Nitrobenzene

Toxicological overview

Key Points

**Kinetics and metabolism**
- Nitrobenzene is readily absorbed following inhalation, ingestion and dermal contact
- Dermal contact with nitrobenzene may give rise to systemic toxicity due to extensive dermal absorption
- Nitrobenzene is slowly excreted in the urine and faeces either unchanged or as the major metabolites \( p \)-aminophenol and \( p \)-nitrophenol

**Health effects of acute exposure**
- Acute exposure to nitrobenzene may cause methaemoglobinaemia, with symptoms including headache, nausea, dizziness, fatigue, shortness of breath, cyanosis and convulsions
- The onset of methaemoglobinaemia may be delayed for 1 to 4 hours post exposure
- Severe acute exposure to nitrobenzene can cause jaundice, renal failure, coma and may be fatal
- Dermal or ocular contact with nitrobenzene may cause mild irritation

**Health effects of chronic exposure**
- Chronic exposure to nitrobenzene can cause methaemoglobinaemia similar to that seen following acute exposure
- Chronic nitrobenzene exposure may give rise to headache, nausea, vertigo, confusion, hyperalgesia, haemolytic anaemia and toxic hepatitis
- Nitrobenzene may cause adverse effects on male fertility due to testicular atrophy
- Nitrobenzene is considered to be a possible human carcinogen

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Toxicological Overview

Summary of Health Effects

Nitrobenzene is readily absorbed following inhalation, ingestion and dermal contact [1]. There is no human information available regarding oral ingestion of nitrobenzene, however, its absorption is expected to be extensive. Nitrobenzene can undergo extensive dermal absorption, therefore dermal contact with nitrobenzene may cause considerable systemic toxicity [1, 2].

The principal characteristic health effect following acute exposure to nitrobenzene is the development of methaemoglobinaemia, which can give rise to symptoms including headache, nausea, dizziness, drowsiness, shortness of breath, fatigue, cyanosis and convulsions [1, 3, 4]. The onset of methaemoglobinaemia may be delayed for 1 to 4 hours post exposure [4]. In some cases of acute nitrobenzene ingestion haemolytic anaemia may develop approximately 5 days post-exposure [1]. Serious acute exposure to nitrobenzene may result in jaundice, liver failure, renal failure, coma and can potentially be fatal [1-3, 5].

Dermal or ocular exposure to vapours or liquid splashes of nitrobenzene may cause mild irritation [4].

Long term occupational exposure to nitrobenzene is associated with similar adverse effects as those observed following a large single acute exposure, such as the development of methaemoglobinaemia [1, 3]. Some symptoms related to chronic nitrobenzene exposure may include headache, nausea, vertigo, confusion and hyperalgesia. Chronic exposure to nitrobenzene may also give rise to the development of haemolytic anaemia and toxic hepatitis [1, 5].

There is evidence from animal studies that nitrobenzene exposure may cause adverse effects on male fertility due to testicular atrophy [1, 3, 5]. Studies in female animals suggest that nitrobenzene does not cause embryotoxicity or teratogenicity at concentrations which do not cause significant maternal toxicity [1]. However, no studies were located regarding reproductive or developmental effects of nitrobenzene following chronic exposure in humans.

The International Agency for Research on Cancer (IARC) have evaluated that there is insufficient evidence for the carcinogenicity of nitrobenzene in humans. However, there is sufficient evidence that nitrobenzene is carcinogenic in experimental animals. Therefore, based on the animal carcinogenicity data, nitrobenzene has been classified overall as possibly carcinogenic to humans (group 2B) [5].
**Kinetics and metabolism**

Nitrobenzene is readily absorbed following inhalation, ingestion and dermal contact [1]. Following a 6-hour inhalation exposure of human volunteers to nitrobenzene the pulmonary absorption was found to be extensive, with uptake in the range of 73-87%. No human studies have been located regarding oral exposure of humans to nitrobenzene, although based on the effects of accidental poisonings, oral absorption is expected to be rapid and extensive. Animal studies have shown significant absorption from the gastrointestinal tract following oral exposure to nitrobenzene [1, 3]. The dermal absorption of nitrobenzene has not been well studied, in either humans or experimental animals. However, there have been numerous incidences of human poisonings following dermal exposure to nitrobenzene, suggesting that dermal absorption is also of considerable importance [1, 2].

Studies involving oral administration of nitrobenzene to rabbits demonstrated distribution to the blood, liver, kidney and lung, and in rats, nitrobenzene was detected in all tissues, excluding stomach and intestines, particularly in kidney and intestinal fat and skeletal muscle. Following accidental nitrobenzene poisoning in humans, the highest concentration was found in the liver, brain, blood and stomach [1]. No studies were located regarding the distribution of nitrobenzene following inhalation or dermal exposure.

There are few studies investigating the metabolism of nitrobenzene in humans. Guinea pigs administered nitrobenzene intraperitoneally showed the main metabolite was p-nitrophenol [1].

The main route of excretion of nitrobenzene is in the urine and to a lesser extent in the faeces. In rats administered nitrobenzene as a single oral dose, approximately 65% of the applied dose had been excreted in the urine and 15% in the faeces by 5 days post-exposure either as metabolites or unchanged [1, 3].

In cases of human toxicity following either dermal or inhalation exposure to nitrobenzene, the major urinary metabolites identified were p-aminophenol and p-nitrophenol [3]. In a human volunteer study following the oral administration of 30 mg nitrobenzene the urinary excretion of p-nitrophenol was slow, with an initial elimination half-life of approximately 5 hours, followed by a secondary slow phase with an elimination half-life of over 20 hours [1]. The presence of p-aminophenol in urine has been used in biomonitoring studies as a marker of occupational exposure to nitrobenzene [1]. Following exposure to nitrobenzene vapours, any absorbed material was excreted in the urine as p-nitrophenol. Similarly, urinary excretion of p-nitrophenol was reported in individuals following inhalation of nitrobenzene [1].

**Sources and route of human exposure**

The major source of exposure to nitrobenzene is from occupational exposure, since its principal use is in industrial processes, such as during the production of aniline [1, 3]. Nitrobenzene is unlikely to be present in significant quantities in domestic situations and does not occur naturally but can be formed at low levels by the reaction of benzene and nitrogen oxides in the atmosphere [1]. In the occupational context, in addition to controlling airborne levels to the occupational exposure standard it is also necessary to protect against skin absorption by the use of appropriate protective clothing [1, 2].

The major routes of occupational exposure to nitrobenzene are by inhalation of vapours or dermal absorption. Nitrobenzene is a considerable occupational exposure hazard as it readily forms a vapour at room temperature and also may undergo significant dermal
absorption. Ingestion of nitrobenzene may also occur, as the relatively pleasant almond
odour may not discourage individuals from drinking water contaminated with it [1, 3].

Nitrobenzene may be present in the environment close to industrial locations where it is
manufactured or used. It has also been reported as being detectable near hazardous waste
sites. However, the levels of nitrobenzene present in such locations would be expected to be
many times lower than those observed during occupational exposure [1, 3].
Health Effects of Acute / Single Exposure

**Human Data**

**General toxicity**

Nitrobenzene is toxic via inhalation, ingestion and dermal absorption. The principal adverse health effect following exposure to nitrobenzene is methaemoglobinaemia [1]. There are very few accounts of human fatalities following nitrobenzene exposure, due to prompt medical intervention in many case studies [3].

**Inhalation**

Acute inhalation of nitrobenzene can result in respiratory irritation, headache, nausea, dizziness, drowsiness, shortness of breath, fatigue, cyanosis and convulsions, due to methaemoglobinaemia [1, 3, 4]. The symptoms of methaemoglobinaemia may develop within 1 to 4 hours post-exposure [4]. Serious acute exposure to nitrobenzene may lead to jaundice, renal failure, unconsciousness and can possibly be fatal [1-3, 5]. However, there are few reports of acute inhalation exposure to nitrobenzene at levels sufficient to cause fatality [1]. In an early study based on toxic symptoms in factory workers, it was considered that exposure to 200 ppm would produce serious adverse effects after a 1 hour exposure, or 60 – 100 ppm for 6 hours [1].

**Ingestion**

There are numerous reports of poisoning from nitrobenzene in the first half of the 20th Century when it was widely available as a substitute for oil of bitter almond or as a dye in shoe polish. Acute oral ingestion of nitrobenzene results in gastric irritation, including nausea and vomiting, the onset of which may be delayed for up to 12 hours post exposure [1]. Methaemoglobinaemia will develop 1 to 4 hours post-exposure, indicated by cyanosis, the symptoms of which may be similar to those following inhalation [4]. Severe exposure to nitrobenzene by ingestion can also give rise to progressive drowsiness and coma which may prove fatal due to respiratory failure [1].

**Dermal / ocular exposure**

Dermal exposure to nitrobenzene either from splashes of liquid or contact from vapours may cause mild irritation [4]. Nitrobenzene will readily undergo dermal absorption and there have been numerous reports of nitrobenzene toxicity in humans, particularly infants, following skin contact [1, 3, 4]. Following dermal absorption of sufficient quantities of nitrobenzene the main adverse effect of nitrobenzene exposure is methaemoglobinaemia similar to that observed following inhalation and ingestion [1, 2].

**Delayed effects following an acute exposure**

The onset of methaemoglobinaemia from all routes of exposure may be delayed for 1 to 4 hours depending upon the severity of exposure [1, 3, 4]. In some cases following severe ingestion of nitrobenzene, haemolytic anaemia may develop around 5 days post exposure [1].
Animal and In-Vitro Data

General toxicity

The acute toxicity of nitrobenzene in experimental animals resembles that seen in humans, with the development of methaemoglobin being the characteristic adverse effect [1, 3, 5, 6].

Inhalation

There are only limited data on acute exposure to nitrobenzene via inhalation. Exposure of rats to an atmosphere saturated with nitrobenzene vapour for 3 hours resulted in no deaths over the 14 day observation period. However, exposure for 7 hours resulted in 25% of the exposed animals (3/12) dying. No information was available on the actual exposure levels [1].

Ingestion

The oral LD$_{50}$ for nitrobenzene administered to rats is 600 mg kg$^{-1}$ body weight [1, 3]. In a study in which female rats were orally administered 640 mg kg$^{-1}$ nitrobenzene the percentage of methaemoglobin formed 30, 60 and 120 mins post-exposure were 11, 19 and 28%, respectively [1]. Histological changes in the liver and testes were noted at autopsy in rats 2 or 5 days after single doses of nitrobenzene at 200 mg kg$^{-1}$ and above [1].

Dermal

The dermal LD$_{50}$ of nitrobenzene in rats has been reported to be 2100 mg kg$^{-1}$ body weight [1]. In this dermal LD$_{50}$ study, the percentage of methaemoglobin formed following an LD$_{50}$ dose, was found to be 16%, 25% and 35% at 30, 60 and 120 minutes, respectively. The dermal LD$_{50}$ of nitrobenzene in rabbits was found to be 760 mg kg$^{-1}$ body weight [1].

Skin and Eye Irritation

Nitrobenzene only produces slight and transient skin and eye irritation. The skin irritation potential of nitrobenzene was investigated in male rabbits using the Draize method and was found to produce only very slight erythema at 24 hours post exposure [1]. Studies investigating ocular irritation in male rabbits using the Draize method found nitrobenzene to produce slight irritation at 1 and 24 hours post-exposure, but had resolved completely at 48 hours [1].
Health Effects of Chronic / Repeated Exposure

**Human Data**

**Inhalation**

Long term occupational exposure to nitrobenzene may be associated with similar symptoms as those observed following excessive levels of acute exposure. The most common adverse effect following occupational inhalation of nitrobenzene is the formation of methaemoglobinemia [1, 3]. A worker exposed to nitrobenzene (no details available on exposure levels) for 17 months developed severe methaemoglobinemia, with headache, nausea, vertigo, confusion and an increased sensitivity to pain (hyperalgesia) from pin-prick. The worker also had enlarged and tender spleen and liver and abnormal results from liver-function tests. The exposure and absorption of nitrobenzene were confirmed by the presence of p-nitrophenol and p-aminophenol in the urine [1]. Chronic exposure to nitrobenzene may also give rise to the development of haemolytic anaemia and toxic hepatitis [1, 5].

**Dermal / Ocular**

Very little information was identified relating to chronic occupational dermal exposure of nitrobenzene. However, as nitrobenzene will undergo rapid dermal absorption the symptoms are expected to be similar to those observed following chronic inhalation.

**Genotoxicity**

No studies were identified regarding genotoxic effects following chronic exposure to nitrobenzene in humans [1, 3]

**Carcinogenicity**

No data was located regarding the carcinogenicity of nitrobenzene in humans. The International Agency for Research on Cancer (IARC) has concluded that there is insufficient evidence for the carcinogenicity of nitrobenzene in humans. However, based on animal carcinogenicity data, nitrobenzene has been classified overall as possibly carcinogenic to humans (group 2B) [5].

**Reproductive and developmental toxicity**

No studies were located regarding reproductive or developmental effects of nitrobenzene in humans [1, 3].

**Animal and In-Vitro Data**

**Inhalation**

The toxicity of nitrobenzene following chronic inhalation was studied in two strains of rat (Fisher-344 and Sprague Dawley) and B6C3F1 mice, exposed to nitrobenzene at concentrations up to 125 ppm for 6 hours day⁻¹, 5 days week⁻¹ for 2 weeks. The Sprague-Dawley rats exposed to 125 ppm nitrobenzene showed severe adverse clinical signs including rapid shallow breathing and wheezing with 40% lethality at the fourth day of exposure. All of the mice exposed at the same concentration showed morbidity which
necessitated the animals being sacrificed on the fourth day of exposure. However, the Fisher-344 rats tolerated this dose for 2 weeks and showed no adverse clinical signs, indicating marked strain differences in susceptibility to nitrobenzene [1]. The study also reported significant concentration-dependent increases in liver, spleen and kidney weights [1]. Rats exposed to nitrobenzene by inhalation at just 5 ppm (25.15 mg m\(^{-3}\)) for 6 hours day\(^{-1}\), 5 days week\(^{-1}\) for 90 days displayed symptoms of methaemoglobinaemia [3].

**Ingestion**

A study of Fisher-344 rats orally exposed to nitrobenzene at 125 mg kg\(^{-1}\) body weight day\(^{-1}\) for 28 days, showed decreased movement, pale skin, gait abnormalities and decreases in body weight or body weight gain. In this study, increases in the weights of the liver, spleen and kidneys were observed along with reductions in the weight of the thymus and the testis in the males. An increase in liver weights of males was also observed at a low dose of 5 mg kg\(^{-1}\) body weight day\(^{-1}\) [1]. In another study in Fisher-344 rats, nitrobenzene was administered (by gavage) for 13 weeks at doses of up to 150 mg kg\(^{-1}\). Some lethality occurred at the top dose. Clinical signs seen at 75 and 150 mg kg\(^{-1}\) body weight day\(^{-1}\) included ataxia, head tilt, lethargy, trembling, circling, dyspnoea as well as cyanosis of the extremities. Marked brain lesions were noted at autopsy [1]. A study in which B6C3F\(_1\) mice were administered nitrobenzene orally at up to 300 mg kg\(^{-1}\) body weight day\(^{-1}\) for 13 weeks showed signs of toxicity including ataxia, lethargy, ataxia, dyspnoea, convulsions, irritability and rapid head-bobbing movements, some lethality was noted at the highest dose [1].

**Dermal**

The effects of repeated skin painting of nitrobenzene have been investigated in a number of studies. In 14 day studies in B6C3F\(_1\) mice and Fisher-344 rats, similar effects were seen in both species. Dose levels in the range of 200 – 3200 mg kg\(^{-1}\) body weight day\(^{-1}\) were used. Dose levels of 1600 mg kg\(^{-1}\) body weight day\(^{-1}\) and above resulted in lethality. Clinical signs reported included ataxia, prostration and dyspnoea. Significant depression of weight gain was noted in mice at all dose levels. At autopsy there was histological evidence of damage to the brain, liver, spleen and testes, with mice being less affected than rats [1].

**Genotoxicity**

Nitrobenzene has been fairly extensively investigated for its genotoxic potential using both in-vitro and in-vivo studies. The data suggest that it is not a genotoxic carcinogen and does not possess any significant mutagenic effects in vivo [1].

Nitrobenzene has been investigated for its ability to produce mutations or DNA lesions in two well established assays. There are numerous reports of studies to investigate the potential of nitrobenzene to produce gene mutations in vitro in the Ames test using strains of *Salmonella typhimurium*, either in the presence or absence of metabolic activation with liver S9 fraction. Negative results were consistently obtained [1].

Nitrobenzene was also found to be negative for unscheduled DNA synthesis (UDS) when tested using primary hepatocytes cultured in vitro from either humans or rats [1].

The ability of nitrobenzene to induce chromosome damage has been investigated in vivo. Negative results were obtained in a micronucleus study using mice and the intraperitoneal route of exposure. Dose levels of up to 250 mg kg\(^{-1}\) body weight were used, with bone marrow harvested after 24 and 48 hours and examined for micronucleated polychromatic erythrocytes. Nitrobenzene has also been investigated in the in-vivo UDS test with rat liver cells. The compound was given orally at dose levels up to 500 mg kg\(^{-1}\). The study was limited by the use of a single harvest time, but there was no increase in UDS [1].
**Carcinogenicity**

The carcinogenicity of nitrobenzene has been investigated in chronic bioassays using the inhalation route in rat and mice studies. In one study in rats an increase in the incidence of hepatocellular neoplasms, thyroid follicular-cell adenomas and adenocarcinomas and renal tubular-cell adenomas was seen in the treated males. In the treated females there was an increase in hepatocellular neoplasms and endometrial stromal polyps. In a second study in which only male rats were used, an increase in the incidence of hepatocellular neoplasm was seen [5]. In male mice exposed to nitrobenzene an increase in alveolar-bronchial neoplasms and thyroid follicular-cell adenomas was observed [5].

Overall, the IARC has concluded that there is sufficient evidence for the carcinogenicity of nitrobenzene in experimental animals [5].

**Reproductive and developmental toxicity**

Numerous studies have established that nitrobenzene is a testicular toxicant. The most sensitive end points were sperm count and motility, followed by progressive motility, viability, presence of abnormal sperm and finally fertility index [1]. In repeated dose studies (13 week) testicular atrophy was seen in mice at daily doses of 19 mg kg\(^{-1}\) body weight and above, and in rats at 150 mg kg\(^{-1}\) body weight and above. In a 2 generation reproductive toxicity study in rats using the inhalation route, 40 ppm (6 hours day\(^{-1}\), 5 days week\(^{-1}\)) produced a marked decrease in fertility index, but was not seen at 20 ppm [1]. These studies suggest that nitrobenzene may cause reproductive toxicity in males due to testicular atrophy [1, 3, 5].

Studies investigating the developmental toxicity of nitrobenzene in rats and rabbits using the inhalation route have not observed fetotoxic, embryotoxic or teratogenic effects at concentrations associated with maternal toxicity [1].
References


This document from the HPA Centre for Radiation, Chemical and Environmental Hazards reflects understanding and evaluation of the current scientific evidence as presented and referenced in this document.