

**WHO/HSE/WSH/09.01/4**  
**English only**

## **Nitrobenzene in Drinking-water**

Background document for development of  
WHO *Guidelines for Drinking-water Quality*

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Printed by the WHO Document Production Services, Geneva, Switzerland

## Preface

One of the primary goals of the World Health Organization (WHO) and its Member States is that “all people, whatever their stage of development and their social and economic conditions, have the right to have access to an adequate supply of safe drinking water”. A major WHO function to achieve such goals is the responsibility “to propose ... regulations, and to make recommendations with respect to international health matters ....”

The first WHO document dealing specifically with public drinking-water quality was published in 1958 as *International Standards for Drinking-water*. It was subsequently revised in 1963 and in 1971 under the same title. In 1984–1985, the first edition of the *WHO Guidelines for Drinking-water Quality* (GDWQ) was published in three volumes: Volume 1, Recommendations; Volume 2, Health criteria and other supporting information; and Volume 3, Surveillance and control of community supplies. Second editions of these volumes were published in 1993, 1996 and 1997, respectively. Addenda to Volumes 1 and 2 of the second edition were published in 1998, addressing selected chemicals. An addendum on microbiological aspects reviewing selected microorganisms was published in 2002. The third edition of the GDWQ was published in 2004, the first addendum to the third edition was published in 2006 and the second addendum to the third edition was published in 2008. The fourth edition will be published in 2011.

The GDWQ are subject to a rolling revision process. Through this process, microbial, chemical and radiological aspects of drinking-water are subject to periodic review, and documentation related to aspects of protection and control of public drinking-water quality is accordingly prepared and updated.

Since the first edition of the GDWQ, WHO has published information on health criteria and other supporting information to the GDWQ, describing the approaches used in deriving guideline values and presenting critical reviews and evaluations of the effects on human health of the substances or contaminants of potential health concern in drinking-water. In the first and second editions, these constituted Volume 2 of the GDWQ. Since publication of the third edition, they comprise a series of free-standing monographs, including this one.

For each chemical contaminant or substance considered, a lead institution prepared a background document evaluating the risks for human health from exposure to the particular chemical in drinking-water. Institutions from Canada, Japan, the United Kingdom and the United States of America (USA) prepared the documents for the fourth edition.

Under the oversight of a group of coordinators, each of whom was responsible for a group of chemicals considered in the GDWQ, the draft health criteria documents were submitted to a number of scientific institutions and selected experts for peer review. Comments were taken into consideration by the coordinators and authors. The draft documents were also released to the public domain for comment and submitted for final evaluation by expert meetings.

During the preparation of background documents and at expert meetings, careful consideration was given to information available in previous risk assessments carried out by the International Programme on Chemical Safety, in its Environmental Health Criteria monographs and Concise International Chemical Assessment Documents, the International Agency for Research on Cancer, the Joint FAO/WHO Meetings on Pesticide Residues and the Joint FAO/WHO Expert Committee on Food Additives (which evaluates contaminants such as lead, cadmium, nitrate and nitrite, in addition to food additives).

Further up-to-date information on the GDWQ and the process of their development is available on the WHO Internet site and in the current edition of the GDWQ.

## Acknowledgements

The first draft of Nitrobenzene in Drinking-water, Background document for development of WHO *Guidelines for Drinking-water Quality* (GDWQ), was prepared by Dr A. Hirose, National Institute of Health Sciences, Japan, to whom special thanks are due.

The work of the following working group coordinators was crucial in the development of this document and others contributing to the fourth edition:

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The draft text was discussed at the Expert Consultation for the fourth edition of the GDWQ, held on 19–23 June 2008. The final version of the document takes into consideration comments from both peer reviewers and the public. The input of those who provided comments and of participants at the meeting is gratefully acknowledged.

The WHO coordinators were Mr R. Bos and Mr B. Gordon, WHO Headquarters. Ms C. Vickers provided a liaison with the International Programme on Chemical Safety, WHO Headquarters. Mr M. Zaim, Public Health and the Environment Programme, WHO Headquarters, provided input on pesticides added to drinking-water for public health purposes.

Ms P. Ward provided invaluable administrative support at the Expert Consultation and throughout the review and publication process. Ms M. Sheffer of Ottawa, Canada, was responsible for the scientific editing of the document.

Many individuals from various countries contributed to the development of the GDWQ. The efforts of all who contributed to the preparation of this document and in particular those who provided peer or public domain review comments are greatly appreciated.

### **Acronyms and abbreviations used in the text**

BMDL <sub>10</sub>	lower limit on the benchmark dose for a 10% response
CIIT	Chemical Industry Institute of Toxicology
DNA	deoxyribonucleic acid
ECD	electron capture detector
LD <sub>50</sub>	median lethal dose
LOAEL	lowest-observed-adverse-effect level
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NTP	National Toxicology Program (USA)
OECD	Organisation for Economic Co-operation and Development
TDI	tolerable daily intake
USA	United States of America
UV	ultraviolet

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This background document is based largely on Environmental Health Criteria 230 on nitrobenzene (IPCS, 2003). The interested reader should refer to that monograph for additional detail and for primary references.

## **1. GENERAL DESCRIPTION**

### **1.1 Identity**

Chemical Abstracts Service Registry Number: 98-95-3  
Molecular formula:  $C_6H_5NO_2$

### **1.2 Physicochemical properties**

Nitrobenzene is a colourless to pale yellow oily liquid that presents a fire hazard. Its chemical and physical properties are shown in Table 1.

**Table 1: Physicochemical properties of nitrobenzene**

Property	Value
Specific gravity	1.2037 at 20 °C
	1.205 at 28 °C
Melting point	5.7 °C
Boiling point	211 °C
Vapour pressure	20 Pa at 20 °C
	38 Pa at 25 °C
	47 Pa at 30 °C
Water solubility	1900 mg/l at 20 °C
	2090 mg/l at 25 °C
Log octanol–water partition coefficient ( $\log K_{ow}$ )	1.85 (1.6–2.0)

Nitrobenzene's odour resembles that of bitter almonds or "shoe polish", with reported odour thresholds of 0.092 mg/m<sup>3</sup> (Amoore & Hautala, 1983) and 0.03 mg/m<sup>3</sup> (Manufacturing Chemists Association, 1968). Its odour threshold in water has been reported as 0.11 mg/l (Amoore & Hautala, 1983) and 0.03 mg/l (USEPA, 1980).

### **1.3 Major uses and sources in drinking-water**

Nitrobenzene is used primarily in the production of aniline, but it is also used as a solvent, as an ingredient of metal polishes and soaps, and in the synthesis of other organic compounds, including acetaminophen. The aniline and other substituted nitrobenzenes produced from nitrobenzene are used primarily for the manufacture of various plastic monomers and polymers (50%) and rubber chemicals (27%). Nitrobenzene can be released to water during these production processes.

Past minor applications of nitrobenzene included uses as a flavouring agent, as a solvent in marking inks and in metal, furniture, floor and shoe polishes, as a perfume, including in perfumed soaps, as a dye intermediate, as a deodorant and disinfectant, in

leather dressing and for refining lubricating oils. It is not known whether nitrobenzene is still used in some countries as a solvent in some consumer products.

#### ***1.4 Environmental fate***

The physical properties of nitrobenzene suggest that transfer from water to air will be significant, although not rapid. Actual data on the evaporation of nitrobenzene from water bodies appear to be somewhat conflicting. A computer model predicted a volatilization half-life of 12–68 days. The shortest estimate cited in the literature was 1 day. In another study of experimental microcosms simulating land application of wastewater, nitrobenzene was reported to be not volatilized, but totally degraded. Owing to its moderate water solubility and relatively low vapour pressure, it might be expected that nitrobenzene would be washed out of the atmosphere by rain to some extent; however, in field experiments, it appeared that washout by rainfall and dryfall of particulates were negligible. Because of nitrobenzene's vapour density, removal processes from the atmosphere may include vapour settling.

Nitrobenzene can undergo degradation by both photolysis and microbial biodegradation. Photodegradation of nitrobenzene in air and water is slow. In water bodies, direct photolysis appears to be the degradation pathway that proceeds most rapidly (half-life: 2.5–6 days). Degradation studies suggest that nitrobenzene is degraded in sewage treatment plants by aerobic processes, with slower degradation under anaerobic conditions. Degradation was generally found to be increased with acclimation of the microbial population and in the presence of other easily degradable substrates. Adaptation of the microflora and the presence of additional substrates also seem to be limiting factors in the decomposition of nitrobenzene in soil.

The measured bioconcentration factors for nitrobenzene in a number of organisms indicate minimal potential for bioaccumulation, and nitrobenzene is not biomagnified through the food-chain.

## ***2. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE<sup>1</sup>***

Concentrations of nitrobenzene in environmental samples, such as surface water, groundwater and air, are generally low. Based on limited data, it appears that the potential for contamination is greater for groundwater than for surface water.

### ***2.1 Surface water***

Nitrobenzene was not detected in any surface water samples collected near 862 hazardous waste sites in the United States of America (USA), according to the Contract Laboratory Program Statistical Database (CLPSD, 1988). Nitrobenzene was not detected (detection limit 4 µg/l) in the Potomac River, USA (Hall et al., 1987). Detailed surveys of Japanese surface waters were undertaken in 1977 and 1991. In the 1977 survey, nitrobenzene was detected in 22 of 115 samples at a level of 0.13–3.8 µg/l (detection limit 0.1–30 µg/l). In the 1991 survey, nitrobenzene was detected in 1 of 153 surface water samples, at a level of 0.17 µg/l (detection limit 0.15 µg/l). The

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<sup>1</sup> Much of the following text was taken from IPCS (2003). No other substantive data were identified in the literature.

samples were taken from both industrialized and rural areas (Kubota, 1979; Environment Agency Japan, 1992).

Staples, Werner & Hoogheem (1985) reported that of the 836 determinations of nitrobenzene in ambient surface water contained in the USA's STORET database, nitrobenzene was detected in 0.4% of the samples, with a median level of <10 µg/l. In a year-long survey of water from two reservoirs near Calgary, Canada, nitrobenzene was not detected in any of the samples taken (detection limit 0.1 µg/l) (Hargesheimer & Lewis, 1987).

Available data indicate that generally low levels (around 0.1–1 µg/l) of nitrobenzene have been measured in surface waters. One of the highest levels reported was 67 µg/l in the river Danube in Europe (Hain et al., 1990). Many of the rivers sampled for nitrobenzene are known to suffer from industrial pollution and so may not represent the general situation. After a temporary failure in an industrial wastewater treatment plant at BASF Aktiengesellschaft in May 1984, a peak nitrobenzene concentration of 25 µg/l was measured in the river Rhine in Germany (BUA, 1994).

## ***2.2 Groundwater***

Nitrobenzene was detected in groundwater at 3 of 862 hazardous waste sites in the USA at a geometric mean concentration of 1400 µg/l, according to the Contract Laboratory Program Statistical Database (CLPSD, 1988). Nitrobenzene was not detected (<1.13 µg/l) in groundwater at an explosives manufacturing site in the USA. The aquifer at the site was known to be contaminated with residues of explosives (Dennis et al., 1990; Wujcik et al., 1992).

Nitrobenzene was detected at a level of 210–250 µg/l in groundwater from Gibbstown, USA (Rosen et al., 1992). No nitrobenzene was detected (minimum detection limit 0.67 µg/l) in three groundwater sources of domestic water in the Mexico City region (Downs, Cifuentes-García & Suffet, 1999).

## ***2.3 Drinking-water***

Nitrobenzene was detected in 1 of 14 samples of treated water in the United Kingdom. The positive sample was water derived from an upland reservoir (Fielding et al., 1981). In a survey of 30 Canadian potable water treatment facilities, nitrobenzene was not detected in either raw or treated water (detection limit 5 µg/l) (Otson, Williams & Bothwell, 1982). Kopfler et al. (1977) listed nitrobenzene as one of the chemicals found in finished tap water in the USA, but did not report its concentrations or locations. According to BUA (1994), the nitrobenzene content in potable water following passage through the soil was 0.1 µg/l (mean), with a maximum value of 0.7 µg/l in 50 samples taken from the river Lek at Hagestein, Netherlands, in 1986.

## ***2.4 Human exposure***

The general population can be exposed to variable concentrations of nitrobenzene in air and possibly drinking-water. Nitrobenzene in drinking-water has been reported in studies conducted in the 1970s and 1980s in the USA and United Kingdom, albeit in only a small proportion of samples, but it was not detected in 30 Canadian samples

(1982 report). There is also potential exposure from consumer products, but accurate information is lacking. Based on air studies and on estimates of release during manufacture, only populations in the vicinity of manufacturing activities and petroleum refining plants are likely to have any significant exposure to nitrobenzene; however, people living in and around abandoned hazardous waste sites may also have potential for higher exposure, due to possible groundwater and soil contamination and uptake of nitrobenzene by plants.

### ***3. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS***

Although no studies have been performed on the extent of uptake of nitrobenzene by humans after oral exposure, oral absorption would appear to be rapid and extensive, based on the very large number of clinical reports of poisonings (IPCS, 2003). Some of these reports showed that metabolites identified in the urine were *p*-aminophenol and *p*-nitrophenol (Von Oettingen, 1941; Ikeda & Kita, 1964; Myślak, Piotrowski & Musialowicz, 1971). In one volunteer given nitrobenzene orally, the half-lives of elimination for *p*-nitrophenol were estimated to be about 5 h (initial phase) and >20 h (late phase) (Piotrowski, 1967).

In rats and rabbits, the major part of orally administered nitrobenzene (about 80% of the dose) was metabolized and eliminated within 8 days at the most (Parke, 1956; Freitag et al., 1982; Rickert et al., 1983; Albrecht & Neumann, 1985). The major route of excretion was urine. Oral doses appeared to be less well absorbed in mice than in rats and rabbits; about 35% was excreted in urine of mice within 3 days (compared with 57–63% in rats). In rats and mice, urinary metabolites were free and conjugated forms of *p*-hydroxyacetanilide, *p*- and *m*-nitrophenol and, in mice only, *p*-aminophenol (Rickert et al., 1983). Metabolic studies using caecal contents of rats showed that nitrobenzene was sequentially reduced to nitrosobenzene, hydroxylaminobenzene and, ultimately, aniline; this anaerobic metabolism occurred 150 times faster than reduction by the hepatic microsomal fraction (Levin & Dent, 1982). Nitrobenzene activation in rats to methaemoglobin-forming metabolites appears to be mediated to a significant degree by intestinal microflora (Reddy, Pohl & Krishna, 1976; Goldstein, Chism & Hamm, 1984), and this may explain species differences in the formation of methaemoglobinaemia. However, further studies are required to allow this information to be used in extrapolating nitrobenzene toxicity between species.

### ***4. EFFECTS ON EXPERIMENTAL ANIMALS AND IN VITRO TEST SYSTEMS***

#### ***4.1 Acute exposure***

The oral median lethal dose (LD<sub>50</sub>) in rats was reported to be 600–640 mg/kg body weight (Sziza & Magos, 1959; Smyth et al., 1969); around this dose, methaemoglobinaemia and histopathological changes in the brain as well as clinical signs of toxicity such as lethargy and ataxia were noted (Sziza & Magos, 1959; Morgan et al., 1985). At lower doses, histopathological changes were found in the liver (at >110 mg/kg body weight) and testes (at >300 mg/kg body weight) (Bond et al., 1981). A minimal fatal dose of 750–1000 mg/kg body weight in dogs by the oral route was stated (Von Oettingen, 1941). For dermal exposure, it was reported that the

LD<sub>50</sub>s were approximately 2100 mg/kg body weight in rats (Sziza & Magos, 1959) and 760 mg/kg body weight in rabbits (Harton & Rawl, 1976), and the minimal fatal dermal dose was about 480 mg/kg body weight in mice (Shimkin, 1939).

#### **4.2 Short-term exposure**

##### **4.2.1 Oral**

A 28-day repeated-dose gavage study was performed in Fischer 344 rats at doses of 0, 5, 25 and 125 mg/kg body weight per day (Shimo et al., 1994). An additional two groups of animals exposed to 0 or 125 mg/kg body weight per day were kept for a 2-week recovery period. In the 125 mg/kg body weight per day group, one of six females died on day 27; decreased movement, pale skin, gait abnormalities and decreases in body weights, body weight gains and food consumption were seen. Haematological examination revealed decreases in red blood cells, haemoglobin and haematocrit and increased white blood cells in the 25 and 125 mg/kg body weight per day groups. In an examination of blood biochemistry, increases in total cholesterol were seen at 5 mg/kg body weight per day and above, and increases in albumin were observed at 25 mg/kg body weight per day and higher. In the 125 mg/kg body weight per day group, increases in total protein, albumin/globulin ratio, alanine aminotransferase and alkaline phosphatase were also observed. At necropsy, increases in the relative weight of the liver at 5 mg/kg body weight per day and above, of the spleen at 25 mg/kg body weight per day and above and of the brain and kidneys at 125 mg/kg body weight per day and decreases in the relative weights of the testes and thymus at 125 mg/kg body weight per day were found. Histopathological changes secondary to haemolytic anaemia were observed in the cerebellum, liver, kidneys, spleen and bone marrow. Congestion, increased brown pigmentation in red pulp and increased extramedullary haematopoiesis in the spleen (control: 0/6; low dose: 3/6, moderate; medium dose: 6/6, moderate; high dose: 6/6, severe; the incidence and severity in both males and females were the same) and increased haematopoiesis of the bone marrow in females (control: 0/6; low dose: 1/6, moderate; medium dose: 3/6, moderate; high dose: 5/5, severe) were detected, even at the lowest dose of 5 mg/kg body weight per day. In addition, spongiotic changes of the cerebellum, epithelial degeneration and atrophy of the seminiferous tubules in the testes, and reduction of spermatozoa in the epididymides were observed at 125 mg/kg body weight per day. Most of the above findings disappeared or tended to decrease during the recovery period, but brown pigmentation in the liver and kidneys and reduction of spermatozoa in the epididymis recovered only slightly. The lowest-observed-adverse-effect level (LOAEL) was established to be 5 mg/kg body weight per day.

In a range-finding United States National Toxicology Program (NTP) study, nitrobenzene was administered to B6C3F1 mice and Fischer 344 rats by gavage at doses in the range 37.5–600 mg/kg body weight per day for 14 days (NTP, 1983). All rats and mice at 600 mg/kg body weight per day and all rats at 300 mg/kg body weight per day died or were sacrificed in a moribund condition prior to the end of treatment. Treated animals were inactive, ataxic, prostrate, cyanotic and dyspnoeic. Significant depression of weight gain was seen in male mice at 37.5 mg/kg body weight per day and in mice of both sexes at 75 mg/kg body weight per day. Haematologically, reticulocyte counts were increased in mice at 75 mg/kg body weight per day, whereas methaemoglobin levels were increased in mice in all dose

groups except the 75 mg/kg body weight per day males and the 37.5 mg/kg body weight per day females. Treated rats showed increases in methaemoglobin and in reticulocyte counts. Histologically, mice and rats showed changes in the brain, liver, lung, kidney and spleen. A no-observed-adverse-effect level (NOAEL) and LOAEL could not be established because detailed data were not available (IPCS, 2003).

In another NTP study, nitrobenzene was administered by gavage to B6C3F1 mice at 0, 18.75, 37.5, 75, 150 or 300 mg/kg body weight per day and to Fischer 344 rats at 0, 9.375, 18.75, 37.5, 75 or 150 mg/kg body weight per day for 13 weeks (NTP, 1983). In mice, three high-dose males died or were sacrificed moribund in weeks 4 and 5. Clinical signs included ataxia, lethargy, dyspnoea, convulsions, irritability and rapid head-bobbing movements. Haematological examination revealed increases in methaemoglobin and reticulocyte counts at 18.75 mg/kg body weight per day and above, with decreases in haemoglobin at 75 mg/kg body weight per day and above and in haematocrit and red blood cells at 150 mg/kg body weight per day and above. Male mice exhibited leukopenia at 18.75 and 150 mg/kg body weight per day and leukocytosis at 300 mg/kg body weight per day. Similarly, lymphopenia was seen in all treated males except at 300 mg/kg body weight per day, at which lymphocytosis was seen. High-dose females exhibited neutrophilia and lymphocytosis. Liver weight in treated mice was increased at 150 mg/kg body weight per day and above in males and at 18.75 mg/kg body weight per day and above in females. On histopathology, liver and spleen haematopoiesis and splenic haemosiderin accumulation at 75 mg/kg body weight per day and above and lymphoid depletion at 150 mg/kg body weight per day and above were observed. In adrenal glands, fatty change was found in the X-zone in high-dose female mice. Testicular atrophy was observed at 18.75, 37.5, 150 and 300 mg/kg body weight per day. One high-dose male had acute necrosis in the area of the vestibular nucleus in the brain.

In high-dose rats, seven males and one female died, and two males and two females were sacrificed moribund during weeks 6–13. Clinical signs included ataxia, left head tilt, lethargy, trembling, circling and dyspnoea, as well as cyanosis of the extremities at 75 mg/kg body weight per day and above. In haematological examination, there were dose-related increases in methaemoglobin, reticulocyte counts, polychromasia and anisocytosis, along with decreases in haemoglobin, haematocrit and red blood cells. In the surviving high-dose animals, there was marked leukocytosis, with lymphocytosis and neutrophilia. Histopathological changes such as pigmentation and/or increased haematopoiesis, which were considered to be secondary to haemolytic anaemia, were observed in the spleen, liver, kidneys and bone marrow. In the spleen, thickening and fibrosis of the splenic capsule were noted at 9.375 mg/kg body weight per day and above, with occasional mast cells, haemosiderin-filled macrophages and fragmented necrotic cells, and hypertrophied and/or hyperplastic mesothelial cells. Brain lesions, characterized by demyelination, loss of neurons, varying degrees of gliosis, haemorrhage and occasional neutrophil infiltration, were also found at 150 mg/kg body weight per day. In addition, the testes were mildly to markedly atrophic at 75 mg/kg body weight per day and above, with varying degrees of hypospermatogenesis and multinucleated giant cell formation. The LOAEL was established to be 18.75 mg/kg body weight per day for mice and 9.375 mg/kg body weight per day for rats (IPCS, 2003).

In accordance with the Organisation for Economic Co-operation and Development (OECD) reproductive toxicology protocol, nitrobenzene was given by gavage to Sprague-Dawley rats at 0, 20, 60 or 100 mg/kg body weight per day throughout pre-mating, mating, gestation and lactation (Mitsumori et al., 1994). Male rats were examined for haematology, blood biochemistry, macroscopic findings, organ weights and histopathology at the completion of the dosing period (41–42 days). At 100 mg/kg body weight per day, animals exhibited clinical signs of toxicity, such as piloerection, salivation, emaciation, torticollis, circling movement and abnormal gait, with deaths of 2 of 10 males and 9 of 10 females. Torticollis and abnormal gait were also observed in 1 of 10 females at 60 mg/kg body weight per day, and one female each from the 20 and 60 mg/kg body weight per day groups died. Depression of body weight gain was found in both sexes of the 100 mg/kg body weight per day group and in females of the 60 mg/kg body weight per day group. Haematological examination revealed decreases in red blood cells, haemoglobin and haematocrit and increases in methaemoglobin at 20 mg/kg body weight per day and above, with elevation in erythroblasts and reticulocyte counts at 60 mg/kg body weight per day and above. Increased white blood cells were also noted at 100 mg/kg body weight per day. On blood biochemical examination, a dose-related increase in total bilirubin was evident. Increased albumin and total protein at 60 mg/kg body weight per day and above and in albumin/globulin ratio and total cholesterol at 100 mg/kg body weight per day were also found. At necropsy, increased liver and spleen weights at 20 mg/kg body weight per day and above and kidney weights at 60 mg/kg body weight per day and above and decreases in the testis and epididymis weights at 60 mg/kg body weight per day and above were noted. Histopathologically, centrilobular swelling of hepatocytes was observed in the liver at 20 mg/kg body weight per day and above. At the same doses, atrophy of the seminiferous tubules was found in the testes, with Leydig cell hyperplasia at 60 mg/kg body weight per day and above. In the epididymides, decreased numbers of cells with round nuclei per seminiferous tubule and loss of intraluminal sperm were detected. Neuronal necrosis and gliosis were observed in certain nuclei in the cerebellar medulla and pons at 60 mg/kg body weight per day and above. In addition, various changes secondary to haemolytic anaemia were observed in the liver, spleen, bone marrow and kidneys at 20 mg/kg body weight per day and above. The LOAEL was established to be 20 mg/kg body weight per day.

#### *4.2.2 Inhalation*

Fischer 344 rats, Sprague-Dawley (CD) rats and B6C3F1 mice were exposed to nitrobenzene at approximately 0, 51, 180 or 640 mg/m<sup>3</sup> via inhalation for 6 h/day, 5 days/week, for 2 weeks in a Chemical Industry Institute of Toxicology (CIIT) study (Medinsky & Irons, 1985). At 640 mg/m<sup>3</sup>, there were severe clinical signs and a 40% rate of lethality in CD rats and morbidity of all B6C3F1 mice, necessitating their early sacrifice; surviving CD rats, exhibiting rapid shallow breathing, wheezing and orange urogenital staining, were sacrificed at the end of the first week. In haematological examination, increases in methaemoglobin were observed at 51 mg/m<sup>3</sup> and above in CD and Fischer 344 rats, with a dose-related reduction in red blood cells, which was reversible after 14 days of recovery. Methaemoglobinaemia was also noted in mice. A marked elevation in circulating white blood cells was noted in male CD rats at 180 and 640 mg/m<sup>3</sup>. Concentration-dependent increases in relative liver, spleen and kidney weights were reported, primarily in Fischer 344 rats. A very prominent decrease in relative testicular weights was also evident in Fischer 344 rats at 640

mg/m<sup>3</sup>. On histopathology, hepatic changes (e.g. necrosis, hydropic degeneration and basophilic degeneration) were observed at 640 mg/m<sup>3</sup> in B6C3F1 mice and at 180 mg/m<sup>3</sup> and above in CD rats. In the kidneys, multifocal degenerative changes in tubular epithelium in male B6C3F1 mice at 180 mg/m<sup>3</sup>, hydropic degeneration of the cortical tubular cells in CD rats at 640 mg/m<sup>3</sup> and hyaline nephrosis in Fischer 344 rats at 640 mg/m<sup>3</sup> were found. Splenic lesions, including haemosiderosis, extramedullary haematopoiesis and sinusoidal congestion, were reported in all treated animals. A capsular hyperplastic lesion was seen in Fischer 344 rats at 180 and 640 mg/m<sup>3</sup>. In mice, a concentration-dependent increase in marginal-zone macrophages and a lymphoid hypoplasia in periarteriolar sheaths were observed. In the testes, increased multinucleated giant cells, Sertoli cell hyperplasia and dysspermiogenesis were detected in Fischer 344 rats at 640 mg/m<sup>3</sup>, with maturation arrested at the level of primary and secondary spermatocytes; this finding was still evident after 2 weeks of recovery. Dysspermiogenesis was also seen in CD rats at 640 mg/m<sup>3</sup>. The testes of mice exposed at 640 mg/m<sup>3</sup> showed a different lesion, with acute testicular degeneration, an absence of spermatozoa in seminiferous tubules and the epididymis, and degeneration of tubular epithelial cells. In mice and CD rats sacrificed during the exposure period, bilateral perivascular haemorrhage in the cerebellar peduncle was also observed. A NOAEL and LOAEL could not be established because detailed data were not available. No clear no-observed-effect level (NOEL) was established as, in addition to testicular and haematological effects, increased relative kidney weights were observed in male Fischer 344 rats at the lowest dose studied (IPCS, 2003).

Fischer 344 rats, Sprague-Dawley (CD) rats and B6C3F1 mice were exposed to nitrobenzene vapour concentrations of 0, 26, 82 and 260 mg/m<sup>3</sup> for 6 h/day, 5 days/week, for 90 days in a CIIT study (Hamm, 1984; Hamm et al., 1984). There was no effect on body weight gain or mortality. In haematological examination, methaemoglobinaemia and haemolysis were observed in mice and rats at 260 mg/m<sup>3</sup> and in both rat strains at 82 mg/m<sup>3</sup>. Spleen and liver weights were increased at 260 mg/m<sup>3</sup> in mice and at 82 mg/m<sup>3</sup> and above in rats. Increased kidney weights in male CD rats and decreased testicular weights in both rat strains were also found at 260 mg/m<sup>3</sup>. On histopathology, hepatocyte hyperplasia and multinucleated hepatocytes were reported in B6C3F1 mice at 82 mg/m<sup>3</sup>. CD rats primarily showed centrilobular hepatocyte hypertrophy, with some cells containing enlarged nucleoli (82 and 260 mg/m<sup>3</sup>), increased cytoplasmic basophilia in periportal hepatocytes and microgranulomas (all exposure levels). Fischer 344 rats exhibited centrilobular necrosis and disorganization of hepatic cords, primarily but not exclusively in animals exposed to 260 mg/m<sup>3</sup>. In the kidneys, dose-related renal toxic nephrosis (an accumulation of hyaline or eosinophilic droplets in the cytoplasm of proximal tubular epithelial cells) was observed in male rats of both strains (not significant at 26 mg/m<sup>3</sup> in CD rats), but not in mice. In addition, bilateral degeneration of the seminiferous epithelium and a reduction or absence of sperm in the epididymides were observed at 260 mg/m<sup>3</sup> in rats, and marginal effects were also noted at 82 mg/m<sup>3</sup> in CD rats. Furthermore, female mice had a dose-related adrenal lesion (prominent cellular vacuolation in the zona reticularis contiguous with the medulla), which was apparent at the lowest concentration. A NOAEL and LOAEL could not be established because detailed data were not available. No clear NOEL was established in the 90-day inhalation study, as female mice at the lowest dose showed cellular vacuolation of the adrenal gland and haematological effects (Hamm, 1984).



### **4.3 Long-term exposure**

No long-term oral administration study has been reported.

In inhalation studies, male and female B6C3F1 mice were exposed to nitrobenzene at 0, 25, 127 or 251 mg/m<sup>3</sup> for 6 h/day, 5 days/week, for 2 years. Survival was not adversely affected by nitrobenzene. Body weight depression was consistently observed from 16 weeks until the final euthanization in males exposed to 251 mg/m<sup>3</sup>. Haematological examination at the end of the exposure period revealed a decrease in red blood cells and haematocrit in females at 25 and 127 mg/m<sup>3</sup> and in males at 251 mg/m<sup>3</sup>; a decrease in haemoglobin in females at 25 and 127 mg/m<sup>3</sup>; and an increase in methaemoglobin levels in females at 127 mg/m<sup>3</sup> and in both sexes at 251 mg/m<sup>3</sup> (Cattley et al., 1994; Holder, 1998).

Male and female Fischer 344 rats were exposed to nitrobenzene at 0, 5, 25 or 127 mg/m<sup>3</sup> for 6 h/day, 5 days/week, for 2 years. Survival was not adversely affected by nitrobenzene. Body weight depression was consistently observed from 16 weeks until the final euthanization of males exposed to 127 mg/m<sup>3</sup>. In haematological examination both at the interim (at the end of 15 months) and final euthanization, decreased red blood cells, haematocrit and haemoglobin and increased methaemoglobin was found in both sexes at 127 mg/m<sup>3</sup>. Exposure to 127 mg/m<sup>3</sup> also increased the frequency of observation of Howell-Jolly bodies and polychromasia in blood smears (Cattley et al., 1994; Holder, 1998).

Male CD rats were exposed to nitrobenzene at 0, 5, 25 or 127 mg/m<sup>3</sup> for 6 h/day, 5 days/week, for 2 years. Survival and body weight were not adversely affected by nitrobenzene. Haematological examination at the interim euthanization (at the end of 15 months) revealed decreased red blood cells at 127 mg/m<sup>3</sup> and increased methaemoglobin at 5 mg/m<sup>3</sup> and above. At the completion of the 24-month exposure period, increased methaemoglobin was found only at 127 mg/m<sup>3</sup>. Exposure to 127 mg/m<sup>3</sup> also increased the frequency of observation of Howell-Jolly bodies and polychromasia in blood smears (Cattley et al., 1994; Holder, 1998).

### **4.4 Reproductive/developmental toxicity**

In the above-mentioned (section 4.2.1) reproductive toxicity study by Mitsumori et al. (1994) (administration by gavage at 0, 20, 60 or 100 mg/kg body weight per day from 14 days before mating to day 4 of lactation), changes in organ weights and the histopathology of male reproductive organs were observed at 60 mg/kg body weight per day and above, but there were no evident effects on copulation, fertility or implantation. Although the survival index (number of pregnant females surviving/number of pregnant females examined; seven females died during the gestation period) was decreased at 100 mg/kg body weight per day, there was no abnormality in the gestation period or delivery conditions in the remaining treated females. On day 0 of lactation, the number of pups alive and the live birth index in the 100 mg/kg body weight per day group and body weights of male and female pups in the 60 and 100 mg/kg body weight per day groups were decreased. No pups were alive on day 4 of lactation in the 100 mg/kg body weight per day group, because the remaining two dams died on days 1 and 3 of lactation, and the viability index at this time was decreased in the 60 mg/kg body weight per day group. The body weights of

male pups at 20 mg/kg body weight per day and above and of female pups at 60 mg/kg body weight per day were decreased on day 4 of lactation. The lack of effect of nitrobenzene on male fertility in this study may be due to the short pre-mating dosing interval and to the fact that rats produce sperm in very large abundance (IPCS, 2003). In fact, Kawashima et al. (1995) reported a decrease in the fertility index after a 21-day administration of nitrobenzene at 60 mg/kg body weight per day to male rats. The LOAEL for reproductive/developmental toxicity was established to be 20 mg/kg body weight per day.

### ***4.5 Genotoxicity***

A number of reverse mutation tests using *Salmonella typhimurium* did not find any significant mutagenic activity with nitrobenzene, either in the presence or absence of S9 mix (Garner & Nutman, 1977; Anderson & Styles, 1978; Chiu et al., 1978; Purchase et al., 1978; Ho, Clark & Guerin, 1981; Haworth et al., 1983; Shimizu, Yasui & Matsumoto, 1983; Suzuki, Koyama & Suzuki, 1983; Hughes, Sparacino & Frazier, 1984; Nohmi et al., 1984; Vance & Levin, 1984; Kawai et al., 1987; Dellarco & Prival, 1989), whereas co-mutagenicity with the co-mutagen norharman was observed (Suzuki, Koyama & Suzuki, 1983). On the other hand, some results suggested mutagenic effects of nitrobenzene: induction of 8-azaguanine resistance with and without metabolic activation and ouabain resistance with metabolic activation in cultured Chinese hamster lung (V79) cells (Kuroda 1986) and a weak increase of sex-linked recessive lethal mutations in *Drosophila* (Rapoport, 1965). In view of the paucity of details, this work provides little meaningful information (IPCS, 2003).

Huang et al. (1996) reported that nitrobenzene caused chromosomal aberrations in cultured human lymphocytes in vitro. Unfortunately, the results were reported only as positive or negative, no data were provided on cytotoxicity, and the dose of nitrobenzene at which positive genotoxicity was reported was 5 times higher than the recommended upper test concentration for relatively non-cytotoxic compounds (OECD Test Guideline 473, adopted 21 July 1997) (IPCS, 2003). On the other hand, a negative result was reported for a clastogenic effect on human sperm chromosomes in vitro (Tateno et al., 1997). In an in vivo study, no effects on chromosomal aberrations or on sister chromatid exchange frequency were observed in cultures of blood and isolated spleen lymphocytes prepared from rats exposed to nitrobenzene via inhalation (26–260 mg/m<sup>3</sup> for 6 h/day, 5 days/week, over a 29-day period [21-day exposure]) (Kligerman et al., 1983). In this study, however, a significant inhibitory effect on the mitotic activity and cell cycle progression of concanavalin A-stimulated peripheral blood lymphocytes was noted (Kligerman et al., 1983).

Although no increase in micronucleated polychromatic erythrocytes was reported in the bone marrow of B6C3F1 mice given a single intraperitoneal injection of nitrobenzene (62.5–250 mg/kg body weight) (BASF, Bayer & Zeneca, 1995), recent studies have shown chromosomal effects of nitrobenzene. For the induction of micronuclei, nitrobenzene exhibited a weak, but definitely positive, test result in V79 cells at remarkably low concentrations (minimal effects concentration: 0.01 µmol/l) (Bonacker et al., 2004). Nitrobenzene induced mostly kinetochor-positive micronuclei, thus characterizing the chromosomal effects as aneugenic. In cell-free assays, a slight effect on tubulin assembly was observed and irregular cluster

formations were found in electron microscopic examination. The microtubule gliding assay showed that nitrobenzene affected the gliding velocity. In addition, the deoxyribonucleic acid (DNA) fragmentation/comet assay and the micronucleus assay on primary cultures of rat and human kidney showed positive results (Robbiano et al., 2004). Increased frequency of DNA breaks and/or alkali-labile sites and micronuclei were also found in the kidneys of rats treated with a single oral dose corresponding to one half the LD<sub>50</sub> (300 mg/kg body weight). Similarly, increases in the frequency of DNA single-strand breaks and alkali-labile sites, as measured by the same comet assay, were obtained in primary cultures of human thyroid cells (Mattioli et al., 2006). Under the same experimental conditions, DNA repair synthesis, as evaluated by quantitative autoradiography, was also found. Consistently, a significant degree of DNA fragmentation was induced in the thyroid, liver and kidneys of rats orally administered nitrobenzene (310 mg/kg body weight).

Nitrobenzene was negative for the induction of unscheduled DNA repair in hepatocytes prepared from different human livers and in rat liver primary hepatocyte cultures (Butterworth et al., 1989). There was also no evidence of any increase in unscheduled DNA synthesis in primary cultures of hepatocytes prepared from rats gavaged with nitrobenzene at 200 or 500 mg/kg body weight (Mirsalis, Tyson & Butterworth, 1982).

Radioactivity was observed in DNA isolated from rat liver and kidney and mouse liver and lung samples collected 24 h after a single subcutaneous injection of <sup>14</sup>C-labelled nitrobenzene at 4 mg/kg body weight (BASF, 1997). Radioactivity, expressed as covalent binding indices, was at the upper end of the range of values typically found with weak genotoxic carcinogens. Recently, the binding of nitrobenzene to hepatic DNA was also shown in mice given lower dose levels of <sup>14</sup>C-labelled nitrobenzene (0.1–10 000 µg/kg body weight) by intraperitoneal injection (Li et al., 2003).

Nitrobenzene did not cause cell transformation in *Bacillus subtilis* (Nohmi et al., 1984), baby Syrian hamster kidney cells (BHK-21 C13) or human diploid lung fibroblasts (WI-38) (Butterworth et al., 1989).

There is some evidence of the genetic activity of some putative metabolites of nitrobenzene, although most data are negative. Ohkuma & Kawanishi (1999) investigated the mechanism of DNA damage induced by nitrosobenzene (in calf thymus DNA in vitro). The authors reported that nitrosobenzene can be reduced non-enzymatically by reduced nicotinamide adenine dinucleotide, and the redox cycle reaction resulted in oxidative DNA damage due to the copper–oxygen complex, derived from the reaction of copper(I) with hydrogen peroxide. Aniline, a reductive metabolite of nitrobenzene, induced gene mutations in V79 cells (Kuroda, 1986) and mouse L5178Y cells (Amacher et al., 1980; Caspary et al., 1988; Wangenheim & Bolcsfoldi, 1988), sister chromatid exchanges (Abe & Sasaki, 1977; Cunningham & Ringrose, 1983; Galloway et al., 1987) and chromosomal aberrations (Galloway et al., 1987) in Chinese hamster cells in vitro and morphological transformation of BALB/c 3T3 cells (Dunkel et al., 1981). It also induced DNA damage in the liver and kidney of rats and sister chromatid exchanges in the bone marrow of mice (Parodi et al., 1981, 1982). Aniline (tested at up to 1 mmol/l in six separate assays) was negative for unscheduled DNA repair in primary cultures of rat and human hepatocytes

(Butterworth et al., 1989). The draft European Union risk assessment report on aniline (EU, 2001) provides further evidence of the genotoxicity of aniline. *p*-Aminophenol induced gene mutations in *Salmonella typhimurium* strain TA1535 and was also reported to be positive in the mouse in vivo micronucleus test (Wild et al., 1980). *p*-Nitrophenol caused DNA damage in *Bacillus subtilis* (Shimizu & Yano, 1986) and *Proteus mirabilis* (Adler et al., 1976). *p*-Nitrosophenol was weakly mutagenic to *Salmonella typhimurium* strain TA1538 (Gilbert et al., 1980).

### **4.6 Carcinogenicity**

The potential carcinogenicity of nitrobenzene administered by the oral route has not been studied.

The potential carcinogenicity of inhaled nitrobenzene was evaluated following a 2-year exposure period in B6C3F1 mice, Fischer 344 rats and Sprague-Dawley (CD) rats (Cattley et al., 1994; Holder, 1998).

Male and female B6C3F1 mice were exposed to 0, 25, 127 or 251 mg/m<sup>3</sup> for 6 h/day, 5 days/week, for 2 years. Increased incidences of alveolar and bronchial tumours (adenoma or carcinoma) in the lungs of males at 25, 127 and 251 mg/m<sup>3</sup> (21/67, 21/65 and 23/66, respectively, compared with 9/68 in the control group), follicular cell adenoma in the thyroids of males at 251 mg/m<sup>3</sup> (7/64, compared with 0/65 in the control group) and adenocarcinoma in the mammary gland of females at 251 mg/m<sup>3</sup> (5/60, compared with 0/48 in the control group) were found. In addition, non-neoplastic lesions were observed in the lung, thyroid, liver, nose, epididymis, bone marrow, thymus, kidney and pancreas. The incidences of bronchiolization of alveolar walls in the lungs, centrilobular hepatocytomegaly and multinucleated hepatocytes in the liver, and pigment deposition and degeneration/loss of olfactory epithelium in the nose were increased even at the lowest concentration of 25 mg/m<sup>3</sup>. The LOAEL was established to be 25 mg/m<sup>3</sup>.

Male and female Fischer 344 rats were exposed to 0, 5, 25 or 127 mg/m<sup>3</sup> for 6 h/day, 5 days/week, for 2 years. Increased incidences of hepatocellular tumours (adenoma or carcinoma) in the liver of males (16/70, compared with 1/69 in the control group), tubular tumours (adenoma or carcinoma) in the kidneys of males (6/70, compared with 0/69 in the control group) and endometrial stromal polyp in the uterus of females (25/69, compared with 11/69 in the control group) were found in the 127 mg/m<sup>3</sup> group. In addition, non-neoplastic lesions were observed in the liver, kidneys, nose and spleen. The incidence of eosinophilic foci and centrilobular hepatocytomegaly in the livers of males increased above 25 mg/m<sup>3</sup>. The incidence of pigment deposition in the olfactory epithelium in the nose, extramedullary haematopoiesis and pigmentation in the spleen increased even at the lowest concentration of 5 mg/m<sup>3</sup>. The LOAEL was established to be 5 mg/m<sup>3</sup>.

Male CD rats were exposed to 0, 5, 25 or 127 mg/m<sup>3</sup> for 6 h/day, 5 days/week, for 2 years. On histopathology, the incidence of hepatocellular tumours (adenoma or carcinoma) (9/65, compared with 2/63 in the control group) was significantly increased in the liver at 127 mg/m<sup>3</sup>. In addition, non-neoplastic lesions were observed in the liver (Kupffer cell pigmentation at 5 mg/m<sup>3</sup> and above, centrilobular hepatocytomegaly at 25 mg/m<sup>3</sup> and above and spongiosis hepatis at 127 mg/m<sup>3</sup>), nose

(squamous epithelial hyperplasia at 5 mg/m<sup>3</sup> and above and pigment deposition in the olfactory epithelium at 25 mg/m<sup>3</sup> and above), testes (atrophy at 127 mg/m<sup>3</sup>) and epididymides (hypospermia at 127 mg/m<sup>3</sup>). The LOAEL was established to be 5 mg/m<sup>3</sup>.

## ***5. EFFECTS ON HUMANS***

Nitrobenzene is toxic to humans by inhalation, dermal and oral routes of exposure. The main systemic effect associated with human exposure to nitrobenzene is methaemoglobinaemia.

Numerous accidental poisonings and deaths in humans from ingestion of nitrobenzene have been reported. In cases of oral ingestion or in which patients were apparently near death due to severe methaemoglobinaemia, termination of exposure and prompt medical intervention resulted in gradual improvement and recovery. Although human exposure to sufficiently high quantities of nitrobenzene can be lethal via any route of exposure, it is considered unlikely that levels of exposure high enough to cause death would occur, except in industrial accidents or suicides.

The spleen is a likely target organ during human exposure to nitrobenzene; in a woman occupationally exposed to nitrobenzene in paint (mainly by inhalation), the spleen was tender and enlarged. Liver effects, including hepatic enlargement and tenderness and altered serum chemistries, have been reported in a woman inhalationally exposed to nitrobenzene. Neurotoxic symptoms reported in humans after inhalation exposure to nitrobenzene have included headache, confusion, vertigo and nausea. Effects in orally exposed persons have also included those symptoms, as well as apnoea and coma.

Available human data are too limited to allow detailed comments about the relationship between the level of exposure and the degree of toxic response.

## ***6. PRACTICAL ASPECTS***

### ***6.1 Analytical methods and analytical achievability***

Nitrobenzene can be analysed by capillary gas chromatography with an electron capture detector or by mass spectrometry. The detection limit is 1.9 ng/l in water by gas chromatography/mass spectrometry (APHA, AWWA & WEF, 2005) and 10 ng/l in water by gas chromatography/electron capture detector (Li, Chen & Du, 2007). Nitrobenzene can also be analysed by high-performance liquid chromatography–atmospheric pressure ionization–mass spectrometry. The detection limit is 10 ng (Xu, van de Craats & de Bruyn, 2004).

### ***6.2 Treatment and control methods and technical performance***

Pilot plant studies of slow sand filtration (0.2 m/h) found the removal of 70–80% of nitrobenzene from a raw water concentration of 85 ng/l (Hrubec et al., 1991).

Isotherms for adsorption of nitrobenzene (9–220 mg/l) onto activated carbon have been published (Haghseresht, Nouri & Lu, 2003). For an equilibrium concentration of 5 mg/l, the adsorption capacity was approximately 60 mg/g.

Nitrobenzene is not oxidized at a significant rate by ozone under water treatment conditions (Hoigné & Bader, 1983). A 36 µg/l solution was treated with ozone at 3 mg/l for 8 min over the pH range 2–12. Effective ( $\geq 80\%$ ) removal was obtained only at pH 10 and above (Ma et al., 2005). In contrast,  $>90\%$  reduction from an initial concentration of 740 µg/l was obtained after 15 min with an ozone dose of 1.5 mg/l per minute (Latifoglu & Gurol, 2003). The presence of humic substances inhibited nitrobenzene removal. Faster removal was obtained in the presence of ultraviolet (UV) irradiation, but not when humic substances were present.

The half-life of a 100 mg/l solution treated with a high dose of ozone (~350 mg/l) was approximately 30 min (Contreras et al., 2001). The rate was unaffected by the application of UV irradiation or the addition of iron(II).

UV irradiation (1.8 W/l) of a 17 mg/l solution gave about 40% conversion in 40 min. Combining ozone (36 mg/l per minute) with UV irradiation resulted in complete removal in 10 min. With ozone alone, complete removal required about 40 min. Ozone with hydrogen peroxide gave similar removal to ozone alone (Beltran, Encinar & Alonso, 1998).

UV/hydrogen peroxide was used to treat petrochemical wastewater spiked with 10.8 mg nitrobenzene/l. The UV intensity was 4.8 mW/cm<sup>2</sup>, and a peroxide dose of 5000 mg/l was applied. Removal of 68.4%, 95.6%, 99.7% and 100% was reported after reaction times of 20, 60, 120 and 240 min, respectively (Juang, Tseng & Yang, 1997).

UV irradiation (12 W) in the presence of iron(II) at 10 mg/l and hydrogen peroxide at 65 mg/l gave complete removal from a 100 mg/l solution after 30 min; the half-life was approximately 5 min (Al Momani, 2006).

Overall, 42–70% removal was achieved from 10–50 mg/l solutions using four different nanofiltration membranes (Kiso et al., 2001). In contrast, another study (Van der Bruggen et al., 1999) reported a maximum of 22% removal from an approximately 350 mg/l solution treated with four different nanofiltration membranes. Over a 150 h treatment period with a 10 mg/l nitrobenzene solution in sodium chloride at 2000–6000 mg/l using a reverse osmosis membrane, 20–60% (average 42%) removal was achieved, whereas sodium chloride rejection was 95–99% (Urama & Mariñas, 1997).

No information was found on the removal of nitrobenzene by point of use or point of entry water treatment systems.

### ***7. CONCLUSION***

Almost all classical mutagenic tests suggest that nitrobenzene is not mutagenic; however, some recent studies have reported positive results. It cannot be excluded that nitrobenzene is a non-genotoxic chemical. No long-term oral administration studies are available. Based on inhalation studies, IARC (1996) concluded that there was

inadequate evidence in humans but sufficient evidence in experimental animals for the carcinogenicity of nitrobenzene. Nitrobenzene was placed in category 2B, possibly carcinogenic to humans.

Because nitrobenzene occurrence in drinking-water at concentrations above trace levels is infrequent, it is not considered necessary to derive a formal guideline value. However, health-based values can be calculated to provide guidance in the event of spills and where there are higher concentrations in industrial areas. Two health-based values, one for short-term exposure and the other for long-term exposure, are derived below based on the limited available information.

### ***7.1 Short-term exposure***

The most sensitive end-point in laboratory animals is the increased extramedullary haematopoiesis in the spleen, which was detected even at the lowest dose of 5 mg/kg body weight per day in a short-term (28-day) gavage study in F344 rats (Shimo et al., 1994). No NOAEL could be derived in this study. In addition, no appropriate benchmark dose can be estimated, as the incidence of extramedullary haematopoiesis reached a maximum at the middle dose.

Using this lowest LOAEL for nitrobenzene administered orally, a tolerable daily intake (TDI) of 5 µg/kg body weight for short-term exposure can be estimated, using an uncertainty factor of 1000 (100 for interspecies and intraspecies differences and 10 for use of a LOAEL instead of a NOAEL). A short-term health-based value of 30 µg/l can be derived for a 60 kg adult drinking 2 litres of water per day, using an allocation factor of 20%.

It should be noted that nitrobenzene is a potent methaemoglobinaemic agent in humans, and this is of particular concern for bottle-fed infants. Currently, data are not adequate to determine a separate value for this end-point.

### ***7.2 Long-term exposure***

No long-term oral administration studies for derivation of a health-based value have been reported. However, as similar severities of toxic effects, including methaemoglobinaemia and testicular toxicity, were observed in short-term studies after both oral gavage and inhalation exposures, it is possible to determine an approximate long-term value for comparison with the short-term value by using long-term inhalation studies. Although there are some uncertainties about kinetic differences between exposure routes, the severity of these systemic effects is considered to be associated with the concentrations of active metabolites in the targeted organs.

The sensitive end-points of decreased red blood cells and increased spleen weight were detected at doses of 5–25 mg/kg body weight per day in the short-term (28 day) oral administration study in rats; a similar level of effect was detected at concentrations of 51–180 mg/m<sup>3</sup> in air in a 14-day inhalation study in rats (Medinsky & Irons, 1985).

Conversion from inhalation exposure to oral dose (in mg/kg body weight) can be achieved using the following dosimetric adjustment formula:

$$\begin{aligned} & [51 \text{ mg/m}^3 \text{ (lowest dose in the 14-day inhalation study)} \times 0.29 \text{ m}^3/\text{day (daily} \\ & \text{respiration volume of rats)} \times (6 \text{ h}/24 \text{ h)} \times (5 \text{ days}/7 \text{ days)} \times 1.0 \text{ (absorption} \\ & \text{ratio)}] / 0.35 \text{ kg body weight (average body weight)} \\ & = 7.5 \text{ mg/kg body weight per day} \end{aligned}$$

Accordingly, the lowest exposure concentration of  $5 \text{ mg/m}^3$  in the 2-year rat inhalation study (Cattley et al., 1994; Holder, 1998) corresponds to a dose of  $0.75 \text{ mg/kg}$  body weight per day.

A LOAEL for the non-carcinogenic end-points of pigmentation of nose epithelium and extramedullary haematopoiesis in spleen of  $5 \text{ mg/m}^3$  ( $0.75 \text{ mg/kg}$  body weight per day) was determined from the chronic rat study. A TDI can be derived from a benchmark dose as a point of departure, since a NOAEL could not be derived. The lowest  $\text{BMDL}_{10}$  (the lower limit on the benchmark dose for a 10% response) for pigmentation of nose epithelium was estimated as  $0.13 \text{ mg/kg}$  body weight per day. This would give a TDI of  $1.3 \text{ }\mu\text{g/kg}$  body weight by applying an uncertainty factor of 100 for interspecies and intraspecies variation. A health-based value of  $8 \text{ }\mu\text{g/l}$  for a 60 kg adult drinking 2 litres of water per day can be derived from this TDI using an allocation to drinking-water of 20%.

However, the toxicological significance of these effects in humans is likely to be equivocal, because pigmentation of nose epithelium is considered to be a secondary effect of haemolysis. Other significant effects, such as eosinophilic foci and centrilobular hepatocytomegaly, were observed in the livers of males in a long-term inhalation study. The NOAEL for these hepatic effects was  $5 \text{ mg/m}^3$  ( $0.75 \text{ mg/kg}$  body weight per day), and the most sensitive  $\text{BMDL}_{10}$  was estimated as  $1.05 \text{ mg/kg}$  body weight per day. Health-based values of  $44 \text{ }\mu\text{g/l}$  and  $63 \text{ }\mu\text{g/l}$  could be determined from the NOAEL and the  $\text{BMDL}_{10}$ , respectively, using the same formulae.

For carcinogenic end-points, the method of linear extrapolation from the BMDL should be applied. Cancer occurrence at 0, 5, 25 and  $127 \text{ mg/m}^3$  in male Fischer 344 male rats was, respectively, as follows (Cattley et al., 1994; Holder, 1998): liver—1, 4, 5 and 16; thyroid—2, 1, 5 and 8; and kidney—0, 0, 0 and 6. Using the number at risk originally set by CIIT of 60, the combined respective incidences are  $3/60$  (0.050),  $5/60$  (0.083),  $10/60$  (0.167) and  $26/60$  (0.433). No time-to-tumour correction needs to be made because there was no differential mortality or significant early death (IPCS, 2003). Using the above values, a  $\text{BMDL}_{10}$  of  $2.70 \text{ mg/kg}$  body weight per day can be estimated (using the United States Environmental Protection Agency's BMDS version 1.4.1). Taking a conservative linear extrapolation approach to the cancer risk assessment, an upper 95% confidence limit of  $3.7 \times 10^{-2}$  per  $\text{mg/kg}$  body weight per day on the unit risk can be determined by dividing 0.1 by the  $\text{BMDL}_{10}$ . The long-term health-based value for nitrobenzene in drinking-water associated with an upper-bound excess lifetime cancer risk of  $10^{-5}$  (i.e. the upper-bound limit risk of one additional cancer per 100 000 of the population ingesting drinking-water containing nitrobenzene at the health-based value for 70 years) can be calculated as follows:



$(60 \text{ kg} \times 10^{-5}) / (3.7 \times 10^{-2} \text{ per mg/kg body weight per day} \times 2 \text{ litres/day})$

$\approx 0.008 \text{ mg/l (8 } \mu\text{g/l)}$

It should be emphasized that the derivation of long-term health-based values includes large uncertainties because of the dose metric conversion from inhalation studies and the possibility of increased metabolism to aniline in the gastrointestinal tract.

It should also be noted that the reported odour threshold for nitrobenzene in water is 30–110  $\mu\text{g/l}$ .

## **8. REFERENCES**

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