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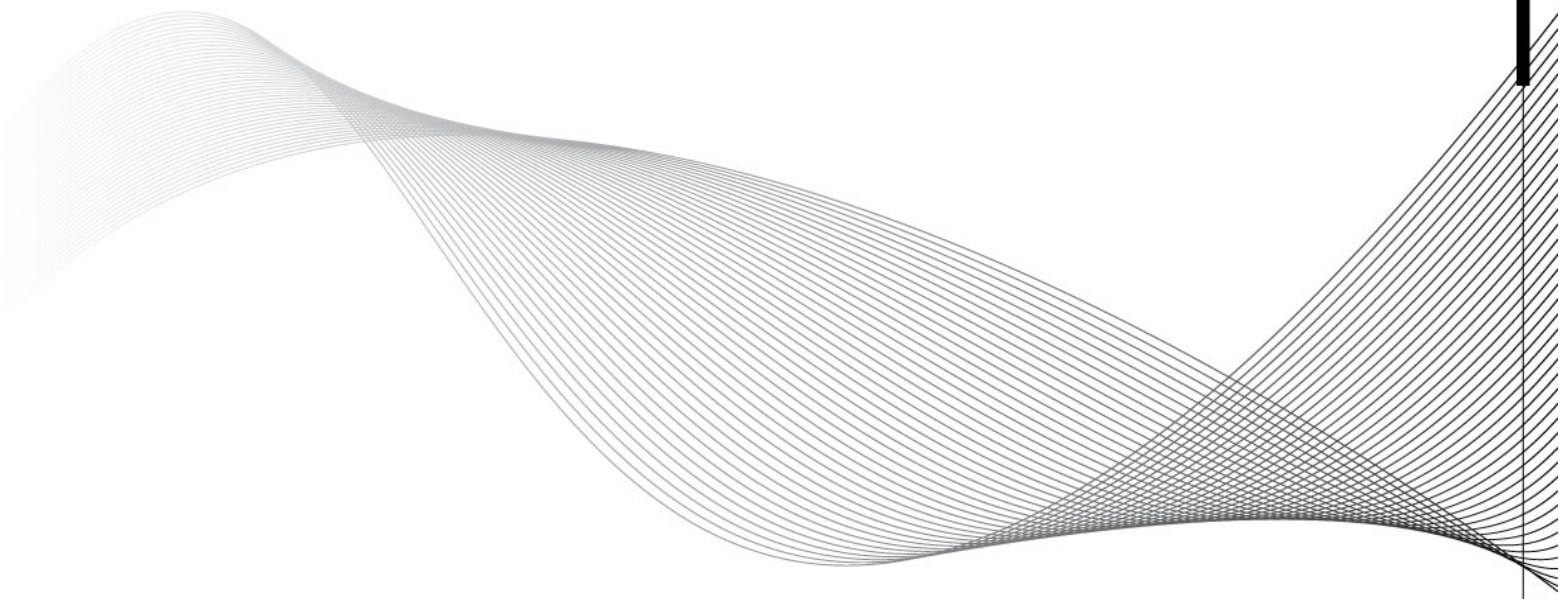

Recommendation from the Scientific Committee on Occupational Exposure Limits for nitrobenzene

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8-hour TWA:	0.2 ppm (1.0 mg/m^3)
STEL:	-
Additional classification:	"skin"

Substance

Nitrobenzene	$C_6H_5NO_2$
EINECS No	202-716-0
CAS No	98-95-3
MWt:	123.11
Melting point:	5.7°C
Boiling point:	210.8°C (760 torr)

1. Occurrence/use

Nitrobenzene is used industrially as an intermediate in the production of aniline and aniline-derived products. Potential for human exposure is principally via inhalation and dermal uptake (Piotrowski 1967, Dorigan and Hushon 1976, Sax 1984). For use and production figures, reference can be made to IARC (1996).

2. Health significance

The toxicity of nitrobenzene to humans and animals has been known for almost a century (review: Beauchchamp et al. 1982). Haematic, neural and hepatic toxicities have been described in both humans and experimental animals. Single oral administrations of nitrobenzene to rats (ca. 200 mg/kg b.w.) resulted in methaemoglobinaemia (Goldstein et al. 1984) while higher oral doses (ca. 500 mg/kg b.w.) resulted in encephalopathy characterised by haemorrhage and malacia of the brainstem and cerebellum (Morgan et al. 1985). Inhalation exposure of rats and mice (10-25 ppm/2 wk., 5-10 ppm/13 wk.) caused methaemoglobinaemia and encephalopathy, and additionally resulted in lesions of the liver, kidney, spleen and testis (Hamm 1984, Medinsky and Irons 1985).

2.1. Metabolism and kinetics

For occupational exposure to nitrobenzene both inhalation and dermal uptake are of practical importance (Seeger and Neumann 1986).

Nitrobenzene is first transformed into nitrosobenzene (Mason 1979; Uehleke 1963, 1964). Nitrosobenzene is transformed in a subsequent step in the liver to phenylhydroxylamine; this may take place in erythrocytes. The reverse reaction (phenylhydroxylamine to nitrosobenzene) in erythrocytes takes place in a coupled reaction whereby methaemoglobin is formed from haemoglobin (Uehleke 1963). In man and animals, nitrobenzene metabolism is considerably more protracted than aniline metabolism; it leads, however, to comparable methaemoglobin levels (Albrecht and Neumann 1985, DFG 1995). Most of the nitrobenzene absorbed by humans is finally metabolized to p-nitrophenol and p-aminophenol (Ikeda and Kita 1964).

Nitrosobenzene, formed as an intermediate in the metabolism of nitrobenzene, is chemically reactive and can react with glutathione and SH-groups of proteins containing cysteine to form a glutathione conjugate or a protein conjugate (Eyer 1979, 1985; Albrecht and Neumann 1985). Thereby, the nitrosobenzene intermediate generated in erythrocytes leads to haemoglobin conjugation (Neumann 1984). This adduct may be used for biological monitoring (DFG 1995). The haemoglobin conjugate of aniline can be detected after exposure, even when nitrobenzene and its metabolites have already been completely eliminated. Unlike the determination of methaemoglobin, the results of the determination of this conjugate are almost independent of the sampling time (Bolt et al. 1985).

2.2. Toxicity in humans

After intake of nitrobenzene, with a certain latency period, cyanosis develops due to the formation of methaemoglobin. In general, a methaemoglobin value of up to 5% has been regarded as tolerable (Bolt et al. 1985).

Chronic intoxication can lead to haemolysis, liver damage and in rare cases also to cutaneous efflorescences (Myslak et al. 1971, Wirth and Gloxhuber 1981, Beauchamp et al. 1982). The lowest lethal dose reported for humans was 35 mg/kg (Sax 1984). The production of methaemoglobin can lead to the formation of Heinz bodies in the erythrocytes.

In persons chronically exposed to nitrobenzene the classic symptoms are characterised as fatigue, lack of appetite, general stomach complaints, weakness, dizziness, depression, and at a later stage anaemia, liver function disorders, Heinz bodies, disorders of kidney function.

2.3. Genotoxicity and carcinogenicity

No clear correlations have been recognised in reproductive effects reported in connection with nitrobenzene (Hatekeyama et al. 1971, Beauchamp et al. 1982, IARC 1996).

In the Ames test with Salmonella typhimurium strains no significant mutagenic activity of nitrobenzene could be detected (Chiu et al. 1978, Beauchamp et al. 1982). IARC (1996), after having evaluated the data on genetic effects of nitrobenzene in experimental systems, arrived at the conclusion that nitrobenzene was non-genotoxic in bacteria and mammalian cells in vitro, and was inactive in mammals in vivo.

Groups of 70 male and 70 female B6C3Fl mice, 63 days of age, were exposed by inhalation to air containing target concentrations of 0, 5, 25 or 50 ppm [0, 25, 125 or 250 mg/m^3 nitrobenzene (>99.8% pure) for 6 h per day on five days per week for 24 months. Body weights of high-dose male mice were approximately 5-8% lower than those of controls throughout the study. Probability of survival at 24 months was 60% for males and 45% for females and was not affected by exposure to nitrobenzene, except that mid-dose females had better survival than controls (70%). The incidence of alveolar-bronchiolar neoplasms was greater in treated males (alveolar-bronchiolar adenomas and carcinomas: 9/68 in controls, 21/67 at the low dose, 21/65 at the mid dose and 23/66 at the high dose; $p < 0.05$, Cochran-Armitage trend test).Though the incidence for adenomas and carcinomas in the control animals of this study was 13%, the mean rates for spontaneous alveolar-bronchiolar adenomas and carcinomas in 21 chamber studies from NTP was higher at 22.9% (10-42%) (Haseman et al 1998). The incidence of alveolar-bronchiolar hyperplasia was also greater in mid- and high-dose males and in mid-dose females. The incidence of thyroid follicular-cell adenomas was greater in treated males (0/65 in controls, 4/65 at the low dose, 1/65 at the mid dose, 7/64 at the high dose; $p < 0.05$ trend test) and that of thyroid follicular-cell hyperplasia was greater in mid-and high-dose males. The incidence of hepatocellular adenomas was greater in treated females (6/51 in controls, 5/61 at the bw dose, 5/64 at the mid dose, 13/62 at the high dose; $p < 0.05$ trend test), although the incidence of hepatocellular adenomas and carcinomas combined was no greater (7/51, 7/61, 7/64, 14/62, respectively). Mammary gland adenocarcinomas were found in 5/60 ($p < 0.05$) high-dose females compared to 0/48 controls (Cattley et al. 1994).

Further, groups of 70 male and 70 female Fischer 344 rats, 62 days of age, were exposed by inhalation to air containing target concentrations of 0, 1, 5 or 25 ppm [0, 5, 25 or 125 mg/m^3 nitrobenzene (>99.8% pure) for 6 h per day on five days per week for 24 months. Groups of 10 rats per sex and per group were killed for an interim evaluation at 15 months. Body weights of high-dose males were slightly lower than those of controls during the study. Probability of survival at 24 months was 75% for males and 80% for females and was not affected by exposure to nitrobenzene. Greater incidences were noted for hepatic

eosinophilic foci in mid- and high-dose males and in high-dose females, and for hepatocellular neoplasms in both treated males (adenomas and carcinomas: 1/69 in controls, 4/69 at the low dose, 5/70 at the mid dose, 16/70 at the high dose; $p < 0.05$, Cochran-Armitage trend test) and treated females (0/70 in controls, 2/66 at the low dose, 0/66 at the mid dose, 4/70 at the high dose; $p < 0.05$ trend test). Most of these tumours were benign. Thyroid follicular-cell hyperplasia occurred with a positive exposure-related trend in males and the incidences of thyroid follicular-cell adenomas and adenocarcinomas were greater in exposed males (2/69 in controls, 1/69 at the low dose, 5/70 at the mid dose, 8/70 at the high dose; $p < 0.05$ trend test). The incidence of endometrial stromal polyps was greater in exposed females (11/69 in controls, 17/65 at the low dose, 15/65 at the mid dose, 25/69 at the high dose; $p < 0.05$); that of renal tubular-cell adenomas was greater in exposed males (0/69 in controls, 0/68 at the low dose, 0/70 at the mid dose, 5/70 at the high dose; $p < 0.05$, Fisher's exact test) and one renal tubular-cell carcinoma occurred in another high-dose male. There was an increased severity of nephropathy in exposed males and females (Cattley et al., 1994).

Moreover, groups of 70 male Charles River CD rats, 62 days of age, were exposed by inhalation to air containing target concentrations of 0, 1, 5 or 25 ppm [0, 5, 25 or 125 mg/m^3] nitrobenzene (>99.8% pure) for 6 h per day on five days per week for 24 months. Groups of 10 rats per sex and per group were killed for an interim evaluation at 15 months. Body weights and survival were not affected by exposure to nitrobenzene during the study. The incidence of hepatocellular neoplasms was greater in treated groups (adenomas and carcinomas: 2/63 in controls, 1/67 at the low dose, 4/70 at the mid dose, 9/65 at the high dose; $p < 0.05$, Cochran-Armatage trend test). The incidence of spongiosis hepatis was greater in high-dose rats, and that of centrilobular hepatocytomegaly was greater in mid- and high-dose groups. The incidence of Kupffer-cell pigmentation was greater in all treated groups (Cattley et al., 1994, 1995).

Based on the bioassays reported by Cattley et al. (1994), IARC (1996) concluded that there was "sufficient evidence in experimental animals for the carcinogenicity of nitrobenzene". By contrast, the evidence in humans for the carcinogenicity of nitrobenzene was considered inadequate (IARC 1996).

Recommendation

The toxicology of nitrobenzene appears complex and it has an unusually high number of target organs of toxicity (including nose, spleen, liver, kidney, lung, erythrocytes). The formation of methaemoglobin in humans and experimental animals after inhalatory, oral or percutaneous exposure to nitrobenzene is well established. Methaemoglobinaemia is regarded as having a serious health effect in humans and in experimental animals. In the past, by analogy to tolerable COHb levels in persons exposed to carbon monoxide, a maximal methaemoglobin level of 5% has been considered tolerable (DFG 1995); a corresponding maximal haemoglobin adduct level has been evaluated as 100 µg aniline, released by acid hydrolysis from isolated haemoglobin, per litre of whole blood (DFG 1996). Nitrobenzene exposure has caused methaemoglobinaemia in animal inhalation studies at 5 ppm (Hamm 1984, Cattley et al. 1995) and in humans at 6 ppm (Pasceri et al. 1958). An air concentration of 1 ppm has mostly been regarded as a No-Observed-Adverse-Effect-Level with respect to methaemoglobin formation (Henderson et al. 1943, Salmova et al. 1963, ACGIH 1996). Accordingly, occupational limit values for nitrobenzene at 1 ppm had been set in most counties, based on a pre-existing evaluation of ACGIH (1996) in the United States.

In 1995, Cattley et al. reported on 2-year bioassays with inhalation exposures (6h/d; 5d/wk) to nitrobenzene in 2 strains of rats (F344, CD; 0, 1, 5, 25 ppm) and one strain of mice (B6C3F1; 0,5, 25, 50 ppm). There was carcinogenicity of nitrobenzene at multiple sites, and tumour rates were elevated at exposure concentrations starting at 5 ppm (v.s.). However, genotoxicity tests with nitrobenzene in vitro (Ames-Test; UDS with human hepatocytes in vitro) and in vivo (UDS, rat hepatocytes; SCE and chromosomal aberrations in lymphocytes of exposed rats) have been evaluated as negative (IARC 1996). This suggests that nitrobenzene is an experimental carcinogen, but with a non-genotoxic mechanism of action.

However, the toxicological mechanisms underlying the development of the experimental tumours are not completely understood. Except for the kidney, the tumours observed in nitrobenzene-exposed animals generally showed a notable background incidence in control animals. This adds support to the assumption that the increased tumour rates were mediated via non-genotoxic mechanisms. There is evidence that the kidney is a target organ for toxicity for nitrobenzene, causing degenerative renal changes in both mice and rats. It appears therefore likely that the one renal carcinoma observed in the high exposure group of male F344 rats arose against a background of chronic cytotoxicity. The increased incidences of thyroid tumours in mice and rats are thought to be an indirect consequence of liver hypertrophy, with consequent disturbances of thyroid hormone metabolism. This mechanism for rodent thyroid tumours is well understood and has little relevance to human health. Although there was a high incidence of alveolar-bronchiolar adenoma and carcinoma in nitrobenzene-exposed mice, there was also a high background incidence of these tumours in the control mice, and the mouse lung tumours appear to be species-specific.

On the basis of these findings and of the data by Cattley et al. (1995) showing experimental tumourogenicity of repetitive long-term inhalations with 5 ppm nitrobenzene, and showing minimal health effects such as pigment deposition in the nasal epithelium at 1 ppm, a health-based Occupational Exposure Limit should be located well below 1 ppm nitrobenzene.

On this background, an OEL (TWA) of 0.2 ppm (1 mg/m^3) is recommended for nitrobenzene. No STEL is set.

The potential of nitrobenzene for skin penetration is well established; exposure scenarios (BUA 1991) point to a ratio of 1/3 dermal absorption and 2/3 inhalation uptake at exposure conditions (airborne levels) of 1 ppm nitrobenzene which had previously been permissible in many countries. This demonstrates the need of classification of nitrobenzene as a skin penetrating compound ("skin notation").

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