Nitrobenzene; CASRN 98-95-3

Human health assessment information on a chemical substance is included in the IRIS database only after a comprehensive review of toxicity data, as outlined in the IRIS assessment development process. Sections I (Health Hazard Assessments for Noncarcinogenic Effects) and II (Carcinogenicity Assessment for Lifetime Exposure) present the conclusions that were reached during the assessment development process. Supporting information and explanations of the methods used to derive the values given in IRIS are provided in the guidance documents located on the IRIS website.

STATUS OF DATA FOR Nitrobenzene

File First On-Line 01/31/1987

Category (section)	Assessment Available?	Last Revised
Oral RfD (I.A.)	yes	02/06/2009
Inhalation RfC (I.B.)	yes	02/06/2009
Carcinogenicity Assessment (II.)	yes	02/06/2009

I. HEALTH HAZARD ASSESSMENTS FOR NONCARCINOGENIC EFFECTS

I.A. REFERENCE DOSE (RfD) FOR CHRONIC ORAL EXPOSURE

Substance Name — Nitrobenzene
CASRN — 98-95-3
Section I.A. Last Revised — 02/06/2009

The RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfD is intended for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action. It is expressed in units of mg/kg-day. Please

refer to the guidance documents at http://www.epa.gov/iris/backgrd.html for an elaboration of these concepts. Because RfDs can be derived for the noncarcinogenic health effects of substances that are also carcinogens, it is essential to refer to other sources of information concerning the carcinogenicity of this chemical substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

The value presented herein replaces the previous RfD posted on the IRIS database in 1991. In the previous IRIS assessment, the RfD for nitrobenzene was 5 x 10^{-4} mg/kg-day based on a 90-day inhalation study in F344 rats and B6C3F1 mice (Chemical Industry Institute of Toxicology [CIIT], 1984). Critical endpoints included methemoglobinemia and histopathologic lesions in the adrenal gland, kidney, and liver. The lowest-observed-adverse-effect level (LOAEL)-no-observed-adverse-effect level (NOAEL) approach was used to derive the RfD. A point of departure (POD) of 25 mg/m^3 (LOAEL) was identified and converted to an equivalent oral dose of 4.6 mg/kg-day, using default assumptions for the mouse breathing rate and body weight. A combined uncertainty factor (UF) of 10,000 was applied, resulting in an RfD of 5 x 10^{-4} mg/kg-day.

I.A.1. CHRONIC ORAL RfD SUMMARY

Critical Effect	Point of Departure*	UF	chronic RfD
Increased methemoglobin levels **Subchronic rat study** **NTP, 1983**	$BMDL_{1SD}$: 1.8 mg/kg-day	1,000	2 x 10^{-3} mg/kg-day

* The animals were gavaged 7 days/week; thus, no adjustment for intermittent exposure was required. See section 5.1.2 of the *Toxicological Review of Nitrobenzene* (U.S. EPA, 2009) for more details. $BMDL_{1SD}$ = 95% lower confidence limit on the maximum likelihood estimate of the dose corresponding to a change in the mean equal to one standard deviation (SD) of the control mean

I.A.2. PRINCIPAL AND SUPPORTING STUDIES

The National Toxicology Program (NTP, 1983) conducted a 90-day oral gavage study of nitrobenzene in F344 rats (10/sex/group) exposed to 0, 9.38, 18.75, 37.5, 75, and 150 mg/kg-day and B6C3F1 mice (10/sex/group) exposed to 0, 18.75, 37.5, 75, 150, and 300 mg/kg-day.

Nine male and three female rats at the 150 mg/kg-day dose level died prior to study completion. In mice, there were no deaths among the highest dose females but three deaths among the highest dose males. Nitrobenzene exposure was associated with changes in absolute and relative organ weights, changes in hematologic parameters, and histopathologic outcomes. The nitrobenzene-induced pathological changes were much less pronounced in mice than in rats. Since the mice were treated with higher doses and were generally more resistant to nitrobenzene toxicity, the mouse data were not considered further for RfD evaluation. The similarity of toxic endpoints in both species, however, had considerable bearing on the choice of critical effect(s).

Organ weights affected by subchronic nitrobenzene exposure included liver and kidney (increase) in both sexes and testis (decrease) in male F344 rats. The statistically significant increases in liver and kidney weights were generally not supported by histopathologic changes or, in the case of the kidney, by changes in clinical endpoints (e.g., blood urea nitrogen or blood creatinine).

There is evidence that nitrobenzene is a male reproductive toxicant. However, a significant effect on testis weight in males was seen only at the two highest doses in rats (75 and 150 mg/kg-day), accompanied by up to 90% lethality (NTP, 1983). Similarly, a significant effect on testis weight was only observed with the highest dose in male mice (300 mg/kg-day) with an accompanying 30% mortality. Because of the high doses required to induce testicular toxicity and the lack of this response in the available human exposure or poisoning data, this endpoint was not used in the RfD assessment for nitrobenzene, since more relevant endpoints were identified at lower levels of exposure.

Statistically significant changes were observed in hematologic parameters in rats, including dose-dependent decreases in hematocrit (Hct), hemoglobin (Hb), and red blood cell (RBC) count and dose-dependent increases in reticulocyte counts and methemoglobin (metHb). In males, these changes achieved statistical significance compared with controls at a dose of 9.38 mg/kg-day for metHb and Hb and 18.75 mg/kg-day for the other parameters. In females, the changes achieved statistical significance compared with controls at 37.5 mg/kg-day and above for RBC count and at 9.38 mg/kg-day for the other parameters. Histopathologic examination revealed splenic congestion. The findings for selected hematologic endpoints and splenic congestion are summarized in Table 5-1 of the *Toxicological Review of Nitrobenzene* (U.S. EPA, 2009).

Based on the changes in absolute and relative organ weights and the dose-dependent increases in reticulocyte count and metHb concentration, all of which were evident at the lowest administered dose, a LOAEL of 9.38 mg/kg-day was identified for the subchronic oral effects of nitrobenzene in F344 rats in this study.

Shimo et al. (1994) administered nitrobenzene by gavage to F344 rats (six/sex/group) at doses of 0, 5, 25, and 125 mg/kg-day nitrobenzene for 28 days. An additional set of control and 125 mg/kg-day rats were allowed to recover for 14 days after the completion of treatment. Clinical signs in high-dose rats included decreased movement, pale skin, and abnormal gait. Additionally, the authors plotted the body weight changes against time and showed a marked treatment-related reduction in body weight increase, even though food consumption was little changed among the groups. Changes in hematologic parameters were evident in nitrobenzene-treated rats, including dose-dependent reductions in RBC count, Hct, and Hb concentration and dose-dependent increases in mean corpuscular volume (MCV) and white blood cell (WBC) count. These changes were not observed in those animals allowed to recover for 14 days after dosing.

Absolute changes in organ weights exhibited similar trends between male and female rats with dose-dependent increases noted for the spleen and liver. Decreases in the thymus (both sexes) and testis (males) were found in the high-dose group. A slight decrease in absolute kidney weights was also noted in the mid-dose males and in the high-dose females. Following the 14 day recovery period, the absolute spleen weights for male and female rats were still increased and absolute testis weights remained statistically significantly reduced in the high-dose males, whereas absolute liver, kidney, and thymus weights returned to control values.

Tissue histopathology findings included dose-dependent increased incidences and severity of splenic congestion and extramedullary hematopoiesis. Livers of both sexes in the high-dose group had increased incidences of extramedullary hematopoiesis and brown pigmentation in Kupffer's cell. The only histopathology finding in the kidney was moderate brown pigmentation in the tubular epithelium in both sexes of the high-dose group. Decreased absolute testis weight correlated with severe degeneration of seminiferous tubular epithelium and severe atrophy of the seminiferous tubules in 100% of male rats receiving 125 mg/kg-day nitrobenzene.

In a reproductive/developmental toxicity study by Mitsumori et al. (1994), Sprague-Dawley rats (10/sex/group) were administered nitrobenzene by gavage (in sesame oil) at doses of 0, 20, 60, or 100 mg/kg-day nitrobenzene for a 14-day premating period, a mating period of up to 14 days, a gestation period of 22 days, and a subsequent lactation period of 4 days, making a potential overall dosing period of 54 days, at which point all animals (males, females, and pups) were necropsied. Because the observed mating period was no more than a single day for most mating pairs, the actual dosing duration for males and females was 40-41 days but could have lasted as long as 54 days for some.

High-dose animals displayed a number of clinical signs, including piloerection, salivation, emaciation, and an apparent anemia from day 13 onward. A number of

behavioral/neurological signs were evident, and body weight and food consumption were reduced by 17% in the high-dose males from day 21 onward. Male rats displayed profound dose-related changes in the levels of some hematologic parameters, including decreases in RBC count, Hb, and Hct and increases in metHb, mean corpuscular hemoglobin (MCH), WBC count, reticulocytes, and erythroblasts. At necropsy, the relative liver, kidney, and spleen weights were statistically significantly increased, and those of testes and epididymides were significantly decreased in the 60 and 100 mg/kg-day animals compared with controls. In rats exposed to 20 mg/kg-day nitrobenzene, however, there was a slight increase in relative testis and epididymis weights compared with controls.

A wide range of histopathologic changes was observed, especially in animals receiving 60 and 100 mg/kg-day of nitrobenzene, including atrophy of the seminiferous tubules, hyperplasia of Leydig cells, loss of intraluminal sperm in the epididymides, and neuronal necrosis/gliosis in the cerebellar medulla. In addition, centrilobular swelling of hepatocytes, hemosiderin deposition in Kupffer cells, and increased extramedullary hematopoiesis in the liver and spleen were seen in all exposed groups.

Copulation and fertility indices were not statistically significantly different from controls at any dose level. However, only two of nine pregnant females in the high-dose group survived to term; the two surviving females died on days 1 and 3 of lactation.

Kawashima et al. (1995a) administered nitrobenzene (60 mg/kg-day in sesame oil by gavage) to male Sprague-Dawley rats for periods ranging from 7-70 days, after which the animals were mated with untreated females and then terminated the following day. Comparative changes in testicular and epididymal weights, sperm count, motility, and viability were evaluated, along with the fertility and copulation indices of treated groups. Significant reductions in testicular (>50%) and epididymal weights, sperm count, and motility were observed in those animals exposed to nitrobenzene for 14 days, while sperm viability and fertility index were severely reduced in those males exposed to nitrobenzene for 21 days or more. There was a concomitant increase in the incidence of abnormal sperm. Although the copulation indices of treated males appeared unchanged with duration of exposure, the numbers of virgin females becoming pregnant by treated males declined markedly with duration of exposure. No mating females became pregnant in groups that were mated with males treated for 28 days or longer, an effect that appeared to result from the production of sperm with poor motility and reduced viability.

Bond et al. (1981) administered a single oral dose of 0, 50, 75, 110, 165, 200, 300, or 450 mg/kg nitrobenzene in corn oil to six male F344 rats/group. Three rats at each dose were sacrificed 2 and 5 days following nitrobenzene administration. Hepatic centrilobular necrosis appeared inconsistently in rats given various doses of nitrobenzene, while hepatocellular nucleolar enlargement was consistently detected in rats given doses of nitrobenzene as low as

110 mg/kg. Lesions occurred in the seminiferous tubules of the testicles, with marked necrosis of primary and secondary spermatocytes following a single oral dose of 300 mg/kg. Furthermore, within 3 days of nitrobenzene administration, multinucleated giant cells were observed, and decreased numbers of spermatozoa were observed in the epididymis. Histopathologic analyses indicated that nitrobenzene had no apparent effects on spermatogonia or the epididymal epithelium. Methemoglobinemia was increased to 25% immediately after dosing at 300 mg/kg, with a subsequent slow decline over the next 10 days. In a control experiment, the administration of sodium nitrite also induced methemoglobinemia but had no histopathologic effects on the testes and liver, suggesting that the histopathologic effects of nitrobenzene occurred through a direct action of the compound or its metabolites at the tissue site rather than as a secondary effect of metHb formation.

Morrissey et al. (1988) evaluated rodent sperm, vaginal cytology, and reproductive organ weight data from a series of NTP 13-week gavage studies, one of which was on nitrobenzene (NTP, 1983). As tabulated by Morrissey et al. (1988), the effects of nitrobenzene on the reproductive organs and the incidence of abnormal sperm were assessed at dose levels of 0, 9.4, 37.5, and 75 mg/kg in rats and at 18.75, 75, and 300 mg/kg in mice. The absolute and relative weights of epididymides and testes were reduced in animals receiving nitrobenzene (data not provided in the report). In addition, sperm motility was adversely affected, and the incidence of abnormal sperm was increased.

Burns et al. (1994) assessed the immunotoxic potential of nitrobenzene for selected immunologic and host resistance responses in female B6C3F1 mice exposed to 0, 30, 100, or 300 mg/kg-day over a 14-day treatment period. This study confirmed the toxic effect of nitrobenzene on the spleen and hematology parameters. Effects on the immune system were mild.

Of the animal studies of oral exposure to nitrobenzene, the 90-day gavage study conducted by NTP (1983) is the most relevant study for deriving an RfD for nitrobenzene. This study used the longest exposure duration and multiple dose levels. Other studies (discussed above) were considered less suitable for developing an RfD (e.g., reproductive toxicity studies using a one-time administration or a single dose level [Kawashima et al., 1995a, b; Levin et al., 1988; Bond et al., 1981] or relatively short exposure duration [Koida et al., 1995; Matsuura et al., 1995; Shimo et al. (1994)]). A reproductive study (Mitsumori et al., 1994) and an immunotoxicology study (Burns et al., 1994) used dose levels higher than the NTP (1983) bioassay.

Benchmark dose software (BMDS) (version 1.4.1c) was applied to estimate a POD for deriving an RfD for nitrobenzene from the male rat metHb data by using a benchmark response (BMR) of 1 SD. As detailed in the BMD technical guidance (U.S. EPA, 2000), a 1

SD BMR provides the exposure level at which 10% of those exposed would be expected to exceed the 98th (or 2nd) percentile of the control group's responses.

I.A.3. UNCERTAINTY FACTORS

UF = 1,000

An intraspecies UF of 10 was applied to account for human variability and protect potentially sensitive humans (e.g., glucose-6-phoshate dehydrogenase deficiency or chronic congenital methemoglobinemia) and lifestages (e.g., children). The default value was selected in the absence of information indicating the degree to which humans might vary in susceptibility to nitrobenzene toxicity.

An interspecies UF of 10 was applied for extrapolation from animals to humans. No suitable data on the toxicity of nitrobenzene to humans exposed by the oral route were identified. Insufficient information is currently available to assess rat-to-human differences in nitrobenzene toxicokinetics or toxicodynamics.

A UF to account for the extrapolation from a LOAEL to a NOAEL was not applied because the BMR of 1 SD was assumed to represent a minimal biologically significant level of change.

A subchronic to chronic UF of 3 was applied to account for less-than-lifetime exposure in the principal study. A chronic oral study is not available. In studies by the inhalation route, the severity of hematologic effects (e.g., metHb, reticulocyte count, and splenic congestion) did not increase between subchronic (CIIT, 1984) and chronic (CIIT, 1993) exposure durations. Nonetheless, other toxicity endpoints may result from chronic oral exposure due to route-specific differences in metabolism, pharmacokinetics, and/or pharmacodynamics that were not observed in the subchronic oral study or the inhalation studies. In particular, several studies of gut bacterial metabolic activation (nitro reduction) support the possibility of higher relative concentrations of active metHb-forming metabolites than would be expected following exposure by the inhalation route.

A database deficiency UF of 3 was applied. The database of oral studies includes the principal study (NTP, 1983), a 90-day gavage study in two species and both sexes; a reproductive/developmental study (Mitsumori et al., 1994) and two male reproductive toxicity studies (Morrissey et al., 1988; Bond et al., 1981); structure-activity relationship studies with dinitro- and trinitrobenzene; and a multidose immunologic study in mice (Burns et al., 1994). Due to the lack of an oral multigeneration reproductive toxicity study and evidence of male reproductive toxicity, a factor of 3 is warranted. There is a two-generation reproductive toxicity study (Dodd et al., 1987) via inhalation exposure, but possible route-specific

differences in metabolism, pharmacokinetics, and/or pharmacodynamics suggest uncertainty in the potential for transgenerational effects from longer-term oral exposures.

I.A.4. ADDITIONAL STUDIES/COMMENTS

Several other studies have been conducted that are not suitable for determining an RfD for nitrobenzene (e.g., reproductive toxicity studies using single administration, single dose [Levin et al., 1988]; multiple administration up to 4 weeks only [Koida et al., 1995; Matsuura et al., 1995]; or administration of a single dose for up to 70 days [Kawashima et al., 1995a, b]). In addition, the studies by Koida et al. (1995) and Matsuura et al. (1995) were presented in abstract form only and were not published in peer-reviewed journals, so they were not considered further. Also, since Kawashima et al. (1995a, b) administered a single dose, no dose-response relationship could be determined.

For more detail on Susceptible Populations, exit to the toxicological review, Section 4.7 (PDF).

I.A.5. CONFIDENCE IN THE CHRONIC ORAL RfD

Study — High
Database — Medium
RfD — Medium

The overall confidence in the RfD is medium. The critical effect on which the RfD is based is well supported by several other oral gavage studies over time periods of up to 70 days (Kawashima et al., 1995a, b). Nitrobenzene also displayed toxicity in reproductive and immunologic studies but at doses higher than those used in the principal study. On the basis of these considerations, confidence in the principal study is high. Confidence in the database is medium because there is no 2-year oral study, no NOAEL in the 90-day gavage study, and no multigeneration reproductive/developmental oral study. The medium confidence rating is driven by such deficits in the database.

For more detail on Characterization of Hazard and Dose Response, exit to the toxicological review, Section 6 (PDF).

I.A.6. EPA DOCUMENTATION AND REVIEW OF THE CHRONIC ORAL RfD

Source Document — U.S. EPA, 2009

This document has been reviewed by EPA scientists, interagency reviewers from other federal agencies, and the public, and peer reviewed by independent scientists external to EPA. A summary and EPA's disposition of the comments received from the independent external peer reviewers and from the public is included in Appendix A of the *Toxicological Review of Nitrobenzene* (U.S. EPA, 2009). ***To review this appendix, exit to the toxicological review, Appendix A, Summary Of External Peer Review And Public Comments And Disposition (PDF)***.

I.A.7. EPA CONTACTS

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at ((202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

I.B. REFERENCE CONCENTRATION (RfC) FOR CHRONIC INHALATION EXPOSURE

Substance Name — Nitrobenzene
CASRN — 98-95-3
Section I.B. Last Revised — 02/06/2009

The RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfC considers toxic effects for both the respiratory system (portal of entry) and for effects peripheral to the respiratory system (extrarespiratory effects). The inhalation RfC (generally expressed in units of mg/m^3) is analogous to the oral RfD and is similarly intended for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action.

Inhalation RfCs are derived according to *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994). Because RfCs can also be derived for the noncarcinogenic health effects of substances that are carcinogens, it is essential to refer to other sources of information concerning the carcinogenicity of this chemical substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

The previous IRIS assessment did not provide an RfC for nitrobenzene.

I.B.1. CHRONIC INHALATION RfC SUMMARY

Critical Effect	Point of Departure*	UF	Chronic RfC
Bronchiolization of the alveoli and olfactory degeneration **Chronic mouse study** **CIIT, 1993**	$BMCL_{10\text{-}HEC}$: 0.26 mg/m^3	30	9×10^{-3} mg/m^3

*Conversion Factors and Assumptions — Bronchiolization of the alveoli and olfactory degeneration are pulmonary and extrathoracic effects of a soluble vapor. The POD was converted to a POD_{ADJ} as follows: $BMCL_{10}$ x daily exposure/24 hours. The POD_{HEC} was calculated based on the POD_{ADJ} x regional gas dose ratio (RGDR). The RGDR is calculated as follows: $(MV_a/S_{a,PU\ or\ ET}) \div (MV_hS_{h,PU\ or\ ET})$, where MV_a = minute volume for mice; MV_h = minute volume for humans; $S_{a,PU\ or\ ET}$ = default pulmonary or extrathoracic surface area for mice; $S_{h,PU\ or\ ET}$ = default pulmonary or extrathoracic surface area in humans. See section 5.2.3 of the *Toxicological Review of Nitrobenzene* (U.S. EPA, 2009) for more details.

I.B.2. PRINCIPAL AND SUPPORTING STUDIES

CIIT (Cattley et al., 1994; CIIT, 1993) conducted a 2-year inhalation study with B6C3F1 mice and F344 rats of both sexes and male CD rats (70 animals/group). Rats were exposed to 0, 1, 5, or 25 ppm nitrobenzene and mice to 0, 5, 25, or 50 ppm nitrobenzene for 6 hours/day, 5 days/week. Animals were sacrificed at 24 months of exposure when blood analyses and complete necropsies were performed. Ten rats/sex/strain/group were terminated 15 months into the study to provide samples for an interim evaluation of hematologic parameters. Cattley et al. (1994) identified the following target tissues: thyroid, spleen, nose, and liver in all strains and species; kidney in rats only; and respiratory tissues in mice only. Testis and epididymis were target tissues in male CD rats; however, statistically significant effects were only observed at the highest concentration tested.

A statistically significant difference in the incidence of centrilobular hepatocytomegaly was observed in a concentration-dependent fashion in both strains of male rats but not at all in female rats. The incidence of renal tubular hyperplasia in male F344 rats showed a statistically significant positive trend. Chronic nephropathy and tubular hyperplasia were observed both in males and females. Bilateral testicular atrophy was reported with effects appearing in the high-

concentration group only in both male CD and F344 rats. Bilateral hypospermia was observed in high-concentration male CD rats.

At interim sacrifice, a statistically significant increase in metHb was observed at all concentrations with male CD rats and only at the highest concentration with male and female F344 rats. At terminal sacrifice, a statistically significant increase in metHb was observed with both sexes of mice and rats at the highest concentrations tested. An approximate twofold increase in metHb was observed with male and female B6C3F1 mice, female F344 rats, and male CD rats, whereas an approximate 1.5-fold increase was observed with male F344 rats (Cattley et al., 1994; CIIT, 1993). Hct and Hb levels were reduced only in female mice, being highly significantly different at the 5 ppm concentration and lower, albeit still statistically significantly reduced, at 25 ppm but not at 50 ppm. Since this effect occurred only in female mice and did not exhibit concentration dependency, it was not considered treatment related.

Exposure-related degeneration and loss of olfactory epithelium were observed in mice of both sexes, with the females being more sensitive than the males. At the highest concentration tested (50 ppm), the incidence was 62% in males and 69% in females. Bronchiolization of the alveoli was also observed at all concentrations in both sexes, with a 94% incidence in males and 100% incidence in females at the highest concentration tested. Follicular cell hyperplasia of the thyroid was observed in both sexes of mice, with males being more sensitive than females. At the highest concentration, this response was reported in 19% of the males. Exposure-related hepatocellular changes (e.g., centrilobular hepatocytomegaly) were observed in males with incidence up to 89% at the highest concentration; in females, the incidence of hepatocellular changes was 11% at the highest concentration. Hypercellularity of the bone marrow, an effect secondary to hemolytic anemia, was recorded for males in a concentration-dependent fashion with low incidence; in females, only the highest concentration animals were examined for this effect, and the response was even lower than in males. There was also evidence for testicular toxicity in males, but only the high-concentration animals were examined.

The most consistent histopathologic findings in mice were degeneration and loss of the olfactory epithelium and bronchiolization of the alveoli. Degeneration and loss of the olfactory epithelium occurred in a concentration-dependent manner, with high incidences (≥62%) at the high dose in both males and females, with females being more sensitive than males. Bronchiolization of the alveoli occurred with high incidence (≥87%) in both males and females in the exposed groups. These lesions were characterized by a pronounced change in the alveolar epithelium in the region of the terminal bronchioles from a simple squamous to a tall columnar epithelium resembling that of the terminal bronchioles. The change was described as being concentration related in severity. In low-concentration animals, bronchiolization was located almost entirely in the region of the terminal bronchioles. In the

mid- and high-concentration animals, the lesions were more florid and involved a large proportion of the lung parenchyma.

Bronchiolization of the alveoli is a histologically distinct lesion that has been seen in various species, including mice and humans, and that may indicate a variety of pathological conditions, including inflammation, chemical irritation, or exposure to carcinogens (Chilosi et al., 2003; Friemann et al., 1999; Jensen-Taubman et al., 1998; Muhle et al., 1995; Pinkerton et al., 1993; Nettesheim and Szakal, 1972).

A 90-day subchronic study was conducted using both sexes of F344 and CD rats and B6C3F1 mice (CIIT, 1984). Exposure concentrations were 0, 5, 16, or 50 ppm 6 hours/day, 5 days/week. Exposure to nitrobenzene had no effect on body weights, but spleen weights were increased and testis weights were decreased in rats. In rats, signs of hemolytic anemia and methemoglobinemia were the most prominent effects observed in both strains. Histopathologic examination of mice and rats demonstrated treatment-related lesions in the spleen, testis, liver, epididymides, kidney, and bone marrow and possibly adrenals, lymph nodes, and lungs.

Dodd et al. (1987) carried out a two-generation reproductive/developmental toxicity study on nitrobenzene in which 30 Sprague-Dawley rats/sex/group were exposed to 0, 1, 10, or 40 ppm nitrobenzene 6 hours/day, 5 days/week for 10 weeks via inhalation, prior to a mating period of up to 2 weeks. After mating, the F_0 males were sacrificed, while the pregnant females were exposed to nitrobenzene through gestation day (GD) 19 and again after delivery on postnatal days (PNDs) 5-20 at which point the pups were weaned. The F_0 females were sacrificed prior to necropsy on PND 21. On this day, 30 pups/sex/group (F_1 generation) were selected (one male and one female from each litter, where possible) and allowed a 2-week growth period during which no nitrobenzene was administered. Subsequently, a repeat of the F_0 exposure and treatment protocol was undertaken, with the exception that, after mating, some F_1 males were not sacrificed. These males were allowed to enter a recovery phase, and after 9 weeks of nonexposure they were mated with virgin, unexposed females to examine potential reversibility of effects on the male gonads.

There were marked reductions in the fertility indices from matings among the 40 ppm animals compared with controls. Most notably, this reduction was also apparent in the matings that involved unexposed females with the high-concentration F_1 males that had been allowed a 9 week period of recovery. In all matings that resulted in live offspring, gestational parameters, such as the number of uterine implantations, resorptions, and postimplantation losses, were unaffected by nitrobenzene in either generation. However, marked spermatocyte degeneration and atrophy of the seminiferous tubules were observed in both generations of high-concentration males, including those that entered the 9 week recovery period.

Morphologically, the lesions were characterized by severe multifocal and diffuse atrophy of the seminiferous tubules in 14/30 animals in the 40 ppm group and by the appearance of giant syncytial spermatocytes in the seminiferous tubules of 22/30 subjects of the F_0 generation. Giant syncytial spermatocytes were much less evident in F_1 males (1/30), and the active stages of spermatocyte degeneration in the seminiferous tubules were less frequent. However, the epididymides of 40 ppm males in the F_0 and F_1 generations displayed degenerative spermatocytes and a reduced number of spermatids. By contrast, there were no apparent lesions in the histopathology of the female reproductive organs at this concentration.

Tyl et al. (1987) exposed 26 pregnant female Sprague-Dawley rats/group to gaseous nitrobenzene at 0, 1, 10, or 40 ppm 6 hours/day on GDs 6-15. No compound-related clinical signs were observed, although maternal body weight gain was reduced by 19% in the high-dose group compared with controls between GDs 6 and 15. However, this parameter had returned to control values by GD 21. Spleen weights increased dose dependently in dams, achieving statistical significance in the 10 and 40 ppm dose groups. Gestational parameters, such as the numbers of corpora lutea, resorptions and dead fetuses, live fetuses per litter, pre- or postimplantation loss rates (as a percent), sex ratio, or fetal body weights, were all unaffected by treatment. Similarly, there were no indications of concentration-dependent developmental toxicity or teratogenicity. There was no effect on fetal body weights, and the incidence of skeletal variations also did not indicate fetal toxicity. The single exception was a significant increase in the incidence of parietal skull plates with an area of nonossification in the 40 ppm group. However, it is unclear whether this isolated effect represents a teratogenic effect of nitrobenzene or whether it is a consequence of maternal toxicity observed in the high-concentration group.

The 2-year study by CIIT (Cattley et al., 1994; CIIT, 1993) was considered the most suitable for an RfC evaluation because of the chronic exposure duration and large group sizes (70 animals/sex/group). The most consistent and extensive histopathologic findings were degeneration and loss of the olfactory epithelium and bronchiolization of the alveoli in mice. Both effects were further evaluated as candidate critical effects for the derivation of the RfC.

Methemoglobinemia was not chosen as a candidate critical endpoint for the inhalation RfC. The biological significance of the hematologic findings, including methemoglobinemia, in the chronic inhalation study (Cattley et al., 1994; CIIT, 1993) is unclear. In several instances, the differences between the dosed groups and the controls were minimal or decreased with increasing length of exposure. In most instances, methemoglobinemia was notably increased only at the highest nitrobenzene exposure, although time-related trends were not evident, possibly due to a compensatory response among all exposed rat groups.

BMD modeling using BMDS (version 1.4.1c) was used to analyze the incidence data for bronchiolization of the alveoli and olfactory degeneration from CIIT (1993). A BMR of 10% was used for these endpoints in the absence of information regarding the level of change considered to be biologically significant and to facilitate a consistent basis of comparison across assessments. Human equivalent concentrations (HECs) were obtained from the POD (95% lower bound of the benchmark concentration at 10% extra risk, $BMCL_{10}$) using the dosimetric procedures in *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994). Because the POD_{HEC}s for the two respiratory effects—bronchiolization of the alveoli and olfactory degeneration—were similar in value at 0.26 and 0.29 mg/m^3, respectively, the effects were considered co-critical effects for purposes of deriving the RfC.

I.B.3. UNCERTAINTY FACTORS

UF = 30

An intraspecies UF of 10 was applied to account for human variability and to protect potentially sensitive humans and lifestages (e.g., children). The default value was selected in the absence of information indicating the degree to which humans might vary in susceptibility to nitrobenzene toxicity.

A UF of 3 was applied to account for uncertainty in extrapolating from laboratory animals to humans. This value is adopted by convention, where a dosimetric adjustment from an animal-specific POD_{ADJ} to a POD_{HEC} already has been incorporated. Application of a full UF of 10 would depend on two areas of uncertainty (i.e., toxicokinetic and toxicodynamic uncertainties). In this assessment, the toxicokinetic component is mostly addressed by the determination of an HEC as described in the RfC methodology (U.S. EPA, 1994). The toxicodynamic uncertainty is also accounted for to a certain degree by the use of the applied dosimetry method.

A UF to account for extrapolation from a LOAEL to a NOAEL was not used because the current approach is to address this extrapolation as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR of a 10% change in either of the respiratory effects was selected under an assumption that it represents a minimal biologically significant change.

A subchronic-to-chronic UF for extrapolation to lifetime exposure was not applied since the data used originated from a 2-year (lifetime) chronic study.

A UF of 1 was applied to account for database deficiencies. The inhalation database is considered complete because it includes developmental toxicity studies in rats (Tyl et al., 1987) and rabbits (Biodynamics Inc., 1984), a two-generation reproduction study in rats (Dodd et al., 1987), and a 2-year toxicity study in mice and two strains of rats (Cattley et al., 1994; CIIT, 1993) in addition to short-term toxicity studies in mice and two strains of rats (Medinsky and Irons, 1985; DuPont, 1981).

I.B.4. ADDITIONAL STUDIES/COMMENTS

Several other studies have been conducted that confirm the findings obtained from subchronic/chronic inhalation studies, including 2-week inhalation studies by DuPont (1981) and Medinsky and Irons (1985), developmental studies by BioDynamics Inc. (1984, 1983) and Bushy Run Research Center (BRRC) (1984), and a reproductive study by BRRC (1985).

For more detail on Susceptible Populations, exit to the toxicological review, Section 4.7 (PDF).

I.B.5. CONFIDENCE IN THE CHRONIC INHALATION RfC

Study — High
Data Base — High
RfC — Medium

Confidence in the principal study is high because it was a 2-year bioassay with a sufficient number of animals and conducted in accordance with good laboratory practices, and it is reasonable to assume that the endpoint is relevant to humans. Confidence in the database is rated high due to the existence of a 2-year inhalation study, a two-generation reproductive and developmental toxicity study, and a subchronic inhalation study. The overall confidence in the RfC evaluation is medium due to a concern that a NOAEL was not identified for the incidence of bronchiolization of the alveoli in all exposure groups.

For more detail on Characterization of Hazard and Dose Response, exit to the toxicological review, Section 6 (PDF).

I.B.6. EPA DOCUMENTATION AND REVIEW OF THE CHRONIC INHALATION RfC

Source Document - U.S. EPA, 2009

This document has been reviewed by EPA scientists, interagency reviewers from other federal agencies, and the public, and peer reviewed by independent scientists external to EPA. A

summary and EPA's disposition of the comments received from the independent external peer reviewers and from the public is included in Appendix A of the *Toxicological Review of Nitrobenzene* (U.S. EPA, 2009). ***To review this appendix, exit to the toxicological review, Appendix A, Summary Of External Peer Review And Public Comments And Disposition (PDF)***.

I.B.7. EPA CONTACTS

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address)

II. CARCINOGENICITY ASSESSMENT FOR LIFETIME EXPOSURE

Substance Name — Nitrobenzene
CASRN — 98-95-3
Section II Last Revised — 02/06/2009

This section provides information on three aspects of the carcinogenic assessment for the substance in question: the weight-of-evidence judgment of the likelihood that the substance is a human carcinogen, and quantitative estimates of risk from oral and inhalation exposure. Users are referred to Section I of this file for information on long-term toxic effects other than carcinogenicity.

The rationale and methods used to develop the carcinogenicity information in IRIS are described in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) and the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b). The quantitative risk estimates are derived from the application of a low-dose extrapolation procedure, and are presented in two ways to better facilitate their use. First, route-specific risk values are presented. The "oral slope factor" is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, a "unit risk" is a plausible upper bound on the estimate of risk per unit of concentration, either per µg/L drinking water (see Section II.B.1.) or per $\mu g/m^3$ air breathed (see Section II.C.1.). Second, the estimated concentration of the chemical substance in drinking water or air when associated with cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000 is also provided.

This assessment of the carcinogenicity of nitrobenzene replaces the previous IRIS assessment on nitrobenzene posted on the IRIS database in 1991. The previous IRIS assessment classified nitrobenzene as Group D ("not classifiable as to human carcinogenicity") under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986). The previous IRIS assessment did not provide quantitative estimates of carcinogenic risk from oral or inhalation exposure.

II.A. EVIDENCE FOR HUMAN CARCINOGENICITY

II.A.1. WEIGHT-OF-EVIDENCE CHARACTERIZATION

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), nitrobenzene is classified as "likely to be carcinogenic to humans" by any route of exposure. While there are no human carcinogenicity data on nitrobenzene, the cancer characterization is based on evidence of the compound's tumorigenicity in a single well-conducted study in two animal species (Cattley et al., 1994; CIIT, 1993).

For more detail on Characterization of Hazard and Dose Response, exit to the toxicological review, Section 6 (PDF).

For more detail on Susceptible Populations, exit to the toxicological review, Section 4.7 (PDF).

II.A.2. HUMAN CARCINOGENICITY DATA

None.

II.A.3. ANIMAL CARCINOGENICITY DATA

Nitrobenzene has been shown to be a carcinogen in rats and mice. Adenomas or carcinomas with a pronounced dose-response relationship were found in livers of male F344 and male CD rats and in thyroids of male F344 rats. Less pronounced dose-related trends were observed for kidney tumors in male F344 rats, endometrial polyps in female F344 rats, cancers of the lung and thyroid in male B6C3F1 mice, and cancers in the mammary gland in female B6C3F1 mice (Cattley et al., 1994; CIIT, 1993).

The 2-year inhalation cancer bioassay (Cattley et al., 1994; CIIT, 1993) was used for development of an inhalation unit risk. Section II.C.2 presents an overview of the incidences of adenomas and carcinomas in male F344 rats that exhibited a positive dose-response trend in the Cochran-Armitage test (Cattley et al., 1994; CIIT, 1993). Positive trends were reported for hepatocellular adenomas or carcinomas in male and female F344 rats and male CD rats,

endometrial stromal polyps in female F344 rats, and kidney and thyroid follicular cell adenoma or carcinoma in male F344 rats as well as cancer of the mammary glands in female B6C3F1 mice. Kidney tubular adenomas or carcinomas in male F344 rats were observed only at the highest dose, and only one carcinoma was detected. No corresponding renal neoplasia occurred in female F344 rats, male CD rats, or any of the B6C3F1 mice from the same study.

Hepatocellular adenomas or carcinomas were consistently seen in male rats (strains F344 and CD) and also in female F344 rats. The incidence of these neoplasms in male CD rats was lower than in male F344 rats. The strongest dose response for this endpoint occurred in male F344 rats; therefore, this data set and the data sets for kidney and thyroid adenomas or carcinomas in male F344 rats were chosen for cancer dose-response assessment. Thyroid and lung adenomas or carcinomas in male B6C3F1 mice were also considered for cancer dose-response assessment.

II.A.4. SUPPORTING DATA FOR CARCINOGENICITY

Nitrobenzene appears to be at most weakly genotoxic. This determination is based on the almost exclusively negative results in salmonella assays (Ames tests; the only exception is TA98 in the presence of a comutagen), as well as negative clastogenic findings from in vivo assays of sister chromatid exchange, unscheduled DNA synthesis, and chromosomal aberrations. In vitro chromosome aberration results were mixed, as were the DNA breakage and micronucleus data. For instance, nitrobenzene was weakly positive for the induction of chromosome aberrations in cultured human peripheral lymphocytes but negative in human spermatozoa. Nitrobenzene induced weak DNA fragmentation but no DNA strand breaks. In addition, nitrobenzene did not cause cell transformation in these cell systems. See the *Toxicological Review of Nitrobenzene* (U.S. EPA, 2009) for more detailed summaries. Based on the available genotoxicity literature, nitrobenzene does not appear to induce tumor formation via a mutagenic mode of action (MOA).

As discussed in section 3.3 of the *Toxicological Review*, nitrobenzene undergoes reductive and oxidative metabolism, including generation of free radicals (e.g., nitro anion and superoxide) and propagation of redox cycling. It is possible that tumors may arise from oxidative stress resulting from nitrobenzene metabolism if the cellular defenses are overwhelmed as proposed recently by Hsu et al. (2007). Under oxidative stress conditions, there may be several possible scenarios by which reactive chemical species (including oxygen radicals) could facilitate tumor development, including direct DNA oxidative damage, lipid peroxidation, protein damage (including DNA repair enzymes), or modulation of DNA methylation (Halliwell, 2007). However, there is no experimental evidence linking any of these processes to nitrobenzene exposure and tumor formation. In addition, the thyroid and kidney tumors observed in experimental animals may be suggestive of rodent-specific MOAs; however, data

are not available to satisfy the criteria specified in EPA guidance for determining human relevance of thyroid and kidney tumors in rodents (U.S. EPA, 1998, 1991) to establish that these tumors occur via MOAs that are not relevant to humans. Additionally, it is not known whether there are any specific qualitative or quantitative differences in nitrobenzene metabolism between rodents and humans, and there is no reason to assume that a cancer MOA exists in animals that might not be relevant to humans. Therefore, a final conclusion on a cancer MOA cannot be determined at this time. This is reflected in the use of a linear approach as a default option in extrapolating the carcinogenic potential of nitrobenzene.

II.B. QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM ORAL EXPOSURE

No oral slope factor for nitrobenzene was derived. Cancer bioassays involving oral exposure to nitrobenzene are not available, and a route-to-route extrapolation is not recommended at this time. Physiologically based toxicokinetic models, which might be useful for route-to-route extrapolation, have not been developed for nitrobenzene.

II.B.1. SUMMARY OF RISK ESTIMATES

Not applicable.

II.C. QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM INHALATION EXPOSURE

II.C.1. SUMMARY OF RISK ESTIMATES

II.C.1.1. Inhalation Unit Risk - 4×10^{-5} per $\mu g/m^3$

The inhalation unit risk is derived from the LEC_{10}, the 95%, the lower bound on the exposure associated with a 10% extra cancer risk of liver, thyroid, or kidney tumors in male F344 rats, by dividing the risk (as a fraction) by the LEC_{10}, and represents an upper bound, continuous lifetime exposure risk estimate:

LEC_{10}, lower 95% bound on exposure at 10% extra risk - 2,610 $\mu g/m^3$

EC_{10}, central estimate (median) of exposure at 10% extra risk - 3,650 µg/m^3

The slope of the linear extrapolation from the central estimate EC_{10} is 0.1/(3,650 µg/m^3) = 3 x 10^{-5} per µg/m^3.

The unit risk for nitrobenzene should not be used with exposures exceeding the POD (LEC_{10} or 3 x 10^3 µg/m^3), because above this level the dose-response is not linear.

Air Concentrations at Specified Risk Levels:

Risk Level	Lower Bound on Concentration Estimate
E-4 (1 in 10,000)	2.5 µg/m3
E-5 (1 in 100,000)	0.25 µg/m3
E-6 (1 in 1,000,000)	0.025 µg/m3

II.C.1.2. Extrapolation Method

Multistage model with linear extrapolation from the POD (LEC_{10}).

II.C.2. DOSE-RESPONSE DATA

Tumor Type - liver hepatocellular adenomas or carcinomas, kidney tubular adenomas or carcinomas, thyroid follicular cell adenomas or carcinomas
Test Species - rat/F344 (male)
Route - Inhalation

Cancer type[a]	Nitrobenzene concentration (ppm)			
	0	1	5	25
Liver, hepatocellular adenoma or carcinoma	1/43 (2.3%)	4/50 (8.0%)	5/47 (10.6%)	16/46 (34.8%)
Kidney, tubular adenoma or carcinoma	0/43 (0%)	0/50 (0%)	0/47 (0%)	6/46 (13.0%)
Thyroid, follicular cell adenoma or carcinoma	1/43 (2.3%)	1/50 (2.0%)	5/47 (10.6%)	8/46 (17.4%)

[a]All incidences shown have positive dose-response trends at $p < 0.05$ (Cochran-Armitage test).

Source: CIIT (1993).

II.C.3. ADDITIONAL COMMENTS

Exposures were converted to human equivalent exposures considering inhalation dosimetry (U.S. EPA, 1994). An upper limit for each unit risk was estimated by linear extrapolation from the lower confidence limit on exposure at the POD. In order to convey the total amount of risk potentially arising from multiple tumor sites, the composite risk of any of the three tumor sites identified was estimated. The resulting (upper bound) unit risk included adjustment for continuous exposure. See the *Toxicological Review of Nitrobenzene* for more information (U.S. EPA, 2009).

II.C.4. DISCUSSION OF CONFIDENCE

There is uncertainty associated with the extrapolation of study data to estimate potential risks to human populations from exposure to nitrobenzene. The uncertainty falls into two major categories: model uncertainty and parameter uncertainty. Model uncertainty "refers to a lack of knowledge needed to determine which is the correct scientific theory on which to base a model," whereas parameter uncertainty "refers to a lack of knowledge about the values of a model's parameters" (U.S. EPA, 2005a). In the absence of a biologically based model, a multistage model was the preferred model because it has some concordance with the multistage theory of carcinogenesis and serves as a benchmark for comparison with other

cancer dose-response analyses. It is unknown how well this model or the linear low-dose extrapolation predicts low-dose risks for nitrobenzene. Also, while the male mice did not appear to have as strong a carcinogenic response as the male rats, it is not known which species is more relevant for extrapolation of risk to humans.

Parameter uncertainty can be assessed through confidence intervals and probabilistic analysis. Each description of parameter uncertainty assumes that the underlying model and associated assumptions are valid. Some uncertainty in the animal dose-response data can be assessed through the ratio of BMCs to their BMCLs. For the liver tumors evaluated here, the ratio was below a factor of 2, which is a typical degree of uncertainty.

II.D. EPA DOCUMENTATION, REVIEW, AND CONTACTS (CARCINOGENICITY ASSESSMENT)

II.D.1. EPA DOCUMENTATION

Source Document - U.S. EPA, 2009

This document has been reviewed by EPA scientists, interagency reviewers from other federal agencies, and the public, and peer reviewed by independent scientists external to EPA. A summary and EPA's disposition of the comments received from the independent external peer reviewers and from the public is included in Appendix A of the *Toxicological Review of Nitrobenzene* (U.S. EPA, 2009). ***To review this appendix, exit to the toxicological review, Appendix A, Summary Of External Peer Review And Public Comments And Disposition (PDF)***.

II.D.2. EPA REVIEW

Agency Completion Date - 02/06/2009

II.D.3. EPA CONTACTS

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

III. [reserved]

IV. [reserved]

V. [reserved]

VI. BIBLIOGRAPHY

Substance Name — Nitrobenzene
CASRN — 98-95-3

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VII. REVISION HISTORY

Substance Name — Nitrobenzene
CASRN — 98-95-3
File First On-Line 01/31/1987

Date	Section	Description
12/01/1990	II.	Carcinogen assessment on-line
02/06/2009	I., II.	Revised the RfD; added an RfC; revised the cancer assessment.

VIII. SYNONYMS

Substance Name — Nitrobenzene
CASRN — 98-95-3
Last Revised — 02/06/2009

- 98-95-3
- Benzene, nitro-
- Essence of mirbane
- Essence of myrbane
- Mirbane oil
- NCI-C60082
- Nitrobenzene

- Nitrobenzol
- Oil of mirbane
- Oil of myrbane