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Provisional Peer Reviewed Toxicity Values for

o-Nitrotoluene (2-Nitrotoluene)
(CASRN 88-72-2)

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

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 *o*-NITROTOLUENE (2-NITROTOLUENE) (CASRN 88-72-2)

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 chronic RfD of 1E-2 mg/kg-day for *o*-nitrotoluene. The assessments were based on a LOAEL of 200 mg/kg-day, corresponding to a duration-adjusted dose of 143 mg/kg-day for spleen lesions in rats treated by gavage for six months (Ciss et al., 1980). The HEAST derivation for the subchronic RfD 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); the total UF for the chronic RfD was 10,000, including an additional UF of 10 for the use of a subchronic study. The source document was a Health and Environmental Effects Profile (HEEP) for nitrotoluenes (U.S. EPA, 1986). *o*-Nitrotoluene was not listed on IRIS (U.S. EPA, 2008) or the Drinking Water Standards and Health Advisories list (U.S. EPA, 2006). Aside from the HEEP, no additional relevant documents were included in the Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1991, 1994).

An RfC for *o*-nitrotoluene was not listed on the HEAST (U.S. EPA, 1997). The HEEP for nitrotoluenes contained no data suitable for deriving an RfC for *o*-nitrotoluene (U.S. EPA, 1986). The American Conference of Governmental Industrial Hygienists (ACGIH, 2001, 2008) established a Threshold Limit Value (TLV)- Time-Weighted Average (TWA) and the National Institute for Occupational Safety and Health (NIOSH 2005) established a REL-TWA of 11 mg/m^3 (2 ppm) with a skin notation for occupational exposure to all isomers of nitrotoluene. The Occupational Safety and Health Administration (OSHA, 2008) had a Permissible Exposure Limit (PEL)-TWA for nitrotoluenes of 30 mg/m^3 (5 ppm) with a skin notation for danger of cutaneous absorption. These occupational exposure limits were intended to protect against the

development of methemoglobinemia and its sequelae (anoxia, cyanosis, headache, lassitude, ataxia, dyspnea, tachycardia, nausea, and vomiting).

The 1997 HEAST did not include a cancer assessment for *o*-nitrotoluene. The 1986 HEEP assigned the nitrotoluenes to weight-of-evidence Group D, not classifiable as to their carcinogenicity. The document reviewed some evidence relative to the carcinogenicity of *o*-nitrotoluene: weakly positive results in a subchronic skin tumor initiation assay in mice, negative results in most short-term *in vitro* genotoxicity tests, and positive results for unscheduled DNA synthesis in the liver of gavaged rats. IARC (1996) considered *o*-nitrotoluene to be not classifiable as to its carcinogenicity in humans (Group 3), because of no evidence in humans, limited evidence in animals, and some evidence of genotoxicity in mammalian systems. Since these assessments, NTP (2002a) completed oral (feeding) carcinogenicity assays in rats and mice for *o*-nitrotoluene.

ATSDR (2008) and the WHO (2008) had not reviewed the toxicology of *o*-nitrotoluene. Toxicity reviews on aromatic nitro compounds (Benya and Cornish, 1994; Weisburger and Hudson, 2001) were consulted for relevant information. Literature searches were conducted for the period from 1985 to July 2008 to identify data relevant for the derivation of provisional RfD, RfC, and cancer assessments for *o*-nitrotoluene. The following databases were searched: TOXLINE, MEDLINE, CANCERLIT, TOXLIT/BIOSIS, Registry of Toxic Effects of Chemical Substances (RTECS), HSDB, GENETOX, CCRIS, TSCATS, EMIC/EMICBACK, and DART/ETICBACK.

This document has passed the Superfund Health Risk Technical Support Center (STSC) quality review and peer review evaluation indicating that the quality is consistent with the SOPs and standards of the STSC and is suitable for use by registered users of the PPRTV system.

REVIEW OF PERTINENT LITERATURE

Human Studies

No data were located regarding oral or inhalation exposure of humans to *o*-nitrotoluene following chronic or subchronic exposure.

Animal Studies

Animal studies for *o*-nitrotoluene included subchronic and chronic feeding bioassays in rats and mice and a subchronic gavage study in rats. The subchronic studies reported some information about reproductive toxicity.

No subchronic or chronic inhalation data were located in the literature search. Acute inhalation exposure to *o*-nitrotoluene caused irritant effects in humans and animals. Eastman Kodak (1981), citing a 1979 version of RTECS, reported that irritation of the mucus membrane occurred in humans exposed to 0.7 mg/m^3 (3.9 ppm) of *o*-nitrotoluene. In an unpublished study,

Haskell Laboratory (1972) reported eye irritation in male rats exposed for one hour to atmospheres containing 1170 mg/m^3 (209 ppm) of *o*-nitrotoluene.

Ciss et al. (1980) reported a 6-month gavage study on mononitrotoluene isomers. Groups of Wistar rats (10 per gender per group) were gavaged with 0 or 200 mg/kg-day of *o*-nitrotoluene (99% purity) in neutralized olive oil, 5 days/week. The duration adjusted daily dose would be 143 mg/kg-day. 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, and did not provide incidence data or details for all toxic effects. 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, but the study did not specify when the blood samples were taken for these tests. Survivors at termination and animals dying prematurely were necropsied and the major organs (at least nine) were weighed and examined histologically; organ weight changes were not reported quantitatively. F_1 animals also were evaluated histologically at termination. There were no compound-related effects on mortality, growth or the incidence of clinical signs. Slight treatment-related reductions in hemoglobin levels (~11%) and erythrocyte counts (~3%), and slight elevations in leukocyte counts (~7-12%), occurred in both genders. Increased size and weight of the spleen (data not shown) were observed in treated males. Histopathologic changes (unspecified) of the spleen was observed in treated adults of both genders. Treatment-related histopathologic changes of the kidney (hyaline tubular lesions) also was observed, but the study did not report the incidence by gender. No histopathological effects of treatment were detected in offspring. Treatment had no effect on reproduction (numbers of litters, litter size) or in the weight or health of offspring. The single duration-adjusted daily dose of 143 mg/kg-day in this study was a LOAEL for spleen effects (histopathologic changes in both genders and splenomegaly in males) and hematological effects (reduced hemoglobin and erythrocytes) in both genders.

NTP (1992; Dunnick et al., 1994) reported short-term toxicity of nitrotoluene isomers in rats and mice. In an initial range-finding study, groups of F344/N rats (5 per gender per dose) were fed diets containing 0, 625, 1250, 2500, 5000 or 10,000 ppm of *o*-nitrotoluene for two weeks. The average daily intakes of *o*-nitrotoluene were reported as 0, 56, 98, 178, 383 or 696 mg/kg-day in male rats and 0, 55, 102, 190, 382 or 779 mg/kg-day in female rats. At necropsy, all rats were examined for gross lesions; the liver and representative portions of gross lesions were examined for histopathologic changes. *o*-Nitrotoluene treatment had no effect on survival or on the incidence of clinical signs of toxicity. Food consumption was reduced by 28% and terminal body weight by 20% in high-dose (696 mg/kg-day) males compared with controls. Smaller decreases in terminal body weight were seen in 383 mg/kg-day males (7% decrease) and 779 mg/kg-day females (6% decrease). No gross lesions were observed in either gender. Four of five high-dose males exhibited minimal oval cell hyperplasia in the liver. No histopathological changes were observed in the livers of female rats.

In the subchronic study (NTP, 1992; Dunnick et al., 1994), groups of F344/N rats (10 per gender per group) were fed diets containing 0, 625, 1250, 2500, 5000 or 10,000 ppm of *o*-nitrotoluene for 13 weeks. The average daily intakes of *o*-nitrotoluene were reported to be 0, 45, 89, 179, 353 or 694 mg/kg-day for male rats and 0, 44, 87, 178, 340 or 675 mg/kg-day for female

rats. Animals were observed twice daily for mortality or moribundity, and weekly for feed consumption, body weight, and clinical signs. Clinical chemistry and hematology evaluations were conducted on satellite groups 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. A complete necropsy was performed on all animals at termination. Organ weights were recorded for heart, liver, lungs, right kidney, thymus and right testicle. All control animals, early death animals, and all animals in the highest dose group with 60% survivors were evaluated for histopathologic changes in gross lesions, tissue masses (or suspect tumors and regional lymph nodes), and 40 tissues.

Subchronic oral exposure to *o*-nitrotoluene had no effect on survival in rats and no clinical signs of toxicity were reported (NTP, 1992; Dunnick et al., 1994). There were dose-related decreases in food consumption and body weight gain. Terminal body weights were 12-13% lower than controls in both genders at 178-179 mg/kg-day, 17% () - 28% () lower at 340-353 mg/kg-day, and 23% ()-44% () lower at 675-694 mg/kg-day. After one week of treatment, analyses of satellite groups revealed significant alterations in hematology parameters. Methemoglobin levels were significantly elevated in males exposed at 353 mg/kg-day and females exposed at 675 mg/kg-day. Other changes included elevated hemoglobin and erythrocyte counts in males exposed at 694 mg/kg-day, elevated leukocyte (primarily lymphocyte) counts and reduced reticulocyte counts in males exposed at 353 mg/kg-day, and elevated platelet counts in males at 353 mg/kg-day and in females at 675 mg/kg-day. After three weeks of treatment, methemoglobin levels were significantly elevated in the lower dose groups, including males exposed at 89 mg/kg-day and all treated female groups except the 87 mg/kg-day group. Signs of anemia (reduced hemoglobin, hematocrit and erythrocyte counts) occurred in males at 694 mg/kg-day and females at 340 mg/kg-day. Other significant changes included elevated leukocyte (lymphocyte) counts in males at 694 mg/kg-day and females at 178 mg/kg-day, elevated platelet counts in males at 89 mg/kg-day and females at 340 mg/kg-day, and increased reticulocytes in females at 675 mg/kg-day. After 13 weeks of treatment, methemoglobin levels were increased in a dose-related manner in the core group males and females at all doses; the increases were statistically significant in males at 179 mg/kg-day and females at 178, 340, and 675 mg/kg-day. At the highest dose (675-694 mg/kg-day), methemoglobin levels were 11 g/dL in males and 4.3 g/dL in females, compared to 2.5 and 2.0 g/dL in respective controls. Signs of anemia (mild decreases in hemoglobin, hematocrit and erythrocyte counts) occurred in the 340-353 mg/kg-day groups in both genders. Other significant increases involved leukocyte counts in males at 89 mg/kg-day and females at 178 mg/kg-day, lymphocyte counts in both genders at 178-179 mg/kg-day, platelet counts in males at 45 mg/kg-day and females at 340 mg/kg-day, and reticulocytes in both genders at 675-694 mg/kg-day.

Analyses of clinical chemistry parameters revealed increasing effects during the course of treatment in rats (NTP, 1992; Dunnick et al., 1994). After one week of treatment, the satellite groups exhibited reductions in total serum protein and albumin in males exposed at 45 mg/kg-day and in females exposed at 353 mg/kg-day. After three weeks of treatment, significant reductions occurred in serum alkaline phosphatase in males at 45 mg/kg-day, total protein in males at 45 mg/kg-day and females at 178 mg/kg-day, albumin in males at 89 mg/kg-day and

females at 353 mg/kg-day, and in alanine transaminase (ALT) in both genders at 675-694 mg/kg-day; serum bile acids, indicative of hepatic damage, were elevated in high-dose males. After 13 weeks of treatment, clinical chemistry changes in the core group animals included slight elevations in serum total protein and albumin in males at 694 mg/kg-day, but reductions in females at 353 mg/kg-day. Changes indicative of liver damage included mild-to-moderate increases in serum bile acids in males at 353 mg/kg-day and in females at 675 mg/kg-day, and mild increases in sorbitol dehydrogenase (SDH) in males at 179 mg/kg-day and in ALT in males at 694 mg/kg-day; ALT was reduced in females at 178 mg/kg-day. Significant changes in organ weights were observed in treated rats. In male rats, the absolute and relative kidney weights were significantly increased at 353 mg/kg-day. The relative liver weights were significantly increased in all treated groups in both genders; dose-related increases in absolute liver weight were significant in males at 179 mg/kg-day, but were not significant in females. The absolute weight of testes (and epididymis and epididymal tail) was significantly reduced at 179 mg/kg-day and the relative testicular weight was reduced in the 694 mg/kg-day group. NTP attributed increased organ-to-body-weight ratios of several organs (heart, lungs and thymus in both genders and the kidney in females) in higher-dose animals to the lower body weights in these groups, since the absolute weights of these organs were reduced.

Treatment-related pathological changes that increased in severity with dose were observed in subchronically exposed rats (NTP, 1992; Dunnick et al., 1994). At necropsy, the livers of high-dose males (694 mg/kg-day) appeared to be enlarged with pale, mottled foci. Histopathological lesions of the liver, including cytoplasmic vacuolization, oval cell hyperplasia, and inflammation, were observed in males at 179 mg/kg-day. Atrophy of the salivary gland was detected histologically in all rats exposed at 87-89 mg/kg-day. Kidney lesions in males included hyaline droplet nephropathy at 89 mg/kg-day and tubular epithelial cell regeneration at 353 mg/kg-day; accumulation of Periodic Acid Schiff (PAS)-positive pigmentation, thought to be lipofuscin, in cortical tubules was observed in males at 694 mg/kg-day and females at 353 mg/kg-day. Significant spleen lesions included hemosiderosis in both genders at 178-178 mg/kg-day, hematopoiesis (in males at 179 and females at 675 mg/kg-day), and capsular fibrosis in males at 694 mg/kg-day. Spleen capsular foci were associated with minimal focal hypertrophy and hyperplasia of mesothelial cells on the serosal surface of the spleen. Cellular hyperplasia of the pancreatic islets was observed in males exposed at 353 mg/kg-day. Atrophy of the preputial gland was observed in male groups exposed at 89 mg/kg-day; all males were affected at the two highest doses. The testes of high-dose males were smaller than controls, with pale, mottled foci. Degeneration of the testis (absence of germinal epithelium) occurred in all male rats treated with 353 mg/kg-day. Epididymal sperm motility and testicular sperm counts also were significantly reduced in these groups. Two out of 10 high-dose males had mesothelial cell hyperplasia of the tunica vaginalis on the surface of the epididymis; mesotheliomas with metastatic foci occurred at the same anatomical location in three out of 10 males from the 353 mg/kg-day group. NTP (1992) cited Huff et al. (1991) in stating that chemical-induced mesotheliomas were relatively rare in rats and had not been associated with treatment in mice. Only eight of 379 chemicals surveyed caused mesotheliomas in rats, all in longer-term studies. In high-dose females (675 mg/kg-day), the estrous cycle was lengthened by 35% (increase in diestrus) and only 4/10 rats appeared to undergo cycling; no treatment-related pathology of the uterus or ovaries was observed, but all high-dose females had atrophy of the clitoral gland.

In summary, the low dose of 625 ppm (44 mg/kg-day) was a LOAEL for increased methemoglobin levels in female rats exposed to *o*-nitrotoluene in this study (NTP, 1992; Dunnick et al., 1994). Methemoglobinemia was the likely cause of anemia and adverse spleen effects observed at higher doses. Carcinogenic effects of *o*-nitrotoluene (mesothelioma of the tunica vaginalis) were observed in male rats exposed to 5000 ppm (353 mg/kg-day).

NTP (1992; Dunnick et al., 1994) conducted similar studies in B6C3F$_1$ mice. For the range-finding assay, groups of mice (5 per gender per dose) were fed diets containing 0, 388, 625, 1250, 2500 or 5000 ppm of *o*-nitrotoluene for two weeks. The average daily intakes of *o*-nitrotoluene were reported as 0, 63, 106, 204, 405 or 854 mg/kg-day for male mice and 0, 134, 217, 397, 631 or 1224 mg/kg-day for female mice. At necropsy, all mice were examined for gross lesions and representative portions of gross lesions and the livers were examined for histopathologic changes. *o*-Nitrotoluene treatment had no significant effect on survival or the incidence of clinical signs, although food consumption appeared to be slightly reduced at the highest dose. Increases in liver weights were observed in males at 204 mg/kg-day, but no other gross or microscopic lesions were observed.

In a subchronic study (NTP, 1992; Dunnick et al., 1994), groups of B6C3F$_1$ mice (10 per gender per group) were fed diets containing 0, 625, 1250, 2500, 5000 or 10,000 ppm of *o*-nitrotoluene for 13 weeks. Average daily intakes of *o*-nitrotoluene were reported to be 0, 104, 223, 415, 773 or 1536 mg/kg-day for male mice and 0, 132, 268, 542, 1007 or 1712 mg/kg-day for female mice. The same measurements as in the rat subchronic study were obtained for mice, except that liver weights included the gall bladder, and no hematology or clinical chemistry analyses were performed on mice. Treatment with *o*-nitrotoluene had no effect on survival or the incidence of clinical signs of toxicity. In both genders, food consumption and body weight gain were significantly lower in the 5000 (773-1007 mg/kg-day) and 10,000 ppm (1536-1712 mg/kg-day) treatment groups. Significant organ weight changes involved the liver (increased relative weight in males at 415 mg/kg-day and females at 268 mg/kg-day), kidney (decreased relative and absolute weight in males at 773 mg/kg-day and increased relative weight in females at 268 mg/kg-day), and lung (increased relative weight in females at 1007 mg/kg-day). No gross lesions were observed in mice at necropsy. Histopathologic alterations of the olfactory nasal epithelium were observed in both genders at 773-1007 mg/kg-day, and occasionally in the 223-268 mg/kg-day and 415-542 mg/kg-day groups; lesions included thinning of the nuclear layer, atrophy of nerve bundles of the lamina propria, dilation of Bowman's glands, and respiratory metaplasia. The researchers suggested that the nasal lesions may have resulted from an irritant effect of inhaled *o*-nitrotoluene volatilized from the feed. Sperm motility was significantly reduced in high-dose males (1536 mg/kg-day); treatment had no effect on other reproductive parameters in either gender. Although no abnormal liver histopathologic changes were detected in the current study, evidence from the chronic study discussed below (NTP, 2002b) suggested that extended exposure to *p*-nitrotoluene at these dose levels might result in changes in liver histopathology. Thus, the liver weight increases observed in this subchronic study might represent an initial sign of hepatic toxicity. In this study, 625 ppm (132 mg/kg-day) was a subchronic NOAEL and 1250 ppm (286 mg/kg-day) was a minimal LOAEL for increased liver weight in female mice.

In a study comparing responses to *o*-nitrotoluene with responses to *o*-toluidine hydrochloride, NTP (1996) fed male F344/N rats a diet containing *o*-nitrotoluene for 26 weeks. Groups of 10 rats were fed control diets and groups of 20 rats were fed diets containing 5000 ppm of the test compound for 13 weeks (interim group) or 26 weeks (continuous group). A third, stop-exposure, group also was exposed to the compound for 13 weeks and received the control diet for 13 additional weeks. The average daily doses were reported as 293, 293, or 292 mg/kg-day for the interim, stop-exposure and continuous groups, respectively. Rats were observed twice daily. Body weights and clinical signs were recorded weekly and at termination; feed consumption was recorded weekly. Complete necropsies were conducted on all animals. The right kidney, liver, spleen, right testis, and epididymis weights were recorded for all control rats and half of the exposed rats. Histopathological examinations of all gross lesions and the weighed organs were conducted on all animals. At termination of the interim, stop-exposure, and continuous groups, liver samples from 9 or 10 rats per group were stained for placental glutathione *S*-transferase-positive foci.

Treatment with *o*-nitrotoluene had no effect on mortality or the incidence of clinical signs of toxicity (NTP, 1996). All groups of treated rats showed decreases in feed consumption and body weight gains compared to controls. Absolute and relative weights of the right testis and epididymis were significantly decreased in all groups of treated male rats. Relative weights of the spleen and right kidney and absolute and relative weights of the liver were significantly increased in all groups of treated rats. Histopathological examination of the interim, stop-exposure and continuously treated rats revealed similar findings. After 13 weeks of treatment, nearly all interim rats treated with *o*-nitrotoluene exhibited increased histopathologic changes in the kidney (hyaline droplet accumulation and tubule epithelial cell regeneration), liver (cytoplasmic vacuolization, oval cell hyperplasia), spleen (hematopoietic cell proliferation, hemosiderin pigmentation), and testis/epididymis (degeneration); one treated rat exhibited fibrosis of the spleen capsule. A few interim control rats exhibited regeneration of the kidney and hematopoietic cell proliferation of the spleen. After 26 weeks, the stop-exposure rats exhibited histopathological changes in the kidney (protein casts and regeneration), liver (cytoplasmic vacuolization, oval cell hyperplasia), spleen (hematopoietic cell proliferation, hemosiderin pigmentation, capsular fibrosis), and testis/epididymis (degeneration and mesothelial hyperplasia). In addition, cholangiocarcinoma of the liver was observed in two of twenty rats and mesothelioma of the tunica vaginalis of the testis or epididymis in five of twenty rats from the stop-exposure group. The rats exposed continuously for 26 weeks exhibited the same lesions as the stop-exposure group, in addition to hyaline droplet accumulation in tubular epithelial cells of the kidney and a slightly higher incidence of mesothelioma of the testis/epididymis (7/20). The livers of rats in treated groups had an increase in glutathione-*S*-transferase-positive foci. In this study, 292 mg/kg-day was a LOAEL for effects in the kidney, liver, spleen, and testis of male rats.

Chronic Data

Groups of F344/N rats (60 per gender per group) were fed diets containing 0, 625, 1250, or 2000 ppm of *o*-nitrotoluene (purity >99%) for two years (NTP, 2002a). Average daily intakes of *o*-nitrotoluene were reported as 0, 25, 50, or 90 mg/kg-day for male rats and 0, 30, 60, or 100 mg/kg-day for female rats. The chronic bioassays did not test dietary levels lower than those

used in the 13-week bioassays (NTP, 1992). In addition, parallel groups of 70 male rats were subjected to diets containing 0, 2000, or 5000 ppm of *o*-nitrotoluene for 14 weeks and then control diets for the remaining 91 weeks; doses in these "stop-exposure" rats were reported as 0, 125 or 315 mg/kg-day. Ten rats from the control and stop-exposure groups were selected for interim evaluation at 3 months. All animals were observed twice daily. Clinical findings were recorded every 4 weeks. Body weights were recorded initially, during week 4, and every fourth week thereafter. No hematology or clinical chemistry parameters were analyzed in this study. A subset of each group (5 per gender per dose) was randomly selected for urinalysis at 2 weeks and at 3, 12, and 18 months, to evaluate excretion of metabolites (*o*-acetamidobenzoic acid, *o*-nitrobenzylmercapturic acid and *o*-nitrobenzoic acid). Necropsies and microscopic examinations of gross lesions, tissue masses, and 36 tissues were performed on all animals. At the 3-month interim evaluation of control and stop-exposure males, organ weights were recorded for heart, right kidney, liver, lungs, right testis, and thymus. The spleen was not weighed, although it was shown to be a target organ in the subchronic bioassay. No organ weight data were recorded for core group animals.

At the 3-month interim evaluation of the male rat "stop-exposure" groups, treatment had no effect on survival, but body weights were significantly reduced compared to controls, by 11% and 27% in the 125 and 315 mg/kg-day groups, respectively (NTP, 2002a). Statistically significant changes in organ weights occurred in both exposure groups. Relative liver weights were increased in both exposed groups and absolute liver weights were increased in the 315 mg/kg-day group. The absolute weights of the right testes in the 315 mg/kg-day group were significantly reduced. Increases in relative heart, thymus, and right kidney weights in the 315 mg/kg-day group, and relative lung weights in both treated groups appeared to be related to reductions in body weight. Significant nonneoplastic lesions observed at 3 months included degeneration of the olfactory epithelium and atrophy of the salivary gland in males treated with 125 mg/kg-day, and hyaline degeneration of renal tubules, hyperplasia of pancreatic islets, atrophy of the preputial glands, congestion and hematopoietic cell proliferation in the spleen, and moderate-to-severe hepatocellular cytoplasmic vacuolization in the liver of males exposed at 315 mg/kg-day.

In the chronic study, there were statistically significant trends for increased mortality in male and female rats exposed to *o*-nitrotoluene (NTP, 2002a). Survival in all exposed male groups (18/60, 3/60 and 0/60 for the 25, 50, and 90 mg/kg-day groups, respectively) was significantly lower than in controls (39/70). Survival in females (47/60, 47/60, 39/60 and 33/60 for the control and 30, 60, and 100 mg/kg-day groups, respectively) was significantly lower than controls only at the high dose. Survival of males in the 125 and 315 mg/kg-day stop-exposure groups (11/60 and 0/60, respectively) also was significantly lower than controls. The early deaths in this study were attributed to the development of tumors (see below). Body weights of males exposed at 50 mg/kg-day were consistently lower than controls throughout the study, but the weight reductions were only significant in the stop-exposure males exposed at 315 mg/kg-day (-19% compared to controls). Body weights of females exposed at 100 mg/kg-day were significantly reduced during the second year (-10% compared to controls). Exposure to *o*-nitrotoluene had no effect on feed consumption. Treatment related clinical findings included large subcutaneous masses throughout the body in both genders, and small ears and thin tails in all males exposed at 90 mg/kg-day.

Treatment-related increases in the incidence of nonneoplastic lesions were observed in the chronically dosed core groups (Table 1) and stop-exposure groups in rats (NTP, 2002a). Hematopoietic cell proliferation of the spleen was significantly increased in all exposed groups; pigmentation of the spleen was increased in males and females exposed to 90-100 mg/kg-day for 2 years. NTP did not discuss the possible relationship of these spleen findings to methemoglobinemia that was observed in the subchronic study (NTP, 1992), so it is uncertain whether the pigmentation observed in the spleen and other organs was hemosiderin. Nonneoplastic lesions of the liver included mixed cell infiltration in males exposed at 50 mg/kg-day and centrilobular necrosis in males exposed at 50 or 90 mg/kg-day. Renal tubule pigmentation was observed in males exposed for two years at 50 and in the 90 mg/kg-day stop-exposure group. Hyperplasia of the bone marrow, characterized as hematopoietic cell proliferation, was increased in all exposed male groups and in females exposed at 50-60 mg/kg-day. Atrophy of several organs (salivary gland in both genders, preputial gland in males, and clitoral gland in females) was increased in groups exposed at 50-60 mg/kg-day. Lymph node effects were observed at 90-100 mg/kg-day and above: mediastinal pigmentation in males and mandibular lymphoid hyperplasia in females. Additional effects in males involved the pancreatic islets (hyperplasia and pigmentation in the 315 mg/kg-day stop-exposure group) and pars distalis of the pituitary gland (cytoplasmic alteration in the 315 mg/kg-day stop-exposure group and the 100 mg/kg-day main group).

Preneoplastic lesions were observed in the liver, mammary gland, and lung of treated rats (NTP, 2002a). The incidence of hepatocellular foci was significantly increased in all exposed groups. Mammary gland hyperplasia, a precursor of fibroadenoma, was significantly increased in females exposed at 30 or 60 mg/kg-day. The incidence of hyperplasia of the alveolar/bronchiolar epithelium was significantly increased in males exposed at 25 and 90 mg/kg-day for 2 years and in both stop-exposure groups; the incidences in females were increased in the 30 and 60 mg/kg-day groups.

In the rat bioassay, all levels of exposure to *o*-nitrotoluene increased the incidences of neoplastic tumors after two years (Table 2); males exposed for 3 months at the higher doses also exhibited increased tumor incidences after two years (NTP, 2002a). For most treatment-related tumor types, the incidences occurred with statistically significant positive trends and tumor latencies decreased with dose. In males, all exposures significantly increased the incidence of malignant testicular mesothelioma compared to concurrent controls and the incidence exceeded the historical control range. Mesotheliomas usually were associated with the tunica vaginalis of the testis or epididymis, but some were associated with the abdominal wall or the surface of abdominal organs. Mesotheliomas were not observed in female rats. The incidences of subcutaneous skin tumors (fibroma, fibrosarcoma, and fibroma or fibrosarcoma combined in all exposed male groups and fibroma and fibroma or fibrosarcoma in females exposed at 60 mg/kg-day) occurred with positive trends and exceeded historical control ranges. The incidence of lipomas was significantly increased in all treated groups of males. The incidence of fibroadenoma of the mammary gland was significantly increased in nearly all treated male and female groups, with the exception of males treated with 100 mg/kg-day for 2 years. The incidences of hepatocellular adenoma and adenoma or carcinoma combined occurred with a significant positive trend in both genders; incidences in both genders exposed at 90-100 mg/kg-day for two years and males exposed at 315 mg/kg-day for 3 months were significantly higher

Table 1. Incidences of Selected Nonneoplastic and Preneoplastic Lesions in a 2-year Feeding Study of *o*-Nitrotoluene in Male and Female F344/N Rats[a]

	Male Rats				Female Rats			
	0 ppm	625 ppm	1250 ppm	2000 ppm	0 ppm	625 ppm	1250 ppm	2000 ppm
Organ: Lesion	Control	25 mg/kg-day	50 mg/kg-day	90 mg/kg-day	Control	30 mg/kg-day	60 mg/kg-day	100 mg/kg-day
Liver: Basophilic focus					51/60	56/59[b]	60/60[b]	54/60
Clear cell focus	29/60	29/60	34/60	31/60	16/60	30/59[b]	28/60[b]	33/60[b]
Eosinophilic focus	7/60	18/60[b]	29/60[b]	24/60[b]	5/60	12/59	25/60[b]	32/60[b]
Mixed cell focus	5/60	7/60	12/60[b]	6/60	6/60	9/59[b]	11/60	28/60[b]
Hematopoietic cell proliferation	0/60	6/60[b]	2/60	2/60				
Mixed cell infiltration	1/60	5/60	11/60[b]	20/60[b]				
Centrilobular necrosis	1/60	3/60	8/60[b]	5/60[b]	3/60	0/59	2/60	2/60
Lung: Alveolar hyperplasia	2/60	8/60[b]	3/60	7/60[b]	6/60	14/60[b]	16/60[b]	9/60
Bone marrow: Hematopoietic cell proliferation	2/60	25/60[b]	43/60[b]	45/60[b]	2/60	7/60	15/60[b]	24/60[b]
Spleen: Pigmentation	6/60	8/60	13/60	16/60[b]	36/60	44/59	43/60	46/60[b]
Hematopoietic cell proliferation	7/60	33/60[b]	38/60[b]	47/60[b]	22/60	38/59[b]	48/60[b]	48/60[b]
Kidney: Renal tubule pigmentation	5/60	12/60[b]	14/60[b]	9/60[b]				
Lymph node: Mandibular lymphoid hyperplasia					3/60	5/60	6/59	15/59[b]
Mediastinal pigmentation	6/60	6/60	3/60	8/60[b]				
Salivary gland: Atrophy	0/60	2/60	18/59[b]	43/60[b]	2/60	3/60	9/59[b]	48/60[b]
Clitoral gland: Atrophy					1/59	3/57	6/54[b]	25/53[b]
Preputial gland: Atrophy	7/60	9/59	35/58[b]	41/56[b]				
Testis: Interstitial cell hyperplasia	10/60	14/60	13/60	31/60[b]				
Germinal epithelial Atrophy	13/60	21/60[b]	12/60	19/60[b]				

[a]NTP, 2002a
[b]Statistically significant in pairwise test versus current controls

Table 2. Incidences of Selected Neoplastic Tumors in a Chronic Feeding Study of *o*-Nitrotoluene in Male and Female F344/N Rats[a]

A- 2-Year Exposure		Male Rats			Female Rats			
	0	625 ppm	1250 ppm	2000 ppm	0	625 ppm	1250 ppm	2000 ppm
Organ: Tumor type	Control	25 mg/kg-day	50 mg/kg-day	90 mg/kg-day	Control	30 mg/kg-day	60 mg/kg-day	100 mg/kg-day
Malignant testicular mesothelioma	2/60[b] (3%)	20/60[c] (33%)	29/60[c] (48%)	44/60[c] (73%)				
Subcutaneous Skin: Fibroma or fibrosarcoma	5/60[b] (8%)	47/60[c] (78%)	55/60[c] (92%)	59/60[c] (98%)	3/60[b] (5%)	3/60 (5%)	21/60[c] (35%)	22/60[c] (37%)
Lipoma	0/60	4/60[c] (7%)	13/60[c] (22%)	13/60[c] (22%)				
Mammary Gland: Fibroadenoma	0/60[b]	7/60[c] (12%)	10/60[c] (17%)	2/60 (3%)	23/60[b] (38%)	47/60[c] (78%)	52/60[c] (87%)	56/60[c] (93%)
Liver: Hepatocellular adenoma	2/60[b] (3%)	3/60 (5%)	3/60 (5%)	7/60[c] (12%)	1/60[b] (2%)	0/59	1/60 (2%)	6/60[c] (10%)
Hepatocellular adenoma or carcinoma	3/60[b] (5%)	3/60 (5%)	3/60 (5%)	8/60[c] (13%)				
Lung: Alveolar/bronchiolar adenoma or carcinoma	2/60 (3%)	5/60 (8%)	1/60 (2%)	2/60 (3%)				
Hemangioma or hemangiosarcoma (all sites)	1/60 (2%)	3/60 (5%)	1/60 (2%)	2/60 (3%)				

B. 3-Month Exposure[d]	Male Rats		
	0 ppm	2000 ppm	5000 ppm
Organ: Tumor type	Control	125 mg/kg-day	315 mg/kg-day
Malignant testicular mesothelioma	2/60[b] (3%)	44/60[c] (73%)	54/60[c] (90%)
Subcutaneous skin: Fibroma or fibrosarcoma	5/60[b] (8%)	47/60[c] (78%)	53/60[c] (88%)
Lipoma	0/60[b]	10/60[c] (17%)	12/60[c] (20%)
Mammary gland: Fibroadenoma	0/60[b]	13/60[c] (22%)	20/60[c] (33%)
Liver: Hepatocellular adenoma	2/60 (3%)	3/60 (5%)	4/60 (7%)
Hepatocellular adenoma or carcinoma	3/60[b] (5%)	3/60 (5%)	6/60[c] (13%)
Lung: Alveolar/bronchiolar adenoma or carcinoma	2/60[b] (3%)	3/60 (5%)	11/60[c] (18%)
Hemangioma or hemangiosarcoma (all sites)	1/60[b] (2%)	0/60	4/60[c] (7%)

[a]NTP, 2002a
[b]Statistically significant trend
[c]Statistically significant in pairwise test versus current controls
[d]Stop-exposure groups were observed for 91 weeks following exposure

than concurrent controls and exceeded the historical control range. NTP considered the isolated hepatocholangiocarcinomas in one 25 mg/kg-day male, one 100 mg/kg-day core male, and three 315 mg/kg-day stop-exposure males to be treatment-related, since the tumors had been observed in a previous study (NTP, 1996) but had not been observed in historical controls. The incidences of alveolar and bronchiolar adenoma, and adenoma or carcinoma combined occurred with a significant positive trend in the stop-exposure groups and were increased compared to controls at 315 mg/kg-day. The incidences of these lung tumors were not significantly increased in the core treatment groups. Similarly, the incidences of hemangioma and hemangioma or hemangiosarcoma combined (at all sites) showed a significant positive trend only in the male stop-exposure groups, and were increased relative to controls only in the 315 mg/kg-day group. NTP attributed significant reductions in the incidences of mononuclear cell leukemia in both genders to the observed spleen toxicity. Similarly, a significant negative trend for testicular tumors (interstitial cell adenoma) was attributed to the increase in testicular atrophy in exposed animals. NTP concluded there was clear evidence for the carcinogenicity of *o*-nitrotoluene to male and female rats.

The lowest dietary level in the chronic rat study (NTP, 2002a), 625 ppm (25 mg/kg-day in males and 30 mg/kg-day in females), resulted in nonneoplastic effects in the testis (atrophy of germinal epithelium), kidney (renal tubule pigmentation), spleen (hematopoietic cell proliferation), and bone marrow (hematopoietic cell proliferation); preneoplastic effects in mammary gland (hyperplasia), lung (hyperplasia), and liver (eosinophilic foci); and increased carcinogenicity in multiple target tissues (malignant mesothelioma in the testis, fibroma or fibrosarcoma of the subcutaneous skin, and fibroadenoma of the mammary gland). There also was a significant increase in mortality among males at this dose, due to tumors.

Chronic oral treatment with *o*-nitrotoluene also resulted in systemic toxicity and carcinogenicity in multiple tissues in male and female mice. Groups of $B6C3F_1$ mice (60 per gender per group) were fed diets containing 0, 1250, 2500, or 5000 ppm of *o*-nitrotoluene (purity >99%) for two years (NTP, 2002a). Average daily intakes of *o*-nitrotoluene were reported as 0, 165, 360, or 700 mg/kg-day for males and 0, 150, 320, or 710 mg/kg-day for females. Mice received the same treatment and analysis as those in the rat chronic study, except that no satellite stop-exposure groups were used; no organ weight data were collected for mice. Treatment with *o*-nitrotoluene increased mortality in all male groups and in high-dose females; the numbers surviving to termination among groups of 60 were 52, 34, 0 and 0 for males, and 52, 46, 47 and 5 for females in the control and low-to-high-dose groups, respectively. All high-dose males had died by week 66 and all mid-dose males had died by week 101. The mortality was due to development of tumors (see below). Throughout the study, body weights in all exposed male groups and the high-dose female group were lower than in control groups; during the second year, mid-dose males lost weight and mid-dose females had reduced weight gains. Feed consumption was reduced in high-dose males.

Treatment-related nonneoplastic and preneoplastic lesions were observed in several organs in mice (Table 3) (NTP, 2002a). Effects in the liver related to chronic dosing included: necrosis at 165 mg/kg-day in males and 710 mg/kg-day in females, hepatocyte focal syncytial alteration in males at 165 mg/kg-day, cytoplasmic vacuolization in females at 710 mg/kg-day,

Table 3. Incidences of Selected Nonneoplastic and Preneoplastic Lesions in a 2-year Feeding Study of *o*-Nitrotoluene in Male and Female B6C3F$_1$ Mice[a]

	Male Mice				Female Mice			
	0 ppm	1250 ppm	2500 ppm	5000 ppm	0 ppm	1250 ppm	2500 ppm	5000 ppm
Organ: Lesion	Control	165 mg/kg-day	360 mg/kg-day	700 mg/kg-day	Control	150 mg/kg-day	320 mg/kg-day	710 mg/kg-day
Liver: Necrosis	1/60	15/59[b]	27/57[b]	30/60[b]	3/60	0/59	2/59	13/60[b]
Hepatocyte focal syncytial alteration	16/60	26/59[b]	43/57[b]	39/60[b]				
Cytoplasmic vacuolization					1/60	2/59	2/59	9/60[b]
Basophilic focus	0/60	6/59[b]	4/57[b]	0/60	1/60	6/59[b]	2/59	6/60[b]
Eosinophilic focus	3/60	14/59[b]	1/57	1/60	2/60	3/59	6/59	28/60[b]
Kidney: Tubular hyaline droplet accumulation	1/58	2/59	5/58[b]	3/60	1/59	3/56	2/58	10/59[b]
Tubular pigmentation	1/58	6/58[b]	32/58[b]	35/60[b]	0/59	1/56	3/58	35/59[b]
Olfactory epithelium: Degeneration	0/60	36/60[b]	60/60	60/60	0/60	28/60[b]	59/59[b]	57/57[b]
Spleen: Hemopoietic cell proliferation	13/60	24/60[b]	49/58[b]	60/60[b]	11/59	19/57	21/58[b]	54/57[b]
Skin: Edema	0/60	3/60	14/60[b]	22/60[b]	0/60	1/60	2/60	4/60[b]

[a]NTP, 2002a
[b]Statistically significant in pairwise test versus current controls

and basophilic or eosinophilic foci, primarily in the 165 mg/kg-day male and 710 mg/kg-day female groups. Kidney lesions included renal tubule pigmentation in males at 165 mg/kg-day and females at 710 mg/kg-day, and hyaline droplet accumulation in the 360 mg/kg-day male and 710 mg/kg-day female groups. Olfactory epithelial degeneration characterized by atrophy, necrosis, regeneration, hyperplasia, hypertrophy and metaplasia was observed in all exposed groups and in every individual mouse exposed at 320-360 mg/kg-day. The incidence of hematopoietic cell proliferation of the spleen was increased in males exposed at 165 mg/kg-day and females exposed at 710 mg/kg-day. NTP considered this lesion to be secondary to the increased incidences of hemangiosarcoma (see below). Incidences of edema of the subcutaneous tissue were elevated in males treated at 165 mg/kg-day and females treated at 710 mg/kg-day. NTP found histopathological evidence that these lesions were secondary to lymphatic obstruction by subcutaneous hemangiosarcomas.

Increased tumor incidences were observed in all exposed male groups and mid- and high-dose female groups of mice (Table 4) (NTP, 2002a). Incidences of hemangiosarcoma in all exposed male groups and in high-dose females were significantly larger than controls and exceeded the historical control ranges; all high-dose males were affected. Hemangiosarcomas

Table 4. Incidences of Neoplastic Tumors in B6C3F$_1$ Mice Exposed to *o*-Nitrotoluene in a 2-Year Feeding Bioassay[a]

	Male Mice				Female Mice			
	0 ppm	1250 ppm	2500 ppm	5000 ppm	0 ppm	1250 ppm	2500 ppm	5000 ppm
Organ: Tumor type	Control	165 mg/kg-day	360 mg/kg-day	700 mg/kg-day	Control	150 mg/kg-day	320 mg/kg-day	710 mg/kg-day
Hemangiosarcoma (all sites)	4/60[b] (7%)	17/60[c] (28%)	55/60[b] (92%)	60/60[c] (100%)	0/60[b]	2/60 (3%)	3/60 (5%)	50/60[b] (83%)
Large intestine (cecum): Carcinoma	0/60[b]	12/60[c] (20%)	9/60[b] (15%)	0/60	0/60[b]	1/60 (2%)	4/60 (7%)	3/60 (5%)
Liver: Hepatocellular adenoma or carcinoma	27/60 (45%)	28/60 (47%)	7/57 (12%)	2/60 (3%)	9/60[b] (15%)	9/60 (15%)	24/59[b] (41%)	39/60[b] (65%)

[a]NTP, 2002a
[b]Statistically significant trend
[c]Statistically significant in pairwise test versus current controls

were associated with the mesentery, skeletal muscle, and subcutis of the skin; in some mice with multiple hemangiosarcomas, the tumors metastasized to the lung and other sites. The incidences of carcinoma of the cecum were significantly increased in the low- and mid-dose male groups, and exceeded the historical control range. NTP attributed the lack of cecal tumors in high-dose males to the early mortality caused by hemangiosarcomas; reduced survival also may have affected cecal tumor incidence in mid-dose males. There was a significant positive trend for cecal tumors in females; NTP considered the slightly increased incidence of cecal tumors in high-dose females to be related to treatment, because the tumor was rare and had never been observed in historical control females. Incidences of hepatocellular adenoma in mid- and high-dose females and hepatocellular carcinoma in high-dose females were significantly greater than in controls and exceeded the historical control range. NTP concluded there was clear evidence for carcinogenicity of *o*-nitrotoluene in male and female mice.

In the chronic mouse bioassay (NTP, 2002a), the lowest dietary level of 1250 ppm (165 mg/kg-day in males and 150 mg/kg-day in females) resulted in toxic effects in the spleen (hematopoietic cell proliferation), olfactory epithelium (degeneration), kidney (tubular pigmentation), and liver (necrosis, focal hepatocyte syncytial alteration, basophilic and eosinophilic foci), and increased incidences of hemangiosarcomas and cecal carcinomas. There was a significant increase in mortality associated with the tumors in males at this dose. Body weight gain also was reduced at this dose in males.

Other Information

On the basis of the skin notations established by ACGIH (2001, 2004), NIOSH (2005), and OSHA (2007), *o*-nitrotoluene is likely to be readily absorbed via skin exposure. Gastrointestinal absorption in rats has been nearly complete (NTP, 2002a).

No pharmacokinetic data were available for *o*-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). This lack of human data did not allow estimations of the relevance of these data to humans.

Studies in rodents indicated rapid absorption of *o*-nitrotoluene in the gastrointestinal tract and rapid excretion, mainly in urine (U.S. EPA, 1986; IARC, 1996). In gavage studies using radio-labeled *o*-nitrotoluene (doses of 2 or 200 mg/kg), gastrointestinal absorption exceeded 99% in rats and 85% in mice (Chism et al., 1984; NTP, 2002a). In rats, most of the radioactivity (>87%) was excreted in urine, feces and expired air during the first 24 hours. During the first 72 hours, >95% of the dose was excreted in urine and <4% in feces.

Chism et al. (1984) and NTP (2002a) reported urinary metabolites of radio-labeled *o*-nitrotoluene quantified in F344/N rats and B6C3F$_1$ mice 24 or 48 hours after a single 200 mg/kg gavage dose. In rats, the metabolites included *o*-nitrobenzoic acid, *o*-nitrobenzyl glucuronide, *o*-aminobenzyl alcohol, S-(2-nitrobenzyl)-N-acetylcysteine, and smaller amounts of *o*-nitrobenzyl alcohol and *o*-toluidine. In male mice, the 24-hour urinary metabolites were: *o*-nitrobenzoic acid and *o*-nitrobenzyl glucuronide. These results and others reviewed in U.S. EPA (1986) indicated that microsomal oxidation of the methyl group to *o*-nitrobenzyl alcohol and *o*-benzoic acid was the major first step in the metabolic pathway in rats. Conjugation with glucuronic acid was a major metabolic pathway. Biliary excretion transferred the hepatic metabolite *o*-nitrobenzyl glucuronide to the intestine, where bacteria converted it to *o*-aminobenzyl alcohol. *In vitro* F344 male rat hepatocytes converted *o*-aminobenzyl alcohol to an unstable *o*-aminobenzyl sulfate by cytosolic sulfotransferases (Chism and Rickert, 1985). A carbonium decomposition product appeared to be the moiety that covalently bound to DNA. The potentially reactive intermediate, mercapturate, also may be formed during the metabolism of *o*-nitrobenzene to S-(2-nitrobenzyl)-N-acetylcysteine (NTP, 2002a).

Biliary excretion mentioned above contributed significantly to the genotoxicity of *o*-nitrotoluene in gavaged F344 rats (Chism and Rickert, 1985). In rats subjected to bile duct cannulation, hepatic macromolecular covalent binding of *o*-nitrotoluene was reduced by 98% in males and 85% in females (Chism and Rickert, 1985). As demonstrated by studies using germ-free rats, intestinal microflora were required for the induction of unscheduled DNA synthesis in hepatocytes of gavaged rats (Doolittle et al., 1983).

o-Nitrotoluene, like other aromatic nitro or amine compounds, has been known to form adducts with hemoglobin (Woo and Lai, 2001). NTP (2002a) reported similar hemoglobin-binding activity for male and female rats 72 hours after a single gavage dose of 200 mg/kg of *o*-nitrotoluene: 26 and 29.9 picomole-equivalents/mg globin for male and female rats, respectively. Sabbioni (1994) evaluated the hemoglobin binding activity of *o*-toluidine (2-methylaniline), a metabolite of *o*-nitrotoluene, in gavaged rats. The hemoglobin binding index [(mmol compound/mol hemoglobin) /(mmol compound/kg body weight)] for *o*-toluidine was 4, about five times lower than the value for aniline, a known inducer of methemoglobinemia in humans.

In vitro, the nitrotoluenes demonstrated some ability to convert hemoglobin to methemoglobin. *o*-Nitrotoluene was less potent than *m*- or *p*-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 20 mM *o*-nitrotoluene, 10 mM *m*-nitrotoluene, or 2.5 mM *p*-nitrotoluene. The presence of an NADP bioactivation system had no significant effect on the activity of *o*- or *p*-nitrotoluene, but slightly increased the activity of *m*-nitrotoluene. The methemoglobin-forming potency of *o*-nitrotoluene in sheep erythrocytes was calculated to be about five times lower than *p*-nitrotoluene and three times lower than *m*-nitrotoluene or aniline.

o-Nitrotoluene was not mutagenic in bacteria, but induced mutagenic responses in several mammalian *in vivo* studies (U.S. EPA, 1986; IARC, 1996). With or without metabolic activation, *o*-nitrotoluene did not induce reverse mutations in *Salmonella typhimurium* strains TA92, TA94, TA97, TA100, TA1535, TA1537, or TA1538, or cause differential toxicity in *Bacillus subtilis rec* strains. *o*-Nitrotoluene induced sister chromatid exchanges in Chinese hamster ovary (CHO) cells with S9 and gave equivocal results without S9 (NTP, 2002a). *o*-Nitrotoluene did not induce chromosomal aberrations in cultured CHO cells, with or without activation, or in Chinese hamster liver cells without activation. Negative results without activation also were reported for unscheduled DNA synthesis in several types of cultured cells: rat primary hepatocytes, rat pachytene spermatocytes or round spermatids, and human hepatocytes. *In vivo,* without exogenous activation, *o*-nitrotoluene yielded positive results for unscheduled DNA synthesis in hepatocytes of gavaged female mice, conflicting results in male and female rats, and negative results in male mice (NTP, 1992). Results of a peripheral blood micronucleus test were equivocal for male mice and negative for female mice exposed to *o*-nitrotoluene in the diet for 13 weeks. In the livers of gavaged male rats, *o*-nitrotoluene covalently bound to DNA, RNA, and protein (IARC, 1996).

NTP (2002a) compared genetic alterations in hemangiosarcoma tissue from male and female $B6C3F_1$ mice that ingested 150-710 mg/kg-day of *o*-nitrotoluene in the 2-year cancer bioassay to spontaneous hemangiosarcomas in control mice from previous NTP assays. All fifteen hemangiomas from mice exposed to *o*-nitrotoluene, but none of thirteen from control mice exhibited immunohistochemically detectable *p53* protein. Immunodetection of *p53* protein in the nucleus was considered an indication of genetic mutation since wild-type *p53* protein has a short life and has not been known to accumulate to levels of detectability in this assay. Using polymerase chain reaction techniques, mutations (primarily point mutations) in K-*ras*, *p53*, or -catenin were detected in 13 of 15 of these hemangiomas. -Catenin protein was detectable in 7 of 15 hemangiosarcomas from exposed mice and 0 of 13 from controls. Expression of -catenin was related to a loss of cell adhesiveness, which is thought to facilitate invasiveness or metastasis.

Dunnick et al. (2003) further elaborated on the NTP (2002) data, noting that the "stop study" data in male rats indicated that critical events leading to tumor formation occurred after 3 months of dosing with *o*-nitrotoluene and irreversibly led to cancer at multiple sites. Hong et al. (2003) examined 15 subcutaneous hemangiosarcomas found in the NTP (2002) mice for genetic alterations in *ras*, *p53* and -Catenin genes. They concluded that *p53* and -Catenin mutations in the *o*-nitrotoluene-induced hemangiomas "most likely occurred as a result of the genotoxic effects" of this chemical, and suggested that these mutations might play a role in the

pathogenesis of these hemangiosarcomas in $B6C3F_1$ mice. Sills et al. (2004) further evaluated the NTP (2002) data on the morphology and molecular profile of oncogenes and tumor suppression genes related to cecal tumors observed in $B6C7F_1$ mice. Mutations in *p53* were identified in 9 of 11 tumors, all in exon 7.

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

No data were available for the chronic or subchronic oral toxicity of *o*-nitrotoluene to humans. Subchronic and chronic oral feeding studies in rodents demonstrated that *o*-nitrotoluene had multiple target tissues. Rats generally appeared to be more sensitive than mice. However, no data were available to determine which species' responses would be more similar to humans. The methemoglobinemic potential of *o*-nitrotoluene appeared to be involved in some of the hematological and spleen effects observed in the NTP studies. In the subchronic rat study (NTP, 1992), significantly elevated methemoglobin levels were observed at the LOAEL of 44 mg/kg-day in female rats following the first three weeks of treatment, although this effect at this dose did not persist to 13 weeks at a statistically significant level. Anemia (reduced hemoglobin, hematocrit, and erythrocyte counts) was observed in males and females treated at 353 or 340 mg/kg-day, respectively, for 13 weeks. Spleen hemosiderosis, resulting from destruction of erythrocytes, occurred in male and female rats treated with 179 and 178 mg/kg-day, respectively. Increased hematopoiesis in the spleen occurred at 179 mg/kg-day in males and at 675 mg/kg-day in females. At 694 mg/kg-day, male spleens exhibited capsular fibrosis. The hematological and spleen findings were supported by the Ciss et al. (1980) 6-month gavage study, in which signs of anemia, splenomegaly, and unspecified spleen histopathologic changes were observed in rats dosed with 200 mg/kg-day, 5 days/week: equivalent to a duration-adjusted dose of 143 mg/kg-day.

Young children are more susceptible to methemoglobinemia, a toxic effect of o-nitrotoluene, than adults. There are several reasons for this. First, newborns still have fetal Hb, which is more susceptible to metHb formation than adult Hb (Goldstein et al., 1969). Next, the activity of NADH-cytochrome b_5 reductase, an enzyme required for the conversion of ferric iron to ferrous iron in Hb, is not fully developed in infants and very young children (Wentworth et al., 1999) and neither is glucose-6-phosphatase dehydrogenase activity, an enzyme required to replenish NADPH (Goldstein et al., 1969). Additionally, the observation of more accidental fatal poisonings in children exposed dermally indicates a potential greater sensitivity to dermal nitrobenzene exposures.

Other toxic effects of *o*-nitrotoluene probably were not related to methemoglobinemia. Clinical chemistry analyses revealed indications of liver toxicity in male rats: decreased total protein at 45 mg/kg-day after one week of treatment and increased SDH at 179 mg/kg-day, bile acids at 353 mg/kg-day, and ALT at 694 mg/kg-day after 13 weeks of treatment. Relative liver weights were increased in males and females at 45 or 44 mg/kg-day, and absolute liver weights were increased in males at 179 mg/kg-day and females at 675 mg/kg-day. Histopathologic changes of the liver (cytoplasmic vacuolization, oval cell hyperplasia, and inflammation) were observed in males treated with 179 mg/kg-day. Other independent effects were atrophy of the

preputial gland in males and the salivary gland in males and females treated at 89 (males) or 87 (females) mg/kg-day for 13 weeks. Reproductive effects were noted at the higher doses: degeneration of the testis and reduced sperm counts at 353 mg/kg-day; impaired estrous cycling in females at 675 mg/kg-day. The gavage study by Ciss et al. (1980), although inadequate because of its small group size, suggested that treatment with a duration-adjusted dose of 143 mg/kg-day had no adverse effect on reproduction in rats.

The LOAEL of 44 mg/kg-day in the 13-week feeding study in rats (NTP, 1992) served as the POD for the subchronic p-RfD for *o*-nitrotoluene. Benchmark dose modeling was not attempted because of the minimal nature of this LOAEL, the variability in the rat methemoglobin data including time-related increases in the control group, and the lack of a well-defined methemoglobin concentration considered adverse in rats. At this dose, methemoglobin levels were significantly increased in female rats that had been treated for three weeks, though the increase over controls was not significant after 13 weeks. Since additional effects of increased methemoglobin were observed only at higher doses, 44 mg/kg-day was considered a LOAEL but the effects at this dose were considered of minimal biological significance. A provisional **subchronic RfD of $1x10^{-2}$ mg/kg-day** for *o*-nitrotoluene was derived by applying the following uncertainty factors to the rat LOAEL of 44 mg/kg-day:

- 3 for the use of LOAEL with minimal biological significance
- 10 to extrapolate from rats to humans
- 10 to protect sensitive individuals
- 10 for the limited data on reproductive and developmental toxicity.

subchronic p-RfD	=	POD / UF
	=	(subchronic minimal LOAEL) / (3x10x10x10)
	=	44 mg/kg-day / 3000
	=	0.0147 or **$1x10^{-2}$ mg/kg-day**

Confidence in the key subchronic study (NTP, 1992) was medium. This well-documented study evaluated adequate numbers of animals in two species and analyzed nearly all critical endpoints. However, the study did not include a NOAEL dose and did not record spleen weights. Confidence in the database was only medium, since the supporting chronic and subchronic studies did not analyze all critical endpoints (e.g., organ weights, hematology, and clinical chemistry) and only limited data were available for potential reproductive and development toxicity. Medium confidence in the subchronic p-RfD followed.

The chronic NTP (2002a) bioassay in rats did not include hematological analysis or an analysis of clinical chemistry parameters. Hematopoietic cell proliferation was observed at the low dose in males (25 mg/kg-day) in the liver, bone marrow, and spleen, and in the spleens of females treated at 30 mg/kg-day. Other nonneoplastic effects observed at 25 mg/kg-day in male rats included atrophy of germinal epithelium in the testis and pigmentation of kidney tubules. Preneoplastic effects included hyperplasia of the lung and eosinophilic foci of the liver. More significantly, this dose resulted in increased carcinogenicity in multiple target tissues (malignant mesothelioma in the testes, fibroma or fibrosarcoma of subcutaneous skin, and fibroadenoma of the mammary gland) and significantly increased cancer-related mortality. Despite the frank

nature of some effects observed at the lowest dose tested, benchmark dose (BMD) analyses of the data from several endpoints provided reasonable bases for derivation of a chronic p-RfD (see Appendix A). The BMD Lower Confidence Limit for a 10% response ($BMDL_{10}$) was chosen based on current BMD guidance (U.S. EPA, 2000). Selected data for several potential critical effects and points of departure (POD) are summarized in Table 5.

Table 5. Potential critical effects and BMD-calculated potential points of departure for *o*-nitrotoluene chronic RfD

Potential critical effects	Control Response	LOAEL mg/kg-day	LOAEL response	BMD_{10} mg/kg-day	$BMDL_{10}$ mg/kg-day
Rat (male) bone marrow hyperplasia	3.3%	25	42%	5.1	0.94
Rat (male) spleen - hematopoietic cell proliferation	12%	25	55%	2.7	0.027
Mouse (male) liver cell necrosis	1.7%	165	25%	33	0.95
Mouse (male) renal tubule pigmentation	1.7%	165	10%	N/A	N/A
Mouse (male) Olfactory epithelial degeneration	0%	165	60%	80	20
Mouse (female) Olfactory epithelial degeneration	0%	150	47%	90	37

The lowest ingested doses of *o*-nitrotoluene in mice (150 and 165 mg/kg-day) were six to nearly seven times higher than those in rats (25 and 30 mg/kg-day). Benchmark dose analyses revealed $BMDL_{10}$-estimated NOAELs of 0.94 mg/kg-day for bone marrow hyperplasia in male rats and 0.95 mg/kg-day for liver cell necrosis of in male mice. These values were more than twenty times below the BMD-estimated NOAELs for olfactory epithelial degeneration in male (20 mg/kg-day) and female (37 mg/kg-day) mice. Table 5 also includes a potential POD (0.027 mg/kg-day) for spleen hematopoietic cell proliferation among male rats that was 35 times below those cited above. However, this value was not selected as the POD because of numerous concerns about the plausibility of the BMD curve (Figure A-4, including the fact that this $BMDL_{10}$ was two orders of magnitude lower than the BMD_{10}.

The $BMDL_{10}$ of 0.94 mg/kg-day for chronic ingestion of *o*-nitrotoluene in male rats (and 0.95 mg/kg-day in male mice) was applied as the POD for calculating the chronic p-RfD. To this POD, the following uncertainty factors were applied:

- 10 to extrapolate from rats to humans
- 10 to protect sensitive individuals
- 10 for the database uncertainty resulting from the limited data on reproductive and developmental toxicity.

The product of these individual UFs was a composite UF of 1000.

chronic p-RfD	=	POD / UF
	=	$BMDL_{10}$ / (10x10x10)
	=	0.94 mg/kg-day / 1000
	=	0.00094 or **$9x10^{-4}$ mg/kg-day**

Confidence in the key chronic study (NTP, 2002a) was low because chronic oral exposure doses of *o*-nitrotoluene did not identify a NOAEL, and the LOAEL for the critical effect resulted in a 42% incidence rate vs. 3.3% among controls. In addition, frank effects were observed at the lowest chronic doses in both the rat and mouse studies. Confidence in the database was medium based on the same considerations as discussed for the subchronic p-RfD. The resulting confidence in the chronic p-RfD was medium.

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

No chronic or subchronic data were located for the subchronic or chronic inhalation toxicity of *o*-nitrotoluene in humans or animals. In addition, no relevant information was available for *m*- or *p*-nitrotoluene, which eliminated the possibility of deriving a p-RfC by analogy to these compounds. Although provisional chronic and subchronic oral RfDs were derived for *o*-nitrotoluene and some pharmacokinetic data were available, observation of irritant portal of entry effects in humans (Eastman Kodak, 1981) and animals (Haskell Labs, 1972) acutely exposed to airborne *o*-nitrotoluene ruled out a route-to-route extrapolation for this compound. Therefore, inhalation p-RfC values for *o*-nitrotoluene were not derived.

DERIVATION OF A PROVISIONAL CARCINOGENICITY ASSESSMENT FOR *o*-NITROTOLUENE

Weight-of-Evidence Descriptor

No human carcinogenicity data were located for *o*-nitrotoluene, but there was strong evidence for carcinogenicity in rats and mice. The subchronic feeding assay by NTP (1992) demonstrated evidence for carcinogenicity in rats, but not in mice, exposed to *o*-nitrotoluene in the diet for 13 weeks. Tumors observed included rare non-malignant mesotheliomas of the tunica vaginalis of the epididymis in three of ten rats fed 353 mg/kg-day. In addition, mesothelial hyperplasia was observed at the epididymal tunica vaginalis in two of ten rats exposed at 694 mg/kg-day for 13 weeks. Mesothelioma in the testes and cholangiocarcinoma in the liver both were observed in rats fed 292 mg/kg-day of *o*-nitrotoluene for 13 or 26 weeks in another subchronic assay (NTP, 1996). Clear evidence for carcinogenicity in multiple organs in both genders was reported in chronic feeding assays in rats and mice (NTP, 2002a). In both rats and mice, there were dose-related decreases in time-to-tumor formation. All doses produced significantly increased tumor incidences in the rat study. The incidence of mesotheliomas of the tunica vaginalis of the epididymis and the serosal surface of abdominal organs was increased in male rats fed 25 mg/kg-day. Subcutaneous fibromas or fibrosarcomas were increased in male rats fed 25 mg/kg-day and female rats fed 60 mg/kg-day. The incidence of fibroadenoma of the

mammary gland was increased in male rats at 25 mg/kg-day and female rats at 30 mg/kg-day. Small but statistically significant increases in hepatocellular adenomas, or adenomas and carcinomas combined, occurred in male rats fed 90 mg/kg-day; similar increases in hepatocellular adenomas occurred in female rats fed 100 mg/kg-day. In the stop-exposure experiments, male rats, fed for three months at 125 mg/kg-day and observed for an additional 21 months while on the control diet, developed malignant testicular mesotheliomas as well as tumors of skin and mammary gland at incidences similar to those in the 90 mg/kg-day group treated for two years. Male rats fed 315 mg/kg-day in the stop-exposure experiments showed, in addition, modest increases in hepatocellular adenoma or carcinoma combined, alveolar/bronchiolar adenoma or carcinoma combined, and hemangioma or hemangiosarcoma combined (all sites). Carcinogenicity of *o*-nitrotoluene in chronically-exposed mice occurred at higher doses in the NTP (2002a) feeding assay. The incidence of hemangiosarcoma at all sites was significantly elevated in male mice fed 175 mg/kg-day and female mice fed 660 mg/kg-day for two years. Male mice fed 175 mg/kg-day had a significant increase in carcinoma of the large intestine, whereas female mice fed 315 mg/kg-day had increased incidences of hepatocellular adenoma or carcinoma (combined). Thus, male and female rats and mice fed *o*-nitrotoluene all exhibited increased incidence of various tumors. Under U.S. EPA (2005) guidelines, the hazard descriptor *likely to be carcinogenic to humans* is applied to *o*-nitrotoluene.

The carcinogenic effects of *o*-nitrotoluene were similar to some of those reported for structurally-related compounds. *o*-Toluidine hydrochloride increased the incidence of mesothelioma in male rats and mammary gland tumors in female rats exposed in feed (NCI, 1979). *p*-Nitrotoluene induced equivocal increases in skin tumors in male rats exposed in the diet (NTP, 2002b). *o*-Nitroanisole induced hepatocellular adenomas and carcinomas in male and female mice exposed in feed (NTP, 1993a). Chronic treatment with *p*-chloroaniline (NTP, 1989) or *p*-nitroaniline (NTP, 1993b) by gavage caused a significant positive trend for hemangiosarcomas at all sites in male $B6C3F_1$ mice.

In vitro and *in vivo* tests suggested that *o*-nitrotoluene was not mutagenic in bacteria and that its mutagenic effects in mammalian systems were dependent on bioactivation. The compound induced sister chromatid exchanges in cultured CHO cells with activation, but gave equivocal results without activation (NTP, 2002a). Adducts to DNA and other macromolecules were formed in the liver of male rats gavaged with *o*-nitrotoluene (NTP, 2002a). In addition, *o*-nitrotoluene caused unscheduled DNA synthesis in the liver of gavaged female mice, whereas results were negative in male mice and equivocal in male and female rats (NTP, 1992). These *in vivo* results were consistent with the increased incidences of hepatocellular tumors reported in the NTP (2002a) feeding assay (Tables 2 and 4) The effects were significant in pairwise and trend tests in female mice, significant trends only in male and female rats, and no increase in male mice. NTP (2002a) and Hong et al. (2003) reported direct evidence for genotoxicity associated with hemangiosarcomas in female $B6C3F_1$ mice treated with *o*-nitrotoluene in the chronic feeding assay in that mutations in several genes associated with cellular regulation (*p53*, K-*ras*, and -catenin) were noted.

Although there were no reports of human carcinogenicity resulting from exposure to *o*-nitrotoluene (ACGIH, 2001), some aromatic amines that induce methemoglobinemia (e.g., 4-aminobiphenyl, benzidine, and 2-naphthylamine) have been implicated as carcinogens in humans

as well as animals (Woo and Lai, 2001). There was evidence that oral exposure might enhance the genotoxicity of *o*-nitrotoluene because of the reduction of the nitro group of hepatic metabolites by intestinal bacteria as a result of biliary circulation (Doolittle et al., 1983; U.S. EPA, 1986; NTP, 2002a). The increase in foci rich in glutathione-S-transferase in livers of male rats gavaged with high doses of *o*-nitrotoluene (Ton et al., 1995) provided some evidence that bioactivation to reactive intermediates may be involved in the carcinogenic mode of action of *o*-nitrotoluene. Metabolites not processed in phase II conjugation reactions could be genotoxic. A genotoxic mode of action for *o*-nitrotoluene also could be inferred from the finding that it induces tumors in a number of tissues. Its mechanism of action may involve common processes regulating cell growth as indicated by the detection of mutations in genes involved in cell regulation in the hemangiosarcomas and cecal tumors induced by *o*-nitrotoluene (NTP, 2002a; Sills et al., 2004; Hong et al., 2003). However, the mode of action of carcinogenicity for o-nitrotoluene is largely unknown, so a linear low-dose extrapolation approach was considered appropriate for the cancer risk quantitation for *o*-nitrotoluene.

Quantitative Estimates of Carcinogenic Risk

Dose-response modeling was performed based on the most prominent tumors in the chronic rat and mouse studies (NTP, 2002a).

- malignant testicular mesothelioma in male rats (Table 6)
- skin fibroma or fibrosarcoma in male and female rats (Table 7)
- mammary fibroadenoma in female rats (Table 8)
- hemangiosarcoma (all sites) in male and female mice (Table 9)
- hepatocellular adenoma or carcinoma in female mice (Table 10).

The following derivation used the methodologies in the U.S. EPA (2005) guidelines for cancer risk assessment. The mode of action (MOA) leading to tumor formation is unknown. Thus, the default linear extrapolation from the point of departure to the origin was used for each cancer risk estimate. In accordance with the 2005 guidelines, the LED_{10} (lower bound on dose estimated to produce a 10% increase in tumor incidence over background) for each tumor type was estimated using the U.S. EPA (2000) benchmark dose methodology, and linear extrapolation to the origin was performed by dividing the LED_{10} into 0.1 (10%). The $0.1/LED_{10}$ provided an estimate of the slope factor in animals. Each unadjusted value, based directly on the animal tumor data, was adjusted to a human value by correcting for differences in body weight between humans and experimental animals. Adjustments from animal to human slope factors were performed by multiplying each animal value by the ratio of human to animal body weight raised to the 1/4 power. No adjustment was needed for the 24-month duration of the experiment, since it was considered equal to the reference life span of 24 months in rodents. This analysis does not account for the dose-related time-to-tumor and early mortality that was observed.

The magnitude of the $0.1/LED_{10}$ value was largest for skin tumors in male rats (Table 7): 2.2×10^{-1} $(mg/kg\text{-}day)^{-1}$ for the human $0.1/LED_{10}$. The slope factors were smaller for tumors in other organs:

- 5.8×10^{-2} $(mg/kg\text{-}day)^{-1}$ for testicular mesotheliomas in male rats (Table 6),

Table 6. LED_{10} Values Based on Incidence of Malignant Testicular Mesothelioma in Male F344/N Rats[a]

Male Rats	Incidence 0 mg/kg-day	Incidence 25 mg/kg-day	Incidence 50 mg/kg-day	Incidence 90 mg/kg-day	rat LED_{10} (mg/kg-day)	rat $0.1/LED_{10}$ $(mg/kg\text{-}day)^{-1}$	human $0.1/LED_{10}$ $(mg/kg\text{-}day)^{-1}$
	2/60	20/60	29/60	44/60	6.3	1.6×10^{-2}	5.8×10^{-2}

[a]NTP, 2002a

Rats were exposed to dietary levels of 0, 625, 1250 or 2000 ppm of *o*-nitrotoluene for 24 months. Doses were reported in NTP (2002a).
Human value ($0.1/LED_{10}$) calculated as: rat value ($0.1/LED_{10}$) x $(W_{hum} / W_{rat})^{1/4}$ x $(L / L_e)^3$ where W_{hum} = 70 kg (human reference body weight), W_{rat} = 0.393 kg (TWA male rat body weight for the lowest affected dose group), L = 24 months (rat life span), L_e = 24 months (duration of experiment)
Rat LED_{10} calculated using polynomial model [polydegree of 1 chosen using algorithm in U.S. EPA (2000)]

Table 7. LED_{10} Values Based on Incidences of Subcutaneous Skin Fibroma or Fibrosarcoma in Male and Female F344/N Rats[a]

Male Rats	Incidence 0 mg/kg-day	Incidence 25 mg/kg-day	Incidence 50 mg/kg-day	Incidence 90 mg/kg-day	rat LED_{10} (mg/kg-day)	rat $0.1/LED_{10}$ $(mg/kg\text{-}day)^{-1}$	human $0.1/LED_{10}$ $(mg/kg\text{-}day)^{-1}$
	5/60	47/60	55/60	59/60	1.7	5.9×10^{-2}	2.2×10^{-1}
Female Rats	Incidence 0 mg/kg-day	Incidence 30 mg/kg-day	Incidence 60 mg/kg-day	Incidence 100 mg/kg-day	rat LED_{10} (mg/kg-day)	rat $0.1/LED_{10}$ $(mg/kg\text{-}day)^{-1}$	human $0.1/LED_{10}$ $(mg/kg\text{-}day)^{-1}$
	3/60	3/60	21/60	22/60	26.5	3.8×10^{-3}	1.6×10^{-2}

[a]NTP, 2002a

Rats were exposed to dietary levels of 0, 625, 1250 or 2000 ppm of *o*-nitrotoluene for 24 months. Doses were reported in NTP (2002a).
Human value ($0.1/LED_{10}$) calculated as: rat value ($0.1/LED_{10}$) x $(W_{hum} / W_{rat})^{1/4}$ x $(L / L_e)^3$ where W_{hum} = 70 kg (human reference body weight), W_{rat} = 0.393 kg or 0.235 kg (TWA male or female rat body weights for the lowest affected dose groups), L = 24 months (rat life span), L_e = 24 months (duration of experiment)
Rat LED_{10} calculated using polynomial model [for males, a polydegree of 1 and for females, a polydegree of 2 (after dropping the high-dose group) chosen using algorithm in U.S. EPA (2000)]

Table 8. LED_{10} Values Based on Incidence of Mammary Fibroadenoma in Female F344/N Rats[a]

Female Rats	Incidence 0 mg/kg-day	Incidence 30 mg/kg-day	Incidence 60 mg/kg-day	Incidence 100 mg/kg-day	rat LED_{10} (mg/kg-day)	rat $0.1/LED_{10}$ $(mg/kg\text{-}day)^{-1}$	human $0.1/LED_{10}$ $(mg/kg\text{-}day)^{-1}$
	23/60	47/60	52/60	56/60	3.2	$3.1x10^{-2}$	$1.3x10^{-1}$

[a]NTP, 2002a

Rats were exposed to dietary levels of 0, 625, 1250 or 2000 ppm of *o*-nitrotoluene for 24 months. Doses were reported in NTP (2002a).
Human value ($0.1/LED_{10}$) calculated as: rat value ($0.1/LED_{10}$) x $(W_{hum} / W_{rat})^{1/4}$ x $(L / L_e)^3$ where W_{hum} = 70 kg (human reference body weight), W_{rat} = 0.236 kg (TWA female rat body weight for the lowest affected dose groups), L = 24 months (rat life span), L_e = 24 months (duration of experiment)
Rat LED_{10} calculated using polynomial model [a polydegree of 1 chosen using algorithm in U.S. EPA (2000)]

Table 9. LED_{10} Values Based on Incidences of Hemangiosarcoma (All Sites) in Male and Female $B6C3F_1$ Mice[a]

Male Mice	Incidence 0 mg/kg-day	Incidence 165 mg/kg-day	Incidence 360 mg/kg-day	Incidence 700 mg/kg-day	mouse LED_{10} (mg/kg-day)	mouse $0.1/LED_{10}$ $(mg/kg\text{-}day)^{-1}$	human $0.1/LED_{10}$ $(mg/kg\text{-}day)^{-1}$
	4/60	17/60	55/60	60/60	63	$1.6x10^{-3}$	$1.0x10^{-2}$
Female Mice	Incidence 0 mg/kg-day	Incidence 150 mg/kg-day	Incidence 320 mg/kg-day	Incidence 710 mg/kg-day	mouse LED_{10} (mg/kg-day)	mouse $0.1/LED_{10}$ $(mg/kg\text{-}day)^{-1}$	human $0.1/LED_{10}$ $(mg/kg\text{-}day)^{-1}$
	0/60	2/60	3/60	50/60	246	$4.1x10^{-4}$	$1.3x10^{-3}$

[a]NTP, 2002a

Mice were exposed to dietary levels of 0, 1250, 2500 or 5000 ppm of *o*-nitrotoluene for 24 months. Doses were reported in NTP (2002a).
Human value ($0.1/LED_{10}$) calculated as: mouse value ($0.1/LED_{10}$) x $(W_{hum} / W_{mouse})^{1/4}$ x $(L / L_e)^3$ where W_{hum} = 70 kg (human reference body weight), W_{mouse} = 0.040 kg or 0.029 kg (TWA male or female mouse body weight for the lowest affected dose groups), L = 24 months (mouse life span), L_e = 24 months (duration of experiment)
Mouse LED_{10} calculated using polynomial model [for males, a polydegree of 2 was chosen and for females, a polydegree of 3 was chosen using algorithm in U.S. EPA (2000)]

Table 10. LED_{10} Values Based on Incidences of Hepatocellular Adenoma or Carcinoma in Female $B6C3F_1$ Mice[a]

Female Mice	Incidence 0 mg/kg-day	Incidence 150 mg/kg-day	Incidence 320 mg/kg-day	Incidence 710 mg/kg-day	mouse LED_{10} (mg/kg-day)	mouse $0.1/LED_{10}$ $(mg/kg\text{-}day)^{-1}$	human $0.1/LED_{10}$ $(mg/kg\text{-}day)^{-1}$
	9/60	9/60	24/59	39/60	70.8	$1.4x10^{-3}$	$9.4x10^{-3}$

[a]NTP, 2002a

Mice were exposed to dietary levels of 0, 1250, 2500, or 5000 ppm of *o*-nitrotoluene for 24 months. Doses were reported in NTP (2002a). Human value ($0.1/LED_{10}$) calculated as: mouse value ($0.1/LED_{10}$) x $(W_{hum} / W_{mouse})^{1/4}$ x $(L / L_e)^3$ where W_{hum} = 70 kg (human reference body weight), W_{mouse} = 0.035 kg (TWA female mouse body weight for the lowest affected dose group), L = 24 months (mouse life span), L_e = 24 months (duration of experiment)
Mouse LED_{10} calculated using polynomial model [polydegree of 1 chosen using algorithm in U.S. EPA (2000)]

- 1.6×10^{-2} $(mg/kg\text{-}day)^{-1}$ for skin tumors in female rats (Table 7),
- 1.3×10^{-2} $(mg/kg\text{-}day)^{-1}$ for mammary tumors in female rats (Table 8),
- 1.0×10^{-2} $(mg/kg\text{-}day)^{-1}$ for hemangiosarcomas in male mice (Table 9),
- 9.4×10^{-3} $(mg/kg\text{-}day)^{-1}$ for hepatic tumors in female mice (Table 10), and
- 1.3×10^{-3} $(mg/kg\text{-}day)^{-1}$ for hemangiosarcomas in female mice (Table 9).

As the largest value is most protective, the $0.1/LED_{10}$ value for skin tumors in male rats (Table 7) was adopted as the provisional **oral slope factor of 2.2×10^{-1} $(mg/kg\text{-}day)^{-1}$** for *o*-nitrotoluene.

There were no human or animal carcinogenicity data from which to derive a provisional inhalation unit risk value for *o*-nitrotoluene.

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Appendix A. Benchmark Dose Analyses of Chronic Dietary o-Nitrotoluene Data for Potential Critical Effects in NTP, 2002

Table A-1. BMD Summaries for o-Nitrotoluene Chronic Male Rat Bone Marrow Hyperplasia Data[a]

BMD Model	P	Σ scaled residuals	AIC	BMD (mg/kg-day)	BMDL (mg/kg-day)
Log-logistic	**0.1566**	**2.3**	**246.083**	**5.09982**	**0.93905**
Log-Probit	0.1449	2.4	246.208	5.49929	1.06454
Multistage	0.1049	1.1	246.427	5.55732	4.67698
Quantal Linear	0.1049	3.3	246.427	5.55732	4.67698
Weibull	0.1049	3.3	246.427	5.55732	4.67698
Gamma	0.1049	3.4	246.427	5.55732	4.67698
Log-normal	0	8.8	264.644	13.7147	11.6601
Probit	0	8.8	264.689	13.4265	11.5968
Quantal quadratic	0	11.1	272.89	20.7592	18.3205

[a]NTP, 2002
LOAEL = 25 mg/kg-day for 42% incidence; Control incidence = 3.3%.

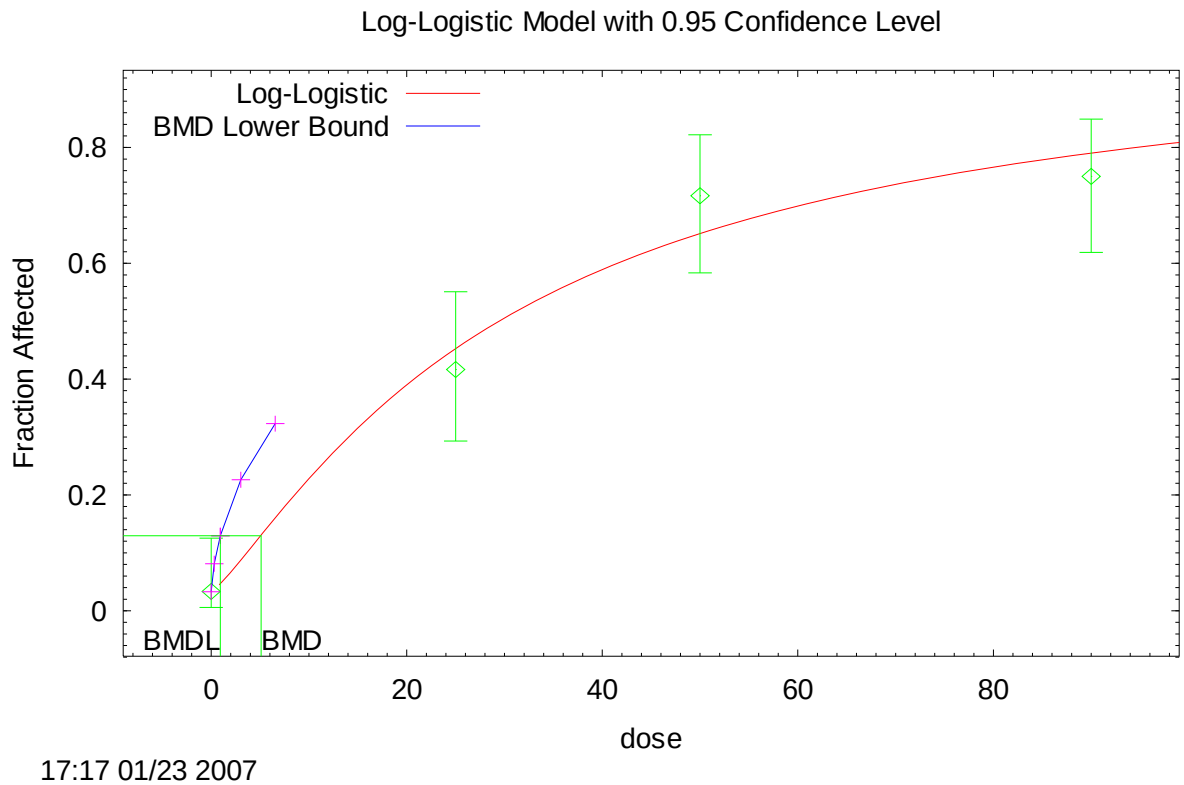

Figure A-1. Plot of Log-Logistic Model of o-Nitrotoluene Chronic Male Rat Bone Marrow Hyperplasia Data (NTP, 2002)

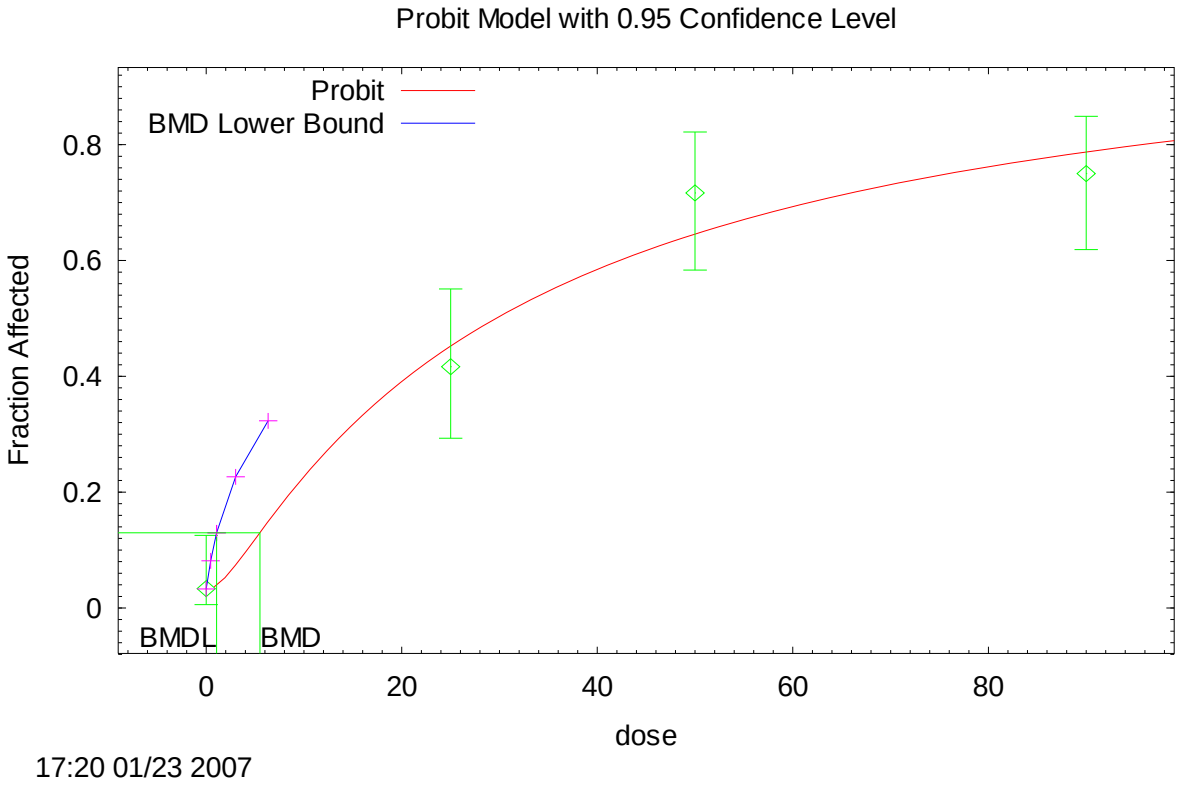

Figure A-2. Plot of Log-Probit Model of o-Nitrotoluene Chronic Male Rat Bone Marrow Hyperplasia Data (NTP, 2002)

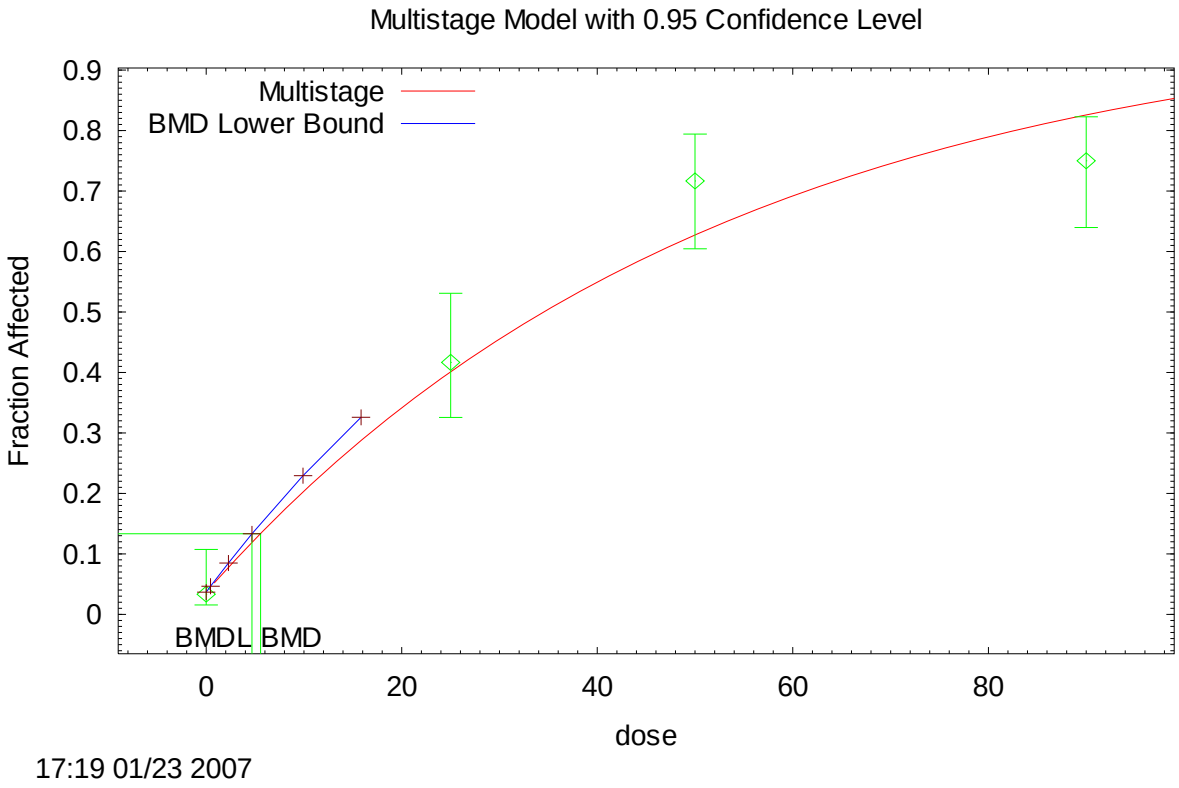

Figure A-3. Plot of Multistage Model of o-Nitrotoluene Chronic Male Rat Bone Marrow Hyperplasia Data (NTP, 2002)

Table A-2. BMD Summaries for o-Nitrotoluene Chronic Male Rat Spleen Hematopoietic Cell Proliferation Data[a]

BMD Model	P	Σ scaled residuals	AIC	BMD (mg/kg-day)	BMDL (mg/kg-day)
Log-Probit	0.5084	1.1	273.817	2.72441	0.0271677
Log-logistic	0.4951	1.1	273.845	2.35451	0.0179337
Multistage	0.1663	1.0	274.917	5.91598	4.82863
Gamma	0.1663	3.2	274.917	5.91598	4.82863
Quantal Linear	0.1663	3.2	274.917	5.91598	4.82863
Weibull	0.1663	3.2	274.917	5.91598	4.82863
Log-normal	0.0028	6.2	283.694	12.1968	10.3748
Probit	0.0026	6.3	283.841	12.1009	10.4504
Quantal quadratic	0	8.5	292.885	23.1198	19.9717

[a]NTP, 2002
LOAEL = 25 mg/kg-day for 33/60 incidence; Control incidence = 7/60

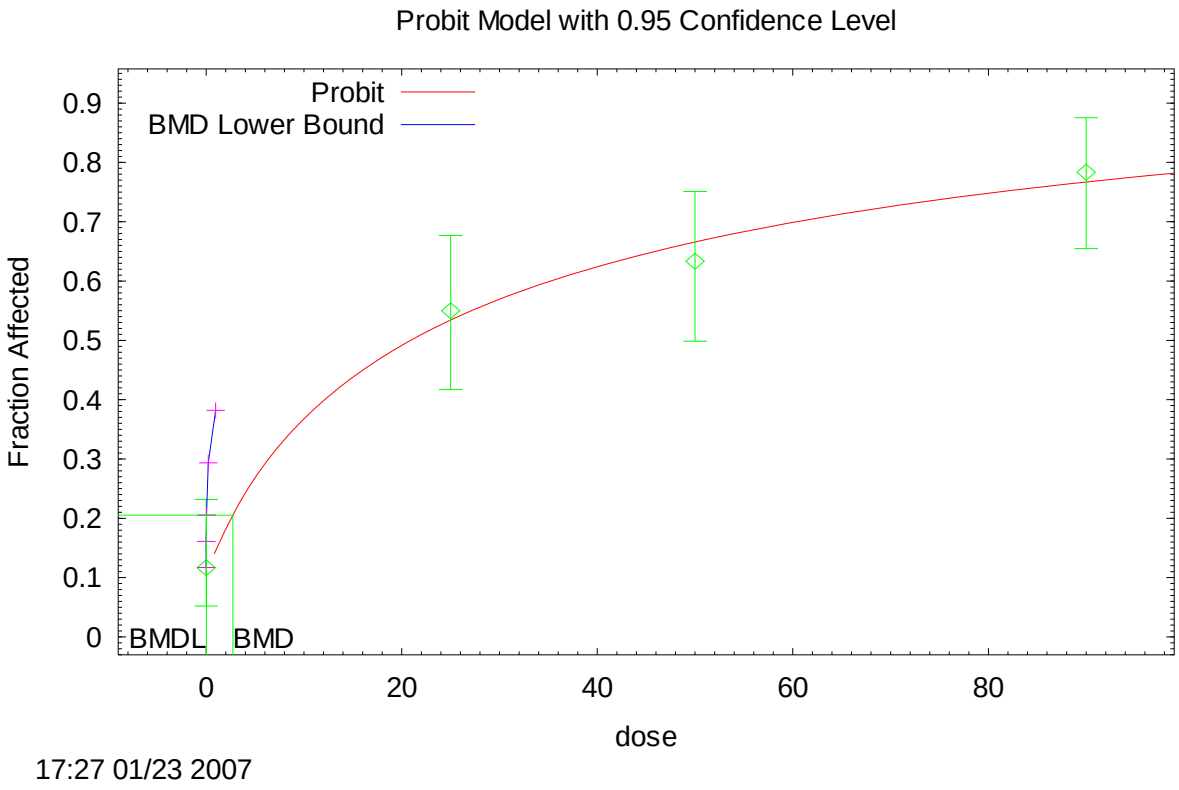

Figure A-4. Plot of Log-Probit Model of o-Nitrotoluene Chronic Male Rat Spleen Hematopoietic Cell Proliferation Data (NTP, 2002)

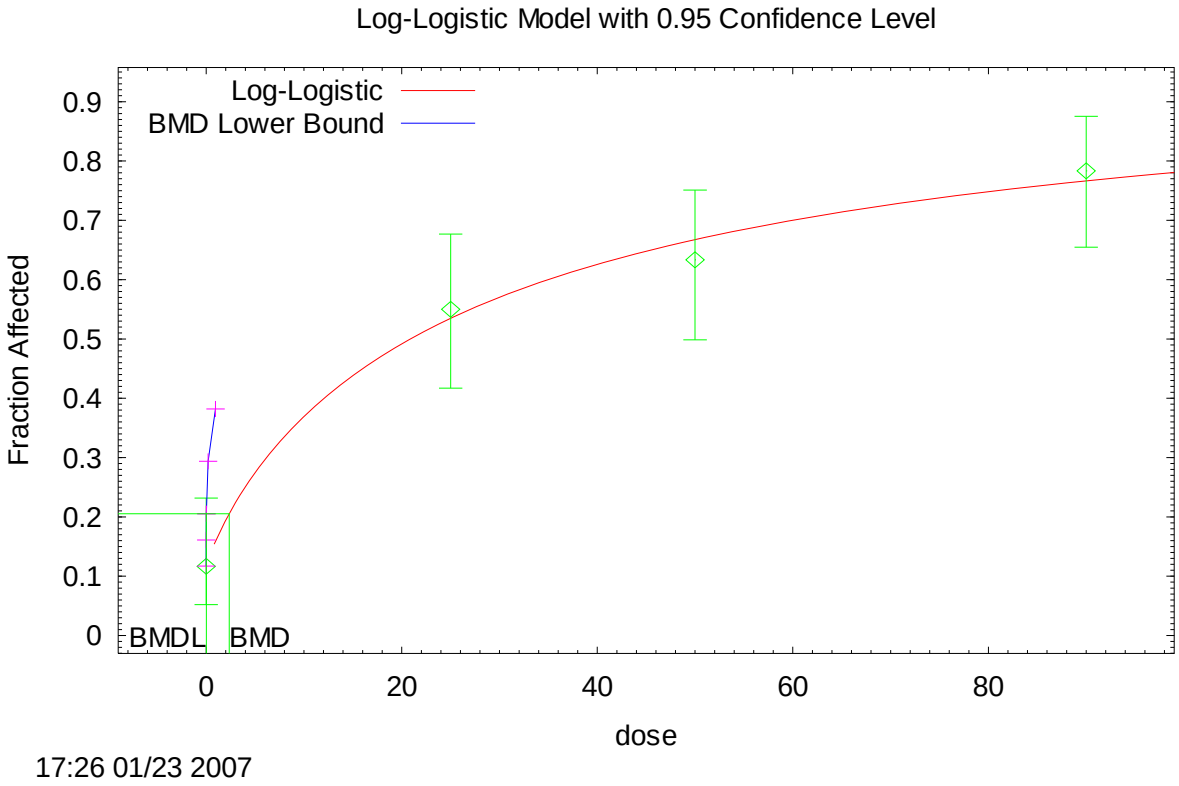

Figure A-5. Plot of Log-Logistic Model of o-Nitrotoluene Chronic Male Rat Spleen Hematopoietic Cell Proliferation Data (NTP, 2002)

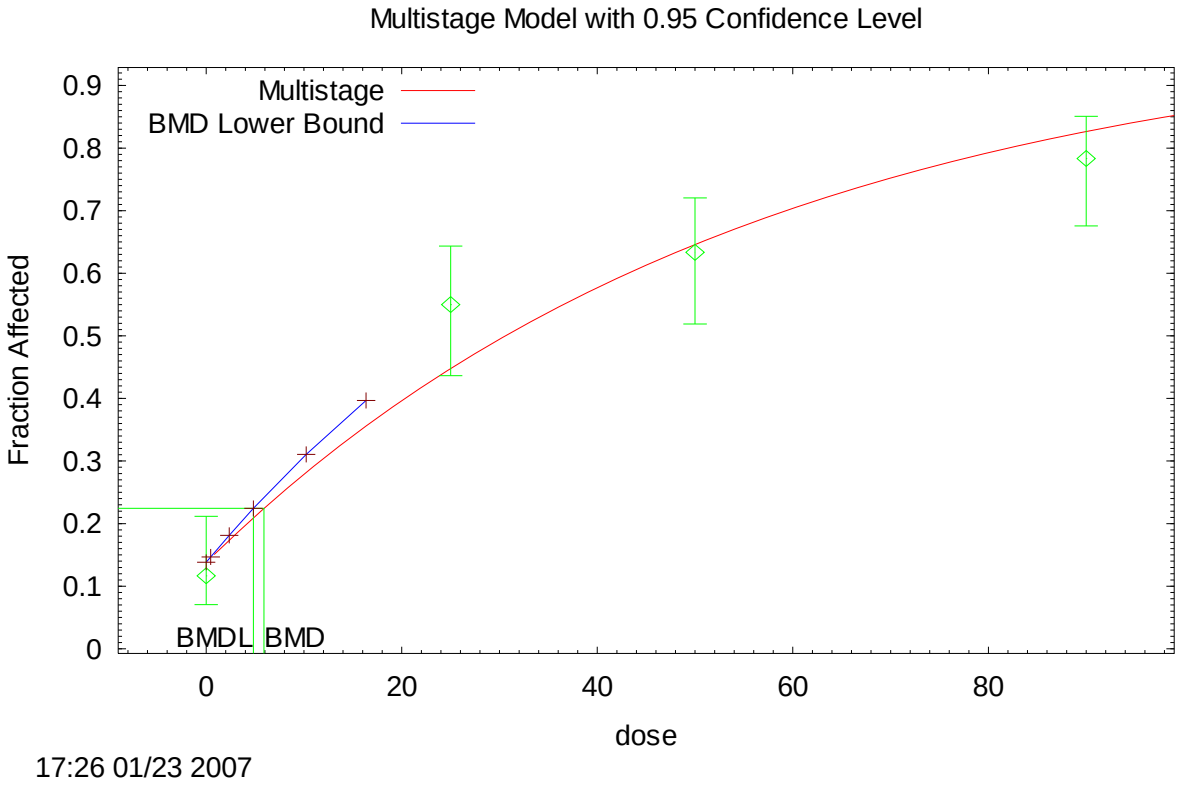

Figure A-6. Plot of Multistage Model of o-Nitrotoluene Chronic Male Rat Spleen Hematopoietic Cell Proliferation Data (NTP, 2002)

Table A-3. BMD Summaries for o-Nitrotoluene Chronic Male Mouse Liver Cell Necrosis Data[a]

BMD Model	P	Σ scaled residuals	AIC	BMD (mg/kg-day)	BMDL (mg/kg-day)
Log-Probit	0.2394	1.9	246.487	39.6047	1.56214
Log-logistic	**0.2318**	**2.0**	**246.532**	**33.1668**	**0.950084**
Multistage	0.0689	1.4	248.337	82.5633	66.9565
Gamma	0.0689	4.2	248.337	82.5633	66.9565
Quantal Linear	0.0689	4.2	248.337	82.5631	66.9565
Weibull	0.0689	4.2	248.337	82.5631	66.9565
Probit	0.0005	7.4	260.693	181.269	153.971
Log-normal	0.0004	7.5	261.822	192.618	162.447
Quantal quadratic	0	9.0	267.13	255.296	215.938

[a]NTP, 2002
LOAEL = 165 mg/kg-day for 25% incidence; Control incidence = 1.7%

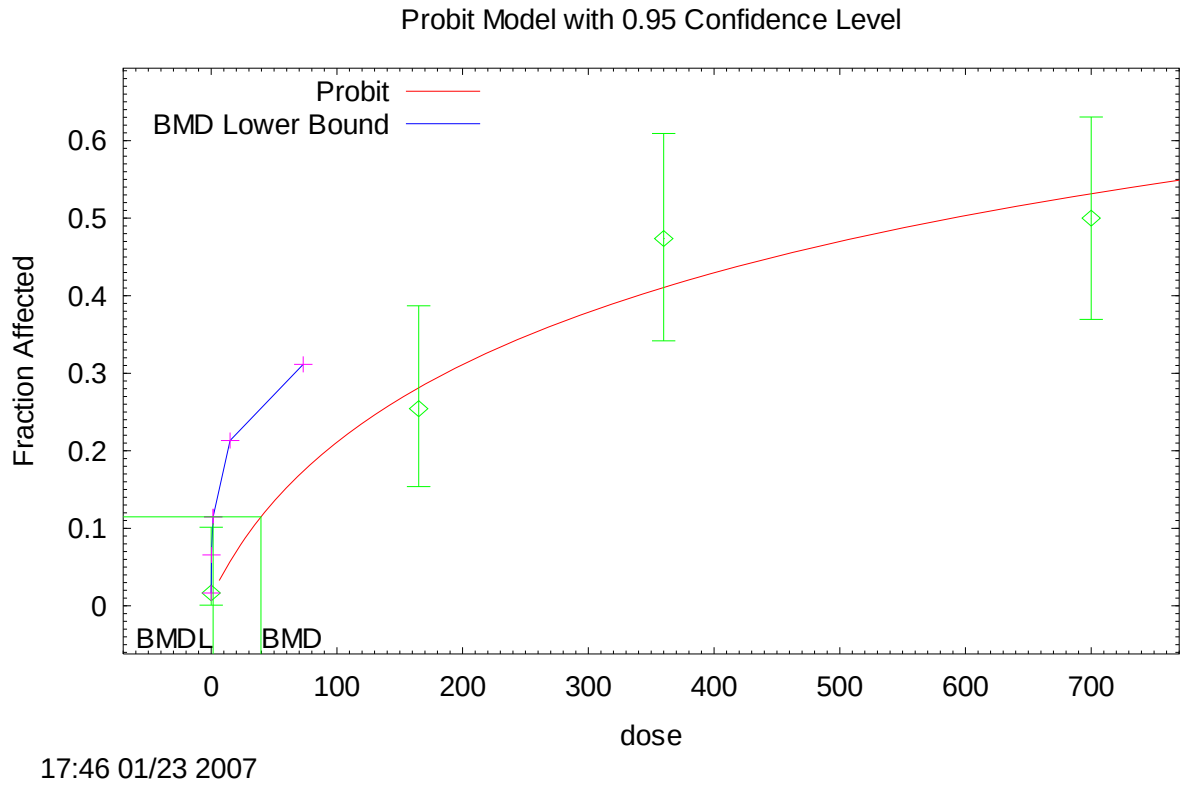

Figure A-7. Plot of Log-Probit Model of o-Nitrotoluene Chronic Male Mouse Liver Cell Necrosis Data (NTP, 2002)

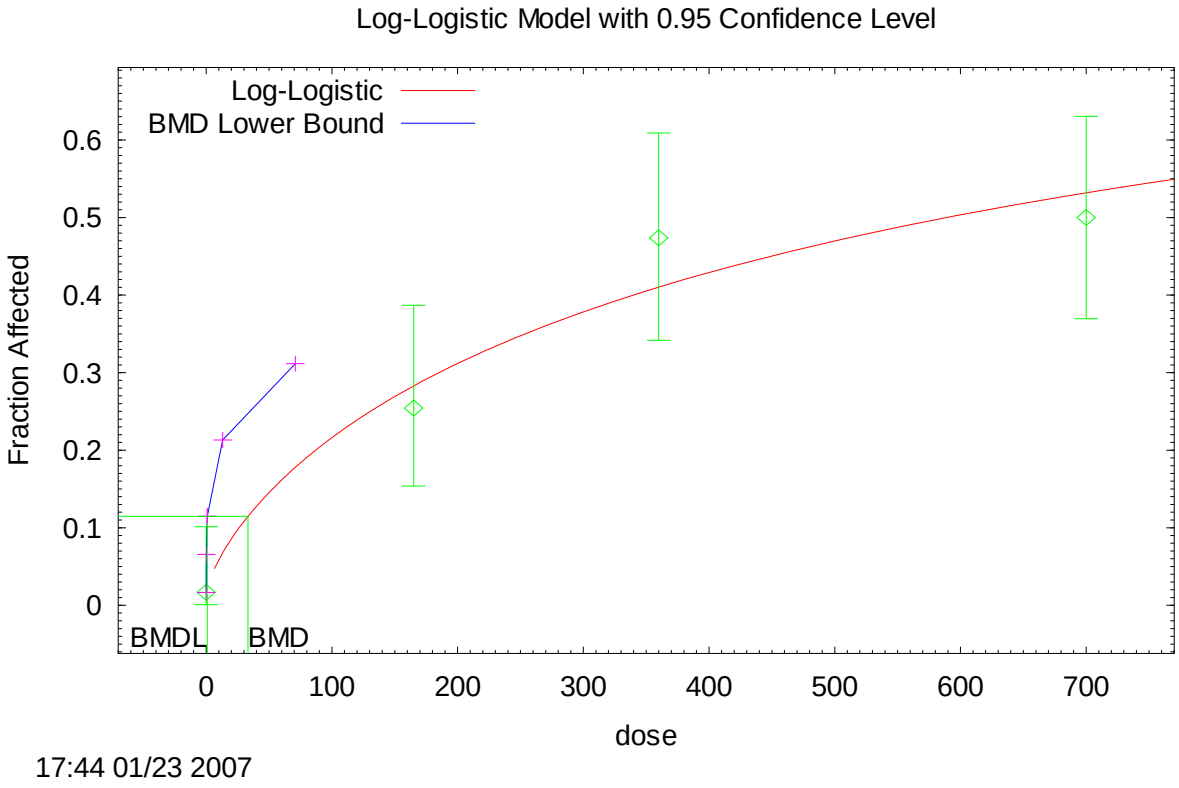

Figure A-8. Plot of Log-Logistic Model of o-Nitrotoluene Chronic Male Mouse Liver Cell Necrosis Data (NTP, 2002)

Table A-4. BMD Summaries: o-Nitrotoluene Chronic Male Mouse Renal Tubule Pigmentation Data[a]

BMD Model	p-value[b]	Σ scaled residuals	AIC	BMD[c] (mg/kg-day)	BMDL (mg/kg-day)
Log-Probit	0.0029	5.1	223.571	126.939	75.3058
Log-log	0.0022	5.3	224.09	116.633	65.0363
Multistage	0.0039	1.8	224.401	75.725	62.1205
Quantal Linear	0.0039	5.5	224.401	75.7253	62.1205
Gamma	0.0009	5.6	225.71	104.824	63.7769
Weibull	0.0009	5.6	225.99	95.2545	63.0692
Quantal quadratic	0	7.5	234.573	201.596	179.804
Probit	0	8.5	234.626	176.12	149.905
Log-normal	0	8.8	236.532	186.003	157.319

[a]NTP, 2002
[b]No acceptable p-values; all p-values <0.1
[c] LOAEL = 165 mg/kg-day for ~10% incidence; Control incidence = 1.7%

Table A-5. BMD Summaries for o-Nitrotoluene Chronic Male Mouse Olfactory Epithelial Degeneration Data[a]

	P	Σ scaled residuals	AIC	BMD (mg/kg-day)	BMDL (mg/kg-day)
Log-log	1	0.01	82.7615	142.787	87.731
Gamma	1	0.04	82.7638	112.811	60.0284
Multistage	**0.9999**	**1**	**82.7702**	**80.2023**	**20.038**
Quantal quadratic	0.8629	1.2	84.0491	53.9012	47.7577
Log-normal	1	0	84.7614	142.822	71.9502
Probit	1	0	84.7614	122.31	60.2904
Log-Probit	1	0	84.7614	132.601	78.0608
Weibull	1	0	84.7614	91.0721	43.6716
Quantal Linear	0.0368	4.6	95.0034	13.6958	11.2888

[a]NTP, 2002
LOAEL = 165 mg/kg-day for 36/60 incidence; Control incidence = 0/60

Table A-6. BMD Summaries for o-Nitrotoluene Chronic Female Mouse Olfactory Epithelial Degeneration Data[a]

	P	Σ scaled residuals	AIC	BMD (mg/kg-day)	BMDL (mg/kg-day)
Log-log	1	0.01	84.9109	147.127	105.901
Gamma	0.9998	0.1	84.9255	122.124	87.0322
Multistage	**0.9580**	**2**	**85.0728**	**90.4122**	**36.8239**
Log-normal	1	0	86.9108	146.563	84.9566
Probit	1	0	86.9108	130.064	72.6025
Log-Probit	1	0	86.9108	140.431	99.7809
Weibull	1	0	86.9108	107.167	67.4248
Quantal quadratic	0.4408	2.3	89.1563	61.2682	54.4693
Quantal Linear	0.001	6.4	107.236	16.4655	13.6653

[a]NTP, 2002
LOAEL = 165 mg/kg-day for 28/60 incidence; Control incidence = 0/60