increased methemoglobin and reticulocyte counts, and decreased hemoglobin concentration and erythrocyte counts, were observed. Elevated methemoglobin was not observed in the 3 month experiment.

Chronic Animal Studies

Chronic studies of *p*-nitrotoluene were conducted by NTP (2002) in rats and mice. Groups of fifty F344/N rats per gender per group were fed diets containing 0, 1250, 2500 or 5000 ppm of *p*-nitrotoluene (purity >99%) for 105-106 weeks (NTP, 2002). Average daily intakes of *p*-nitrotoluene were reported as 0, 55, 110 or 240 mg/kg-day for male rats and 0, 60, 125 or 265 mg/kg-day for female rats. Treatment had no adverse effect on survival, which was

high in all dose groups. Body weight was reduced through most of the study in high-dose males, with mean terminal body weights 11% less than controls, and high-dose females, with mean terminal body weights 25% less than controls. A smaller reduction occurred in females at 60 mg/kg-day and 125 mg/kg-day during the second year. Feed consumption was reduced compared to controls only among high-dose females during the second year. The only clinical sign reported was nasal and eye discharge in treated male and female rats; incidences were not reported. The excretion ratio of urinary metabolites *p*-nitrobenzoic acid and *p*-acetamidebenzoic acid compared to creatinine had a linear relationship to exposure level. Treatment-related non-neoplastic lesions occurred in the kidney, spleen, liver, testis, and uterus (Table 2). Significant kidney lesions included hyaline (proteinaceous) droplet accumulation and lipofuscin pigment accumulation in both genders at ≥55-60 mg/kg-day, mineralization of the distal tubules in females at ≥125 mg/kg-day, and oncocytic hyperplasia of the renal tubules in females at 265 mg/kg-day. NTP considered the hyaline droplets observed in the 2-year study to be unrelated to α2u-globulin nephropathy because female rats generally produce little α2u-globulin, male rats cease production of α2u-globulin by 18 months of age, and the droplets in the 2-year study appeared slightly different from those in the 13-week study (NTP, 1992). Significant increases in the incidences or severity of hematopoietic cell proliferation and hemosiderosis occurred in the spleens of male and female rats exposed to ≥110 and 125 mg/kg-day, respectively. The incidence and severity of atrophy of the testicular germinal epithelium was increased in males at 240 mg/kg-day. The incidence of endometrial cystic hyperplasia of the uterus was significantly elevated in females at ≥125 mg/kg-day. Treatment-related hepatic lesions included basophilic and clear cell foci in males at ≥110 mg/kg-day, and eosinophilic foci in males at 240 mg/kg-day and females at ≥125 mg/kg-day. NTP suggested that the apparent increase in hepatic foci was related to the decreased incidences of leukemic infiltration of the liver that normally obscures detection of foci.

Neoplastic findings among rats are shown in Table 3 (NTP, 2002). The incidence of combined adenomas and carcinomas of the clitoral gland was significantly increased in 125 mg/kg-day female rats compared to concurrent and historical controls, but not in 265 mg/kg-day females. NTP considered this to be a significant treatment-related lesion, since it also was observed in a previous NTP (1994) bioassay on *p*-nitrobenzoic acid, a major metabolite of *p*-nitrotoluene. Furthermore, NTP suggested, based on studies of dietary restriction effects, that the low incidence of clitoral neoplasms in the 265 mg/kg-day group was attributable to low body weight in that group. NTP concluded that there was some evidence for p-nitrotoluene related carcinogenicity in female rats. In males, the incidences of subcutaneous fibroma and of combined subcutaneous fibroma and sarcoma were elevated in the 110 mg/kg-day group compared to concurrent and historical controls. Since neoplasm incidences were not increased in the 240 mg/kg-day group and these types of tumors were not known to be sensitive to body weight reduction, the NTP considered the evidence for skin tumors in male rats to be equivocal. There were significant negative trends for the incidences of testicular adenoma in males, fibroadenoma of the mammary gland in females, and mononuclear cell leukemia in both genders.

The low dose level of 1250 ppm (55 mg/kg-day in males and 60 mg/kg-day in females) in the chronic rat study (NTP, 2002) was a LOAEL for non-neoplastic lesions in the kidney, including hyaline droplet accumulation and lipofuscin pigment accumulation, in both genders.

Table 2. Incidence of Selected Non-neoplastic Lesions in a 2-year Feeding Study of *p*-Nitrotoluene in Male and Female F344/N Rats (NTP, 2002)

	Male Rats				Female Rats			
	0 ppm	1250 ppm	2500 ppm	5000 ppm	0 ppm	1250 ppm	2500 ppm	5000 ppm
Organ: Lesion	Control	55 mg/kg-day	110 mg/kg-day	240 mg/kg-day	Control	60 mg/kg-day	125 mg/kg-day	265 mg/kg-day
Kidney: Tubular hyaline droplet accumulation	2/50 (4%) [2.0][b]	23/50[a] (46%) [1.9]	27/50[a] (54%) [2.0]	18/50[a] (36%) [2.5]	8/50 (16%) [1.8]	41/50[a] (82%) [2.1]	49/50[a] (98%) [2.2]	46/50[a] (92%) [2.4]
Tubular pigmentation	10/50 (20%) [2.3][b]	28/50[a] (56%) [1.5]	47/50[a] (94%) [1.8]	46/50[a] (92%) [2.4]	9/50 (18%) [1.7]	43/50[a] (86%) [1.5]	49/50[a] (98%) [1.9]	50/50[a] (100%) [2.6]
Mineralization					15/50 (30%) [1.1][b]	21/50 (42%) [1.1]	32/50[a] (63%) [1.3]	40/50[a] (80%) [1.8]
Tubular oncocytic hyperplasia	0/50	0/50	0/50	3/50 (6%)	0/50	2/50 (4%)	4/50 (8%)	6/50[a] (12%)
Spleen: Hemosiderin deposition	10/50 (20%)	12/50 (24%)	24/50[a] (48%)	38/50[a] (76%)	24/50 (48%)	32/50 (64%)	45/50[a] (90%)	48/50[a] (96%)
Hematopoietic cell proliferation	9/50 (18%)	13/50 (26%)	19/50[a] (38%)	25/50[a] (50%)	26/50 (52%)	26/50 (52%)	45/50[a] (90%)	43/50[a] (86%)
Liver: Basophilic focus	31/50 (62%)	39/50 (78%)	42/50[a] (82%)	45/50[a] (90%)				
Clear cell focus	20/50 (40%)	27/50 (54%)	30/50[a] (60%)	32/50[a] (62%)				
Eosinophilic focus	5/50 (10%)	5/50 (10%)	5/50 (10%)	19/50[a] (38%)	1/50 (2%)	2/50 (4%)	7/50[a] (14%)	9/50[a] (18%)
Testis: Interstitial cell hyperplasia	8/50 (16%)	15/50[a] (30%)	7/50 (14%)	23/50[a] (26%)				
Germinal epithelial atrophy	7/50 (14%)	11/50 (22%)	8/50 (16%)	30/50[a] (60%)				
Uterus: Endometrial cystic hyperplasia					5/50 (10%)	10/50 (20%)	13/50[a] (26%)	19/50[a] (38%)
Clitoral gland: Hyperplasia					3/50 (6%)	5/50 (10%)	4/50 (8%)	6/49 (12%)

[a] Statistically significant in pairwise test versus current controls

[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

Table 3. Incidence of Selected Neoplastic Tumors in a 2-year Feeding Study of *p*-Nitrotoluene in Male and Female F344/N Rats (NTP, 2002)

	Male Rats				Female Rats			
	0 ppm	1250 ppm	2500 ppm	5000 ppm	0 ppm	1250 ppm	2500 ppm	5000 ppm
Lesion	Control	55 mg/kg-day	110 mg/kg-day	240 mg/kg-day	Control	60 mg/kg-day	125 mg/kg-day	265 mg/kg-day
Clitoral Gland Adenoma or carcinoma					8/50 (16%)	12/50 (24%)	20/50[a] (40%)	8/49 (16.9%)
Historical Incidence Mean Range					86/636 (12.8%) (2-24%)			
Skin Fibroma or fibrosarcoma	1/50 (2%)	2/50 (4%)	9/50 (18%)[a]	1/50 (2%)				
Historical Incidence Mean Range	41/609 (6.3%) (2-14%)							

[a] Statistically significant in pairwise test versus current and historical controls
[b] Statistically significant in pairwise test versus current controls

In the chronic mouse study (NTP, 2002), groups of fifty $B6C3F_1$ mice per gender per group were fed diets containing 0, 1250, 2500 or 5000 ppm of *p*-nitrotoluene (purity >99%) for 105-106 weeks. Average daily intakes of *p*-nitrotoluene were reported as 0, 170, 345 or 690 mg/kg-day for males and 0, 155, 315 or 660 mg/kg-day for females. Treatment with *p*-nitrotoluene had no effect on survival, feed consumption or the incidence of clinical signs in mice. Mean body weights were reduced in the high-dose groups in both genders during most of the study, and in mid-dose males after week 92. Treatment with *p*-nitrotoluene resulted in adverse effects in the liver and lung of mice (Table 4). In the liver, the incidence of hepatocyte focal syncytial alteration was increased in all exposed groups of males in a dose-related manner. This lesion was considered to be of minimal severity in all groups. In the lung, the incidence of alveolar epithelial bronchiolization, an uncommon non-neoplastic lesion, was markedly increased in treated groups in both genders. Not found at all in controls, the incidence increased in a dose-related fashion from 40-66% at the low dose (155-170 mg/kg-day) to near 100% at the high dose (660-690 mg/kg-day). Severity scores also increased slightly with dose in both males and females; however, even in the high-dose groups, the lesions were considered to be of minimal severity, with a maximum score of 1.5/4. NTP considered this lesion to be a metaplastic change that was not a precursor lesion to neoplasia. The combined incidence of alveolar and bronchiolar adenoma or carcinoma of the lung was significantly increased in high-dose males, but was within the historical control range. The incidence of alveolar epithelial hyperplasia, which was considered a precursor lesion to these types of tumors, also was increased in high-dose males, but the increase was not statistically significant. NTP concluded that there was equivocal evidence of carcinogenicity in male mice. The low-dose level of 1250

Table 4. Incidence of Selected Neoplastic Tumors and Non-neoplastic Lesions in a 2-year Feeding Study of p-Nitrotoluene in Male and Female B6C3F$_1$ Mice (NTP, 2002)

	Male Mice				Female Mice			
	0 ppm	1250 ppm	2500 ppm	5000 ppm	0 ppm	1250 ppm	2500 ppm	5000 ppm
Lesion	Control	170 mg/kg-day	345 mg/kg-day	690 mg/kg-day	Control	155 mg/kg-day	315 mg/kg-day	660 mg/kg-day
Neoplastic Tumors								
Lung: Alveolar/bronchiolar Adenoma or carcinoma	8/50[a] (16%)	14/50 (28%)	12/50 (24%)	19/50[b] (38%)	6/50 (12%)	2/50 (4%)	4/50 (8%)	8/50 (16%)
Historical Incidence Mean Range	176/659 (27%) (12-44%)							
Non-neoplastic Lesions								
Lung: Alveolar epithelium Bronchiolization	0/50	20/50[b] (40%) [1.1][d]	30/50[b] (60%) [1.2]	48/50[b] (96%) [1.4]	0/50	33/50[b] (66%) [1.0]	41/50[b] (82%) [1.3]	49/50[b] (98%) [1.5]
Hyperplasia	1/50 (2%)	1/50 (2%)	4/50 (8%)	6/50 (12%)	2/50 (4%)	1/50 (2%)	2/50 (4%)	1/50 (2%)
Liver: Focal syncytial alteration	2/50 (4%)	13/50[c] (26%)	17/50[c] (34%)	33/50[c] (66%)				

[a] Statistically significant positive trend
[b] Statistically significant in pairwise test versus concurrent control
[c] Statistically significant in pairwise test versus concurrent control-(Fisher Exact Test- SRC)
[d] Average severity score of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

ppm (170 mg/kg-day in males and 155 mg/kg-day in females) was a LOAEL for non-neoplastic changes in the liver (hepatocyte focal syncytial alteration) and lung (alveolar epithelial bronchiolization) of both genders.

Reproductive and Developmental Animal Studies

In addition to the data noted in the subchronic animal studies, one dedicated reproductive study was identified. Aso et al. (2005) administered oral gavage doses of 0, 40, 80, or 160 mg/kg-day to groups of 24 Crj:CD(SD)IGS rats of each gender, over two generations. In this comprehensive and well-described study, Aso et al. (2005) noted increased liver and kidney weights in F0 and F1 parents, increased spleen weights in F0 females, and increased salivation in F0 and F1 animals in all groups other than controls. The authors postulated that the increased

salivation was an irritation response. Histopathology of the liver, spleen, and kidney revealed only the spleen and kidney effects noted below in the higher dose groups. Other effects noted at higher doses included the following:

- 80 and 160 mg/kg-day:
  - Lower body weights among F2 pups and F1 male pups
  - Lower brain weights among F1 and F2 males
  - Increased renal hyaline droplets in F0 and F1 males
  - Increased spleen hemosiderin deposition in F0 and F1 females
- 160 mg/kg-day
  - Decreased viability of F1 and F2 pups
  - Lower brain weights among F1 and F2 females
  - Perinatal reduced locomotor activity, eyelid closure, and death among F0 and F1 females
  - Reduced body weights among F1 males
  - Prolonged gestation in F0 females
  - Smaller vaginal opening in F1 females
  - Decreased serum T4 concentration in F1 females
  - Black-colored spleens among F0 an F1 females, and F1 males
  - Increased renal eosinophilic microbodies in F0 and F1 males
  - Dotted pattern on renal surfaces among F1 males

All F1 parent groups, including controls, exhibited a low fertility index. However, no other adverse endocrine or reproductive effects were noted. Other changes noted either were not considered significant or were described as being within the normal range for this strain of rat and, thus, were considered to be incidental.

The investigators (Aso et al., 2005) described the lowest dose, 40 mg/kg-day, as a NOAEL for growth and development of offspring, but concluded that the NOAEL for parental animals was less than 40 mg/kg-day. F0 and F1 adults exhibited increased liver and kidney weights at all doses tested, suggesting that 40 mg/kg-day should be considered a LOAEL because of the combination of effects reported at all dose levels.

Other Information

An injection study reported equivocal evidence for carcinogenicity of *p*-nitrotoluene in mice. Slaga et al. (1985) intraperitoneally injected groups of 30 male A/Jax mice with 0, 1800, 4500 or 9000 mg/kg of *p*-nitrotoluene in corn oil 3 days/week for 8 weeks and observed the mice for an additional 16 weeks. There was a dose-related increase in the percentage of survivors with lung tumors and in the number of tumors per lung. However, tumor rates were not statistically different from the controls. The short treatment and observation periods might have contributed to the statistical non-significance of this study.

Ishido et al. (2004) observed significantly hyperactive behavior among 4 to 5-week old male Wistar rats following a single intracisternal administration of 10 µg *p*-nitrotoluene at 5 days of age. At 8 weeks, DNA array analyses revealed changes in gene expression of the striatum

glutamate and GABA transaminase, the mesencephalic dopamine transporter, and peptide coding.

No pharmacokinetic data were available for *p*-nitrotoluene in humans, but diazo-positive metabolites have been detected in the urine of workers exposed to a mixture of aromatic nitro compounds (IARC, 1996). Studies in rodents indicated rapid absorption of *p*-nitrotoluene in the gastrointestinal tract and rapid excretion, mainly in urine (U.S. EPA, 1986; IARC, 1996). In gavage studies using radio-labeled *p*-nitrotoluene (doses of 2 or 200 mg/kg), gastrointestinal absorption exceeded 82% in rats and 94% in mice (Chism et al., 1984; NTP, 2002). Most of the radioactivity (>80%) was excreted in urine, feces, and expired air during the first 24 hours. During the first 72 hours, >90% of the dose was excreted in urine and <7% in feces.

Urinary metabolites of radio-labeled *p*-nitrotoluene were quantified in F344/N rats and $B6C3F_1$ mice 48 hours after a single 200 mg/kg gavage dose (NTP, 2002). In rats, the metabolites included *p*-nitrobenzoic acid, *p*-acetamidebenzoic acid, *p*-nitrohippuric acid, and *p*-nitrobenzyl mercapturate. In mice, the 48-hour urinary metabolites were: *p*-nitrohippuric acid, 2-methyl-5-nitrophenyl sulfate, 2-methyl-5-nitrophenyl glucuronide, *p*-nitrobenzoic acid, and *p*-acetamidebenzoic acid. These results and others reviewed in U.S. EPA, 1986 indicated that microsomal oxidation of the methyl group to form *p*-nitrobenzyl alcohol and subsequently *p*-nitrobenzoic acid was the major first step in the metabolic pathway in rats, whereas ring-hydroxylation was significant in mice; in both species, phase 2 conjugation reactions occurred. NTP (2002) considered the mercapturate metabolite in rats to be an indication that a potentially reactive benzylating intermediate was formed during metabolism of *p*-nitrobenzene in rats.

Biliary excretion of *p*-nitrotoluene has been investigated in rats. Six hours after a single gavage dose of 200 mg/kg of radio-labeled *p*-nitrotoluene, 7.7% of the dose was recovered in the bile of male F344/N rats (NTP, 2002). The biliary metabolite profile included, as a percentage of dose, 4.4% S-(*p*-nitrobenzyl)-glutathione, 2.5% *p*-nitrobenzoic acid, and 0.4% *p*-nitrobenzyl glucuronide. In a 12-hour study, male F344 rats excreted 9.8% and females excreted 1.3% of a single oral dose of 200 mg/kg of *p*-nitrotoluene into bile (Chism and Rickert, 1985). In addition to those previously mentioned, the major biliary metabolites included *S*-(nitrobenzyl)-*N*-acetylcysteine, nitrohippuric acid, acetamidebenzoic acid, and 2-methyl-5-nitrophenyl glucuronide. In rats subjected to bile duct cannulation, hepatic macromolecular covalent binding of *p*-nitrotoluene was reduced by 78% in males and 45% in females, indicating that the enterohepatic circulation contributed to the bioactivation of *p*-nitrotoluene (Chism and Rickert, 1985). This gender difference may have contributed to the different patterns of toxicity in male and female rats.

Potential uterotropic activity was analyzed in groups of 5-18 CD Sprague-Dawley rats that received single intraperitoneal doses of 0.01-1000 mg/kg of *p*-nitrotoluene in corn oil (Smith and Quinn, 1992). Doses of 10 mg/kg or less of p-nitrotoluene were without effect, but doses of 30 and 100 mg/kg increased uterine weights, compared to controls, without producing overt toxicity; doses of 1000 mg/kg were clearly toxic. Rats receiving 1000 mg/kg became heavily sedated and remained in that condition until termination 24 hours later. The authors concluded that *p*-nitrotoluene had weak estrogenic activity.

In vitro, the nitrotoluenes demonstrated some ability to convert hemoglobin to methemoglobin. *p*-Nitrotoluene was more potent than m- or *o*-nitrotoluene in inducing methemoglobin formation in freshly-drawn sheep erythrocytes (French et al., 1995). Methemoglobin levels about three times higher than in controls were produced by treatment with 2.5 millimolar (mM) *p*-nitrotoluene, 10 mM *m*-nitrotoluene or 20 mM *o*-nitrotoluene. The presence of a nicotinamide adenine dinucleotide phosphate (NADP) bioactivation system had no significant effect on the activity of *p*- or *o*-nitrotoluene, but slightly increased the activity of *m*-nitrotoluene. The methemoglobin-forming potency of *p*-nitrotoluene in sheep erythrocytes was calculated to be about five times higher than *o*-nitrotoluene, and 1.6 times higher than *m*-nitrotoluene or aniline.

In vitro, *p*-nitrotoluene yielded primarily negative, but a few positive, results for mutagenicity in bacteria and mammalian systems (U.S. EPA, 1986; IARC, 1996). *p*-Nitrotoluene induced differential toxicity in *Bacillus subtilis rec* strains without metabolic activation, and gave conflicting results in reverse mutation assays with *Salmonella typhimurium* strain TA100 with or without activation. Mutation assays were negative in other strains of *S. typhimurium* (TA92, TA98, TA1535, TA1537 or TA1538). *p*-Nitrotoluene induced gene conversion in *Saccharomyces cerevisiae* and mutations in the *tk* locus of cultured mouse L5187Y cells only with activation.

p-Nitrotoluene also induced sister chromatid exchanges in Chinese hamster ovary (CHO) cells with or without metabolic activation. Assays for chromosomal aberration were weakly positive in CHO cells with activation, and negative in CHO cells and Chinese hamster liver cells without activation. *In vitro*, *p*-nitrotoluene induced chromosomal aberrations in human peripheral lymphocytes at a concentration of ≥5 mmol/L (Huang et al., 1996). Without activation, *p*-nitrotoluene did not induce unscheduled DNA synthesis in cultured rat primary hepatocytes, pachytene spermatocytes or round spermatids, or in hepatocytes of gavaged rats. *p*-Nitrotoluene did not induce micronucleus formation in mice exposed *in vivo*. In livers of gavaged male rats, *p*- nitrotoluene covalently bound to RNA and protein, but did not bind to DNA. These results provided limited evidence of the genotoxicity of *p*-nitrotoluene.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR *p*-NITROTOLUENE

No human data were available for the chronic or subchronic oral toxicity of *p*-nitrotoluene. Subchronic and chronic oral exposure studies in rodents demonstrated multiple organ toxicity, presumably related to the bioactivation of *p*-nitrotoluene *in situ*. Non-neoplastic lesions in rats involved the kidney, liver, testis, and spleen. In rats, the conversion of hemoglobin to methemoglobin, followed by erythrocyte destruction and enhanced erythropoiesis was a significant result of exposure, which led to secondary effects in the spleen. In mice, the liver and lung were the primary target organs. It seemed prudent to consider any significant increase in methemoglobin-related effects in laboratory rodents to be a potentially adverse effect because rats and mice appeared to be more efficient than humans in reducing methemoglobin to hemoglobin (Smith, 1991).

Derivation of a Subchronic RfD

In the 2-generation study in rats, Aso et al. (2005) described the lowest dose, 40 mg/kg-day, as a NOAEL for growth and development of offspring. However, in F0 and F1 adults, the data suggested that 40 mg/kg-day should be considered a LOAEL because of the following combination of effects reported at all dose levels:

- Increased liver weights in F0 and F1 rats
- Increased kidney weights in F0 and F1 rats
- Increased pituitary weights in F0 female rats
- Increased salivation, suggesting irritation, throughout administration in F0 and F1 rats

Aso et al. (2005) and Yamaski et al. (2005) reported that gavage administration of the same doses of *p*-nitrotoluene to rats resulted in no obvious effects on the endocrine system or reproductive toxicity.

In the 13-week feeding study, the lowest dietary level, 625 ppm (42 mg/kg-day in male rats and 44 mg/kg-day in females) was a subchronic LOAEL with high incidences of spleen lesions including hematopoiesis, hemosiderosis, and congestion in male and female rats (NTP, 1992; Dunnick et al., 1994). At this dose, 60% - 80% of the rats exhibited the critical spleen effects, suggesting it would not be a useful point of departure for deriving a p-RfD. In female rats exposed at this dietary level, there was evidence of erythrocyte destruction, including reduced erythrocyte count, hemoglobin concentration, and hematocrit, and lesions of the kidney, including karyomegaly and lipofuscin accumulation. In male rats, these erythrocyte effects were observed at doses of $\geq$5000 ppm (342 mg/kg-day), and relevant kidney effects were observed at 1250 ppm (82 mg/kg-day).

NTP (1992) reported testicular degeneration at the highest subchronic dose (723 mg/kg-day) in male F344/N rats. The highest dose in females (680 mg/kg-day) disrupted the estrous cycle but did not cause ovarian histopathology. These findings were supported by data from a subchronic gavage study in rats (Ciss et al., 1980) in which nine of ten male rats gavaged with 400 mg/kg-day, 5 days/week for 6 months, exhibited testicular atrophy.

Other researchers (Kovalenko, 1973; Vasilenko and Kovalenko, 1975) observed increased methemoglobin and Heinz bodies, and decreased hemoglobin and erythrocytes in rats gavaged with 392 mg/kg-day for 30 days. Exposure at the same dose for 3 days/week for 3 months resulted in less severe effects: Heinz bodies, but no increase in methemoglobin concentrations. Ciss et al. (1980) observed splenomegaly and testicular atrophy in Wistar rats gavaged with 400 mg/kg-day for 6 months, but found that a 3-month exposure had no adverse effect on reproductive function in rats.

In the 13-week feeding study in $B6C3F_1$ mice (NTP, 1992; Dunnick et al., 1994), 625 ppm (202 mg/kg-day in males and 388 mg/kg-day in females) was a LOAEL for increased liver weight in mice of both genders. No reproductive effects were observed in mice exposed to up to 10,000 ppm in the diet (1548 mg/kg-day in males and 2010 mg/kg-day in females).

Since available data reported relevant effects in rat spleens at lower doses than effects in mice, the appropriate basis for the subchronic p-RfD for *p*-nitrotoluene was the 13-week rat data, which demonstrated a LOAEL of 625 ppm or 42 mg/kg-day in males or 44 mg/kg-day in females (NTP, 1992; Dunnick et al., 1994). Although the Aso et al. (2005) study also demonstrated minimal effects at 40 mg/kg-day, the much higher incidence of more pronounced effects noted in the NTP (1992) study suggested this study and the subsequent paper by Dunnick et al. (1994) should be considered the key data sources for deriving the subchronic p-RfD.

Benchmark dose modeling was performed for derivation of the subchronic p-RfD for *p*-nitrotoluene. The modeling was conducted according to draft EPA guidelines (U.S. EPA, 2000) using Benchmark Dose Software (BMDS) Version 1.4.1. The benchmark dose modeling provided the benchmark dose (BMD) and its 95% lower confidence limit (BMDL) associated with a benchmark response (BMR) of 10%. The BMDL then was used as the as the point of departure (POD) in determining the subchronic p-RfD. A BMR of 10% extra risk was considered appropriate for derivation under the assumption that it represented a minimally significant biological response level for the observed spleen effects. Details of BMD analyses for the subchronic spleen effects data are in Appendix 1.

Benchmark dose modeling was used to analyze the data sets for spleen hematopoiesis and pigmentation in male rats. In addition, the LOAEL of 42 mg/kg-day was considered for the spleen congestion data. Attempts to model the spleen congestion data were model-dependent (Table A1-2), as might be expected when attempting to extrapolate from a very high incidence rate (80%) at the lowest dose (U.S. EPA, 2000). These endpoints in male rats were selected because the data (Table 1) demonstrated consistently higher response rates in males than females at equivalent doses. All BMD models were used with standard defaults to analyze each data set. Once the models were assessed based on the goodness-of-fit p-values, the absolute values of relevant scaled residuals corresponding to the data point closest to BMR, the Akaike Information Criterion (AIC), and visual analyses of the BMD curves, it was concluded that the quantal-linear model best fit the data for spleen hematopoiesis and the log-logistic model best fit the spleen pigmentation data. These models were used to calculate the following $BMDL_{10}$ values:

- Spleen hematopoiesis: 2.6 mg/kg-day
- Spleen pigmentation: 1.1 mg/kg-day

For each endpoint, another model could have been selected, based on the criteria for goodness-of-fit. However, the BMDLs calculated using these alternate models were nearly five to six times higher.

- The quantal-quadratic model of the hematopoiesis data fit the data as well as the quantal-linear model, but it calculated a $BMDL_{10}$ nearly five times higher than any of the other well-fitted models for this or the other endpoints. BMD technical guidance (US EPA, 2000) recommends choosing the model with the lowest BMDL when the BMDLs from models with acceptable fit ($p>0.1$) differ by more than a factor of 3.
- Based on curve fit, either the Log-logistic or Probit-log models could have been chosen to represent the spleen pigmentation data. The log-logistic curve had a larger p-value and comparable local scaled residuals and AIC values. We chose the log-logistic model primarily because it calculated a $BMDL_{10}$ nearly six times lower than the probit-log model.

Uncertainties in this analysis provided additional support for using this more conservative approach.

The fact that these $BMDL_{10}$ values were 16 to nearly 40 times below the lowest tested doses raised concerns about the reliability of the curves used in their calculation and suggested that the BMDs and lower bounds calculated in these cases are not well defined and thus highly model dependent. Despite these uncertainties, the $BMDL_{10}$ of 1.1 mg/kg-day for spleen pigmentation was selected as the point of departure (POD) because it was the lower $BMDL_{10}$ value among the two modeled endpoints.

To calculate the subchronic p-RfD, we used the $BMDL_{10}$ of 1.1 mg/kg-day for spleen pigmentation as the POD and applied the following uncertainty factors:

- 10 for using animal data
- 10 for human variability
- 3 for database limitations. The database for *p*-nitrotoluene included comprehensive subchronic studies in two animal species that were supported by chronic studies in two animal species and a 2-generation reproductive study. However, an UF greater than one was applied because of the lack of sufficient developmental studies.

Subchronic p-RfD = (1.1 mg/kg-day)/300 = **$3.7x10^{-3}$ mg/kg-day**

Rounding this value to one significant figure resulted in a subchronic p-RfD = 0.004 mg/kg-day or **$4x10^{-3}$ mg/kg-day**.

Confidence in the key subchronic study (NTP, 1992) was medium. The well-documented study evaluated ten animals per group in two species, analyzed nearly all potential critical endpoints, and the subchronic data was supported by observations from a chronic study in the same animal species (NTP, 2002). However, the subchronic study did not include a NOAEL dose and several spleen lesions were observed in 60%-80% of animals at the lowest dose tested. Confidence in the database was medium. The key subchronic study was supported by adequate chronic studies in rats and mice (NTP, 2002), and by subchronic gavage studies in rats (Ciss, et al, 1980; Kovalenko, 1973; Vasilenko and Kovalenko, 1975) that were not adequately designed or reported. The Aso et al. (2005), two-generation study in rats, the key subchronic study (NTP, 1992), and one supporting study (Ciss, et al, 1980) provided some information about effects on reproductive function in rats. However, no developmental toxicity studies were available. The overall weakness of the database was somewhat mitigated by the fact that the toxic effects of *p*-nitrotoluene were similar to those reported for analogous arylnitro or arylamino compounds (Weisburger and Hudson, 2001). Overall confidence in the provisional subchronic p-RfD for *p*-nitrotoluene was medium.

Derivation of a Chronic RfD

In the 2-year NTP (2002) study, exposure of F344/N rats to the lowest dietary concentration of *p*-nitrotoluene, 1250 ppm, corresponding to 55 mg/kg-day in males and 60 mg/kg-day in females, resulted in lesions of the kidney including tubular hyaline droplet accumulation and lipofuscin pigmentation in both genders. NTP considered the hyaline droplet

nephropathy observed in this study to be unrelated to male rat-specific α2u-globulin nephropathy. For this reason, the kidney lesions in male and female rats were considered to be potentially relevant to humans. The middle dose in this study, 2500 ppm, corresponding to 110 or 125 mg/kg-day for males and females, respectively, caused an increase in spleen lesions similar to those observed in the subchronic study (NTP, 1992), including hemosiderin deposition and hematopoietic cell proliferation, and liver foci in both genders, and endometrial cystic hyperplasia of the uterus and mineralization of kidney tubules in females. At the highest dietary concentration, 5000 ppm, females (265 mg/kg-day) showed an increase in tubular oncocytic hyperplasia of the kidney, and males (240 mg/kg-day) exhibited testicular atrophy.

The chronic NTP (2002) study in $B6C3F_1$ mice reported uncommon lesions in the lung (alveolar epithelial bronchiolization) in both genders at the lowest dietary concentration, 1250 ppm, corresponding to 170 or 155 mg/kg-day for males and females, respectively, and an increase in focal syncytial alteration in the livers of males at the same level.

Benchmark dose modeling (U.S. EPA, 2000) was used calculate potential points of departure (POD) for deriving a chronic RfD for *p*-nitrotoluene using the data sets for kidney tubule pigmentation in female rats and hyaline droplet nephropathy in female and male rats. Details of the BMD analyses for chronic exposures are presented in Appendix 2. These endpoints were selected because they appeared to be the most sensitive effects in the chronic study for *p*-nitrotoluene toxicity (see Table 2). All BMD models were used with standard defaults to analyze each data set. Following review of p-values and absolute values of scaled residuals for the data points closest to the 10% benchmark response, and visual analyses of BMD curves, it was concluded that the high dose data for hyaline droplet nephropathy in both genders was causing the BMD curves to fit the data poorly in the low dose region. One explanation for this may be that gross toxicity at the highest doses might be confounding these high dose effects. BMD analyses for these endpoints were repeated using only data from the two lower dose groups and controls.

Based on review of p-values, absolute values of the scaled residuals, Akaike Information Criterion (AIC), and visual analyses of BMD curves, it was concluded that a quantal-linear model best fit the data for both kidney endpoints in female rats, while the log-logistic model best fit the hyaline droplet nephropathy in male rats. These models were used to calculate the following $BMDL_{10}$ values:

- Kidney tubule pigmentation in female rats: 2.7 mg/kg-day
- Hyaline droplet nephropathy in female rats: 3.0 mg/kg-day
- Hyaline droplet nephropathy in male rats: 6.3 mg/kg-day

The fact that these $BMDL_{10}$ values were approximately ten to twenty times below the lowest tested doses raised concerns about the reliability of the curves used in their calculation. Based on the estimated $BMDL_{10}$ values, the kidney tubule pigmentation in female rats and the hyaline droplet nephropathy BMDLs were essentially the same. The relative biological significance of these effects is unknown, thus the $BMDL_{10}$ of 2.7 mg/kg-day for kidney tubule pigmentation was chosen as the POD because it represented the lower of the two values.

A chronic p-RfD of $8x10^{-3}$ mg/kg-day, based on the $BMDL_{10}$ POD of 2.7 mg/kg-day for the kidney tubule pigmentation in female rats was derived with the application of the following uncertainty factors:

- 10 for using animal data
- 10 for human variability
- 3 for database limitations. The database for p-nitrotoluene included comprehensive subchronic and chronic studies in two animal species, and a two-generation reproductive study. However, a UF of 3 was used because of the lack of sufficient developmental studies.

However, this value was higher than the subchronic p-RfD of $4x10^{-3}$ mg/kg-day, derived using data from the spleen effects observed at all doses following 13 weeks of exposure in rats. Similar spleen effects were observed in the chronic study only at higher doses, 110 and 125 mg/kg-day for males and female rats, indicating the subchronic p-RfD should protect against spleen toxicity, even following long term exposure. Therefore, the subchronic p-RfD was considered for use as the chronic p-RfD. Application of an UF for extrapolation from subchronic to chronic exposure was considered unnecessary. In addition, the subchronic value would be more protective than the potential chronic value ($8x10^{-3}$ mg/kg-day) for kidney pathology observed at all doses in the chronic study. Thus, the subchronic p-RfD of $4x10^{-3}$ mg/kg-day was selected as the chronic p-RfD.

Confidence statements for the subchronic p-RfD also generally applied to the chronic value, because the subchronic data were used to derive the chronic p-RfD. Confidence was further enhanced by the well-documented chronic study (NTP, 2002), which evaluated 50 animals per group in two species, and analyzed nearly all potential critical endpoints. However, the doses used in the chronic studies were higher than doses in the subchronic studies and neither study identified a NOAEL, requiring BMD modeling to calculate a POD much lower than the range of experimental data. Confidence in the database was medium. The database included comprehensive subchronic (NTP, 1992) and chronic (NTP, 2002) studies in rats and mice, a two-generation study in rats (Aso et al., 2005), and several supporting studies that provided some information about effects on reproductive function in rats. However, no developmental toxicity studies were available. The overall weakness of the database was somewhat mitigated by the fact that the toxic effects of *p*-nitrotoluene were similar to those reported for analogous arylnitro or arylamino compounds (Weisburger and Hudson, 2001). Confidence in the provisional chronic RfD value for *p*-nitrotoluene was medium.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR *p*-NITROTOLUENE

No data were located for the subchronic or chronic inhalation toxicity of *p*-nitrotoluene in humans or animals. In addition, no relevant information was available for *m*- or *o*-nitrotoluene, which eliminated the possibility of deriving a p-RfC by analogy to these compounds. Although provisional oral RfDs were derived for *p*-nitrotoluene and some pharmacokinetic data were available, the observation that irritant, portal of entry effects had been observed in male rats exposed for one hour to atmospheres containing 230 mg/m^3 (41 ppm) of *p*-nitrotoluene (Haskell

Labs, 1972) ruled out a route-to-route extrapolation for this compound. Therefore, it was not feasible to derive subchronic or chronic inhalation p-RfC values for *p*-nitrotoluene.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR *p*-NITROTOLUENE

Weight-of-Evidence Descriptor

No human carcinogenicity data were located for *p*-nitrotoluene. The NTP (2002) chronic feeding study concluded there was some evidence for increased tumors, adenomas, and carcinomas of the clitoral gland in female F344/N rats exposed at 125 mg/kg-day. NTP noted that these tumors typically were sensitive to reductions in body weight, and concluded that the lack of tumor increase at the higher dose (265 mg/kg-day) was caused by the reduced body weight, 25% lower than controls at termination, in that group. The NTP (2002) chronic feeding study also concluded there was equivocal evidence for skin tumors, fibromas and fibrosarcomas in male rats exposed at 110 mg/kg-day; the evidence for these tumors was considered equivocal because high-dose males were not affected and these tumor types were not known to be sensitive to reduced body weight. However, these NTP conclusions did not seem to have considered the possibility that high dose tumors were lower than expected because of gross toxicity.

NTP (2002) also reported a significant increase of alveolar and bronchiolar adenomas and carcinomas in male mice exposed at the highest dose, 690 mg/kg-day. However, NTP (2002) considered the relationship between these tumors and exposure to p-nitrotoluene equivocal because the incidence of these tumors was within the range for historical controls on the same diet, although it exceeded the range for historical controls on the previous standard NTP diet. The finding of lung tumors in male mice was supported somewhat by the nonsignificant increases in lung tumors in mice injected for 8 weeks and observed for only 16 weeks (Slaga et al., 1985) and by the small but dose-dependent increases in alveolar epithelial hyperplasia in male mice, which NTP (2002) considered a precursor lesion. NTP (2002) found no evidence for carcinogenicity in female mice exposed at $\leq$660 mg/kg-day.

In vitro tests provided some evidence that *p*-nitrotoluene was mutagenic. *p*-Nitrotoluene gave positive results in the *B. subtilis rec* assay without metabolic activation and conflicting results for reverse mutation in *S. typhimurium* strain TA100 with or without activation. With activation, *p*-nitrotoluene induced gene mutations in mouse L5187Y cells. The compound was a weak inducer, with metabolic activation, of chromosomal aberrations in CHO cells and human lymphocytes *in vitro*. In livers of gavaged rats, *p*-nitrotoluene bound to RNA and protein, but not to DNA.

The NTP (2002) finding of increased incidence of clitoral neoplasms in female F344/N rats exposed to *p*-nitrotoluene was supported by the similar increase observed in the NTP (1994) feeding bioassay for *p*-nitrobenzoic acid, one of its metabolites. This finding suggested that metabolic activation of *p*-nitrotoluene might be required for the induction of clitoral tumors. Studies of the metabolites of *p*-nitrotoluene also provided evidence for a mutagenic mode of action. The compound was readily absorbed through the gastrointestinal tract and oxidized by

mutagen P450 in the liver and other tissues. Although the parent compound did not appear to be a potent mutagen, metabolites were potentially mutagenic. NTP (2002) considered the finding of mercapturate metabolites in the urine of gavaged rats to be an indication that a potentially reactive benzylating intermediate was formed during the metabolism of *p*-nitrotoluene. The results of bile duct cannulation studies by Chism and Rickert (1985) also indicated that bioactivation of *p*-nitrobenzoic acid in the liver increased the amount of covalent binding to hepatic macromolecules.

The findings of increased tumor incidences in both male and female rats, as well as in male mice, a second animal species, plus the positive findings in three different organs (clitoral gland, skin, and lung) indicated that p-nitrotoluene is "*likely to be carcinogenic to humans*", according to the U.S. EPA (2005) guidelines. However, p-nitrotoluene was on the low end of the range for this descriptor.

Quantitative Estimates of Carcinogenic Risk

Cancer dose-response modeling was performed on the incidences of clitoral tumors in female rats (Table 5), skin tumors in male rats (Table 6), and lung tumors in male mice (Table 7) found in the NTP (2002) chronic feeding study. Dose-response modeling was performed using the U.S. EPA (2005) methodologies. Since the weight of evidence suggested, but did not clearly demonstrate, that tumors might be related to a mutagenic mode of action involving bioactivated *p*-nitrotoluene, the default linear model was used for dose-response modeling for each tumor type. In accordance with the 2005 Guidelines, the lower bound on the dose estimated to produce a 10% increase in tumor incidence over background (LED_{10}) was estimated for each tumor type, using the U.S. EPA (2000) benchmark dose methodology. Linear extrapolations to the origin were performed by dividing each LED_{10} into 0.1 (10%). The slope factor ($0.1/LED_{10}$) from the BMD methodology provided estimates of the slope factor for each animal tumor type. The slope factor values, based directly on the animal tumor data, were adjusted to human values by multiplying the animal value by the ratio of human to animal body weight raised to the 1/4 power.

As summarized in Table 5, the human slope factor estimated from incidence data for clitoral tumors observed in rats was 1.6×10^{-2} $(mg/kg\text{-}day)^{-1}$. Modeling of these data was accomplished by dropping data from the high dose group because clitoral tumors in rats were known to be sensitive to body weight, and terminal body weight was reduced 25% in the high-dose females. The human equivalent slope factor based on incidence of skin tumors in male rats, was 7.7×10^{-3} $(mg/kg\text{-}day)^{-1}$ (Table 6). The data for the high-dose group were dropped to achieve adequate model fit with the algorithm.

The calculation based on lung tumors in male mice, summarized in Table 7, resulted in a slope factor of 4.3×10^{-3} $(mg/kg\text{-}day)^{-1}$.

Since the clitoral tumors in female rats appeared most sensitive to induction by p-nitrotoluene, these data were used to estimate a provisional oral slope factor of **1.6×10^{-2} $(mg/kg\text{-}day)^{-1}$** for *p*-nitrotoluene.

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Table 5. LED_{10} Values Based on Clitoral Tumor Incidences in Female F344/N Rats (NTP, 2002)

Tumor Location & Type	Incidence 0 mg/kg-day	Incidence 60 mg/kg-day	Incidence 125 mg/kg-day	Incidence 265 mg/kg-day		rat LED_{10} (mg/kg-day)	rat $0.1/LED_{10}$ $(mg/kg\text{-}day)^{-1}$	Human $0.1/LED_{10}$ $(mg/kg\text{-}day)^{-1}$
Adenoma or carcinoma (Clitoris)	8/50	12/50	20/50	8/49		25.6	$3.9x10^{-3}$	$1.6x10^{-2}$

Doses listed are daily average doses
For the purposes of modeling, the highest dose data were omitted since this tumor type was sensitive to the reduced body weight at this dose (NTP, 2002).
Human value ($0.1/LED_{10}$) calculated as: rat value ($0.1/LED_{10}$) x $(W_{hum} / W_{rat})^{1/4}$ where W_{hum} = 70 kg (human reference body weight), W_{rat} = 0.220 kg (time weighted average female rat body weight in this study)
Rat LED_{10} calculated using multistage model (polydegree of 1 chosen using algorithm in U.S. EPA, 2000)

Table 6. LED_{10} Values Based on Skin Tumor Incidences in Male F344/N Rats (NTP, 2002)

Tumor Location & Type	Incidence 0 mg/kg-day	Incidence 55 mg/kg-day	Incidence 110 mg/kg-day	Incidence 240 mg/kg-day		rat LED_{10} (mg/kg-day)	rat $0.1/LED_{10}$ $(mg/kg\text{-}day)^{-1}$	Human $0.1/LED_{10}$ $(mg/kg\text{-}day)^{-1}$
Fibroma or fibrosarcoma (Skin)	1/50	2/50	9/50	1/50		46.7	$2.1x10^{-3}$	$7.7x10^{-3}$

Doses listed are daily average doses
Human value ($0.1/LED_{10}$) calculated as: rat value ($0.1/LED_{10}$) x $(W_{hum} / W_{rat})^{1/4}$ where W_{hum} = 70 kg (human reference body weight), W_{rat} = 0.378 kg (time weighted average male rat body weight in this study)
Rat LED_{10} calculated using multistage model (polydegree of 1 chosen using algorithm in U.S. EPA, 2000)

Table 7. LED_{10} Values Based on Lung Tumor Incidences in Male $B6C3F_1$ Mice (NTP, 2002)

Tumor Location & Type	Incidence 0 mg/kg-day	Incidence 170 mg/kg-day	Incidence 345 mg/kg-day	Incidence 690 mg/kg-day		mouse LED_{10} (mg/kg-day)	mouse $0.1/LED_{10}$ $(mg/kg\text{-}day)^{-1}$	Human $0.1/LED_{10}$ $(mg/kg\text{-}day)^{-1}$
Alveolar/bronchiolar Adenoma or carcinoma (Lung)	8/50	14/50	12/50	19/50		150.9	$6.6x10^{-4}$	$4.3x10^{-3}$

Doses listed are daily average doses
Human value ($0.1/LED_{10}$) calculated as: mouse value ($0.1/LED_{10}$) x $(W_{hum} / W_{mouse})^{1/4}$ where W_{hum} = 70 kg (human reference body weight), W_{mouse} = 0.039 kg (time weighted average male mouse body weight in this study)
Mouse LED_{10} calculated using multistage model (polydegree of 1 chosen using algorithm in U.S. EPA, 2000)

No data were available for the quantitative estimate of cancer risk resulting from inhalation exposure to *p*-nitrotoluene.

Confidence in the quantitative assessment of risk was low based on the NTP conclusion that evidence of carcinogenicity was equivocal

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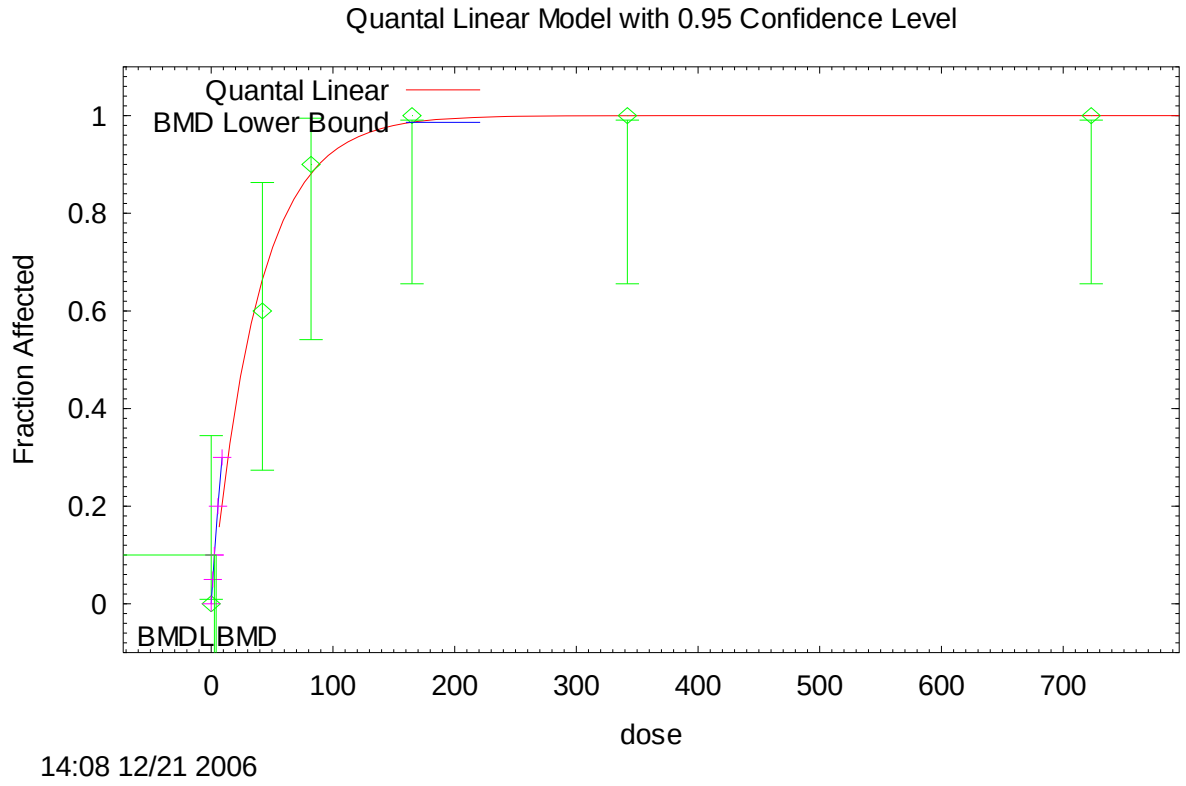
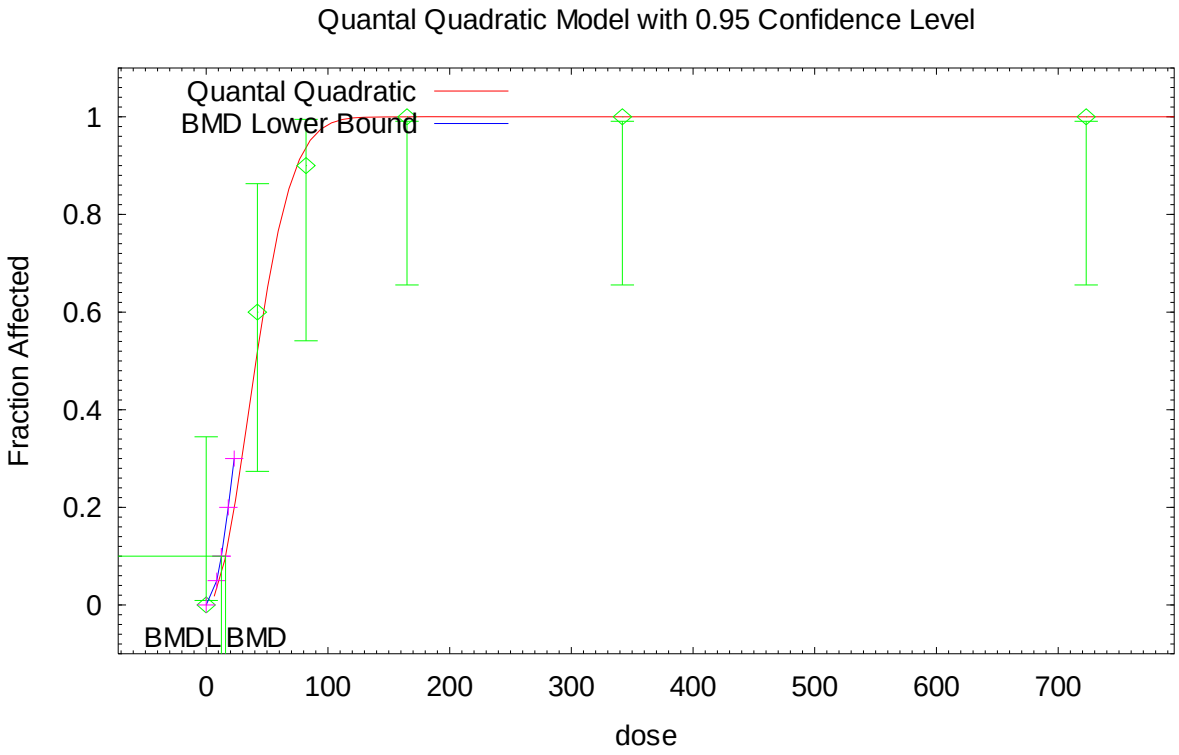
Appendix 1
Benchmark Dose Modeling Summary Data for Subchronic Oral Exposure to p-Nitrotoluene

Table A1-1: BMD_{10} data for p-nitrotoluene 13-week male Rat spleen hematopoiesis

	P	Σ scaled residuals	AIC	BMD (Mg/kg-d)	BMDL (Mg/kg-d)	LOAEL (Mg/kg-d)	Curve observations
Quantal quadratic	.9913	1.0	22.4464	15.9375	12.49		Very Good
Quantal Linear	**.9964**	**1.0**	**22.4566**	**4.05262**	**2.64028**		**Very Good**
Weibull	.9999	0.2	23.998	9.57883	2.74115		Excellent
Gamma	.9998	0.3	24.0221	11.9586	2.7355	42	Excellent
Probit- Log	.9991	2	24.0973	17.4096	4.85743		Excellent
Log-log	.9963	0.8	24.2352	18.7219	2.99056		Very good
Probit	.8577	2.0	25.6193	15.9964	8.94633		Good
Log-normal	.8544	2.0	25.6655	16.8853	9.07673		Good
Multistage	.9997	2	25.9807	6.84451	2.74671		Excellent





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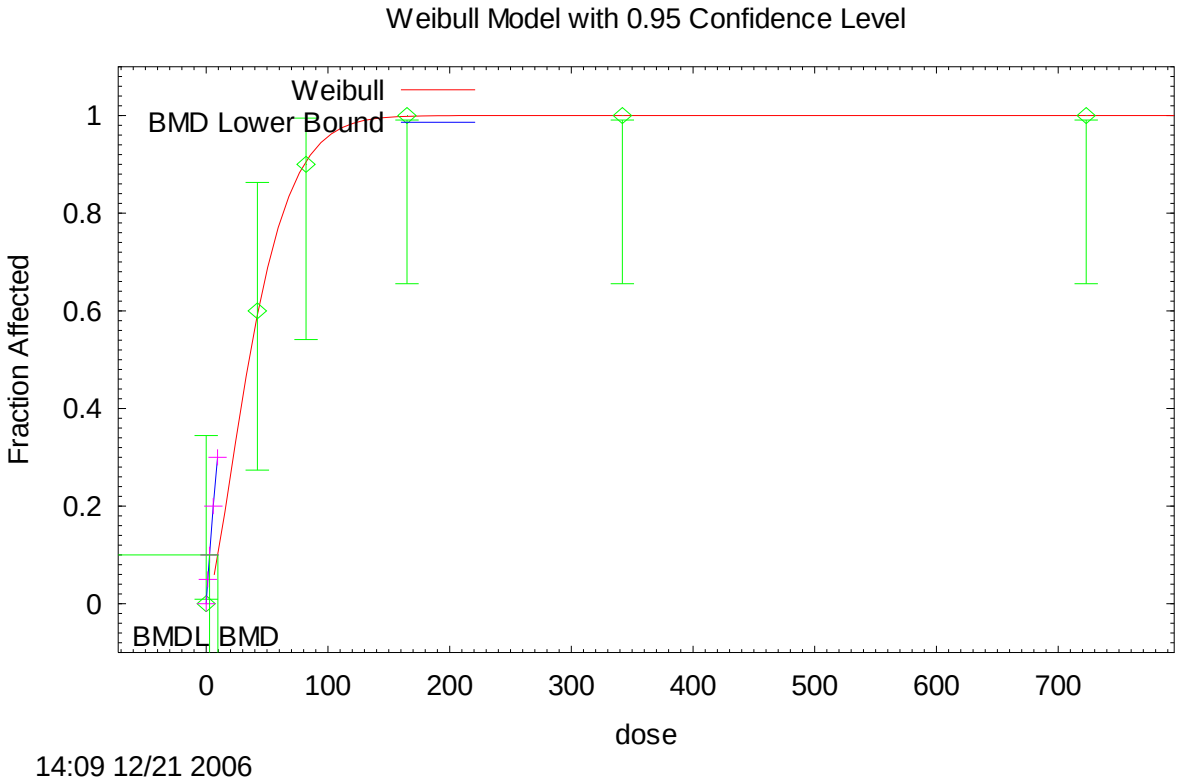

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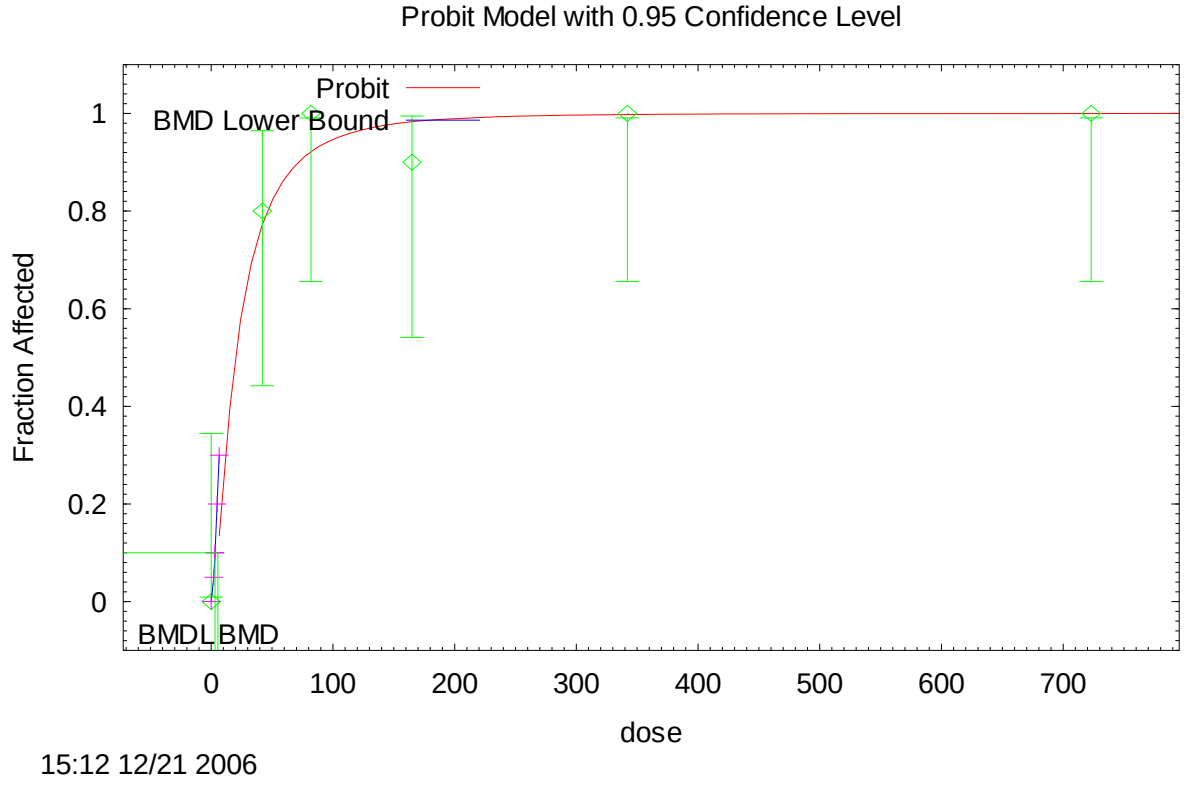
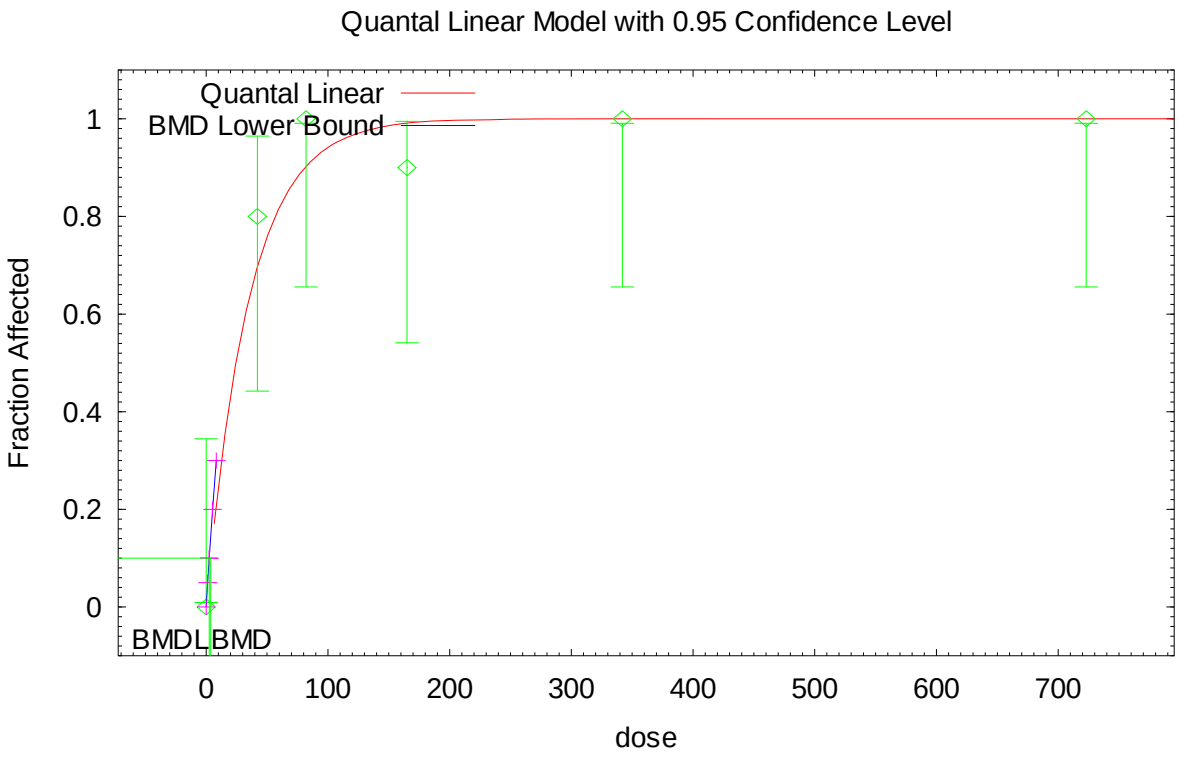
Table A1-2: BMD_{10} data for p-nitrotoluene 13-week male Rat spleen congestion

	P	Σ scaled residuals[2]	AIC	BMD (Mg/kg-d)	BMDL (Mg/kg-d)	LOAEL (Mg/kg-d)	Curve observations
Probit- Log	.4117	1.1	22.1702	5.49419	3.10715	42	OK
Log-log	.6394	1.2	23.4911	2.46051	0.259649		Overest slope?
Quantal Linear	.0601	1.7	24.145	3.71088	2.45895		Underest slope?
Weibull	.0601	1.7	24.145	3.71088	2.45895		Underest slope?
Gamma	.0601	1.7	24.145	3.71088	2.45895		Underest slope?
Multistage	.0601	1.7	24.145	3.71087	2.45928		Underest slope?
Log-normal	.0000	3.8	33.9411	10.2205	6.34687		Underest slope?
Probit	.0005	4.9	36.7537	11.3314	7.7989		Underest slope
Quantal quadratic	.0000	4.0	39.5409	18.848	15.3305		Underest slope?



[2] For controls and two lowest treated groups, only



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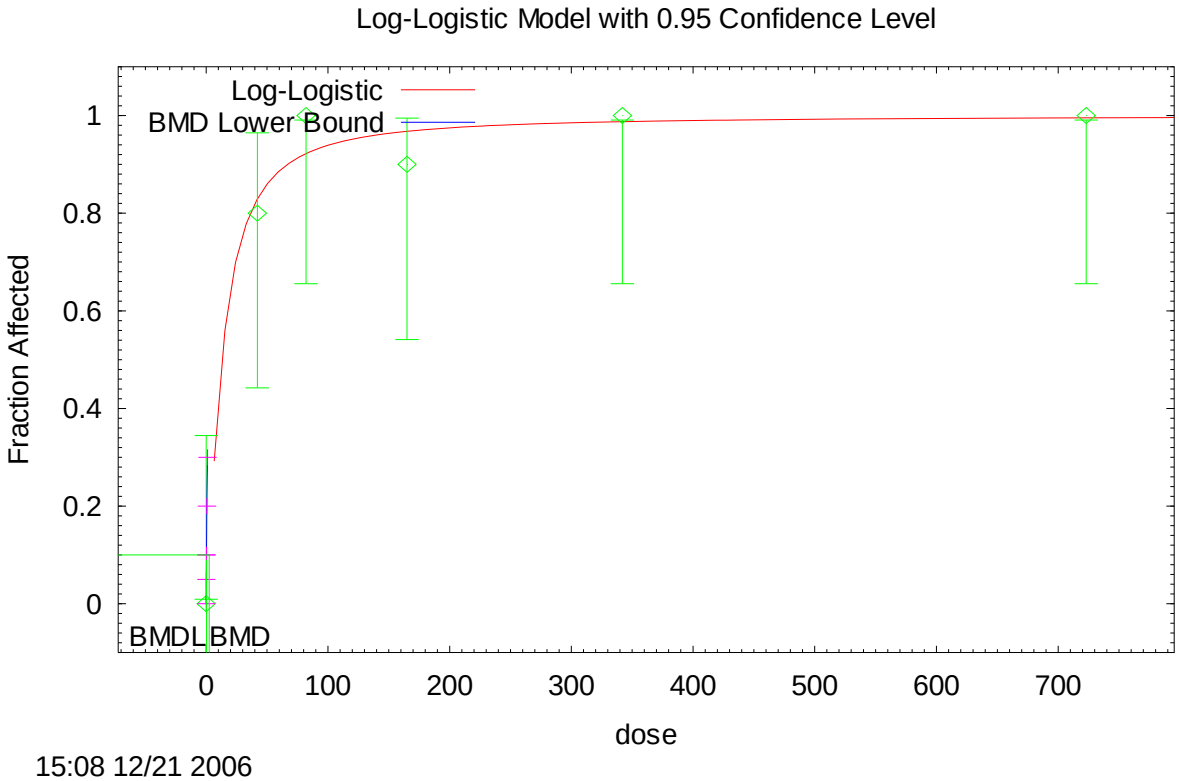

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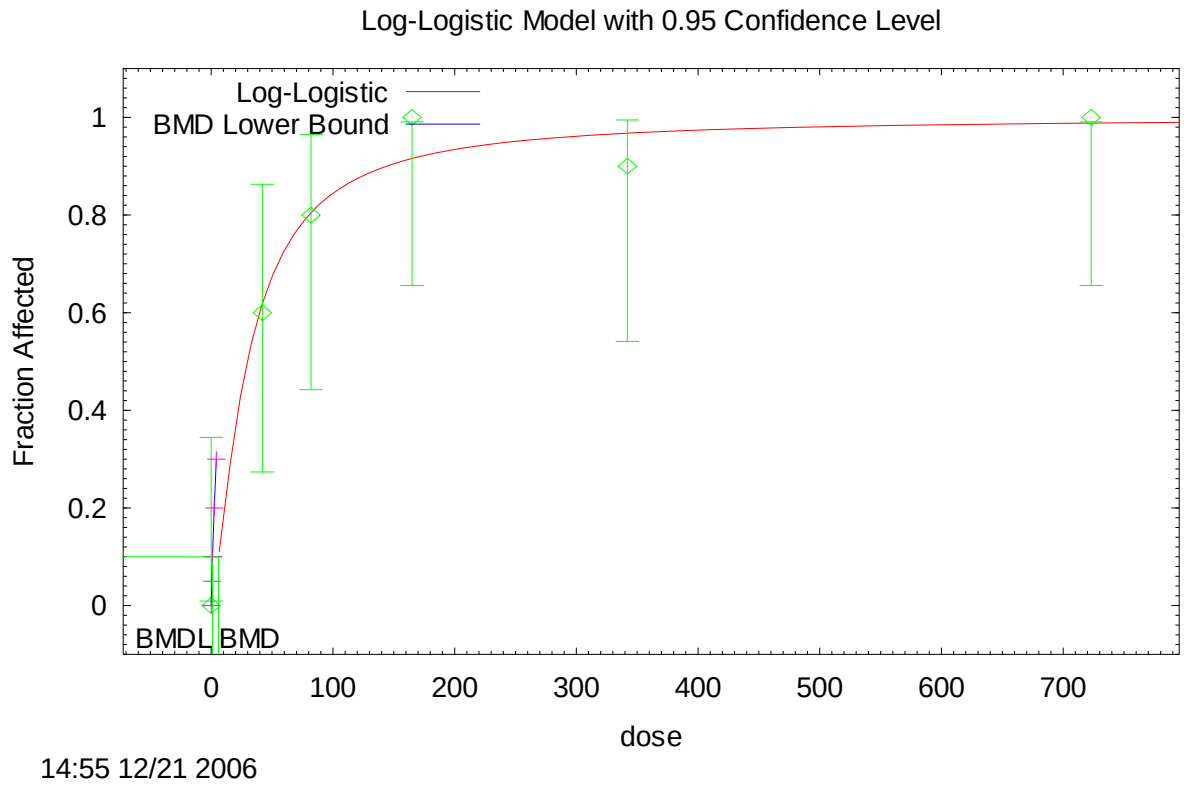
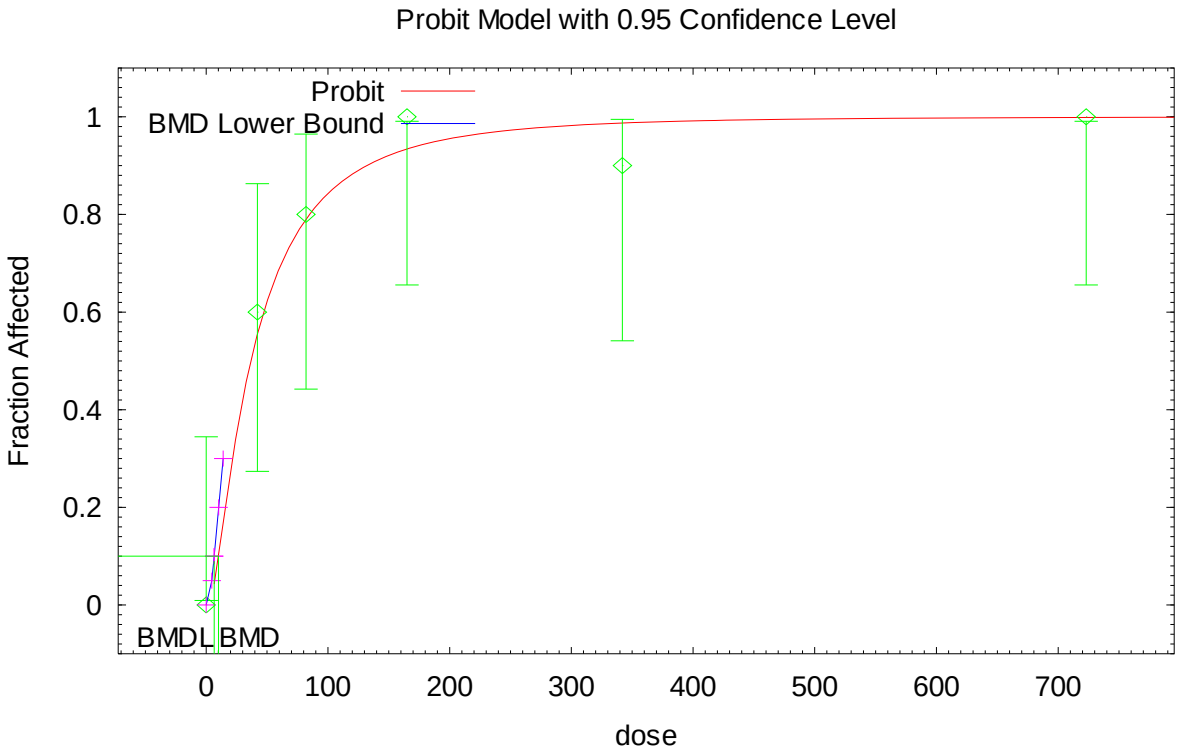
Table A1-3: BMD_{10} data for p-nitrotoluene 13-week male Rat spleen pigmentation

	P	Σ scaled residuals[3]	AIC	BMD (Mg/kg-d)	BMDL (Mg/kg-d)	LOAEL (Mg/kg-d)	Curve observations
Probit- Log	.2269	1.2	35.9131	10.1373	6.55793		Good
Log-log	**.6364**	**1.1**	**36.9339**	**6.08047**	**1.09704**		**Good**
Gamma	.0009	2.2	38.7103	6.74149	4.70516	42	Underest slope
Multistage	.0009	1.9	38.7103	6.74149	4.70516		Underest slope
Quantal Linear	.0009	2.2	38.7103	6.74148	4.70516		Underest slope
Weibull	.0009	2.2	38.7103	6.74148	4.70516		Underest slope
Log-normal	.0000	4.8	49.1851	17.2698	11.5457		Underest slope
Probit	.0008	508	51.7363	21.2574	15.02		Underest slope
Quantal quadratic	.0000	6.9	56.6149	52.2592	36.3924		Poor



[3] Lowest 4 doses, including controls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Appendix 2
Benchmark Dose Modeling Summary Data for Chronic Oral Exposure to p-Nitrotoluene

Table A2-1: BMD_{10} data for p-nitrotoluene 2-yr Rat Female kidney tubule pigmentation

	P	**Σ scaled residuals**	**AIC**	BMD (Mg/kg-d)	**BMDL** (Mg/kg-d)	**LOAEL** (Mg/kg-d)	**Curve observations**
Multistage	N/A	N/A	N/A	N/A	N/A		N/A
Quantal Linear	**.9916**	**0.16934**	**101.472**	**3.55393**	**2.73669**	**60**	**Excellent**
Log-normal	.4962	~ 1.8	102.505	8.95413	6.82015	(43/50)	Very good
Weibull	.9048	~ 0.18	103.465	3.94729	2.73763	(vs 9/50)	Excellent
Gamma	.9034	< 0.2	103.467	4.14975	2.73744		Excellent
Log-Probit	.8210	> 0.3	103.528	13.105	4.35425		Odd BMDL slope
Log-log	.7354	~ 0.5	103.632	18.1088	2.81042		Odd BMDL slope
Probit	.1046	~ 3.3	104.456	9.04199	7.24125		Good
Quantal quadratic	.0089	> 4.1	105.638	16.5518	14.2473		Good

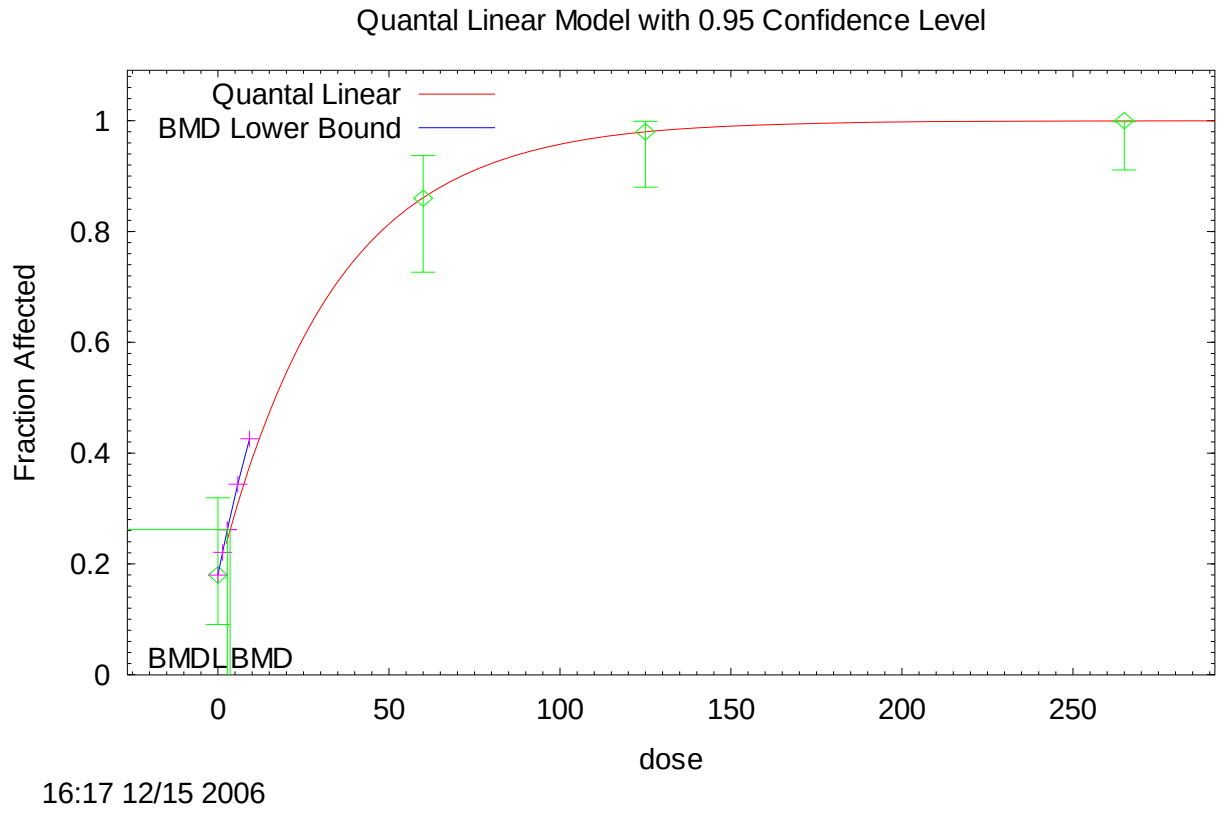


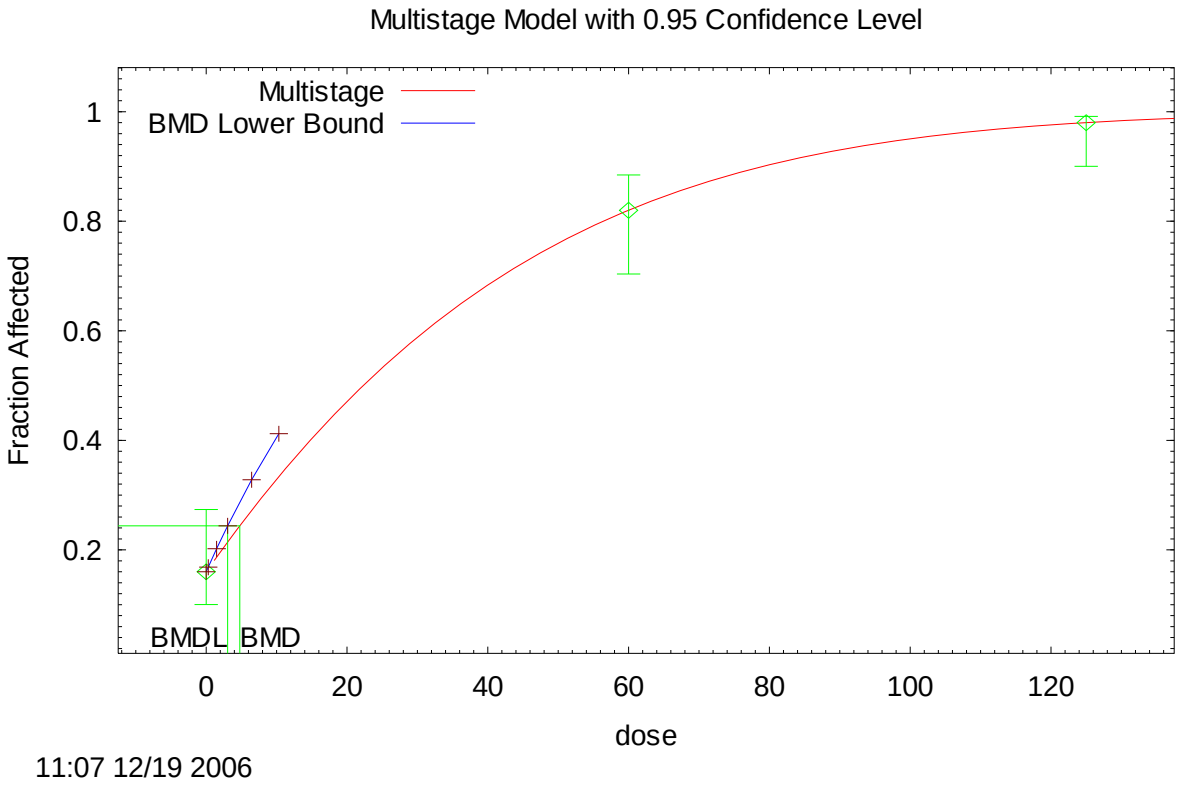
Table A2-2: BMD_{10} data for p-nitrotoluene 2-yr Female Rat renal hyaline droplets

	P	Σ scaled residuals	AIC	BMD	BMDL	Curve notes
Log-log	**.0943**	**~ 3.5**	**138.157**	**1.51893**	**0.903554**	**(delete high dose**
Log-Probit	0.0001	~ 6	143.486	8.86006	**6.72375**	Better than Probit (delete high dose)
Gamma	.0000	~ 9.5	151.93	6.09944	**4.89492**	(delete high dose)
Multistage	.0000	N/A	151.93	6.09944	**4.89492**	(delete high dose)
Quantal Linear	.0000	~ 9.5	151.93	6.09944	**4.89492**	(delete high dose)
Weibull	.0000	~ 9.5	151.93	6.09944	**4.89492**	(delete high dose)
Log-normal	.0000	~ 14.8	167.461	12.6708	**10.2836**	(delete high dose)
Probit	.0000	~ 14	176.691	15.5739	**13.0306**	(delete high dose)
Quantal quadratic	.0000	~ 18	195.59	39.8793	**32.942**	Not good

Table A2-3: BMD_{10} data for p-nitrotoluene 2-yr Female Rat renal hyaline droplets, excluding high dose group

	P	Σ scaled residuals	AIC	BMD (Mg/kg-d)	BMDL (Mg/kg-d)	LOAEL (Mg/kg-d)	Curve observations
Quantal Linear	**.6499**	**< 0.7**	**105.128**	**3.88231**	**3.01487**	**60**	**Excellent**
Log-normal	.4120	< 1.3	105.476	10.1521	7.7554	(41/50)	Very good
Probit	.1080	~ 2.6	106.883	10.0016	8.01071	(vs 8/50)	Very good
Multistage	NA	NA	106.91	4.77127	3.04607		Excellent
Log-log	NA	$2x10^{-14}$	106.91	20.7256	2.63383		Excellent Underestimated slope?
Probit-Log	NA	$5x10^{-14}$	106.91	16.688	5.19785		Excellent Underestimated slope?
Weibull		~ $1.4x10^{-5}$	106.91	6.50895	3.04607		Excellent
Gamma	NA	< 10^{-5}	106.91	8.28715	3.04607		Excellent
Quantal quadratic	.0280	~ 3.1	107.703	17.3347	14.9725		Good

BMD_{10} data for p-nitrotoluene 2-yr Female Rat renal hyaline droplets, excluding high dose group



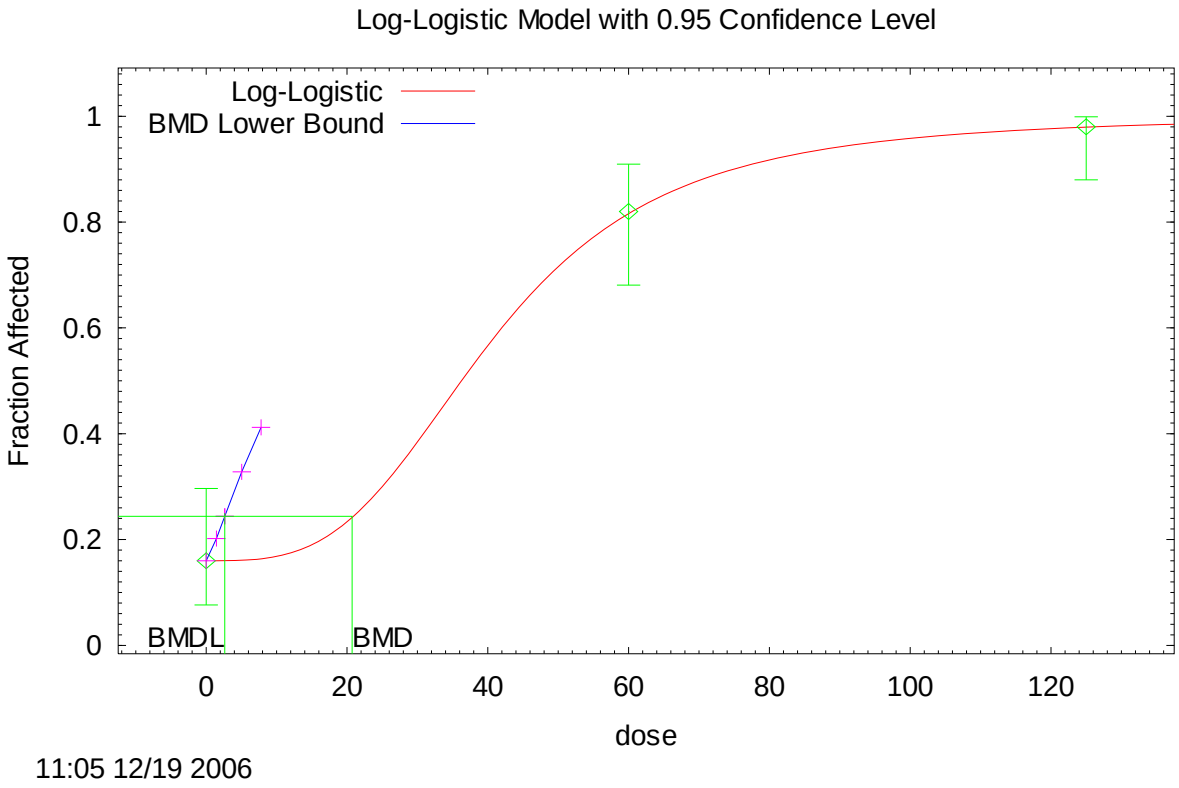

Log-Logistic Model with 0.95 Confidence Level
Log-Logistic
BMD Lower Bound
Fraction Affected
1
0.8
0.6
0.4
0.2
0
BMDL
BMD
0
20
40
60
80
100
120
dose
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Table A2-4: BMD_{10} data for p-nitrotoluene 2-yr male Rat renal hyaline droplets, excluding high dose group

Note: these effects were considered possibly relevant to humans because they probably did not result from alpha-2u-globulin in male rats, which does not occur in humans, but also had not been observed in male rats beyond 18 months of age.

	P	**Σ scaled residuals**	**AIC**	**BMD** (Mg/kg-d)	**BMDL** (Mg/kg-day)	**Curve observations**	**LOAEL** (Mg/kg-d)	Curve notes-all data
Log-log	**.5304**	**< 1.2**	**159.175**	**9.005**	**6.30784**	**Good**	**55**	**Fair**
Gamma	.1169	~2.4	161.192	14.9012	11.5536	Fair	23/50	Terrible
Multistage	.1169	~2.4	161.192	14.9012	11.5536	Fair	vs 0/50	Terrible
Quantal Linear	.1169	~2.4	161.192	14.9012	11.5536	Fair +		Bad
Weibull	.1169	~2.4	161.192	14.9012	11.5536	Fair		Bad
Probit- Log	.0442	~3	162.713	25.7976	20.5259	Fair		Bad
Probit	.0026	~4.9	168.124	31.5147	26.0264	Fair -		Bad
Log-normal	.0019	~5.1	168.991	33.3954	27.2947	Fair -		Bad
Quantal quadratic	.0003	~5.9	171.477	41.6837	35.5918	Poor		Terrible