



Nitrobenzene

Toxicological Overview

Key Points

Kinetics and metabolism

- nitrobenzene is readily and extensively absorbed following exposure by any route
- following absorption nitrobenzene is distributed throughout the body
- the two major routes of metabolism of nitrobenzene include reduction to aminophenols and oxidation to form nitrophenols
- p-nitrophenol and p-aminophenol are urinary metabolites of nitrobenzene which are biomarkers of exposure

Health effects of acute exposure

- nitrobenzene causes methaemoglobinaemia following acute exposure; symptoms may develop within 1 to 4 hours post-exposure
- low methaemoglobin levels cause apparent cyanosis, fatigue, dizziness headaches, with weakness tachypnoea, tachycardia at increasing levels
- high levels may cause stupor, coma, convulsions, respiratory depression, cardiac arrhythmias, acidosis and death

Health effects of chronic exposure

- chronic inhalation of nitrobenzene in occupation has been associated with similar symptoms as those observed following acute exposure
- the IARC have classified nitrobenzene as possibly carcinogenic in humans (group 2B)
- nitrobenzene is a reproductive toxicant in male animals but does not cause developmental toxicity

Summary of Health Effects

Nitrobenzene is readily and extensively absorbed by all routes of exposure. Dermal absorption is not as high as ingestion or inhalation, but is still significant. Nitrobenzene is widely distributed following absorption. Human autopsies following fatal ingestion of nitrobenzene showed the highest concentrations to be in the liver, brain, blood and stomach. There are thought to be two major routes of nitrobenzene metabolism; reduction to aminophenols and oxidation to form nitrophenols. The main route of excretion for nitrobenzene is in the urine.

The formation of methaemoglobin is the primary (but not the only) mechanism by which nitrobenzene causes health effects. Methaemoglobinaemia may be delayed up to 4 hours following exposure. The severity of features increases with methaemoglobin concentration in the blood. Effects progress from cyanosis, fatigue, dizziness, headaches, weakness tachypnoea, tachycardia, stupor, coma, convulsions, respiratory depression, cardiac arrhythmias, acidosis and death. Inhalation of nitrobenzene can lead to coughing, wheezing, headache, nausea, dizziness, drowsiness, shortness of breath, fatigue, cyanosis and dyspnoea, convulsions and respiratory distress. Ingestion may result in gastric irritation, including nausea, diarrhoea and vomiting; the onset of which may be delayed for up to 12 hours post exposure. Nitrobenzene is readily absorbed through unbroken skin and there have been numerous reports of nitrobenzene toxicity in humans following skin contact. Pain, blepharospasm, lacrimation, conjunctivitis, palpebral oedema and photophobia may follow ocular exposure to nitrobenzene. Nitrobenzene is of low acute toxicity in experimental animals.

Methaemoglobinaemia has been observed in workers chronically exposed to nitrobenzene. Chronic exposure to nitrobenzene may also give rise to the development of haemolytic anaemia and toxic hepatitis. Carcinogenicity, genotoxicity and reproductive toxicity following long term exposure to nitrobenzene has been poorly studied in humans.

There is significant interspecies variation in the response to nitrobenzene exposure. Humans are more sensitive to the formation of methaemoglobin and thereby nitrobenzene toxicity than rats. Data from in-vitro and in-vivo studies suggest that nitrobenzene may be weakly genotoxic, but not mutagenic. Cancer bioassays in rats and mice have suggested a carcinogenic potential for nitrobenzene, however in light of the aforementioned assays on genotoxicity, this is unlikely to be due to a direct action on DNA by nitrobenzene.

In experimental animals nitrobenzene has been established as a testicular toxicant following exposure from all routes. This effect has caused a marked reduction in the fertility index. The specific toxic effects on the testes appear to be independent of nitrobenzene's ability to induce methaemoglobinaemia.

Kinetics and Metabolism

Nitrobenzene is readily absorbed following exposure by any route [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% (decreasing over time with assumed blood saturation) [2]. Data from human and animal studies and reports following human poisoning suggest absorption following the oral route is near absolute [3].

The numerous incidences of human poisonings following dermal exposure to nitrobenzene suggest that dermal absorption is of considerable importance. In vitro experiments on human skin suggest dermal absorption of up to 40% where evaporation was prevented; in vivo and in vitro studies suggest an absorption rate of up to 8% where the skin was not obscured [3].

Studies involving oral administration of nitrobenzene to rats demonstrated distribution to the blood, liver, kidney and lung [4]. In rabbits, nitrobenzene was distributed to various tissues including kidney and intestinal fat and skeletal muscle [2]. Following accidental nitrobenzene poisoning in humans, the highest concentration was found in the liver, brain, blood and stomach [4]. There is no evidence to suggest that nitrobenzene or its metabolites are significantly retained in the body [2].

The metabolism of nitrobenzene involves both reduction and oxidation pathways. Nitrobenzene is reduced to form nitrosobenzene, phenylhydroxylamine and aniline. Oxidative metabolites of aniline include o-, m- and p-aminophenol which conjugate with glucuronide or sulphate. Alternately nitrobenzene itself may be ring oxidised to form nitrophenols which also conjugate with glucuronide or sulphate. The toxicological effects of nitrobenzene i.e. methaemoglobinaemia are caused by the metabolites [2, 5].

The main route of excretion of nitrobenzene is in the urine and to a lesser extent in the faeces and via exhalation [2, 5]. Metabolites nitrophenol and aminophenol have been detected in the urine of humans and experimental animals exposed to nitrobenzene [2, 5, 6]. Elimination of nitrobenzene is not considered to be rapid. In a study in rats it took 3 days to eliminate 80% of a 22.5 mg/kg dose of nitrobenzene [5]. In some cases of nitrobenzene poisoning it has taken individuals over 7 days to recover from the clinical signs of methaemoglobinaemia [4, 5].

Sources and Route of Human Exposure

Nitrobenzene does not occur naturally, although it may be formed at low levels by the reaction of benzene and nitrogen oxides in the atmosphere [4]. Nitrobenzene is typically produced by the nitration of benzene and in the EU the vast majority of this is used as an intermediate in the production of aniline. Nitrobenzene also has minor uses in the manufacture of pharmaceuticals and other chemicals [2].

Populations in the vicinity of industrial areas that produce or use nitrobenzene may be exposed to higher levels of nitrobenzene in air [4, 7].

Exposure to nitrobenzene is most likely to occur in the workplace, by either inhalation of vapours or dermal absorption. Workplace Exposure Limits (WELs) are enforced to protect workers from the harmful effects of nitrobenzene; in the UK the long-term WEL is 1 mg/m^3 (0.2 ppm) [8].

Health Effects of Acute/Single Exposure

Human data

General toxicity

Methaemoglobinaemia is the principle adverse health effect following exposure (from all routes) to nitrobenzene [4, 9]. Methaemoglobin forms when the iron component (Fe^{2+}) of haemoglobin is oxidised to the ferric (Fe^{3+}) state, which is unable to bind oxygen. Under normal conditions low levels of methaemoglobin are continuously produced in the body. Methaemoglobinaemia occurs when the level of methaemoglobin is greater than 1% of total haemoglobin. This leads to reduction in the amount of oxygen available to tissues and can result in tissue hypoxia [9].

Infants and those having consumed ethanol may be more susceptible to methemoglobinemia and thus nitrobenzene toxicity [4]. Young children may still have some foetal haemoglobin, which is more susceptible to the formation of methaemoglobin and key metabolic enzymes able to clear methaemoglobin may not be fully developed [2]. Studies in experimental animals suggest that sensitivity to the effects of nitrobenzene is increased with concurrent exposure to ethanol, although the toxicokinetics responsible for this effect have not been elucidated [6].

Table 1 below summarises clinical signs and symptoms observed at blood methaemoglobin concentrations. The onset of methaemoglobinaemia from all routes of exposure may be delayed for 1 to 4 hours depending upon the severity of exposure [4, 6, 10].

Table 1: Expected clinical effects and corresponding blood methaemoglobin concentrations

Methaemoglobin concentration (%)	Clinical signs and symptoms
0-10	Features unlikely
10-30	Milder effects: apparent cyanosis (blue to grey lips, tongue, mucous membranes and slate grey skin), fatigue, dizziness, headaches
30-50	Moderate effects: weakness, tachypnoea, tachycardia
50-70	Severe effects: stupor, coma, convulsions, respiratory depression, cardiac arrhythmias, acidosis
More than 70	Potentially fatal
Reference	
TOXBASE. Methaemoglobinaemia, 06/2012. http://www.toxbase.org (accessed 03/2018).	

Exposure to nitrobenzene is also associated with damage to the bone marrow, lymphoid organs, and nervous system [2]. Haemolytic anaemia, jaundice and renal failure are common in severe cases [1].

Inhalation

Inhalation of nitrobenzene may lead to coughing, wheezing, dyspnoea and respiratory distress before systemic effects (as described above) develop [6, 10]. However, there are few reports of acute inhalation exposure to nitrobenzene at levels sufficient to cause fatality [4]. In an early study based on toxic symptoms in factory workers, it was considered that exposure to 200 ppm (1,000 mg/m³) would produce serious adverse effects after a 1 hour exposure, or 60 – 100 ppm (300 -500 mg/m³) for 6 hours [4].

Ingestion

Acute oral ingestion of nitrobenzene results in gastric irritation, including nausea, diarrhoea and vomiting, the development of systemic features (as described above) is common but may be delayed [1].

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. Common features following these ingestion cases include marked cyanosis, unconsciousness, irregular breathing and the smell of bitter almonds on the patient's breath [2].

Dermal/ocular exposure

Dermal exposure to nitrobenzene either from splashes of liquid or contact from vapours may cause mild irritation. Skin contact may also cause dermatitis [1]. Nitrobenzene is readily absorbed through skin and there have been numerous reports of nitrobenzene toxicity (as described above) in humans, particularly in infants, following skin contact [4, 6, 10].

Pain, blepharospasm, lacrimation, conjunctivitis, palpebral oedema and photophobia may follow ocular exposure to nitrobenzene [1].

Animal and in-vitro data

General Toxicity

As seen in humans, the main adverse effect in animals following exposure to nitrobenzene is methaemoglobinaemia. There appears to be a significant interspecies variation between humans and rats for the formation of methaemoglobin, with humans being more sensitive. This variation may be explained in part by the activity of methaemoglobin reductase, which is 5 times higher in rat erythrocytes than in human erythrocytes [11].

Inhalation

There are only limited data on acute exposure to nitrobenzene via inhalation [3].

In rats exposed to nitrobenzene (head only) for single 4 hour periods the LC₅₀ was reported to be 2,850 mg/m³ [3].

Ingestion

Acute studies have indicated that there is a wide range of lethal doses in experimental animals. In its background document for the opinion on the harmonised classification for Nitrobenzene, the European Chemicals Agency Committee for Risk Assessment cites 4 rat studies giving an oral LD₅₀ range of 450 to 732 mg/kg bw/day [11].

In a study in which female rats were administered 640 mg/kg nitrobenzene by gavage the percentage of methaemoglobin formed 30, 60 and 120 minutes post-exposure were 11, 19 and 28%, respectively [4].

Dermal/ocular exposure

The dermal LD₅₀ of nitrobenzene in rats has been reported to be 2,100 mg/kg bw [4]. In this dermal LD₅₀ study, the percentage of methaemoglobin formed following an LD₅₀ dose, was found to be 16%, 25% and 35% at 30, 60 and 120 minutes, respectively.

Dermal LD₅₀ values of 301 and 760 mg/kg bw/day have been reported for rabbits and a LD_{Lo} of 480 mg/kg bw for mice [4, 11].

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. 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 [4].

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 acute exposure. Methaemoglobinaemia has been observed in chronically exposed workers. A worker exposed to nitrobenzene (no details available on exposure levels) for 17 months developed severe methaemoglobinaemia, with headache, nausea, vertigo, confusion and an increased sensitivity to pain (hyperalgesia). 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 [4, 5]. Chronic exposure to nitrobenzene may also give rise to the development of haemolytic anaemia and toxic hepatitis [4, 12].

A study of workers in a factory producing nitrobenzene and dinitrochlorobenzene reported daily air concentrations of 15-29 mg/m³. Increased methaemoglobin levels and Heinz bodies, but not anaemia, were observed. Levels as high as 196 mg/m³ had been measured in the same factory in the past and at this level cases of anaemia and intoxication were reported [4].

Ingestion

No studies were identified regarding the effects of chronic ingestion of nitrobenzene in humans.

Dermal/ocular exposure

There are no data on the effects of long term dermal exposure to nitrobenzene in humans.

Genotoxicity

No studies were identified regarding genotoxic effects following exposure to nitrobenzene in humans [4, 6].

Carcinogenicity

There are limited data available on 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) [12].

Reproductive and developmental toxicity

No studies were located regarding reproductive or developmental effects of nitrobenzene in humans.

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 (625 mg/m³) for 6 hours a day, 5 days a week for 2 weeks. The Sprague-Dawley rats exposed to 125 ppm (625 mg/m³) 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. The study also reported significant concentration-dependent increases in liver, spleen and kidney weights [4].

Rats exposed to nitrobenzene by inhalation at just 5 ppm (25.2 mg/m³) for 6 hours a day, 5 days a week for 90 days displayed symptoms of methaemoglobinaemia [6].

Ingestion

A gavage study of male and female Fisher-344 rats administered nitrobenzene at 0, 5, 25, or 125 mg/kg bw/day for 28 days reported decreased movement, pale skin, gait abnormalities and decreases in body weight or body weight gain in the highest dose group. Increases in the weights of the liver, spleen and kidneys and reductions in the weight of the thymus and the testis were also seen in the 125 mg/kg bw/day group. In addition, an increase in liver weights was observed in the males administered 5 mg/kg bw/day and increases in liver and spleen weights were reported in both sexes of the 25 mg/kg bw/day group [4].

In another study in Fisher-344 rats, nitrobenzene was administered (by gavage) for 13 weeks at doses of 0, 9.4, 19, 38, 75 or 150 mg/kg bw/day. Some lethality occurred at the top dose. Clinical signs seen at 75 and 150 mg/kg bw/day included ataxia, head tilt, lethargy, trembling, circling, dyspnoea as well as cyanosis of the extremities. Marked brain lesions were noted in the highest dose group at autopsy [4].

In a 13 week gavage study B6C3F1 mice were administered 0, 19, 38, 75, 150 or 300 mg/kg bw/day nitrobenzene. Some lethality was noted in the highest dose group. Clinical signs included ataxia, lethargy, dyspnoea, convulsions, irritability and rapid head-bobbing movements. Increases in liver weight were statistically significant at all doses in females and in the two highest doses in males [4].

Dermal

The effects of repeated skin painting of nitrobenzene have been investigated in a number of studies. In 14 day studies in B6C3F1 mice and Fisher-344 rats, similar effects were seen in both species. Dose levels in the range of 200 – 3,200 mg/kg bw/day were used. Dose levels of 1,600 mg/kg bw/day 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 [4].

In a similar 90 day study, 17 of 20 mice died by the end of the study in the 800 mg/kg bw/day groups, with survivors showing ataxia, dyspnoea, circling, lethargy and insensitivity to pain [5].

Genotoxicity

Nitrobenzene gave negative results in various bacterial mutagenicity assays with or without metabolic activation. In in-vitro genotoxicity tests of mammalian cells, nitrobenzene has given largely negative or equivocal results [11].

Following in-vivo studies in experimental animals, mixed results for genotoxicity have been reported. Negative results have been obtained for assays of micronucleus formation, unscheduled DNA synthesis, sister chromatid exchange and chromosomal aberrations. Two studies report the formation of DNA adducts, however in these studies the routes of exposure were of intraperitoneal and subcutaneous and so their relevance is questionable [11].

Taken together the evidence suggests that nitrobenzene may be weakly genotoxic. It does not however appear to be a mutagen and does not cause cancer through mutagenic effects [11].

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. In male mice exposed to nitrobenzene, an increase in alveolar-bronchial neoplasms and thyroid follicular-cell adenomas was observed [12]. In light of the difference in response between mice and rats, and the gender disparity in rats, this evidence is of limited value for the assessment of human carcinogenicity [11].

Reproductive and developmental toxicity

Numerous studies have established that nitrobenzene is a testicular toxicant in experimental animals following exposure from all routes. Commonly reported effects include bilateral testicular atrophy, atrophy of the seminiferous tubules, hyperplasia of the interstitial cells and hypospermatogenesis [11].

Studies assessing reproductive ability following paternal nitrobenzene exposure have found decrements in the fertility index of the animals. In a 2 generation reproductive toxicity study in rats using the inhalation route, there was a marked decrease in fertility index for the F0 and F1 generations, associated with the testicular effects described above [4]. These studies suggest that nitrobenzene may cause reproductive toxicity due to effects on the male

reproductive organs [4, 12]. This effect is believed to be a direct result of nitrobenzene toxicity rather than a secondary effect of methaemoglobinaemia [11].

Following a single exposure to 60 mg/kg bw nitrobenzene by gavage the fertility of male rats diminished over the observation period. Following this exposure sperm counts dropped progressively; fertility was affected below 10% of sperm count and the rats became infertile after 28 days [11].

Developmental toxicity has not been observed in the offspring of animals affected by the male reproductive toxicity of nitrobenzene. Developmental toxicity has not been observed in the absence of and in the presence of mild maternal toxicity following nitrobenzene toxicity [2, 4, 6].

In conclusion, the data indicates nitrobenzene is a reproductive toxicant in male animals but does not cause developmental toxicity.

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