European Union Risk Assessment Report

NITROBENZENE

CAS No: 98-95-3
EINECS No: 202-716-0

RISK ASSESSMENT

GENERAL NOTE

This document contains two different reports:

- **Volume 77 Part I Environment** (Publication: EUR 22480 EN) – pages 2-106
- **Part II Human Health** (Final approved version awaiting for publication) – pages 107-259

European Chemicals Bureau

Institute for Health and Consumer Protection

European Chemicals Bureau

Existing Substances

European Union Risk Assessment Report

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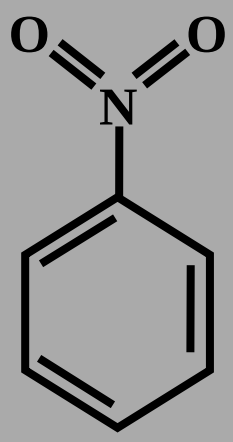
nitrobenzene
Part I - environment



3rd Priority List

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European Union Risk Assessment Report
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77

The mission of the IHCP is to provide scientific support to the development and implementation of EU polices related to health and consumer protection. The IHCP carries out research to improve the understanding of potential health risks posed by chemical, physical and biological agents from various sources to which consumers are exposed.

The Toxicology and Chemical Substances Unit (TCS), commonly known as the European Chemicals Bureau (ECB), provides scientific and technical input and know-how to the conception, development, implementation and monitoring of EU policies on dangerous chemicals including the co-ordination of EU Risk Assessments. The aim of the legislative activity of the ECB is to ensure a high level of protection for workers, consumers and the environment against dangerous chemicals and to ensure the efficient functioning of the internal market on chemicals under the current Community legislation. It plays a major role in the implementation of REACH through development of technical guidance for industry and new chemicals agency and tools for chemical dossier registration (IUCLID5). The TCS Unit ensures the development of methodologies and software tools to support a systematic and harmonised assessment of chemicals addressed in a number of European directives and regulation on chemicals. The research and support activities of the TCS are executed in close co-operation with the relevant authorities of the EU Member States, Commission services (such as DG Environment and DG Enterprise), the chemical industry, the OECD and other international organisations.

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RISK ASSESSMENT

Final Report, 2007

Germany

The rapporteur for the risk assessment of Nitrobenzene is Germany

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Review of report by MS Technical Experts finalised:	**April 2005**
Final report:	**2007**

Foreword

We are pleased to present this Risk Assessment Report which is the result of in-depth work carried out by experts in one Member State, working in co-operation with their counterparts in the other Member States, the Commission Services, Industry and public interest groups.

The Risk Assessment was carried out in accordance with Council Regulation (EEC) 793/93[1] on the evaluation and control of the risks of "existing" substances. "Existing" substances are chemical substances in use within the European Community before September 1981 and listed in the European Inventory of Existing Commercial Chemical Substances. Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of these substances if they are produced or imported into the Community in volumes above 10 tonnes per year.

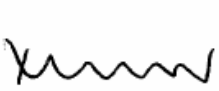
There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each substance on a priority list, a Member State volunteers to act as "Rapporteur", undertaking the in-depth Risk Assessment and recommending a strategy to limit the risks of exposure to the substance, if necessary.

The methods for carrying out an in-depth Risk Assessment at Community level are laid down in Commission Regulation (EC) 1488/94[2], which is supported by a technical guidance document[3]. Normally, the "Rapporteur" and individual companies producing, importing and/or using the chemicals work closely together to develop a draft Risk Assessment Report, which is then presented at a Meeting of Member State technical experts for endorsement. The Risk Assessment Report is then peer-reviewed by the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) which gives its opinion to the European Commission on the quality of the risk assessment.

If a Risk Assessment Report concludes that measures to reduce the risks of exposure to the substances are needed, beyond any measures which may already be in place, the next step in the process is for the "Rapporteur" to develop a proposal for a strategy to limit those risks.

The Risk Assessment Report is also presented to the Organisation for Economic Co-operation and Development as a contribution to the Chapter 19, Agenda 21 goals for evaluating chemicals, agreed at the United Nations Conference on Environment and Development, held in Rio de Janeiro in 1992.

This Risk Assessment improves our knowledge about the risks to human health and the environment from exposure to chemicals. We hope you will agree that the results of this in-depth study and intensive co-operation will make a worthwhile contribution to the Community objective of reducing the overall risks from exposure to chemicals.



Roland Schenkel
Acting Director-General
DG Joint Research Centre

Catherine Day
Director-General
DG Environment

[1] O.J. No L 084, 05/04/1993 p.0001 – 0075

[2] O.J. No L 161, 29/06/1994 p. 0003 – 0011

[3] Technical Guidance Document, Part I – V, ISBN 92-827-801 [1234]

0 OVERALL RESULTS OF THE RISK ASSESSMENT

CAS Number: 98-95-3
EINECS Number: 202-716-0
IUPAC Name: Nitrobenzene

Environment

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

No **conclusion (iii)** was drawn.

Conclusion (i) There is a need for further information and/or testing.

No **conclusion (i)** was drawn

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (ii) applies to surface water, sediment, the atmosphere and the terrestrial compartment for the production and/or processing of nitrobenzene. All PEC/PNEC ratios are below 1. This conclusion also applies to the industrial WWTPs of all sites.

Human health

(to be added later).

CONTENTS

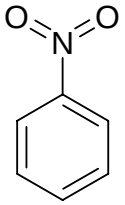
Euses Calculations can be viewed as part of the report at the website of the European Chemicals Bureau: http://ecb.jrc.it

TABLES

1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS No:	98-95-3
EINECS No:	202-716-0
IUPAC-Name:	nitrobenzene
Synonyms:	Nitrobenzol, Benzene, Nitro-Essence of Mirbane; Essence of Myrbane, Mirbane Oil; Myrbane Oil, Mononitrobenzene
Molecular formula:	$C_6 H_5 N O_2$
Molecular weight:	123 g/mol
Structural formula:	



1.2 PURITY/IMPURITIES, ADDITIVES

Purity: > 98.9%

Impurities: Benzene
o-Nitrotoluene
p-Nitrotoluene
p-Dinitrobenzene
m-Dintirobenzene
o-Dinitrobenzene
Toluene
Water

1.3 PHYSICO-CHEMICAL PROPERTIES

Table 1.1 Summary of physico-chemical properties

Property	Value	Reference
Physical state	liquid	
Melting point	5.26°C	BASF AG (1986)
Boiling point	210.8°C	Lide (1991)
Relative density	1.2037	Lide (1991)
Vapour pressure	0.2 hPa at 20°C[1)] 32.6 Pa at 25°C[1)]	Auer (1988) Daubert and Danner, 1989
Water solubility	1,900 mg/l at 20°C [2)]	Bayer AG (1998)
Partition coefficient n-octanol/water (log value)	1.86 at 24.5°C [3)]	BASF AG (1987)
Granulometry		
Conversion factors		
Flash point	88°C	BAM (1997)
Autoflammability	480°C (DIN 51794)	BAM (1997)
Flammability	Not extremely flammable Not highly flammable Not flammable [4)]	BAM (1997)
Explosive properties	No explosive properties	BAM (1997)
Oxidizing properties	Not applicable (liquid)	
Viscosity		
Henry's constant	1.296 Pa·m^3·mol^{-1} at 20°C 2.16 Pa·m^3·mol^{-1} at 25°C [5)]	calculated
Surface tension	43.9 mN/m at 20°C (pure substance)	Lide (1991)

1) The vapour pressure of 0.2 hPa at 20°C was confirmed by entries in safety data sheets of various companies. US EPA confirmed also this value (http://www.who.int/pcs/ehc/full-text/ehc230/part_I.pdf). Daubert and Danner present an experimental vapour pressure as 0.245 mm Hg equivalent to 32.6 Pa at 25ºC (Daubert and Danner 1989).
2) The flask method was used for the determination of the water solubility. In the safety data sheets of Bayer and Hoechst a water solubility of 2.0 g/l at 20°C is cited. No information about the purity of the test substance, the test method and the test conditions are available. Therefore the water solubility of 1.9 g/l at 20°C (pH 6.5) is recommended for the risk assessment.
3) The shaking flask method was used for the determination of the partition coefficient n-octanol/water. The calculation according to Leo and Hansch resulted in a logPow of 1.81. For the risk assessment the experimental value is preferred.
4) The tests according to A.12 and A.13 were not conducted. Due to the properties and the handling of the substance it has not to be assumed that flammable gases format in contact with water or the substance has pyrophoric properties.
5) The Henry law constant is based on the Water solubility-Vapour Pressure Method. Calculation models (both bond and group method) always assume an idealized form of a substance and therefore an experimental determination should be preferred. An experimental Henry's Law constant of $2.4 \cdot 10^{-5}$ atm · m^3/mol at 25ºC exists (Warner et al. 1987) but this value is higher than expected with respect to a temperature difference of 5°C.The data presented in this Draft Risk Assessment Report do not support the current classification as N R51-53. According to this data the current classification should be changed from N R51-53 to R52-53

1.3.1 Current classification

For the environment nitrobenzene is classified with N; R51-53 (had been included in the 22nd ATP).

The current classification is T, N; R 23/24/25-48/23/24-40-62-51/53.

1.3.2 Proposed classification

The data presented in this Draft Risk Assessment Report do not support the current classification as N R51-53. According to this data the current classification should be changed from N R51-53 to R52-53.

2 GENERAL INFORMATION ON EXPOSURE

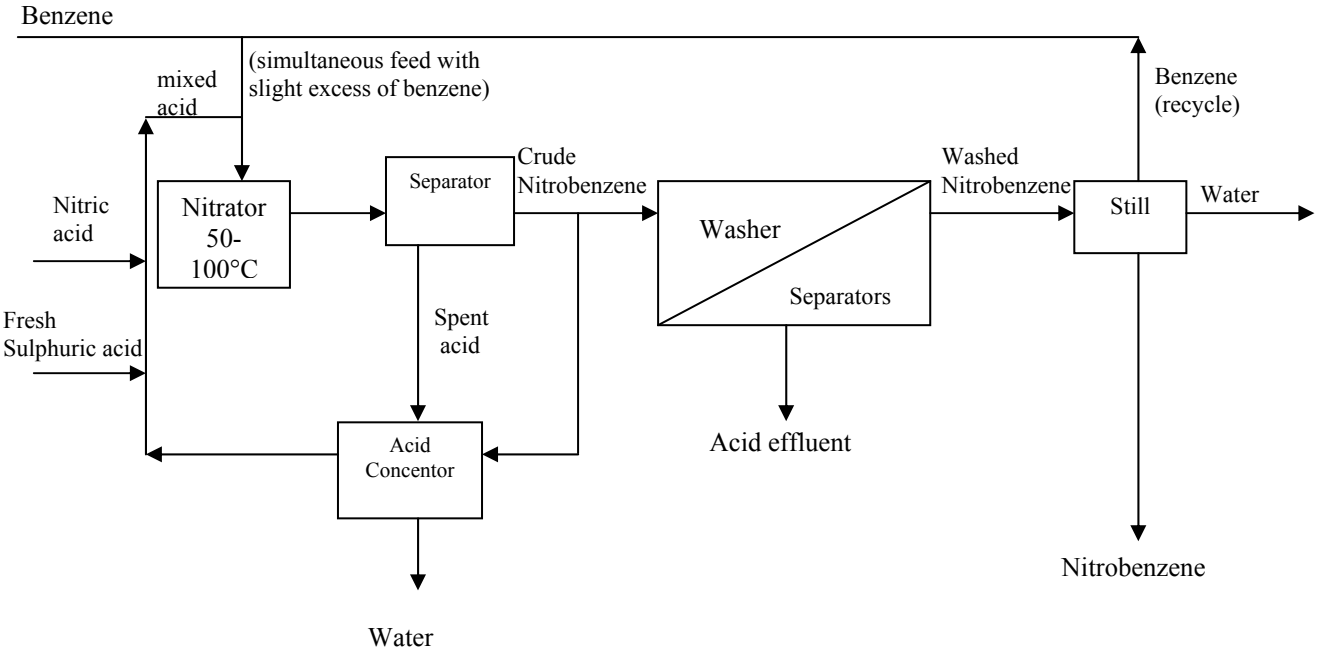
2.1 PRODUCTION

There is no natural source of nitrobenzene known. However, nitrobenzene may be formed by OH-initiated photooxidation of benzene which could theoretically be of natural origin. This possible source is not considered to be significant. Nitrobenzene is almost exclusively produced by nitration of benzene. Nitrobenzene is mainly used as an intermediate in the manufacture of aniline.

2.1.1 Production processes

Nitrobenzene is produced by nitration of benzene with nitrating acid, which is a mixture of nitric acid, sulphuric acid and water. It is usually performed continuously in stirred-vessel cascades or in loop-type reactors. Clean-up of the reaction mixture takes place in static separators or in centrifuges. The organic phase is washed with water, dilute alkali and then with water again to free it from acid and by-products containing hydroxyl groups. The washed nitrobenzene is then freed from un-nitrated components, such as benzene, by steam stripping. Finally, it is dried by azeotropic distillation. Spent acid is continuously concentrated and reintroduced to the cycle. Also fresh acid is added.

Figure 2.1 Production of nitrobenzene – continuous process (Ullmann´s Encyclopedia of Industrial Chemicals, 1995).



2.1.2 Production capacity

According to available data there are 8 production and/or processing sites of nitrobenzene within the EU. The data are based on company information for the year 2000, except for one site which refers to 2002. Taking into account these actual statements, the resultant quantity of nitrobenzene produced in the EU amounts to be $1.18 \cdot 10^6$ tonnes/year.

2.2 USES

2.2.1 Introduction

Almost all nitrobenzene is primarily used for the production of aniline and, to a much lesser extent, for the production of pharmaceuticals and various other chemicals. According to new exposure data from IND nitrobenzene is no longer used as a solvent.

Table 2.1 Use pattern

Type of use	Tonnage [tonnes/annum]	Appr. % in this application*
Processing to aniline	1,162,900	99
Processing to pharmaceuticals	9,300	0.8
Processing to other chemicals	2,800	0.2
Total	1,175,000	100

* These figures refer to the company information given in this report

There is a difference of about 5,000 tonnes/annum between production and processing which amounts only to around 0.42% of the total production volume. It could not be clarified whether this amount is further used at all and if so for which application it might be used. There is no evidence that this missing tonnage in the mass balance is actually further processed and it is hence considered to be due to inaccuracies in estimates rather than due to a missing tonnage as it amounts It is not known that any quantities of nitrobenzene are imported from outside the EU or exported into it.

In Germany nitrobenzene was used for perfuming soaps in the past as the so called Mirbanoil. However, the use of nitrobenzene in cosmetic products has been forbidden in Germany since the 1980s. (Cosmetic Regulation from 19th June 1985). No information is available whether nitrobenzene is or was used in soaps in other EU countries than Germany and whether this possible use has maybe been discontinued in other EU countries.

There are a number of sources that indicate nitrobenzene may be present as a solvent in floor/furniture polishes, shoe polish and some paints[1,2,3]. But these sources seem not to be relevant for Europe.

1 http://www.epa.gov/seahome/housewaste/house/nitroben.htm
2 http://www.arb.ca.gov/toxics/tac/factshts/nitroben.pdf
3 Environmental Hazard Assessment: Nitrobenzene. TSD/24, Toxic Substances Division, Department of the En vironment, 1996

The substance is further subject to annual emissions reporting requirements for the Pollution Inventory in the UK. According to the Pollution Inventory fact sheet, nitrobenzene is also used in small amounts to make explosives, aniline dyes and pesticides.

The content of nitrobenzene in different products is listed in the Danish Product Register. In 2003 nitrobenzene was present in 23 adhesive or binding products and reprographic agents in a range of 0-2% with an approximate quantity of less than 1 tonne/year. These products might be used by professionals or consumers.

The Rapporteur has no information on any of these uses in Europe at present. It can be assumed that they are of historical relevance only and that they can be neglected in the Risk Assessment Report.

This assumption is supported by the SPIN database where in the year 2001 nitrobenzene was only present in 41 products in Denmark (reprographic agents) but with an amount of 0 tonnes/year. In Sweden, Norway or Finland no nitrobenzene containing products were listed at this time.

2.2.2 Scenarios

According to the information given by the companies 99% of the produced nitrobenzene is used for the production of aniline. Another 1% is processed to pharmaceuticals and other chemicals.

Table 2.2 Main, industrial and use category according to the TGD

	Main category	Industry category	Use category	Percentage of total use
Production	Non-dispersive use 1b	Chemical industry: chemicals used in synthesis (3)	Intermediate (33)	100
Processing: Production to aniline and various other chemicals	Non-dispersive use 3	Chemical industry: chemicals used in synthesis (3)	Intermediate (33) Oxidizer (37)	100

2.3 TRENDS

There is no data available.

2.4 LEGISLATIVE CONTROLS

In Germany the use of nitrobenzene in cosmetic products has been forbidden since the 1980s by the Cosmetic Regulation from 19th June 1985.

Industrial releases of nitrobenzene in the UK are controlled under the Pollution Prevention and Control (PPC) Regulation 2000, which implement EC Directive 96/61 on Integrated Pollution Prevention and Control.

Nitrobenzene is on the List of potential Substances of Concern to be considered by HELCOM, but not as a substance for which immediate priority action is necessary. It is on the

Reference List of Substances agreed by the Third and Fourth North Sea Conference (e.g. Annex 1D to The Hague Declaration), for further selection of priority substances.

Nitrobenzene is not on the OSPAR list of substances of possible concern.

Nitrobenzene is a Volatile Organic Compound (VOC) according to the Solvents Emissions Directive 1999/13/EC but its uses do not fall under the categories of activity referred to in Article 1.

3 ENVIRONMENT

3.1 ENVIRONMENTAL EXPOSURE

3.1.1 General discussion

Nitrobenzene is industrially produced on a large scale and primarily manufactured to aniline. Other smaller applications are the use as an intermediate for the production of other various chemicals and pharmaceuticals.

3.1.2 Environmental releases

Releases of nitrobenzene into the environment are to be expected during production and processing with waste water and, to a lesser extend, exhaust gases.

Further emissions to air are expected from waste water treatment plants.

Direct releases to agricultural or natural soil were not identified.

A further source of nitrobenzene may be its formation in the atmosphere by the OH-initiated photooxidation of benzene (Rogozen MB, Rich HE and Guttmann MA, 1987). The quantification of the amount of nitrobenzene formed in the atmosphere is not possible and is not considered within the scope of this risk assessment.

3.1.2.1 Release from production

Nitrobenzene is produced by nitration of benzene with nitrating acid, which is a mixture of nitric acid, sulphuric acid and water.

Within the EU there are 8 sites where production and/or processing takes place. The total production volume is $1.18 \cdot 10^6$ tonnes of nitrobenzene/year. According to the information given by the companies 99% of the produced nitrobenzene is used for the production of aniline and another 1% is processed to pharmaceuticals and other chemicals.

Processing of nitrobenzene takes place on site at 5 production sites, respectively. About 50,000 tonnes/year are processed at external sites.

The highest production volume on a single site is about 380,000 tonnes/year. Companies, where nitrobenzene is processed only, buy it from production companies within the EU. It is not known of any quantities of nitrobenzene imported from outside of the EU or exported to it. All these data refer to the year 2000.

3.1.2.2 Release from formulation

No formulation takes place.

3.1.2.3 Release from private use

No releases of nitrobenzene from private use are to be expected.

3.1.2.4 Release from disposal

As almost all nitrobenzene is used as an intermediate in the production of aniline and various other chemicals no release from disposal is expected.

3.1.2.5 Summary of releases

Releases of nitrobenzene into the environment occur during production and processing via waste water and, to a lesser extend, exhaust gases. Further emissions to air are expected from waste water treatment plants.

Direct releases to agricultural or natural soil are not expected.

3.1.3 Environmental fate

Using the distribution model according to Mackay (level 1) results in the following theoretical distribution figures:

Table 3.1 Level I environmental partitioning of nitrobenzene

Compartment	Percentage
Air	30.7
Water	68.5
Soil	0.39
Sediment	0.40

The hydrosphere, followed by the atmosphere is therefore the target compartments for nitrobenzene in the environment.

3.1.3.1 Degradation in the environment

3.1.3.1.1 Atmospheric degradation

Direct Photolysis

The direct photolysis behaviour of nitrobenzene in air was examined by Freitag et al. (1982). Nitrobenzene, which is adsorbed on silica gel (60 ng/g silica gel), was irradiated with a high pressure mercury lamp at 290 nm, which can be considered as environmentally relevant. After 17 hours 6.7% were mineralised to carbon dioxide. This result indicates that direct photolysis is not an important degradation pathway for nitrobenzene.

Nitrobenzene shows a broad absorption maximum at 260 nm and absorbs UV-light up to 400 nm. When nitrobenzene is irradiated with light in the wavelength of 260 nm the primary reaction products are nitrosobenzene and p-nitrophenol (Hastings and Matsen, 1948). Since the sunlight on earth contains only a very small amount of irradiation with a wavelength of 260 nm this reaction has no relevance for the environment.

Both o-and p-nitrophenol were found when irradiating nitrobenzene for 5 hours at 25-30°C with a xenon lamp ($\lambda > 300$ nm) in the presence of O_2, whereas phenol was also found when O_2 was absent (Nojima and Kanno, 1977). The authors state that 38% of the initial nitrobenzene was degraded within 5 hours. But these figure was obtained by extracting the residue in the reaction vessel with methanol after the reaction and subsequent analysis of the extract. So only the residue which was adsorbed to the vessel sides was recorded. This result is therefore not considered to be valid.

Photooxidation in the Troposphere

The photo-reaction of nitrobenzene with hydroxyl radicals or ozone in the troposphere was also examined by Atkinson et al. (1987). In order to investigate the reaction with OH-radicals nitrobenzene (0.005 mg/l at 23°C) was irradiated in a Teflon-chamber with a Xenon lamp (λ>290 nm). No information is given about the reaction time. In this experimental system no decay of nitrobenzene was observed outside of the analytical uncertainties. Hence, the upper limit of the reaction rate constant was determined to be $7 \cdot 10^{-13}$ $cm^3/(molecules \cdot sec)$. Given an OH-concentration of $5 \cdot 10^5$ $molecules \cdot cm^{-3}$ this leads to an atmospheric half-life of more than 23 days.

Further investigations resulted in half-lives of 62 to 100 days (Zetzsch, 1982) based on a rate constant of $(0.21 \pm 0.05) \cdot 10^{-12}$ $cm^3/(molecules \cdot sec)$, 115-125 days (Witte et al., 1986) based on a rate constant of $(1.32 \pm 0.07) \cdot 10^{-13}$ $cm^3/(molecules \cdot sec)$ and 107 days (Becker et al., 1984) based on a rate constant of $0.15 \cdot 10^{-12}$ $cm^3/(molecules \cdot sec)$. An OH-concentration of $5 \cdot 10^5$ $molecules \cdot cm^{-3}$ was used to calculate these half-lives.

Calculation of the reaction rate constant and the half-life of nitrobenzene in air with the AOP v.1.9-model, using a hydroxyl radical concentration of $5 \cdot 10^5$ $molecules \cdot cm^{-3}$, results in a reaction rate constant of $2.44 \cdot 10^{-13}$ $cm^3 \cdot molec.^{-1} \cdot s^{-1}$ and a half-life of 65.8 days ($kdeg_{air}=1.06 \cdot 10^{-2}$ d^{-1}). For calculation see **Appendix F**. This value is within the range of the experimentally determined half-lives and is used in the further calculations.

3.1.3.1.2 Aquatic degradation (incl. sediment)

Aerobic biodegradation in waste water treatment plants

The biodegradability of nitrobenzene has been investigated in some standard tests on ready biogradability and in similar test configurations as well.

In a MITI I test (OECD 301C) (CITI, 1992) nitrobenzene at a concentration of 100 mg/l was tested with an inoculum (30 mg/l) containing activated sludge from a municipal sewage plant and 10 samples from 10 different sites in Japan. A degradation of 3.3% related to BOD after an incubation of 14 days has been measured.

In another standard test the biodegradability of nitrobenzene was studied according to the modified OECD screening test (OECD 301E) (BASF AG, 1989a). At a nitrobenzene concentration of 38.5 mg/l an elimination of 100% related to DOC after 21 days was measured, but in the physico-chemical batch an elimination of 88% has also been determined. Because nitrobenzene is not likely to adsorb to organic matter it can be assumed that it evaporated in this test system and that this test is consequently not appropriate for testing semi-volatile substances.

In a manometric respirometry test (similar to OECD 301F) (BASF AG, 1989c) two test concentrations were tested, 60 and 120 mg/l. Concerning the test concentration of 120 mg/l there is conflicting information. The text of the test description stated that the concentration is 120 mg/l whereas the marking of the diagram says 100 mg/l. At a concentration of 60 mg/l a biodegradation rate of 48% related to BOD after an incubation of 35 days has been determined. The lag phase was 25 days. At the higher test concentration 5 parallel assays were run and the biodegradation rate varied between 0 and 16% related to BOD. Only few experimental details are given in the report. Due to the long lag phase it can be concluded that adaptation has taken place. In fact this study cannot be considered valid but it confirms the prediction from the other studies that nitrobenzene is not readily biodegradable.

In a study on ready biodegradability (Gomólka and Gomólka, 1979) using a Warburg respirometry test system, it was shown that at initial concentrations up to 300 mg/l, nitrobenzene was degraded slowly. 33% related to BOD were degraded by day 14 at an initial concentration of 100 mg/l test substance with biodegradation starting after 90 hours lag time. At an initial dosage of 300 mg/l 30% were degraded after 10 days. At this concentration nitrobenzene slowly dissolves in water so nitrobenzene concentration increases during the first 80 hours. After that the concentration declines. At an initial concentration of 1,400 mg/l the nitrobenzene concentration increased at first due to slow solution in water. No decrease and no elimination of nitrobenzene were reported. The authors state that at concentrations above 1,000 mg/l micro-organisms are inhibited.

Nitrobenzene at a concentration of 100 mg/l was only degraded to 10% related to BOD after 10 days of incubation with domestic activated sludge in an electrolytic respirometer system similar to the MITI procedures (Urano and Kato, 1986). BOD, DOC and biomass were monitored, whereas no substance-specific analytic procedure was performed.

It can be concluded that nitrobenzene is not readily biodegradable.

A test on inherent biodegradation according to a modified Zahn-Wellens test system (OECD 302B), (BASF AG, 1989b) was conducted using activated sludge from an industrial waste water treatment plant (WWTP), that has to be regarded as adapted to nitrobenzene. The test batch with a nitrobenzene concentration of 221 mg/l shows 86% elimination after 10 days whereas in the physico-chemical control (without inoculum) using the same nitrobenzene concentration, 79% were eliminated by day 10. It can be assumed that a removal through evaporation may have taken place. The test system has not to be considered valid because of the high removal rate in the physico-chemical batch. Nitrobenzene is therefore not biodegradable in this test.

In a Sapromat respirometric test system with an inoculum originating from industrial adapted activated sludge nitrobenzene concentrations between 5.4 mg/l to 541.5 mg/l were tested. The test period lasted for 28 days and nitrobenzene was the only carbon source. The inoculum concentration was 200 mg/l (BASF AG, 1985). The biodegradation ranged between 34% at a

concentration of 54 mg/l and 1,600% at 5.4 mg/l. At 180 mg/l nitrobenzene was degraded to approximately 50% after 12 days. No further degradation was observed after that day. At concentrations 18 mg/l and 541 mg/l no biodegradation was observed. All biodegradation data are related to BOD. No explanations are given for these inconsistencies. As the results are so extremely contradictory they have only a restricted reliability.

In a modified OECD-Confirmatory test (OECD 303 A) (BASF AG, 1984) nitrobenzene concentrations between 0.1 and 10.5 mg/l have been added to a bench scale WWTP with sewage cultivated in bench scale treatment systems over a period of 4 months. The system was designed to simulate conditions in waste water treatment plants featuring activation basins and sludge recycled from clarifiers. During the first phase (35 days) a nitrobenzene concentration of 3 mg/l had been maintained in the influent of the bench scale WWTP. In the second phase which lasted for 7 days it was maintained at 10 mg/l. The authors stated that 23 days elapsed between starting the test and complete elimination of nitrobenzene in the effluent. However, elimination rates of 72-93% were already recorded during this period of adaptation. After the adaptation period a feed concentration of 3.5 mg nitrobenzene/l was totally eliminated in one day and 10 mg/l within 3 days. Inhibitors in the form of 1 to 3 mg/l hydroxylamine and 3 to 6 mg/l potassium cyanide did not impair the elimination efficiency. Interrupting the addition of nitrobenzene for seven to nine days and then restarting it, showed that the activated sludge was still able to totally eliminate nitrobenzene after a lag phase of two days after this pause. It can be recorded that even if nitrobenzene was mostly eliminated by 100% there are considerable fluctuations in the elimination behaviour (20-100%) independent of the tested concentration. Mere primary degradation has taken place as only nitrobenzene concentrations were measured in the effluent. It is unclear whether the losses are due to real biodegradation or to volatilisation and/or adsorption, so the biodegradation rates cannot be explicitly determined. Evaporation might play an important role. The fact that the inhibitors did not affect adversely the elimination of nitrobenzene confirms this.

The biodegradation rates are more or less in accordance with monitoring results from an adapted industrial activated sludge treatment plant. Within a period of 7 months over 90% of nitrobenzene were eliminated most of the time. However, at some sampling days the elimination rate was significantly lower, sometimes only 34% (BASF AG, 1983). Reasons for these fluctuations are not known. Maybe they were due to toxic effects of nitrobenzene to microorganisms.

Gomólka (1979) investigated the biological degradation of nitrobenzene in a two stage bench-scale waste water treatment plant. In this pilot plant nitrobenzene concentrations were determined by spectrophotometry at 252 nm and hence the elimination rates are based on the parent substance. The first stage of the WWTP (activated sludge was prior adapted to pyridine) received increasing nitrobenzene concentrations from 5 to 60 mg/l during a first step. The elimination ranged from 98.6–100% after 22 days. At a concentration of 60 mg/l, the elimination was 89.4%. The influent concentrations of the secondary reactor were lower (2.5-33.5 mg/l) and elimination rates of 88.8% (at 60 mg/l) to 98.1% (at 10 mg/l) were found. In these tests it could not be clarified, whether the high removal rates of nitrobenzene were due to its biodegradation or to evaporation resulting from the intensive aeration of the wastewater in a system open to the atmosphere.

An increasing degradation capacity after a stepwise adaptation to increasing nitrobenzene concentrations was also observed by the same authors (Gomólka and Gomólka, 1979) in a Warburg respirometry test system. The maximum concentration of 300 mg was decomposed

to < 0.1 mg/l within 3 days. The sludge inoculum originated from the above mentioned two stage bench-scale pilot plant system.

Nitrobenzene removal and fate has been furthermore determined in pilot-scale activated sludge systems in two separate studies, one with nitrobenzene concentrations of about 0.13 mg/l and the other with about 0.45 mg/l (Bhattacharya et al., 1989). These treatment systems both consisted of primary clarification followed by conventional plug flow activated sludge treatment and sedimentation. In each study two pilot-scale activated sludge systems (sludge retention time was 4 days for 0.4473 mg/l and 8 days for 0.129 mg/l) were operated in parallel. One system was continuously fed with sewage spiked by nitrobenzene at concentrations of 0.129 mg/l and 0.4473 mg/l, respectively ("acclimated biomass"), the other one was fed intermittently 24 hours with sewage spiked by nitrobenzene (at the same nominal concentration as in the continuously fed assay) followed by 24 days without nitrobenzene spiking („unacclimated biomass"). Mass balances were carried out. However, special problems have been encountered with the analytical procedures, so recovery rates could not be determined and variations in results are high. Nitrobenzene concentrations were detected with GC/MS, so only primary biodegradation was measured. Air emission sampling was reported to be performed in the study with 0.13 mg nitrobenzene/l but no results on air concentrations are listed in the study report. In the study with 0.45 mg /l no air emissions were measured.

Table 3.2 Nitrobenzene elimination in two bench scale pilot plants (Bhattacharya et al., 1989)

	Continuous feed system		Intermittent feed system	
initial concentration [mg/l]	0.129	0.4473	0.129	0.4473
elimination [%]	93 ± 6	91.3 ± 3.4	73 ± 13	50 ±18

It can be seen, that the elimination in the acclimated system was significantly higher. This result is in good accordance with the outcomes of the other studies which show that adapted microorganisms have the ability to biodegrade nitrobenzene. Although nitrobenzene is only a semi-volatile substance, evaporation seems to have taken place in several of the biodegradation studies. As in this study no results on air emissions are available, it is unclear how much of the nitrobenzene evaporated. Results can therefore only be declared as elimination and not primary degradation. Adsorption to sludge turned out only to be 1-2% in both studies.

The elimination of nitrobenzene in a complete-mix, bench-scale, continuous-flow activated sludge reactor was also examined (Stover and Kincannon, 1982). The reactor was fitted with stainless steel covers to facilitate off-gas analysis. Nitrobenzene was added to synthetic waste water containing ethylene glycol, ethyl alcohol, glucose, glutamic acid, acetic acid, phenol, ammonium sulphate, phosphoric acid and salts. Activated sludge from a municipal activated sludge sewage treatment plant was acclimated to the nitrobenzene-containing waste water and then used as inoculum. The hydraulic retention time was 8 hours. Mean cell residence times of the activated sludge system were 2, 4 and 6 days. Over a period of 60 days elimination of nitrobenzene was measured by GC analysis. With an influent concentration of 100 mg/l the nitrobenzene concentration was reduced by 76% (sludge age: 2 days) and by 97.8% (sludge age: 6 days). 0% of nitrobenzene were found to be stripped from the test system. BOD_5, COD and TOC of the synthetic waste water were also measured over a period of 60 days. With a sludge age of 6 days the BOD_5 was reduced by 99.6%, COD by 95.7% and TOC by 90%.

Biodegradation in surface water

Biodegradability of several organic compounds in river and sea water was tested by Kondo et al. (1988b). The test chemical was added to a mixture of river or sea water from an unpolluted area and an autoclaved solution of 0.2% peptone (and 3% NaCl for sea water) in a test tube with a tight plug. The test tubes were incubated in the dark at 30°C. After 3 days of incubation a primary degradation of nitrobenzene of 0% were found in river water and of 13% in sea water (Kondo et al., 1988a). According to the classification of Kondo nitrobenzene has to be considered as to be of "hard degradability". No information was given on the analytical method.

Anaerobic Biodegradation

The anaerobic biodegradation behaviour of nitrobenzene was examined (Kameya et al., 1995). Nitrobenzene (30 mg C/l) with glucose and glutamic acid as a coexistent organic medium (15 mg C/l each) had been incubated for 28 days. After every week a sample was analysed. DOC-decrease is taken as parameter for biodegradation and 33% nitrobenzene were degraded after 28 days.

The anaerobic degradation rates and toxic effects of nitrobenzene on acetate utilizing methanogens were investigated (Bhattacharya et al., 1996). An acetate enrichment culture in combination with a nutrient solution and yeast extract with stable gas production was spiked with nitrobenzene. Acetic acid was added daily. 10 mg/l of nitrobenzene did not inhibit total gas production in the acetate enrichment methanogenic culture. 20 and 30 mg/l both caused reversible inhibition of methanogenesis. Nitrobenzene was analysed by HPLC. As a result of the anaerobic degradation study 90% of the nitrobenzene in the presence of the acetate enrichment culture was eliminated within 6 days. The initial nitrobenzene concentration was 20 mg/l. But control samples showed that 45% of the elimination was due to abiotic processes. No nitrobenzene was detected in the extracted samples from solids in the methanogenic systems. This indicates that primarily biodegradation of nitrobenzene (45%) in the methanogenic system has taken place.

In another study (Dickel et al., 1993) biodegradation of nitrobenzene by a sequential anaerobic-aerobic process was investigated. Increasing nitrobenzene concentrations (98-678 mg/l) in a 20 mmol/l glucose solution were pumped through a closed fixed–bed column filled with glass beads which were inoculated with anaerobic sewage sludge. Adaptation to the increasing nitrobenzene concentrations took place in 4 weeks during which the concentration was steadily raised. Nitrobenzene and its reduction product aniline were monitored analytically. At concentrations up to 344 mg/l nitrobenzene was completely converted to aniline and no nitrobenzene was detected in the effluent of the column (retention time: 2 days). At the highest concentration tested 7% nitrobenzene were found in the effluent of the column and 78% were recovered as aniline. A control experiment without inoculum under the same conditions, however, showed no chemical reduction of nitrobenzene. After the anaerobic treatment of nitrobenzene the formed aniline was metabolised by aerobic microorganisms in a subsequent aeration tank.

Bacterial strains of *Methanococcus* sp. were tested under anaerobic conditions during static incubation at a nitrobenzene concentration of 61.6 mg/l (Boopathy, 1994). An elimination of 65% was achieved after 20 days under formation of aniline. This elimination may be biologically mediated as without inoculum or after inactivation by heating, no elimination of nitrobenzene occurred.

Hydrolysis

No investigations are available with regard to the hydrolytic degradation behaviour of nitrobenzene. However, the substance category of the aromatic nitro compounds is generally resistant to hydrolysis (Harris JC, 1990), so that nitrobenzene is not expected to hydrolyse under environmental conditions.

Photodegradation

In principle there are two pathways of nitrobenzene degradation in the hydrosphere, the direct photolysis and the photo oxidation by hydroxyl radicals.

In view of the poor biodegradability and the stability to hydrolysis, photolysis may be the only relevant degradation pathway for nitrobenzene in the hydrosphere even if it only occurs in the upper layer of the water.

Direct photolysis in water was studied both in distilled water and in the presence of humic substances (Simmons and Zepp, 1986). Firstly, a very diluted aqueous nitrobenzene solution (0.00001 mol/l distilled water) at pH 5.5 was exposed to irradiation with a wavelength of 313 nm. No specific information on exposure time is available. It is only stated that the exposure times varied depending on which nitroaromatic was tested, but achieving approximately 30% reaction for each exposure. For nitrobenzene a half-life of 133 days was calculated from the direct photolysis constant of $5.2 \cdot 10^{-3}$ d^{-1}. Then the influence of humic substances on photoreactions were determined by comparing results in humus-containing water (13.6 mg C/l) with those in distilled water. In contrast to most of the examined nitroaromatic compounds, which showed enhanced photolysis rates, nitrobenzene was hardly affected by the presence of humic substances.

Nitrobenzene in the upper layers of the water can also undergo photochemical reactions with OH-radicals (Anbar et al., 1966). A rate constant of $2.0 \cdot 10^{9}$ l/(mol·sec) was experimentally determined and a half-life of 40-400 days was calculated depending on the concentration of OH-radicals which is given as 10^{-16}-10^{-17} mol/l in this study. Only few data are given, so that the result has to be treated with caution.

A rate constant of $3.2 \cdot 10^{9}$ l/(mol·sec) was determined (Neta and Dorfman, 1968) and half-lives of 25-250 days were calculated, again depending on the varying concentrations of OH-radicals (10^{-16}-10^{-17} mol/l). The nitrobenzene concentration tested was 0.0005 mol/l, the pH was 7.

The nitrate-induced photooxidation of trace organic chemicals in water was examined (Zepp et al., 1987). 1 µmol/l nitrobenzene, 4,000 µmol/l nitrate and 1-octanol in an aqueous phosphate buffer at pH 6.2 were irradiated in a merry-go-round reactor at 313 nm for about 5-6 hours. After irradiation the solution was analysed by HPLC and it was found that 63% nitrobenzene were degraded. The OH-radical concentration was not reported.

Biodegradation in sediment (aquifer microcosms)

During incubation of nitrobenzene in an aquifer system (groundwater plus fine material of sediment) no biodegradation was observed during the exposure period of 150 days. The incubation was performed at 10°C in the dark in a slowly rotating box with aeration taking place (minimum oxygen concentration 9 mg/l). The test substance was monitored by GC-FID and GC-ECD, the initial concentration was 0.15 mg/l (Nielsen et al., 1996).

In an earlier study performed by the same author (Nielsen and Christensen, 1994) sediment and groundwater were incubated with a mixture of organic compounds containing nitrobenzene at a concentration of 0.15 mg/l. After a lag phase of 70 days nitrobenzene was primarily degraded by 100% within 20 days in only 2 out of 16 experiments. No degradation took place in the other experiments. No explanation for this inconsistency is given. These results are considered not valid, however they confirm that nitrobenzene is persistent under environmental conditions.

20% primary degradation of nitrobenzene in an aquifer test system with an initial concentration of 0.1 mg/l were found after 50 days (Albrechtsen et al., 1997). Again a mixture of chemicals was tested. This time a control sample was examined. The decrease of nitrobenzene in this control was 15%. Hence the loss of nitrobenzene was mainly due to abiotic elimination processes such as evaporation.

It is not possible to derive a degradation rate constant for the sediment. All that can be said is that nitrobenzene in sediment is not biodegradable in the tests described above.

3.1.3.1.3 Degradation in soil

Aerobic soil micro-organisms have been tested for their potency to degrade nitrobenzene during incubation in soil columns (Kincannon and Lin, 1985). Nitrobenzene as a component of different types of waste sludge was given to different types of soil. The origin and composition of these different types of sludge were not further specified. A column filled with sandy loam soil was loaded with DAF sludge (an industrial waste not further described). The nitrobenzene concentration dropped from 2,400 mg/kg soil to 800 mg/kg within 97 days (67% elimination). Another sandy loam soil column was loaded with slop oil sludge and the nitrobenzene concentration dropped by 98% within 76 days (from 2,746 mg/kg to 54 mg/kg). In silt loam soil, loaded with wood preserving sludge the nitrobenzene degradation was 87% (from 393 mg/kg to 54 mg/kg) within 78 days and started at day 151. Nitrobenzene was monitored by gas chromatography of extracts of treated soils. In a sterilised control assay a nitrobenzene concentration of 122 mg/kg soil dropped to 19 mg/kg within 21 days (84% removal). It can be assumed that the loss is due to volatilisation.

To simulate a rapid infiltration land treatment system for wastewater microcosms were used (Piwoni et al., 1986). The microcosms consisted of 1.5 metre soil columns filled with a fine sandy soil with sampling ports at various depths. The top of the column was closed in a ‘green house’ and air was replaced every 8 minutes. Nitrobenzene containing wastewater was added to the soil during a 12 week acclimatisation period. After that the columns received wastewater containing nitrobenzene at a concentration of 271 µg/litre each day (every 4 hours at a dosage of 4.4 ± 0.17 cm^3/day). The water samples were analysed by extraction and GC-analysis. As a result, only less than 0.1% of the nitrobenzene volatilised from the column and less than 0.1% were found in the final effluent which means that more than 99.9% were degraded. As only primarily biodegradation was determined in adapted soil samples, results cannot be taken for the derivations of kinetic biodegradation rates in soil.

There are contradictory results on the volatilisation behaviour and biodegradability of nitrobenzene in soil. In one study the elimination of nitrobenzene was due to volatilisation and the other study shows that more than 99% of the nitrobenzene was primarily degraded and almost no nitrobenzene evaporated. No explanation for this inconsistency can be given.

3.1.3.1.4 Summary of environmental degradation

Summary Photodegradation in water and air

In summary photodegradation of nitrobenzene both in the hydrosphere and in the atmosphere seems to be a slow process. Nitrobenzene is expected to be a long lived contaminant in urban air. It is expected to be slowly removed from the atmosphere by physical processes, including dry deposition ($t_{1/2}$ > 2 months) (Grosjean, 1991). But this reaction mechanism is considered to be speculative and the study of Grosjean is therefore not considered valid.

The nitrate-induced photooxidation maybe a significant transformation mechanism for nitrobenzene in shallow, clear water bodies with high ratios of nitrate to DOC concentrations (Zepp et al., 1987). But in deep water bodies oxidation of organic chemicals by this process is much slower because of the strong attenuation of the UV light required to initiate nitrate photolysis.

The half-life of nitrobenzene in water due to photo-oxidation with OH-radicals can vary in a rather wide range depending on the hydroxyl radical concentration in water and the second order rate constant.

Besides the poor biodegradability and the stability to hydrolysis, photolysis of nitrobenzene also does not play an important role under environmental conditions.

Both direct photolysis and the photochemical reaction with OH-radicals only take place in the upper layers of surface water. Experimentally determined photodegradation half-lives of nitrobenzene range from 25-400 days. These laboratory results, carried out with distilled or tap water, do not represent environmental conditions, where the surface water is normally deeper and muddier. Considering the total water body, the environmental half-lives are expected to be significantly higher than the results from laboratory studies.

Table 3.3 Summary of photodegradation

Compartment	Degradation process	Half-life period [d]
Air	Photo-oxidation with OH radicals (calculation)	65.8
Water	Direct photoysis	133
Water	Photo-oxidation with OH radicals	25-400 (∞)

Summary of biodegradation results

The available biodegradation tests show a great variation of the results.

It can be stated that nitrobenzene is not biodegradable with unadapted inoculum. Regarding the various non-standard biodegradation tests there are contradictory results. Some tests show high elimination rates without stripping of nitrobenzene while in other tests the elimination appears almost completely to be due to evaporation.

The Zahn-Wellens test conducted with industrial inoculum showed no biodegradation within 10 days but volatilisation of nitrobenzene from the physico-chemical batch. Hence for reasons of precaution a rate constant of 0 h^{-1} is derived according to the TGD as an input parameter for Simple Treat calculations.

However, the tests and the monitoring data show clearly that adapted microorganisms in industrial WWTP have the ability to biodegrade nitrobenzene at high levels (over 90%). But occasionally there are significant fluctuations in the degradation behaviour and then nitrobenzene is partly degraded at only 34%. The reason for this has not yet been found out so far. Toxic effects to microorganisms may play a role.

Anaerobic biodegradation seems to be a more efficient way of eliminating nitrobenzene from wastewater. Even with unadapted microorganisms relatively high elimination rates are achieved. With adapted microorganisms the elimination rate is still higher. But as only primary degradation takes place, aniline as a reduction product is formed. This is not further anaerobically biodegradable and has to undergo subsequent aerobic biodegradation. Under aerobic conditions aniline is readily biodegradable. This substance has been subject to a recent risk assessment under the Existing Substances Regulation (see EU Risk Assessment Report on Aniline).

Nitrobenzene is considered to be not inherently biodegradable. Hence the rate constants $kbio_{wwtp}$, $kbio_{water}$, $kbio_{soil}$ and $kbio_{sed}$ *) are set to be 0 d^{-1}.

*) As the sediment in general consists of a relatively thin oxic top layer (10%) and anoxic deeper layers (90%), anaerobic conditions dominate there. Nitrobenzene can be primarily degraded to aniline under anaerobic conditions. Aniline again forms covalent bonds to humic acids under anaerobic conditions and can therefore accumulate in the sediment. But as the sediment is not the targeted compartment for nitrobenzene, this mechanism has only limited relevance for the environment. So the approach to allocate a rate constant of 0 d^{-1} to the sediment seems to be fully justified.

3.1.3.2 Distribution

3.1.3.2.1 Adsorption

A chemical's ability to bind or adsorb to soils is characterised by its organic-carbon partition coefficient Koc. On the basis of the log Pow value (1.86) and according to the TGD equation for phenols, anilines, benzonitriles and nitrobenzenes ($\log Koc = 0.63 \log Kow + 0.90$) the Koc value is calculated as 118 l/kg (for calculation see **Appendix D**). This calculated Koc value is located within the range of the experimentally determined values and is taken in all model calculations of this report. The value of 118 l/kg does not indicate a significant potential for geoaccumulation. If nitrobenzene is released or deposited to soil most of the substance is expected to leach through the soil into the groundwater. To a smaller extend nitrobenzene is likely to volatilise to the atmosphere.

The adsorption of nitrobenzene to 2 different soils (organic carbon content of 2.58% for soil 1 and 1.82% for soil 2) at a initial concentration range from 2-100 mg/l was tested (Loekke, 1984). The adsorption behaviour could sufficiently be described by a Freundlich isotherm and Koc-values of 170-370 l/kg were calculated. The tests were carried out at two different temperatures (5°C and 21°C). At the lower temperature the experiments were performed for 72 hours, the test duration at the higher temperature was 48 hours. The results are as follows:

Soil 1: 5°C: 210 l/kg
21°C: 170 l/kg

Soil 2: 5°C: 170 l/kg
21°C: 370 l/kg

The results show a broad distribution and no temperature dependence. It seemed not justified to calculate a mean value out of these 4 results.

The soil sorption capacity of three different types of soils was examined (Seip et al., 1986). The three soil columns were supplied with water containing nitrobenzene (1.0 mg/l) and eluted with tap water, respectively. For the sandy forest soil with 0.2% organic carbon a Koc of 30.6 was calculated. The silty agriculture soil (2.2% organic carbon) and the silty forest soil (3.7% organic carbon) result in Koc values of 88.8 and 103 l/kg, respectively.

In another test soil columns (sandy soil, 0.087% organic C) were fed with aqueous solutions of nitrobenzene (Wilson et al., 1981). With the concentration in the effluent the migration velocity of nitrobenzene relative to water was obtained. The retardation factor experimentally determined was 1.5–2.3 (calculated value 1.4). This leads to the conclusion that nitrobenzene is mobile in soil which is in accordance with the conclusion drawn from the calculated and measured Koc-values.

The Koc-value was also calculated from the water solubility and from the Kow (Roy and Griffin, 1985). The resulting Koc-values are 79 and 62 l/kg, respectively. Although the equations used for calculations are not specifically designed for substituted benzenes, the calculated Koc-values lie in the same order of magnitude as the other calculated and experimentally determined values.

The solid-specific partition coefficients Kp were estimated for soils, sediments, suspended matter based on the Koc value of 118 l/kg.

Table 3.4 presents the calculated soil/water partition coefficients of these compartments based on the Koc value.

Table 3.4 Calculated partition coefficients for nitrobenzene

Compartment	Partition coefficient*)	
Soil-water	Kp_{soil} = 2.36 l/kg	$Kp_{soil\text{-}water}$ = 3.74
Sediment-water	Kp_{sed} = 5.90 l/kg	$Kp_{sediment\text{-}water}$ = 3.75
Suspended matter-water	Kp_{susp}= 11.8 l/kg	$Kp_{suspension\text{-}water}$= 3.85
Sewage sludge-water, calculated	Kp_{sludge}= 43.7l/kg	

*) For the calculation see Appendix G

3.1.3.2.2 Volatilisation

With a vapour pressure of 20 Pa and a water solubility of 1,900 mg/l a Henry´s law constant of 1.296 $Pa \cdot m^3 \cdot mol^{-1}$ at 20°C was calculated. This value is used for all model calculations in this report. A Henry constant of 2.23 $Pa \cdot m^3 \cdot mol^{-1}$ is estimated by Thomas (1990), who stated that in the range of $1.013 < H < 101.3$ $Pa \cdot m^3 \cdot mol^{-1}$, liquid phase and gas phase resistances are both important. Volatilisation for compounds in this range is less rapid than for compounds in

a higher range of H but is still a significant transfer mechanism. The substance therefore remains preferably in water, but it can also be assumed that a slight volatilisation from an aqueous solution takes place.

The volatilisation rate constant and half life of nitrobenzene due to evaporation from two lakes near Istanbul were predicted (Ince, 1992). For lake 1 a half life of 8 days (rate constant was 0.0036 h^{-1}) and for lake 2 a half life of 20 days (rate constant = 0.00147 h^{-1}) were predicted. The differences were due to the different depths of the lakes (lake 2 is 2.5 times deeper than lake 1) and due to different water evaporation rates (in lake 2 the evaporation rate is about two times lower than in lake 1).

Table 3.5 Air/Water Partition Coefficients

Compartments	Partition Coefficient	Value	Source
Henry's law constant	H	1.296 Pa m^3 mol^{-1}	calculated
Henry's law constant	log H	0.1126	calculated
Air/Water partitioning	K_{air_water}	$5.32 \cdot 10^{-4}$	calculated

3.1.3.2.3 Distribution in wastewater treatment plants

Based on the physico-chemical properties of nitrobenzene and the rate constant for biodegradation of 0 h^{-1} the elimination of nitrobenzene in municipal WWTPs is calculated with the SIMPLETREAT 3.0 model (debugged version, February 1997) in accordance with the TGD (see **Appendix G**).

Table 3.6 Behaviour of Nitrobenzene in WWTP according to the Simple Treat Model

	$kbio_{stp}$ = 0 h^{-1}
Evaporation to air [%]	2.5
Release (dissolved) to water [%]	96.7
Adsorption to sewage sludge [%]	0.8
Degradation [%]	0
total elimination from waste water [%]	3.3

These values calculated with SimpleTreat are only of theoretical interest as no nitrobenzene is supposed to enter municipal waste water treatment plants.

Considering the high elimination rates of the industrial WWTPs (over 90%) the regional and continental concentrations were calculated with an average elimination rate of the industrial WWTPs (see also Section 3.1.8).

3.1.3.3 Accumulation and metabolism

Bioaccumulation

In the MITI-list (CITI 1992) the bioaccumulation of nitrobenzene in the fresh water species *Cyprinus carpio* was ascertained. The used guideline corresponds to the guideline OECD

305 C "Bioaccumulation: Test for the degree of bioconcentration in fish". The test concentrations were 0.125 and 0.0125 mg/l, respectively, at 25 ± 2°C and the lipid content of the test organisms varied between 2 and 6%. At a nitrobenzene concentration of 0.125 mg/l a BCF in the range of 3.1-4.8 was determined during an exposure period of 42 days. At the concentration of 0.0125 mg/l the BCF varied between 1.7 and 7.7.

The bioaccumulation of nitrobenzene in fish, algae and activated sludge was also examined (Freitag et al. 1982). Experimental protocols were described in detail in Korte et al., 1978. For the fish test the golden orfe *Leuciscus idus melanotus* was chosen as test organism. Five fish weighing about 1.5 g each were exposed to 50 µg/l of ^{14}C-labelled nitrobenzene for three days in a closed system. The fish were not fed during this time and no aeration took place. After three days the radioactivity in the whole fish was determined and referred to the average constant concentration of nitrobenzene in the water. A BCF of < 10 (related to wet weight) was calculated. For the algae test the green alga *Chlorella fusca* was used. Algae (20 mg d.w./200ml) were exposed to 50 µg/l ^{14}C-labelled nitrobenzene for 24 hours. After this time algal cells were separated by centrifugation and the radioactivity was measured in the algae and in the supernatant. A BCF of 24 (related to wet weight) could be determined. In the third test activated sludge from a municipal sewage treatment plant (1 g dw/l) was exposed to 50 µg/l ^{14}C-labelled nitrobenzene in a nutrient solution for five days. Then an aliquot was taken and filtered. From measurement of the radioactivity in the filtrate and in the residue the distribution of nitrobenzene between activated sludge and water was obtained. An enrichment factor of 40 (related to dry weight) could be calculated.

In another study (Geyer et al., 1984) bioaccumulation of nitrobenzene in the alga *Chlorella fusca var. vacuolata* was examined. Algae were exposed to a nitrobenzene concentration of 50 µg/l in nutrient solution at room temperature (20–25°C). The experimentally determined bioconcentration factor is 24.

Also experiments with female guppies (*Poecilia reticulata,* 5 to 8 months old) were performed (Deneer et al., 1987). The mean fat content was 8 ± 2%. The test concentration was 1/5 of the LC_{50} (100 µmol/l=12.3 mg/l). Nitrobenzene solutions were renewed daily. After 3 days the nitrobenzene content of the individual fish was determined. The BCF_{fish} on the basis of fat weight varied from 22.4 to 38.9. The authors state that the relatively low BCF for nitrobenzene might be due to experimental difficulties in the determination of nitrobenzene in fish, due to the relatively high volatility of this compound.

According to the relationship developed by Veith et al. (1979) and proposed in the Technical Guidance Documents, a BCF of 7.6 $l \cdot kg^{-1}_{wet\ fish}$ can be estimated from log BCF = 0.85 · log Kow. This value is in good accordance with the measured values.

On a worst case basis, the experimentally derived mean BCF of 30.6 (Deneer et al., 1987, see above) is used for all further calculations.

Summary of bioaccumulation

The different experiments show that nitrobenzene seems to have a low bioaccumulation potential. In all available tests conducted with fish BCF values were clearly below 100.

Geoaccumulation

Both the calculated and measured solid-specific partition coefficients do not indicate a significant potential for geoaccumulation. If nitrobenzene is released or deposited to soil, most of the substance is expected to leach through soil into the groundwater and partly volatilise to the atmosphere.

3.1.4 Aquatic compartment (incl. sediment)

3.1.4.1 Calculation of predicted environmental concentrations (PEC_{local})

In the Technical Guidance Document a generic (i.e. non site-specific) exposure scenario for the release of intermediates to surface water during production and processing is proposed. This scenario is described in the Emission Scenario Document (ESD) IC -3- "chemicals used in synthesis; intermediates" and reflects a realistic worst case situation. For this generic local exposure estimation an average production volume of 300,000 tonnes/annum, which is processed on site, is used, as the production volume of the three greatest producers is between 200,000 and about 380,000 tonnes/annum.

3.1.4.1.1 Calculation of PEC_{local} for production and processing

a) Estimation of C_{local} for production and processing / Generic approach

The generic exposure scenario for production and processing of 300,000 tonnes nitrobenzene/year leads to a $C_{localwater}$ = 1.790 mg/l.

Default emission factors of 0.3% for production and 0.7% for processing and 300 days of emission per year were used for the calculation. According to the TGD $C_{localwater}$ was calculated with a default effluent discharge rate of an industrial STP of 10,000 m^3/day and a default dilution factor of 40.

The WWTP elimination rate used for calculation was 92.8%. It is the mean value of the six known elimination rates of the companies industrial waste water treatment plants (data from companies B, D, E, F, G and H).

b) Estimation of C_{local} for production and processing/Site-specific approach

The discharges of nitrobenzene during production and processing are assessed as point source emissions because the individual production/processing sites are identifiable. For the site-specific scenarios all nitrobenzene producers as well as all known nitrobenzene processing sites are considered. The emission factors are calculated from site-specific yearly releases into the respective waste water treatment plants or, at the site(s) without WWTP, to surface water and the production/processing amount. For the sites where production and processing takes place these factors are in the range of 0.006 to 0.04 kg/tonne and hence are considerably lower than the TGD default values. Two of the mere processing sites have the highest emission factors. These are higher than the TGD defaults. However, there results no concern for the environment out of this relatively high emission factors as the corresponding industrial WWTPs have high elimination rates.

For the Ceffluent, which is the basis for the assessment of treatment plants, the 90 percentile value or, if the compound was not detected, the detection limit is used.

For releases into rivers, the C_{local} is calculated with the $C_{effluent}$ and a dilution factor resulting from waste water and river low flow (10 percentile or 1/3 of the mean flow). According to new TGD the maximum dilution factor is 1,000.

The PEC_{local} includes the $PEC_{regional}$ of 0.01 µg/l (see Section 3.1.8).

There are 5 sites where production and processing takes place. At 3 sites only processing occurs. The following table lists the calculated PECs and the respective data used in the calculations (for calculation see confidential files). The detailed information provided and the calculations themselves are not included in this report. They can be made available to Member States Competent Authorities, as a confidential annex, on request.

The PEC for microorganisms in the STP (PEC_{stp}) equals the concentration in the effluent of a STP. The concentrations in the specific STPs vary from < 1 µg/l to 140 µg/l. However, at site E, a much higher PEC_{stp} of 1,000 µg/l occurs.

The total discharge during nitrobenzene production and processing for all known production and processing sites are summarised to be 6.6 tonnes/annum released directly to surface water and about 14 tonnes/annum directed to industrial waste water treatment plants.

In the TGD a calculation method for the estimation of PEC_{local} in sediment is proposed. As nitrobenzene is not expected to adsorb to organic matter a $PEC_{sediment}$ is only calculated for site C because it shows the highest PEC_{local} for water. Using the corresponding $PEClocal_{water}$ of 8.35 µg/l and the calculated partition coefficient between suspended matter and water (ksusp-water = 3.849 $m^3 \cdot m^{-3}$) the following $PEC_{sediment}$ was calculated (see **Appendix B**)

$$PEC_{sediment} = 0.03 \text{ mg/kg ww}$$

Table 3.7 Data used in local aquatic exposure assessment

Site		Site specific information	Release Factor [kg/t] *)	Clocal $_{water}$ [µg/l]	PEClocal $_{water}$ [µg/l]
A	Production Processing	Effluent concentration, river flow rate	$3.5 \cdot 10^{-4}$	0.86	0.87
B	Production Processing	Effluent concentration and effluent discharge rate, river flow rate	0.03	0.07	0.08
C	Production Processing	No WWTP	0.017	8.33	8.35
D	Production Processing	Effluent concentration and effluent discharge rate, river flow rate, emission days	0.007	0.02	0.03
E	Production Processing	Effluent concentration and effluent discharge rate, river flow rate	0.01	2.77	2.78
F	Processing	Effluent discharge rate, river flow rate	0.05	$1 \cdot 10^{-3}$	0.01
G	Processing	generic	3.5	$6 \cdot 10^{-3}$	0.02
H	Processing	Effluent concentration, river flow rate	26.1	0.003	0.02

*) For sites where both production and processing takes place release factors have not been calculated separately for production and processing, respectively,.

Site B $Clocal_{water}$ was calculated on the basis of the 90 percentile value of the WWTP effluent. There were only 16 random sampling measured over a period of 18 months. Hence the value for $clocal_{water}$ might be an underestimation of the real situation, also due to the fact that there are inexplicable fluctuations in the biodegradation behaviour of nitrobenzene. However, all measured values are under the $PNEC_{surfacewater.}$

Site E At this site, not a conventional WWTP is used but a set of four reed beds (constructed wetland). The effluent of this constructed wetland is emitted into a channel, for which only an average flow is available.

Site F $clocal_{water}$ was calculated on the basis of the annual discharge rate.

3.1.4.1.2 Calculation of PEC_{local} for formulation

As no formulation takes place a calculation for a separate PEC_{local} is not necessary.

3.1.4.1.3 Calculation of PEC_{local} for the use in consumer products

As no information is available on the use of nitrobenzene in consumer products no such scenario was calculated.

3.1.4.2 Measured levels

The large amount of nitrobenzene monitoring data provides a basis for comparing the calculated exposure data with measured ones. However, the available monitoring data are partly relatively old and cannot be assigned to the individual emission sources. They only provide an indication of the orders of magnitude which are to be expected.

Most of these monitoring data refer to the German river Rhine, but data on other rivers, as Elbe, Main, Ruhr etc. also exist. **Tables C1** and **C6** in **Appendix C** present summaries of published data for surface water (**Table C1**) and ground water (**Table C6**). Additional monitoring data for the hydrosphere from other countries (Japan, USA and the Czech Republic) are also compiled in these tables.

Most of the 90-percentile values in surface water are below 1 µg/l. There are a few measured maximum values that exceed 1 µg/l in the river Rhine which are in the range between 1.2 µg/l and 22.5 µg/l. The highest value was measured in the river Rhine at Mainz. The monitoring data for the river Elbe show values of about 0.5 µg/l at Zollenspieker (the city Hamburg is situated there) and Semannshöft (also close to Hamburg), respectively. Only in the Czech Republic higher concentrations for the river Elbe of maximum 5.2 µg/l were measured.

Nitrobenzene was detected in groundwater near a former ammunition plant in Leverkusen (Germany) at a concentration of 1 µg/l.

Sediment

Sediment monitoring data are scarcely found in the literature (see **Appendix C**, **Table C2**) because nitrobenzene is not likely to adsorb to organic matter. The low water/soil and water/sediment partition coefficients of 3.7-3.8 m^3/m^3 (**Table 3.4**) confirm this.

Sediment concentrations in samples of the river Rhine in Germany at different locations are found to be less than 10 µg/ kg dry substance with two exceptions of 18 and 26 µg/kg dry substance (LWA NRW, 1989).

3.1.4.3 Comparison between predicted and measured levels

The available monitoring data cannot be assigned to the individual emission sources. They only provide an indication of the orders of magnitude which are to be expected. However, the comparison of monitored and predicted water concentrations in fresh water rivers show good accordance. Both the measured and the predicted concentrations are mostly below 1 µg/l.

The default TGD calculation on production/processing shows a nitrobenzene concentration in the aquatic compartment that cannot be confirmed by monitoring data.

Monitoring data show regional differences in pollution levels. The river Rhine is the only river in Germany with monitoring data of nitrobenzene exceeding 1 µg/l.

The calculated $PECregional_{surfacewater}$ of 0.01 µg/l is confirmed by these monitoring investigations.

3.1.5 Terrestrial compartment

The release of nitrobenzene to soil occurs through atmospheric deposition after local releases to the atmosphere at the production and processing sites. The input through sludge application on agricultural soil is considered negligible. Nitrobenzene does not partition to a significant extent to sewage sludge in the WWTP (according to Simple Treat only 0.8% of the nitrobenzene in a WWTP enters the sludge). The log Pow of 1.86 (Koc = 118 l/kg) also indicates a low potential for adsorption to organic matter. In addition all nitrobenzene enters industrial WWTPs which sludges are not applied to agricultural soil.

3.1.5.1 Calculation of PEC_{local}

3.1.5.1.1 Calculation of PEC_{local} for production and processing

With the worst case deposition rate of $DEPtotal_{ann}$ of 0.84 $\mu g \cdot m^2 \cdot d^{-1}$ calculated for site C in Section 3.1.6.1.1 the maximum equilibrium soil concentration in the vicinity of a production/processing plant can be calculated according to the procedure proposed in the TGD. The calculations are presented in **Appendix H**, the resulting concentrations in natural soil and in agricultural soil are equal.

bulk soil concentration: $PEClocal_{soil} = 2.3 \cdot 10^{-3}$ mg/kg ww
porewater concentration: $PEClocal_{soil\text{-}porew} = 1.1$ µg/l

3.1.5.1.2 Calculation of PEC_{local} for formulation

No PEC_{local} for formulation has to be calculated because no formulation takes place.

3.1.5.1.3 Calculation of PEC_{local} for the use in consumer products

As no nitrobenzene is used in consumer products no PEC_{local} was calculated for this scenario.

3.1.5.2 Measured levels

Measured nitrobenzene values in soil are only available for 3 sites in Ontario and Quebec (Canada). There 100-150 µg/kg dry soil were found in agricultural soil (Webber and Wang, 1995).

These values are considerably higher than the calculated values in this report. But there is no information available whether possible entries of nitrobenzene via sludge in these soils took place. Of the 10 sites examined in different provinces of Canada nitrobenzene was only found in these 3 samples. In the other seven soil samples no nitrobenzene could be detected.

3.1.6 Atmosphere

Direct releases of nitrobenzene into the atmosphere occur during production and processing . Releases from industrial waste water treatment plants can be expected. With a Henry constant of 1.3 $Pa \cdot m^3 \cdot mol^{-1}$(calculated) the substance can be regarded as moderate volatile from aqueous solution. Using the SIMPLETREAT model releases from WWTPs are estimated to be 2.4% of the total quantity entering the waste water treatment plant.

3.1.6.1 Calculation of PEC_{local}

3.1.6.1.1 Calculation of PEC_{local} for production

For all production/processing sites specific air emission data are available. All data refer to the year 2000, with the exception of site H, for which data refer to 1996.

As for sites A and G no current figures were available the emission data from former years, which refer to other production/processing figures, were used and with the production/processing figures of the year 2000 converted to the respective emission data.

The emission factors (kg/tonne) are calculated from site-specific yearly release into air and the production/processing amount. For the sites where production and processing take place these factors are in the range between $3 \cdot 10^{-6}$ and $2 \cdot 10^{-3}$ kg/tonne and hence are considerably lower than the TGD default values. The calculations are presented in confidential files.

The results of the calculations are summarised in the following table.

Table 3.8 Data used in local atmospheric exposure assessment

Site		Site specific release information	Release Factor [kg/t]	$clocal_{air\_ann}$ [mg/m³]	$PEClocal_{air\_ann}$ [mg/m³]	$DEPtotal_{ann}$ [mg/(m² · d)]
A	Production Processing	x	$2.3 \cdot 10^{-5}$	$2.3 \cdot 10^{-4}$	$2.3 \cdot 10^{-4}$	$3.4 \cdot 10^{-4}$
B	Production Processing	x	$1.5 \cdot 10^{-5}$	$1.3 \cdot 10^{-4}$	$1.3 \cdot 10^{-4}$	$1.9 \cdot 10^{-4}$
C	Production Processing	x	$1.6 \cdot 10^{-3}$	$4.6 \cdot 10^{-4}$	$4.6 \cdot 10^{-4}$	$8.4 \cdot 10^{-4}$
D	Production Processing	x	$4.6 \cdot 10^{-4}$	$5.6 \cdot 10^{-5}$	$5.7 \cdot 10^{-5}$	$1.1 \cdot 10^{-4}$
E*)	Production Processing	x	$3.0 \cdot 10^{-6}$	$3.0 \cdot 10^{-5}$	$3.0 \cdot 10^{-5}$	$4.4 \cdot 10^{-5}$

Table 3.8 continued overleaf

Table 3.8 continued Data used in local atmospheric exposure assessment

Site		Site specific release information	Release Factor [kg/t]	clocal$_{air_ann}$ [mg/m^3]	PEClocal$_{air_ann}$ [mg/m^3]	DEPtotal$_{ann}$ [mg/(m^2 · d)]
F	Processing	x	$6.0 \cdot 10^{-7}$	$4.4 \cdot 10^{-5}$	$4.4 \cdot 10^{-5}$	$6.3 \cdot 10^{-5}$
G	Processing	TGD, B-table	0.001	$2.1 \cdot 10^{-5}$	$2.1 \cdot 10^{-5}$	$3.5 \cdot 10^{-5}$
H	Processing	x	0.82	$6.2 \cdot 10^{-5}$	$6.3 \cdot 10^{-5}$	$1.6 \cdot 10^{-4}$

*) At this site a set of four reed beds (constructed wetland) is used as a WWTP, covering a total area of 10,000 m^2. It can be assumed that considerably higher air emissions occur at this site than at the other sites with conventional WWTPs. But these air emissions are not quantifiable and hence all figures were calculated with the fraction of emission directed to air (2.5 %) which was obtained by Simple Treat calculation.

3.1.6.1.2 Calculation of PEC_{local} for formulation

No PEC_{local} for formulation has to be calculated because no formulation takes place.

3.1.6.1.3 Calculation of PEC_{local} for the use in consumer products

As nitrobenzene is not used in consumer products no PEC_{local} was calculated for this scenario.

3.1.6.2 Measured levels

For Europe no measured air concentrations are available.

For the US some data for exist. In an industrial area in New Jersey (US) values in the same order of magnitude as the predicted values were measured; the average value was 0.4 µg/m^3 and the maximum value was 3.5 µg/m^3 (Bozzelli and Kebbukus, 1982). In urban areas of New Jersey nitrobenzene in a concentration range of 0.36-0.51 µg/m^3 was measured in summer (average values). In winter the average values were less than the detection limit of 0.26 µg/m^3. (Harkov et. al, 1984). Measured data from a smog episode over Los Angeles, California, gave 0.05 µg/m^3 as the highest value (Fraser et. al, 1998).

3.1.6.3 Comparison between predicted and measured levels

The predicted air concentrations are in the range of 0.02-0.5 µg/m^3. These values refer to all industrial sites and not to residential areas. These data are in good accordance with the measured values from the US.

3.1.7 Secondary poisoning

Nitrobenzene has some potential for persistence. But there are no indications for bioaccumulation potential of nitrobenzene. Neither has nitrobenzene a log Kow ≥ 3 nor is it highly adsorptive or belongs to a class of substances known to have a potential to accumulate in living organisms. The TGD indicates that substances which have a potential to cause toxic

effects if accumulated in higher organisms should be considered in the effects assessment for secondary poisoning only if there is an indication of their bioaccumulation potential.

As nitrobenzene has only a low bioaccumulation potential it is not necessary to carry out a risk characterization for secondary poisoning.

3.1.8 Calculation of $PEC_{regional}$ and $PEC_{continental}$

All releases from point sources are considered in the determination of a regional background concentration. The calculations for the regional PECs are performed with Simple Box 2.0 (see **Appendix E**).

Sources for the release into the aquatic compartment and the atmosphere

The local emissions from the production and/or processing of nitrobenzene (8 sites within the EU) are summarised and distributed to the regional and continental area in a ratio of 10% to 90%.

Table 3.9 shows releases of nitrobenzene to the aquatic compartment and atmosphere.

As it is described in Section 3.1.3.1.4 "Summary of environmental degradation" nitrobenzene is considered to be not inherently biodegradable and the rate constant for the degradation of nitrobenzene in WWTPs is therefore 0 d^{-1}.

Nevertheless, industry data show clearly that nitrobenzene is efficiently eliminated in industrial WWTPs. Because of the high elimination rates in the industrial WWTPs the calculations of the regional PECs were conducted with the mean value (92.8%) of the six known elimination rates of the companies industrial waste water treatment plants (data from companies B, D, E, F, G and H).

Table 3.9 Releases to aquatic compartment and atmosphere

Site/scenario	Total release into the hydrosphere [t/a]		Total release into the atmosphere [t/a]
	Directly to surface water	via WWTP	
regional	0.66	1.44	0.08
continental	5.93	12.95	0.74
total	6.59	14.39	0.82

Point releases to soil:

No direct point releases to soil were identified.

In **Appendix E** the input and output figures of the Simple Box 2.0 calculation adapted to the TGD and EUSES 1.00 are presented. (The results of this calculation are consistent with EUSES) The resulting regional concentrations are:

$PECregional_{surface\ water} = 0.01\ \mu g/l$
$PECregional_{air} = 0.05\ ng/m^3$
$PECregional_{agr\ soil} = 5.4\ ng/kg_{ww}$
$PECregional_{agr\ soil\ porewater} = 2.5\ ng/l$
$PECregional_{sediment} = 0.04\ \mu g/kg_{ww}$

3.2 EFFECTS ASSESSMENT: HAZARD IDENTIFICATION AND DOSE (CONCENTRATION) - RESPONSE (EFFECT ASSESSMENT)

3.2.1 Aquatic compartment (incl. sediment)

Many investigations are available concerning the toxicity of nitrobenzene to aquatic organisms from different systematic classes including also several non-standard tests. Tests were regarded as valid if they were performed according to national or international test guidelines or if they are sufficiently documented and scientifically acceptable.

3.2.1.1 Toxicity test results

3.2.1.1.1 Fish

Acute toxicity

The following table gives an overview of the sensitivity of different fish species to nitrobenzene in short-term tests. It covers the full range of species tested. For each species the lowest available valid test was selected, respectively.

Table 3.10 Acute toxicity data to fish

Species	Endpoint	Effect concentrations [mg/l]	Test system	Reference
Brachydanio rerio	96-hour LC_{50}	112.5 (nc)	Static (no standard method)	(Wellens H, 1982)
Brachydanio rerio	96-hour LC_{50}	92 (mc)	Flow-through (OECD Guideline)	(Roederer, 1990)
Brachydanio rerio	14-day NOEC	5 (mc)	Flow-through (OECD Guideline)	(Roederer, 1990)
Leuciscus idus	48-hour LC_0 48-hour LC_{100}	50 (nc) 100 (nc)	Static (no standard method)	(Wellens H, 1982)
Leuciscus idus	48-hour LC_{50}	60 (nc)	Static (no standard method)	(Juhnke and Luedemann, 1978)
Oryzias latipes	48-hour LC_{50}	20 (nc)	static (Japanese Industrial Standards Committee)	(Tonogai et al., 1982)
Oryzias latipes	48-hour LC_{50}	70 (nc)	Semi-static(OECD Guideline)	(Yoshioka and Ose, 1993)
Oryzias latipes	48-hour LC_{50}	125 (nc)	Semi-static	(CITI, 1992)
*Oryzias latipes**	48-hour LC_{50}	1.8 (no information)	No information	(Yoshioka et al., 1986b)

Table 3.10 continued overleaf

* Only very few information are given on the test design and test conditions. The results are presented only in tabular form and can therefore not be validated.

Table 3.10 continued Acute toxicity data to fish

Species	Endpoint	Effect concentrations [mg/l]	Test system	Reference
Pimephales promelas	96-hour LC_{50}	117 (mc)	Flow-through (method: US-EPA)	(Holcombe et al., 1984)
Pimephales promelas	96-hour LC_{50}	119 (mc)	Flow-through (no standard method)	(Geiger et al., 1985)
Pimephales promelas	96-hour LC_{50} 7-day LC_{50} 7-day NOEC (growth)	44 (nc) 39 (nc) < 10.2 (nc)	Flow-through larval test (method: US-EPA) Effect: growth, survival	(Marchini et al., 1992)
Lepomis macrochirus	48-hour LC_{50}	43 (nc)	Static (method: US-EPA)	(Buccafusco et al., 1981)
Cyprinodon variegatus (saltwater)	96-hour LC_{50}	59 (nc)	Static (method: US-EPA)	(Heitmuller et al., 1981)
Poecilia reticolata	14-day LC_{50}	61.7 (mc)	Semi-static (no standard method)	(Deneer et al., 1987)

In acute toxicity tests to fresh (and one salt) water species values in the range from 20 mg/l (*Oryzias latipes*) to 125 mg/l (*Oryzias latipes*) were obtained.

The most sensitive fish species in fresh water seems to be *Oryzias latipes*. A 48-hour LC_{50} of 20 mg/l based on nominal concentrations was calculated (Tonogai et al., 1982). The test was conducted according to the procedure of Japan Industrial Standards (Japanese Industrial Standards Committee, 1971). Fish of the same age were chosen and acclimated for 10 days in tap water before the start of the test. The nitrobenzene test solution was prepared without the use of solvents.

Prolonged toxicity tests with *Pimephales promelas*, exposition at larval stage, showed a 7-day NOEC less than 10.2 mg/l for the endpoint growth and a NOEC (survival) of 38.3 mg/l (Marchini et al., 1992).

In a 14-day prolonged toxicity test, conducted according to OECD Guideline 204, *Brachydanio rerio* showed a NOEC of 5 mg/l (Roederer, 1990).

The experimental values are in reasonable agreement with the QSAR estimation according to the TGD (1996) which results in a fish (96-hour) LC_{50} of 37 mg/l for polar narcotic acting substances.

Long-term toxicity

Only one long-term toxicity test with *Oncorhynchus mykiss* exists (Black et al., 1982).

Log probit analysis was used by the authors to determine the LC_{50} at hatching and 4 days after hatching. Values of 0.002 mg/l for both points of time were obtained.

Table 3.11 Embryo-larval test with *Oncorhynchus mykiss* (Black et al., 1982).

Nitroenzene conc. [mg/l]	Percent hatchability	Percent survival normal organisms	Percent survival normal organisms
		at hatching	4 days posthatching
0.001	64	62	62
0.010	24	21	21
0.12	5	3	3
0.36	0	0	0
0.91	0	0	0
11.9	0	0	0

The effect values found by Black et al. (1982) for several substances other than nitrobenzene (e.g. benzene, toluene) are usually very low compared to effect values found by other authors. No explanation for these large discrepancies could be found. A careful examination of the entire information provided by Black et al. gave no plausible reason for the inconsistency of the data. However, as it was not possible to reproduce the effect values found by Black and his co-workers, it was decided by the EU member states not to use these data for a derivation of a PNECaqua if other valid fish early life stage tests are available. Concerning nitrobenzene no other fish early life stage tests exists. Nevertheless, the effect values found by Black et al. for *Oncorhynchus mykiss* are not employed in the further effects assessment because for other substances it was not possible to confirm the low effect values and it can be assumed that the value for nitrobenzene is also not representative.

3.2.1.1.2 Aquatic invertebrates

Acute toxicity

Table 3.12 shows the available test results for nitrobenzene obtained in short-term tests with aquatic invertebrates.

Table 3.12 Acute toxicity data to aquatic invertebrates

Species	Endpoint	Effect concentrations [mg/l]	Test system	Reference
Daphnia magna	24-hour EC_{50}	50 (nc)	Static (German DIN method) Endpoint: immobilisation	(Bringmann and Kühn, 1982)
Daphnia magna	48-hour EC_{50}	35 (nc)	Semistatic (OECD proposal 1979) Endpoint: behaviour	(Canton et al., 1985)
Daphnia magna	48-hour LC_{50}	27 (nc)	Static (method: US-EPA) Endpoint: mortality	(LeBlanc, 1980)

Table 3.12 continued overleaf

Table 3.12 continued Acute toxicity data to aquatic invertebrates

Species	Endpoint	Effect concentrations [mg/l]	Test system	Reference
Daphnia magna	24-hour EC_{50}	11.2 (mc)	Static (OECD Guideline 202) Endpoint: immobilisation	(Tosato et al., 1991)
Ceriodaphnia dubia	24-hour LC_{50}	54 (mc)	Static (method: US-EPA) Endpoint: mortality	(Marchini et al., 1993)
Mysidopsis bahia[*1]	96-hour EC_{50}	6.68		(LeBlanc, 1984)
Dugesia japonica[*2]	7-day EC_{50} 7-day LC_{50}	1.5 2.0	Endpoint: head regeneration	(Yoshioka et al. 1986b)

Short-term effect values for fresh-water invertebrates between 11 mg/l and 54 mg/l were reported. The most sensitive species seems to be Daphnia magna with a 24-hour EC_{50} of 11 mg/l. The test was conducted according OECD-Guideline 202 in a static test system with analytical monitoring. The concentration of the test solution was determined at the beginning and the end of the test. Test solutions were prepared without solubilising agents. For the further risk assessment the 24-hour EC_{50} of 11 mg/l is used as effect value for short-term toxicity of nitrobenzene to invertebrates.

The experimental EC_{50} values (48-hour) for *Daphnia* are in reasonable agreement with QSAR estimations according to the TGD (1996) which result in a Daphnia (48-hour) EC_{50} of 18 mg/l for polar narcotic acting substances

Long-term toxicity

There are three valid tests on chronic toxicity to Daphnia magna available. A LC50 of 24 mg/l and a LOEC of 18 mg/l were determined in a 21-day reproduction rate test (Maas-Diepeveen and van Leeuwen, 1986). In a semi-static chronic test to daphnia magna a 21-day NOEC of 12.5 mg/l based on nominal concentrations was found. Based on the measured concentration at day 3 after renewal of the test solution the NOEC is 2.6 mg/l (Kühn et al., 1988).

The lowest long-term effect value for *Daphnia magna* is a 21-day NOEC of 1.9 mg/l (measured) with the endpoint reproduction rate (Canton et al., 1985). No information about test conditions is given in this article, but for the performance of the standard tests the authors refer to their former publications (Canton and Slooff, 1982) and Slooff and Canton, 1983. According to this all daphnids (one day old) had been obtained from standardised laboratory cultures, whereas the tests were carried out in analogy to the rules of the Dutch Standardisation Organisation (NEN 6501, 6502, 6504 and 6506 DSO 1980). 25 organisms per group were used and the test volume per group was 1 litre. Daphnids were fed with Chlorella and the test solution was renewed three times a week. In addition to the test description of the Dutch Standardisation Organisation, where only nominal concentrations were reported, the actual concentrations of the test substance were measured in the present test.

[*1] This value is only a numeric value in a table in the cited study. It refers to an unpublished US-EPA study (68-01-4646, 1978). No further information concerning test design and test conditions is given in the EPA-study, hence the value can not be validated.

[*2] Relevant information are missing which allow to assess the validity and reliability of the results of the study. The results are presented only as graphs and in tabular form and can therefore not be validated.

3.2.1.1.3 Algae

In the following table the toxicity data to algae are listed.

Table 3.13 Toxicity data to algae

Species	Endpoint	Effect concentrations [mg/l]	Test system	Reference
Chlorella pyrenoidosa	96-hour EC_{50}	18 (no data whether nc or mc)	Static (OCED-Guideline 201) Endpoint: growth inhibition	(Maas-Diepeveen and van Leeuwen, 1986)
Chlorella pyrenoidosa	72-hour EC_{10} 72-hour NOEC	8.5 (mc) 9.2 (mc)	Static (no standard method) Endpoint: growth inhibition	(Ramos et al., 1999)
Microcystis aeruginosa *Scenedesmus quadricauda*	8-day EC_3 8-day EC_3	1.9 (nc) 33 (nc)	Static (no standard method) Endpoint: growth inhibition	(Bringmann and KühnKühn, 1978)
Scenedesmus obliquus	48-hour EC_{50}	67.7 (nc)	Static (OCED-Guideline 201) Endpoint: growth inhibition	(Liu and Lang, 1995)
Selenastrum capricornutum	96-hour EC_{50}	23.8 (no data whether nc or mc)	Static (US-standard test) Endpoint: growth inhibition	(Bollmann et al., 1989)
Skeletonema costatum	96-hour EC_{50}	10.3*)	Endpoint: photosynthesis effects	(LeBlanc, 1984)

*) This value is only a numeric value in a table in the cited study. It refers to an unpublished US-EPA study (68-01-4646, 1978). No further information concerning test design and test conditions is given in the EPA-study, hence the value can not be validated.

EC_{50}-values for different algal species are in the range from 18 mg/l to 68 mg/l. The lowest effect value from a test with a standardized exposure time of 96 hours was found by Maas-Diepeveen with *Chlorella pyrenoidosa* with a 96-hour EC_{50} of 18 mg/l.

For *Microcystis aeruginosa* Bringmann and Kühn found an 8-day EC_3 of 1.9 mg/l. However, after 8 days the algae may no longer be in the exponential growth phase and this can have a negative influence on the test result. Therefore this low effect value should be used with care.

There are additional toxicity data to algae in the USEPA report "Nitrobenzene Ambient Water Quality Criteria. EPA 440/5-80-061". A 96-hour EC_{50} for the freshwater alga *Selenastrum capricornutum* of 44.1 and 42.8 mg/l based on chlorophyll-A and cell numbers respectively, and a 96-hour EC_{50} for the salt-water alga *Skeletonema costatum* of 10.3 and 9.7 mg/l based on chlorophyll-A and cell numbers are mentioned. These algal data are only figures in a table and have apparently not been published (US EPA 1978, Contract No. 68-01-4646). No test protocol or test description is available and hence the data can not be validated.

3.2.1.1.4 Microorganisms

The following table shows the effect values available for microorganisms. The effect concentrations for bacteria range from 24-hour IC_{50}=0.92 mg/l (*Nitrosomonas*) to 49-hour IC_{50}=370 mg/l (aerobic heterotrophs).

For protozoa the effect data show a range from 1.9 mg/l (72-hour TGK *Entosiphon sulcatum*) up to 98 mg/l (24-hour EC_{50} *Tetrahymena pyriformis*).

Table 3.14 Toxicity to microorganisms

Species	Duration	Effect concentrations [mg/l]	Effect	Reference
Aerobic heterotrophs	49 hours	IC_{50} = 370 (nc)	Inhibition of oxygen uptake	(Blum and Speece, 1991)
Activated sludge	3 hours	EC_{50} = 100 (nc)	Inhibition of oxygen uptake	(Yoshioka et al., 1986a)
Vibrio harveyi	5 hours	EC_{50} = 17.5 (nc)	growth inhibition	(Thomulka et al., 1992)
Methanogens	13 days	NOEC = 10 (mc)	Inhibition of gas production	(Bhattacharya et al., 1996)
Methanogens	96 hours	IC_{50}= 13 (nc)	Inhibition of gas production	(Blum and Speece, 1991)
Nitrosomonas	24 hours	IC_{50} = 0.92 (nc)	Inhibition of ammonia consumption	(Blum and Speece, 1991)
Chilomonas paramaecium (cryptomonad)	48 hours	TGK = 17 (nc) 1)	Inhibition of cell multiplication	(Bringmann et al., 1980)
Entosiphon sulcatum (euglenoid)	72 hours	TGK = 1.9 (nc) 1)	Inhibition of cell multiplication	(Bringmann and Kühn, 1980a)
Tetrahymena pyriformis (ciliate)	24 hours	EC_{50} = 98 (nc)	growth inhibition	(Yoshioka et al., 1985)
Uronema parduczi (ciliate)	20 hours	TGK = 15 (nc) 1)	Inhibition of cell multiplication	(Bringmann and Kühn, 1980b)

1) TGK= toxic threshold concentration, defined as 5% effect compared to the control

There is one study with activated sludge of one of the production sites (BASF AG, 1979). Industrial activated sludge was incubated with nitrobenzene in the presence of nutrient salts, but the exposure period was not reported. An EC_{20} of 1,000 mg/l was found.

The most sensitive microorgamism species is *Nitrosomonas*. The test was conducted in a closed system. Sealed serum bottles were prepared with ammonia feed and 20 ml of surcharged oxygen. Ammonia was measured at the end of the assay period using an ammonia selective electrode. Nitrite was checked to ensure that only toxicity to *Nitrosomonas* and not toxicity to *Nitrobacter* was controlling the rate of metabolic activity. Therefore, the effect value from this test will be used for the risk assessment of sewage treatment plants.

However, according to expert judgement the process of nitrification is not the main mechanism in a constructed wetland like the one used as a WWTP at site E. The activated sludge respiration inhibition test is considered more relevant for the reed bed system and therefore this test will be used for assessing the risk at this specific site.

3.2.1.1.5 Amphibians

There are two studies on amphibians available.

An acute test to *xenopus laevis* (African clawed frog) resulted in a 96-hour LC_{50} of 121 mg/l and in a 96-hour EC_{50} of 54 mg/l (Canton et al., 1985). The description of the method was given by another publication (Canton and Slooff, 1982). Ten animals per group (3-4 weeks old) were exposed to nitrobenzene test solutions over a period of 96 hours in a semi-static test system (renewal of the test substance once a day). Besides the endpoint mortality the effect "behaviour" was not further explained.

In another test to *rana pipiens* (northern leopard frog) a 9-day LC_{50} of 0.640 mg/l was obtained (Black et al., 1982). These effect values found by Black et al. for *rana pipiens* are not employed in the further effects assessment (for explanation please see Section 3.2.1.1.1 Fish – long-term toxicity).

3.2.1.2 Calculation of Predicted No Effect Concentration (PNEC)

Calculation of $PNEC_{aqua}$

For vertebrates, invertebrates and algae no significant differences in sensitivity between marine and freshwater species are recorded.

Results from acute toxicity tests with species from 3 trophic levels are available. The most sensitive organisms from standard tests (EC_{50}) are *Oryzias latipes, Daphnia magna* and *Chlorella pyrenoidosa.*

Reliable long-term NOECs are available for invertebrates (*Daphnia magna*) and several algae species. For fish and other vertebrates only prolonged tests results are available which are not regarded as long-term tests.

Thus, according to the EU Technical Guidance Document the assessment factor is set at 50 for the aquatic compartment as data from valid long-term tests on 2 trophic levels are available. The most sensitive value has been determined for *Daphnia magna* with a 21-day NOEC of 1.9 mg/l.

The PNECaqua is calculated as follows:

$$PNECaqua = 1.9 \text{ mg/l} / 50 = 0.038 \text{ mg/l} = 38 \text{ µg/l}$$

Calculation of the $PNEC_{WWTP}$

For the determination of the $PNEC_{WWTP}$ different tests with microorganisms, bacteria and protozoa, are available.

The lowest effect concentration found was for *Nitrosomonas* with a 24-hour IC_{50} of 0.92 mg/l. Applying an assessment factor of 10 leads to a $PNEC_{WWTP}$ = 92 µg/l. This PNEC is used for the risk characterisation of WWTP at all sites except site E.

For the assessment of the reed bed system at site E the activated sludge respiration inhibition test was considered. It leads to a 3-hour EC_{50} of 100 mg/l. Applying an assessment factor of 100 results in a $PNEC_{WWTP}$ of 1 mg/l for the assessment of this specific site.

3.2.1.3 Toxicity test results for sediment organisms

No sediment tests with nitrobenzene and sediment-dwelling organisms are available.

Nitrobenzene reaches waste water by production and processing industries. From Henry's law constant and the log Pow it can be concluded that nitrobenzene will remain mostly in water and only a small part of it will adsorb to the sediment.

3.2.1.4 Calculation of Predicted No Effect Concentration (PNEC) for sediment organisms

As there is a lack of tests on sediment-dwelling organisms the equilibrium partitioning method can be used as surrogate to calculate the $PNEC_{sediment}$.

$PNEC_{sediment} = K_{susp\text{-}water} / RHO_{susp} \cdot PNEC_{water} \cdot 1{,}000$

where $K_{susp\text{-}water} = 3.85\ m^3/m^3$
$RHO_{susp} = 1{,}150\ kg/m^3$
$PNEC_{water} = 0.038\ mg/l$

The $PNEC_{sed.}$ is 0.127 mg/kg wet weight obtained by this calculation. Using the default water content of sediment from the Technical Guidance Document of 80% by volume or 61.54% by weight this value can be converted to a $PNEC_{sed.}$ = 0.331 mg/kg dry weight.

3.2.2 Terrestrial compartment

3.2.2.1 Toxicity test results

There are toxicity test results both on plants and on terrestrial invertebrates (earthworms) available.

3.2.2.1.1 Plants

In a 72 hours phytotoxicity test eight species of plants were exposed to nitrobenzene in exposure chambers (McFarlane et al., 1990). All plant species examined in this study were provided with nutrient medium containing nitrobenzene. Phytotoxicity to nitrobenzene varies considerably between species. When roots were dosed at 8 mg/l the photosynthesis and transpiration responses vary from no effect to complete suppression.

No visible symptoms or changes in the transpiration or photosynthetic rates occurred with soybeans (*glycine maximus*), barley (*hordeum vulgare*), honeysuckle (*lonicera tatarica*) and poplar (*populus robusta*). For these species the 72-hour NOEC ≥ 8 mg/l. Green ash (*fraxinus pennsylvanica*) and lettuce (*lactuca sativa*) showed no visible symptoms but suffered an initial decrease in both transpiration and photosynthesis rate. The ash plants started recovery after about 10 hours. Lettuce plants recovered much more slowly, the photosynthetic rate started to increase after about 60 hours. Two *Elaeagnus* species seem to be the most sensitive to nitrobenzene. Autumn olive (*Elaeagnus umbellata*) did not survive the dosing of 8 mg/l (LC_{100} = 8 mg/l). Shortly after dosing the transpiration and photosynthetic rate decreased rapidly and did not recover, leaves dropped spontaneously and by the end of the study all remaining leaves dropped when the plants were touched. Russian olive plants (*Elaeagnus angustifolia*) were similar to the autumn olive in that some of the leaves on some of the plants dropped. However, the newest leaves and all leaves on one plant remained intact and continued to function (photosynthesis and transpiration), although at reduced rates. Recovery started after about 10 hours and was complete at the end of the experiment.

Inhibition of root growth of soybean plants (*glycine maximus*) without an accompanied impairment of transpiration and photosynthesis rate was also observed (Fletcher J et al., 1990). The plants were exposed to nitrobenzene concentrations of 0.02 to 100 mg/l via roots and

harvested after 72 hours. The lower concentration of nitrobenzene did not appear to cause plant damage or alter shoot growth. But a visual examination of roots before and after nitrobenzene exposure indicated that the highest concentration inhibited root growth.

3.2.2.1.2 Earthworm

The following table shows the results of the toxicity tests to four earthworm species (Neuhauser et al., 1986). All of the reported concentrations are nominal concentrations.

Table 3.15 Toxicity towards earthworm

Species	Duration	Effect concentrations [mg/l]	Effect
Allolobophora tuberculata	48 hours	LC_{50} = 11.6 µg/cm² filter paper	Mortality
	14 days	LC_{50} = 362 mg/kg soil dw	Mortality
Eisenia fetida	48 hours	LC_{50} = 16 µg/cm² filter paper	Mortality
	14 days	LC_{50} = 319 mg/kg soil dw	Mortality
Eudrilus eugeniae	48 hours	LC_{50} = 5.5 µg/cm² filter paper	Mortality
	14 days	LC_{50} = 226 mg/kg soil dw	Mortality
Perionyx excavatus	48 hours	LC_{50} = 10.4 µg/cm² filter paper	Mortality
	14 days	LC_{50} = 343 mg/kg soil dw	Mortality

In the 48-hour test filter paper soaked with 1 ml nitrobenzene solution was put into a glass vial and one animal per glass vial was added. After 48 hours at 20°C in the dark mortality was determined

For the 14 day tests 10 worms per assay were exposed to soil containing various concentrations of nitrobenzene. The artificial soil consisted of 10% peat, 20% kaolinite clay, 69% fine sand and 1% calcium carbonate. An aqueous nitrobenzene solution was then added (water content = 35% of dry weight).

3.2.2.2 Calculation of Predicted No Effect Concentration (PNEC)

From the tests to plants no EC_{50} or NOEC can be derived. Hence for the calculation of the $PNEC_{soil}$ there is only one terrestrial test result available (earthworm). It is calculated with the most sensitive of the earthworm species *Eudrilus eugeniae* with a 14-day LC_{50} of 226 mg/kg soil dw and an assessment factor of 1,000, as results from long-term tests are not available.

$$PNEC_{soil} = 0.226 \text{ mg/kg dw}$$

As there is only one terrestrial test result available the TGD instructs that the risk assessment should be performed both on this test result and on the basis of the outcome of the aquatic toxicity data in this case. A PNECsoil can be derived by using the equilibrium partitioning method.

$$PNEC_{soil} = K_{soil\text{-}water} / RHO_{soil} \cdot PNEC_{water} \cdot 1{,}000$$

With:

$K_{soil\text{-}water} = 3.74 m^3/m^3$
$RHO_{soil} = 1{,}700 \text{ kg/m}^3$
$PNEC_{water} = 0.038 \text{ mg/l}$

A $PNEC_{soil}$ of 0.084 mg/kg ww is obtained by this method. Using the default water content of soil from the Technical Guidance Document of 20% by volume or 11.8% by weight this value can be converted to $PNEC_{soil}$ = 0.10 mg/kg dry weight.

The use of the equilibrium partitioning method based on the $PNEC_{water}$ results in a lower $PNEC_{soil}$. Thus, the $PNEC_{soil}$ derived from the equilibrium partitioning is used for risk assessment.

3.2.3 Atmosphere

There is one short-term test with wheat plants (*Tritium aestivum*) reported (Christ, 1996).

Ten days old plants were kept under greenhouse conditions using a flow-through system with a flow of air containing various concentrations of nitrobenzene (2 m^3/hour) in an exposure chamber of 260 litres. The test lasted over three hours. The maximum light intensity was about 50% compared to outdoor conditions. The endpoint photosynthetic rate was measured as the differences in the CO_2-content between incoming and outgoing air compared to a control without nitrobenzene. Apparently no analytical measurement took place. The following LOEC was found:

Triticum aestivum endpoint: photosynthetic rate 3-hour LOEC = 150 mg/m^3

The author classifies nitrobenzene as a substance which is dangerous to plants at low concentrations.

3.2.4 Secondary poisoning

Nitrobenzene has some potential for persistence. However, there are no indications for bioaccumulation potential of nitrobenzene. Neither has nitrobenzene a log Kow ≥ 3 nor is it highly adsorptive or belongs to a class of substances known to have a potential to accumulate in living organisms. The TGD indicates that substances which have a potential to cause toxic effects if accumulated in higher organisms should be considered in the effects assessment for secondary poisoning only if there is an indication of their bioaccumulation potential. Nitrobenzene does not present an indication of a bioaccumulation potential. An effect assessment for secondary poisoning is therefore not considered necessary.

3.3 RISK CHARACTERISATION

3.3.1 Aquatic compartment (incl. sediment)

Surface water

The PEC/PNEC ratios are below 1 for all production and/or processing sites. The currently available data do not indicate any risk to the aquatic biocenosis. Regarding the $PNEC_{aqua}$ of 38 µg/l the following PEC/PNEC ratios can be calculated:

Table 3.16 PEC/PNEC ratios for the aquatic compartment

Scenario	Site specific PEC_{aqua} [µg/l]	PEC/PNEC
Production/processing sites:		
A	0.87	0.02
B	0.08	< 0.01
C	8.35	0.22
D	0.03	< 0.01
E	2.78	0.07
F	0.01	< 0.01
G	0.02	< 0.01
H	0.02	< 0.01

Sediment

The highest PEC/PNEC ratio for sediment is the same as for water at site C as both the PEC and the PNEC are calculated from the respective water values. Hence no risk for sediment dwelling organisms can be detected.

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (ii) applies to surface water and sediment regarded for the production and/or processing of nitrobenzene. All PEC/PNEC ratios are below 1.

Waste-water treatment plants

A possible risk to microorganisms is only evaluated for industrial waste water treatment plants as no nitrobenzene is expected to enter municipal waste water treatment plants.

Effluent concentrations (=PEC_{stp}) between < 1 µg/l and 140 µg/l were given for the industrial WWTPs at the production/processing sites except for site E where a PEC_{WWTP} of 1,000 µg/l is reported. All data are based on measurements at the respective sites.

Applying the $PNEC_{microorganisms}$ of 92 µg/l for the industrial WWTPs at all sites except for site E and the $PNEC_{microorganisms}$ of 1,000 µg/l for the constructed wetland at site E all ratios of $PEC/PNEC_{microorganisms}$ are ≤ 1.

The maximum effluent concentration at one site is above the PNEC (140 µg/l). At this site daily measurements of the effluent concentrations of the industrial WWTP took place in the year 2000. Only 5 out of 352 values were above the detection limit (12 µg/l) and thereof only two were in the range of the $PNEC_{WWTP}$. Hence, it can be assumed that there is no risk to the WWTP microorganisms at this site.

Conclusion (i) There is a need for further information and/or testing.

No **conclusion (i)** was drawn

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Conclusion (ii) applies to all of the industrial WWTPs at sites A, B, D, E, F, G and H.

3.3.2 Terrestrial compartment

The comparison of $PEClocal_{soil}$ at site C of $2.3 \cdot 10^{-3}$ mg/kg ww with the $PNEC_{soil}$ of 0.084 mg/kg ww indicates no risk for the terrestrial compartment.

Conclusions to the risk assessment for the terrestrial compartment:

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

3.3.3 Atmosphere

There are no representative fumigation tests for nitrobenzene available. An quantitative effect assessment for this compartment therefore can not be performed.

The only result is a 3-hour LOEC of 150 mg/m^3. Comparing this result with the highest $PEClocal_{air\_annual}$ of $4.6 \cdot 10^{-4}$ mg/m^3 at site C no risk to terrestrial plants via air emissions of nitrobenzene can be found as the ratio of $PEClocal_{air\_annual}$ /LOEC is very small ($3 \cdot 10^{-6}$).

Nitrobenzene may be dangerous for the atmospheric environment at low concentrations as it is classified as R48 (”Danger of serious damage to health by prolonged exposure”). There are no long-term or chronic data available that could help decide whether further plant fumigation toxicity data were needed. However, the comparison of the PEC with the LOEC, which leads to a very small risk quotient, indicates in a first approach that nitrobenzene might pose no risk to plants.

The potential of a contribution of nitrobenzene to the formation of harmful ground-level ozone is an aspect which can not be excluded. Nitrobenzene has a relative long half life in the atmosphere ($t_{1/2}$ = 66 days) but, on the other hand, releases to the atmosphere take only place from point sources and no additional entry into the atmosphere, e.g. from traffic emissions, occur. There are no data available on the ozone formation potential of nitrobenzene and no measured air concentrations exist for Europe.

Conclusions to the risk assessment for the atmosphere:

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

3.3.4 Secondary poisoning

Nitrobenzene has some potential for persistence. But there are no indications for bioaccumulation potential of nitrobenzene. Neither has nitrobenzene a log Kow ≥ 3 nor is it highly adsorptive or belongs to a class of substances known to have a potential to accumulate in living organisms. The TGD indicates that substances which have a potential to cause toxic effects if accumulated in higher organisms should be considered in the effects assessment for secondary poisoning only if there is an indication of their bioaccumulation potential.

As nitrobenzene has only a low bioaccumulation potential no risk characterization for secondary poisoning has to be conducted.

3.3.5 PBT-assessment

The following table shows the PBT/vPvB criteria as defined in the TGD and the values relevant for nitrobenzene. The description of the relevant tests can be found in Section 3.1.3.1 (B), 3.1.3.3 (P) and in Section 3.2.1.1 (T).

Table 3.17 Data for nitrobenzene and PBT/vPvB criteria according to TGD

Criterion	PBT-criteria	vPvB-criteria	Nitrobenzene
P	Half-life > 60 days in marine water or > 40 days in freshwater or half-life > 180 days in marine sediment or > 120 days in freshwater sediment	Half-life > 60 days in marine- or freshwater or half-life > 180 days in marine or freshwater sediment	non biodegradable (surface water) no degradation rate constant for the sediment could be derived.
B	BCF > 2,000	BCF > 5,000	BCF (fish) = 30.6
T	Chronic NOEC < 0.01 mg/l or CMR or endocrine disrupting effects	Not applicable	72-hour NOEC (algae): 9.2 mg/l 21-day NOEC (*Daphnie Magna*) = 1.9 mg/l Carcinogenic Cat. 3 Toxic for Reproduction Cat. 3

Nitrobenzene has to be considered as non biodegradable in surface water. No degradation rate constants for degradation of nitrobenzene in sediment or soil could be derived.

The highest measured BCF in fish is 30.6.

The lowest long-term effect value of 1.9 mg/l was found for *Daphnia magna*.

Nitrobenzene is classified as Carcinogenic (Cat. 3) and Toxic for Reproduction (Cat. 3). There is evidence of serious damage to health by prolonged exposure through inhalation and contact with skin (T, R48/23/24).

It can be concluded that nitrobenzene could possibly fulfil the P/vP-criteria. The B- and T-criteria are not fulfilled. Overall nitrobenzene does not meet the PBT criteria.

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 General discussion

4.1.1.2 Occupational exposure

4.1.1.3 Consumer exposure

4.1.1.4 Humans exposed via the environment

Indirect exposure via the environment is calculated using data for oral intake via food, drinking water and air (for calculation see **Appendix A**). One local scenario, site C with the highest PEClocal for surface water, was considered. Following the data for the regional scenario the total daily dose is smaller. The resultant daily doses for the uptake of nitrobenzene are:

DOSEtot = 0.42 µg / kg bodyweight and day (local scenario site C)

DOSEtot = 0.57 ng/kg bodyweight and day (regional background concentrations)

Table 4.1 Results of calculation of the indirect exposure

Intake route	% of total intake	
	Local site C	Regional
Drinking water	47.0	64.9
Air	23.7	1.95
Stem	6.31	0.56
Root	2.44	4.23
Meat	< 0.01	< 0.01
Milk	< 0.01	< 0.01
Fish	20.6	28.4

5 RESULTS

5.1 INTRODUCTION

5.2 ENVIRONMENT

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

No **conclusion (iii)** was drawn.

Conclusion (i) There is a need for further information and/or testing.

No **conclusion (i)** was drawn

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Conclusion (ii) applies to surface water, sediment, the atmosphere and the terrestrial compartment regarded for the production and/or processing of nitrobenzene. All PEC/PNEC ratios are below 1. This conclusion also applies to the industrial WWTPs of all sites.

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Abbreviations

ADI	Acceptable Daily Intake
AF	Assessment Factor
ASTM	American Society for Testing and Materials
ATP	Adaptation to Technical Progress
AUC	Area Under The Curve
B	Bioaccumulation
BBA	Biologische Bundesanstalt für Land- und Forstwirtschaft
BCF	Bioconcentration Factor
BMC	Benchmark Concentration
BMD	Benchmark Dose
BMF	Biomagnification Factor
BOD	Biochemical Oxygen Demand
bw	body weight / *Bw*, *bw*
C	Corrosive (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
CA	Chromosome Aberration
CA	Competent Authority
CAS	Chemical Abstract Services
CEC	Commission of the European Communities
CEN	European Standards Organisation / European Committee for Normalisation
CEPE	European Committee for Paints and Inks
CMR	Carcinogenic, Mutagenic and toxic to Reproduction
CNS	Central Nervous System
COD	Chemical Oxygen Demand
CSTEE	Scientific Committee for Toxicity, Ecotoxicity and the Environment (DG SANCO)
CT_{50}	Clearance Time, elimination or depuration expressed as half-life
d.wt	dry weight / dw
dfi	daily food intake
DG	Directorate General
DIN	Deutsche Industrie Norm (German norm)
DNA	DeoxyriboNucleic Acid
DOC	Dissolved Organic Carbon
DT50	Degradation half-life or period required for 50 percent dissipation / degradation
DT90	Period required for 90 percent dissipation / degradation
E	Explosive (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
EASE	Estimation and Assessment of Substance Exposure Physico-chemical properties [Model]

EbC50	Effect Concentration measured as 50% reduction in biomass growth in algae tests
EC	European Communities
EC10	Effect Concentration measured as 10% effect
EC50	median Effect Concentration
ECB	European Chemicals Bureau
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECVAM	European Centre for the Validation of Alternative Methods
EDC	Endocrine Disrupting Chemical
EEC	European Economic Communities
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EN	European Norm
EPA	Environmental Protection Agency (USA)
ErC50	Effect Concentration measured as 50% reduction in growth rate in algae tests
ESD	Emission Scenario Document
EU	European Union
EUSES	European Union System for the Evaluation of Substances [software tool in support of the Technical Guidance Document on risk assessment]
F(+)	(Highly) flammable (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
FAO	Food and Agriculture Organisation of the United Nations
FELS	Fish Early Life Stage
foc	Organic carbon factor (compartment depending)
GLP	Good Laboratory Practice
HEDSET	EC/OECD Harmonised Electronic Data Set (for data collection of existing substances)
HELCOM	Helsinki Commission -Baltic Marine Environment Protection Commission
HPLC	High Pressure Liquid Chromatography
HPVC	High Production Volume Chemical (> 1000 tonnes/annum)
IARC	International Agency for Research on Cancer
IC	Industrial Category
IC50	median Immobilisation Concentration or median Inhibitory Concentration
ILO	International Labour Organisation
IPCS	International Programme on Chemical Safety
ISO	International Organisation for Standardisation
IUCLID	International Uniform Chemical Information Database (existing substances)
IUPAC	International Union for Pure and Applied Chemistry
JEFCA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues

Koc	organic carbon normalised distribution coefficient
Kow	octanol/water partition coefficient
Kp	solids-water partition coefficient
L(E)C50	median Lethal (Effect) Concentration
LAEL	Lowest Adverse Effect Level
LC50	median Lethal Concentration
LD50	median Lethal Dose
LEV	Local Exhaust Ventilation
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
LOED	Lowest Observed Effect Dose
LOEL	Lowest Observed Effect Level
MAC	Maximum Allowable Concentration
MATC	Maximum Acceptable Toxic Concentration
MC	Main Category
MITI	Ministry of International Trade and Industry, Japan
MOE	Margin of Exposure
MOS	Margin of Safety
MW	Molecular Weight
N	Dangerous for the environment (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC
NAEL	No Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NOEC	No Observed Effect Concentration
NTP	National Toxicology Program (USA)
O	Oxidising (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
OC	Organic Carbon content
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational Exposure Limit
OJ	Official Journal
OSPAR	Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic
P	Persistent
PBT	Persistent, Bioaccumulative and Toxic
PBPK	Physiologically Based PharmacoKinetic modelling

PBTK	Physiologically Based ToxicoKinetic modelling
PEC	Predicted Environmental Concentration
pH	logarithm (to the base 10) (of the hydrogen ion concentration $\{H^+\}$
pKa	logarithm (to the base 10) of the acid dissociation constant
pKb	logarithm (to the base 10) of the base dissociation constant
PNEC	Predicted No Effect Concentration
POP	Persistent Organic Pollutant
PPE	Personal Protective Equipment
QSAR	(Quantitative) Structure-Activity Relationship
R phrases	Risk phrases according to Annex III of Directive 67/548/EEC
RAR	Risk Assessment Report
RC	Risk Characterisation
RfC	Reference Concentration
RfD	Reference Dose
RNA	RiboNucleic Acid
RPE	Respiratory Protective Equipment
RWC	Reasonable Worst-Case
S phrases	Safety phrases according to Annex IV of Directive 67/548/EEC
SAR	Structure-Activity Relationships
SBR	Standardised birth ratio
SCE	Sister Chromatic Exchange
SCHER	Scientific Committee on Health and Environmental Risks
SDS	Safety Data Sheet
SETAC	Society of Environmental Toxicology And Chemistry
SNIF	Summary Notification Interchange Format (new substances)
SSD	Species Sensitivity Distribution
STP	Sewage Treatment Plant
T(+)	(Very) Toxic (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
TDI	Tolerable Daily Intake
TG	Test Guideline
TGD	Technical Guidance Document
TNsG	Technical Notes for Guidance (for Biocides)
TNO	The Netherlands Organisation for Applied Scientific Research
ThOD	Theoritical Oxygen Demand
UC	Use Category
UDS	Unscheduled DNA Synthesis
UN	United Nations

UNEP	United Nations Environment Programme
US EPA	Environmental Protection Agency, USA
UV	Ultraviolet Region of Spectrum
UVCB	Unknown or Variable composition, Complex reaction products of Biological material
vB	very Bioaccumulative
VOC	Volatile Organic Compound
vP	very Persistent
vPvB	very Persistent and very Bioaccumulative
v/v	volume per volume ratio
w/w	weight per weight ratio
WHO	World Health Organisation
WWTP	Waste Water Treatment Plant
Xn	Harmful (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
Xi	Irritant (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)

Appendix A Indirect exposure via the environment

INDIRECT EXPOSURE VIA THE ENVIRONMENT

(TGD On New and Existing Chemicals, chapter 2)

Parameter [Unit]	*Symbol*
Definitions (for the use in this document)	
definition of the unit 'kg$_{bw}$' for body weight	$kg_{bw} := 1 \cdot kg$
definition of the unit 'd' for day	$d := 1 \cdot Tag$
	$scenario := 1 .. 2$
	$local := 1$ $regional := 2$
Constants	
gas - constant R	$R := 8.314 \cdot J \cdot K^{-1} \cdot mol^{-1}$
Defaults	
volumefraction air in plant tissue [-]	$Fair_{plant} := 0.3$
volumefraction water in plant tissue [-]	$Fwater_{plant} := 0.65$
volumefraction lipids in plant tissue [-]	$Flipid_{plant} := 0.01$
bulk density of plant tissue [kg$_{wet\ plant}$ *m$_{plant}$ $^{-3}$]	$RHO_{plant} := 700 \cdot kg \cdot m^{-3}$
leaf surface area [m^2]	$AREA_{plant} := 5 \cdot m^2$
conductance (0.001 m*s^{-1}) [m*d^{-1}]	$g_{plant} := 0.001 \cdot m \cdot s^{-1}$
shoot volume [m^3]	$V_{leaf} := 0.002 \cdot m^3$
transpiration stream [m^3*d^{-1}]	$Q_{transp} := 1 \cdot 10^{-3} \cdot m^3 \cdot d^{-1}$
correction exponent for differences between plant lipids and octanol [-]	$b := 0.95$
growth rate constant for dilution by growth [d^{-1}]	$kgrowth_{plant} := 0.035 \cdot d^{-1}$
pseudo-first order rate constant for metabolism in plants [d^{-1}]	$kmetab_{plant} := 0 \cdot d^{-1}$
pseudo-first order rate constant for photolysis in plants [d^{-1}]	$kphoto_{plant} := 0 \cdot d^{-1}$

concentration in meat and milk

daily intake of grass
[$kg_{wetgrass}*d^{-1}$]

$IC_{grass} := 67.6 \cdot kg \cdot d^{-1}$

daily intake of soil
[$kg_{wet\ soil}*d^{-1}$]

$IC_{soil} := 0.46 \cdot kg \cdot d^{-1}$

daily intake of air
[$m_{air}{}^{3}*d^{-1}$]

$IC_{air} := 122 \cdot m^3 \cdot d^{-1}$

daily intake of drinkingwater
[$l*d^{-1}$]

$IC_{drw} := 55 \cdot l \cdot d^{-1}$

daily intake for human

daily intake for the several pathways
[$kg_{chem}*d^{-1}$] or [$m^{3}*d^{-1}$]

$IH_{drw} := 2 \cdot l \cdot d^{-1}$

$IH_{fish} := 0.115 \cdot kg \cdot d^{-1}$

$IH_{stem} := 1.2 \cdot kg \cdot d^{-1}$

$IH_{root} := 0.384 \cdot kg \cdot d^{-1}$

$IH_{meat} := 0.301 \cdot kg \cdot d^{-1}$

$IH_{milk} := 0.561 \cdot kg \cdot d^{-1}$

$IH_{air} := 20 \cdot m^3 \cdot d^{-1}$

bioavailability through route of intake
[-]

$BIO_{inh} := 0.75$

$BIO_{oral} := 1.0$

average body weight of human
[kg]

$BW := 70 \cdot kg_{bw}$

Name: **Nitrobenzene** CAS - No.: 98 – 95 – 3

Input

chemical properties

octanol-water partitioning coefficient [-]	$\log K_{OW} := 1.86$ $K_{OW} := 10^{\log K_{OW}}$
Henry - partitioning coefficient [Pa*m^3*mol^{-1}]	$HENRY := 1.296 \cdot Pa \cdot m^3 \cdot mol^{-1}$
air-water partitioning coefficient [-]	$K_{air\_water} := 5.32 \cdot 10^{-4}$
fraction of the chemical associated with aerosol particles [-]	$F_{ass\_aer} := 5 \cdot 10^{-6}$
half-life for biodegration in surface water [d]	$DT_{50\_bio\_water} := 1000000\,d$

environmental concentrations

annual average local PEC in surface water (dissolved) [mg$_{chem}$ * l$_{water}^{-1}$] , (from open use)	$PEClocal_{water\_ann} := 6.85 \cdot 10^{-3} \cdot mg \cdot l^{-1}$
annual average local PEC in air (total) [mg$_{chem}$ * m$_{air}^{-3}$] (from open use)	$PEClocal_{air\_ann} := 4.6 \cdot 10^{-4} \cdot mg \cdot m^{-3}$
local PEC in grassland (total), averaged over 180 days [mg$_{chem}$ * kg$_{soil}^{-1}$]	$PEClocal_{grassland} := 2.36 \cdot 10^{-3} \cdot mg \cdot kg^{-1}$
local PEC in porewater of agriculture soil [mg$_{chem}$ * l$_{porewater}^{-1}$]	$PEClocal_{agr\_soil\_porew} := 1.05 \cdot 10^{-3} \cdot mg \cdot l^{-1}$
local PEC in porewater of grassland [mg$_{chem}$ * l$_{porewater}^{-1}$]	$PEClocal_{grassland\_porew} := 1.07 \cdot 10^{-3} \cdot mg \cdot l^{-1}$
local PEC in groundwater under agriculture soil [mg$_{chem}$ * l$_{water}^{-1}$]	$PEClocal_{grw} := 1.05 \cdot 10^{-3} \cdot mg \cdot l^{-1}$
regional PEC in surface water (dissolved) [mg$_{chem}$ * l$_{water}^{-1}$]	$PECregional_{water} := 1.3 \cdot 10^{-5} \cdot mg \cdot l^{-1}$
regional PEC in air (total) [mg$_{chem}$ * m$_{air}^{-3}$]	$PECregional_{air} := 5.2 \cdot 10^{-8} \cdot mg \cdot m^{-3}$
regional PEC in agriculture soil (total) [mg$_{chem}$*kg$_{soil}^{-1}$	$PECregional_{agr\_soil} := 5.4 \cdot 10^{-6} \cdot mg \cdot kg^{-1}$
regional PEC in porewater of agriculture soils [mg$_{chem}$*l$_{water}^{-1}$	$PECregional_{agr\_soil\_porew} := 2.5 \cdot 10^{-6} \cdot mg \cdot l^{-1}$

Definition of the concentrations used for indirect exposure

$C_{water_{local}} := PEClocal_{water\_ann}$ $C_{water_{regional}} := PECregional_{water}$

$C_{air_{local}} := PEClocal_{air\_ann}$ $C_{air_{regional}} := PECregional_{air}$

$C_{grassland_{local}} := PEClocal_{grassland}$ $C_{grassland_{regional}} := PECregional_{agr\_soil}$

$C_{agr\_porew_{local}} := PEClocal_{agr\_soil\_porew}$ $C_{agr\_porew_{regional}} := PECregional_{agr\_soil\_porew}$

$C_{grass\_porew_{local}} := PEClocal_{grassland\_porew}$ $C_{grass\_porew_{regional}} := PECregional_{agr\_soil\_porew}$

$C_{grw_{local}} := PEClocal_{grw}$ $C_{grw_{regional}} := PECregional_{agr\_soil\_porew}$

bioconcentration in fish

bioconcentration factor for fish
[$m_{water}^3 * kg_{chem}^{-1}$]

$$BCF_{fish} := 10^{0.85 \cdot \log K_{OW} - 0.7} \cdot l \cdot kg^{-1}$$

modified equation for logKow > 6

$$BCF_{fish} := wenn\left[\log K_{OW} > 6, \left[-0.278 \cdot \left(\log K_{OW}\right)^2 + 3.38 \cdot \log K_{OW} - 5.94\right] \cdot l \cdot kg^{-1}, BCF_{fish}\right]$$

$$C_{fish_{scenario}} := BCF_{fish} \cdot C_{water_{scenario}}$$

bioconcentration in plants

$$K_{plant\_water} := Fwater_{plant} + Flipid_{plant} \cdot K_{OW}^{b}$$

$$Croot_{agr\_plant_{scenario}} := \frac{K_{plant\_water} \cdot C_{agr\_porew_{scenario}}}{RHO_{plant}}$$

$$TSCF := 0.784 \cdot e^{\frac{-\left(\log K_{OW} - 1.78\right)^2}{2.44}}$$

remark: for $\log K_{OW}$ out of the range from -0.5 to 4.5
the TSCF is limited by the values for $\log K_{OW}$ = -0.5 resp. 4.5

$$TSCF := wenn\left(\log K_{OW} < -0.5, 0.903, TSCF\right)$$

$$TSCF := wenn\left(\log K_{OW} > 4.5, 0.832, TSCF\right)$$

$$K_{leaf\_air} := Fair_{plant} + \frac{K_{plant\_water}}{K_{air\_water}}$$

$$kelim_{plant} := kmetab_{plant} + kphoto_{plant}$$

$$\alpha := \frac{AREA_{plant} \cdot g_{plant}}{K_{leaf\_air} \cdot V_{leaf}} + kelim_{plant} + kgrowth_{plant}$$

$$\beta_{agr\_plant_{scenario}} := C_{agr\_porew_{scenario}} \cdot TSCF \cdot \frac{Q_{transp}}{V_{leaf}} + \left(1 - F_{ass\_aer}\right) \cdot C_{air_{scenario}} \cdot g_{plant} \cdot \frac{AREA_{plant}}{V_{leaf}}$$

$$C_{leaf\_crops_{scenario}} := \frac{\beta_{agr\_plant_{scenario}}}{\alpha \cdot RHO_{plant}}$$

$$\beta_{grass\_plant_{scenario}} := C_{grass\_porew_{scenario}} \cdot TSCF \cdot \frac{Q_{transp}}{V_{leaf}} + \left(1 - F_{ass\_aer}\right) \cdot C_{air_{scenario}} \cdot g_{plant} \cdot \frac{AREA_{plant}}{V_{leaf}}$$

$$C_{leaf\_grass_{scenario}} := \frac{\beta_{grass\_plant_{scenario}}}{\alpha \cdot RHO_{plant}}$$

purification of drinking water

system may defined dependent from the aerobic biodegradation

$$system := wenn\left(DT_{50\_bio\_water} < 10 \cdot d, 0, 1\right)$$

select a column on dependence from log K_{OW}

$$FIndex := wenn\left(logK_{OW} < 4, 0, wenn\left(logK_{OW} > 5, 2, 1\right)\right)$$

$$Fpur_{logKow} := \begin{bmatrix} 1 & \frac{1}{4} & \frac{1}{16} \\ 1 & \frac{1}{2} & \frac{1}{4} \end{bmatrix}$$

$$Fpur := \frac{Fpur_{logKow_{system, FIndex}}}{wenn(HENRY > 100 \cdot Pa \cdot m^3 \cdot mol^{-1}, 2, 1)}$$

$$C_{drw_{scenario}} := wenn\left[C_{grw_{scenario}} > \left(C_{water_{scenario}} \cdot Fpur\right), C_{grw_{scenario}}, C_{water_{scenario}} \cdot Fpur\right]$$

Biotransfer to meat and milk

$$BTF_{meat} := 10^{-7.6 + logK_{OW}} \cdot kg^{-1} \cdot d$$

remark: for $logK_{OW}$ out of the range from 1.5 to 6.5
the BTF_{meat} is limited by the values for $logK_{OW}$ = 1.5 resp. 6.5

$$BTF_{meat} := wenn\left(logK_{OW} < 1.5, 7.943 \cdot 10^{-7} \cdot kg^{-1} \cdot d, BTF_{meat}\right)$$

$$BTF_{meat} := wenn\left(logK_{OW} > 6.5, 0.07943 \cdot kg^{-1} \cdot d, BTF_{meat}\right)$$

$$C_{meat_{scenario}} := BTF_{meat} \cdot \left(\begin{array}{l} C_{leaf\_grass_{scenario}} \cdot IC_{grass} + C_{grassland_{scenario}} \cdot IC_{soil} \cdots \\ + C_{air_{scenario}} \cdot IC_{air} + C_{drw_{scenario}} \cdot IC_{drw} \end{array}\right)$$

$$BTF_{milk} := 10^{-8.1 + logK_{OW}} \cdot kg^{-1} \cdot d$$

remark: for $logK_{OW}$ out of the range from 3 to 6.5
the BTF_{milk} is limited by the values for $logK_{OW}$ = 1.5 resp. 6.5

$$BTF_{milk} := wenn\left(logK_{OW} < 3, 7.943 \cdot 10^{-6} \cdot kg^{-1} \cdot d, BTF_{milk}\right)$$

$$BTF_{milk} := wenn\left(logK_{OW} > 6.5, 0.02512\, kg^{-1} \cdot d, BTF_{milk}\right)$$

$$C_{milk_{scenario}} := BTF_{milk} \cdot \left(\begin{array}{l} C_{leaf\_grass_{scenario}} \cdot IC_{grass} + C_{grassland_{scenario}} \cdot IC_{soil} \cdots \\ + C_{air_{scenario}} \cdot IC_{air} + C_{drw_{scenario}} \cdot IC_{drw} \end{array} \right)$$

total daily intake for human

daily dose through intake of several pathways

[$kg_{chem}*kg_{bw}^{-1}*d^{-1}$]

$$DOSE_{drw_{scenario}} := \frac{C_{drw_{scenario}} \cdot IH_{drw}}{BW} \qquad DOSE_{air_{scenario}} := \frac{C_{air_{scenario}} \cdot IH_{air} \cdot BIO_{inh}}{BW \cdot BIO_{oral}}$$

$$DOSE_{stem_{scenario}} := \frac{C_{leaf\_crops_{scenario}} \cdot IH_{stem}}{BW} \qquad DOSE_{root_{scenario}} := \frac{Croot_{agr\_plant_{scenario}} \cdot IH_{root}}{BW}$$

$$DOSE_{meat_{scenario}} := \frac{C_{meat_{scenario}} \cdot IH_{meat}}{BW} \qquad DOSE_{milk_{scenario}} := \frac{C_{milk_{scenario}} \cdot IH_{milk}}{BW}$$

$$DOSE_{fish_{scenario}} := \frac{C_{fish_{scenario}} \cdot IH_{fish}}{BW}$$

total daily intake for human

total daily intake for human as sum of each pathway

[$kg_{chem}*kg_{bw}^{-1}*d^{-1}$]

$$DOSE_{tot_{scenario}} := DOSE_{drw_{scenario}} + DOSE_{fish_{scenario}} + DOSE_{stem_{scenario}} + DOSE_{root_{scenario}} \ldots + DOSE_{meat_{scenario}} + DOSE_{milk_{scenario}} + DOSE_{air_{scenario}}$$

relative doses of specific different pathway (%)

$$RDOSE_{drw_{scenario}} := \frac{DOSE_{drw_{scenario}} \cdot 100 \cdot \%}{DOSE_{tot_{scenario}}} \qquad RDOSE_{air_{scenario}} := \frac{DOSE_{air_{scenario}} \cdot 100 \cdot \%}{DOSE_{tot_{scenario}}}$$

$$RDOSE_{stem_{scenario}} := \frac{DOSE_{stem_{scenario}} \cdot 100 \cdot \%}{DOSE_{tot_{scenario}}} \qquad RDOSE_{root_{scenario}} := \frac{DOSE_{root_{scenario}} \cdot 100 \cdot \%}{DOSE_{tot_{scenario}}}$$

$$RDOSE_{meat_{scenario}} := \frac{DOSE_{meat_{scenario}} \cdot 100 \cdot \%}{DOSE_{tot_{scenario}}} \qquad RDOSE_{milk_{scenario}} := \frac{DOSE_{milk_{scenario}} \cdot 100 \cdot \%}{DOSE_{tot_{scenario}}}$$

$$RDOSE_{fish_{scenario}} := \frac{DOSE_{fish_{scenario}} \cdot 100 \cdot \%}{DOSE_{tot_{scenario}}}$$

Results of calculation

$DOSE_{tot_{local}} = 4.16297 \cdot 10^{-4} \circ \frac{mg}{kg_{bw} \cdot d}$

$DOSE_{tot_{regional}} = 5.72412 \cdot 10^{-7} \circ \frac{mg}{kg_{bw} \cdot d}$

$RDOSE_{drw_{local}} = 47.013124 \circ \%$

$RDOSE_{drw_{regional}} = 64.888324 \circ \%$

$RDOSE_{air_{local}} = 23.678143 \circ \%$

$RDOSE_{air_{regional}} = 1.94665 \circ \%$

$RDOSE_{stem_{local}} = 6.305251 \circ \%$

$RDOSE_{stem_{regional}} = 0.561165 \circ \%$

$RDOSE_{root_{local}} = 2.440699 \circ \%$

$RDOSE_{root_{regional}} = 4.226292 \circ \%$

$RDOSE_{meat_{local}} = 1.010229 \cdot 10^{-3} \circ \%$

$RDOSE_{meat_{regional}} = 1.006768 \cdot 10^{-3} \circ \%$

$RDOSE_{milk_{local}} = 8.218654 \cdot 10^{-3} \circ \%$

$RDOSE_{milk_{regional}} = 8.190497 \cdot 10^{-3} \circ \%$

$RDOSE_{fish_{local}} = 20.553555 \circ \%$

$RDOSE_{fish_{regional}} = 28.368371 \circ \%$

Appendix B Calculation of PEC_{local} for sediment

Calculation of PEC_{local} for Sediment

Chemical: Nitrobenzene - Prod.

concentration in surface water during emission period $PEClocal_{water} := 0.00835 mg \cdot l^{-1}$

partition coefficient suspended matter-water: $K_{susp\_water} := 3.849 \cdot m^3 \cdot m^{-3}$

bulk density of (wet) suspended matter $RHO_{susp} := 1150 \cdot kg \cdot m^{-3}$

$$PEClocal_{sed} := \frac{K_{susp\_water}}{RHO_{susp}} \cdot PEClocal_{water}$$

$$PEClocal_{sed} = 2.79 \cdot 10^{-2} \ mg \cdot kg^{-1}$$

Appendix C Monitoring data of surface water (Table C1), sediment (Table 2), wastewater treatment plants (Table C3), sewage of agricultural soils (Table C4), air (Table C5) and soild and groundwater (Table C6)

Table C1 Surface Water

Location	Concentration [µg/l]	Period	Remark	Reference
Germany Rhine at Götterswickerhamm	1.0	1985	detection limit: 0.1 µg/l	LWA NRW 1986
Germany Rhine at mouth from the Emscher	0.3	1985	detection limit: 0.1 µg/l	LWA NRW 1986
Germany river Rhine at Leverkusen	max.: 0.7	1984	detection limit: 0.1 µg/l extraction with Hexan GC with ECD	LWA NRW 1985
Germany river Rhine tributarie: Lippe	0.1	1984	dto.	LWA NRW 1985
Germany river Rhine at Bad Honnef	percentil-50: < 0.1 percentil-90: 0.3	1984	dto.	LWA NRW 1985
Germany river Rhine at Düsseldorf-Flehe	 0.15 0.32 0.08 0.11 0.28 0.33 0.18 0.19 0.10 0.47 0.07	1986 Feb. March April May June July Aug. Sept. Oct. Nov. Dec.		ARW 1986
Germany river Rhine at Wiesbaden	 < 0.02 0.20 < 0.02 < 0.02 0.10 0.66 0.55	1986 Feb. March April May June Aug. Dec.		ARW 1986

Table C1 continued overleaf

Table C1 continued Surface Water

Location	Concentration [µg/l]	Period	Remark	Reference
Germany river Rhine at Wesel	 0.17 0.04 0.05 0.10 0.03 0.12 0.26 0.15 0.05 0.06 0.02	1986 Feb. March April May June July Aug. Sept. Oct. Nov. Dec.		ARW 1986
Germany river Rhine at Köln	 0.12 0.36 0.07 0.10 0.06 0.12 0.21 0.25 0.16 0.05 0.65 0.15	1986 Jan. Feb. March April May June July Aug. Sept. Oct. Nov. Dec.		ARW 1986 ARW 1986
Germany river Rhine at Wiesbaden	0.05-1.6	1987	concentration fluctuations max.-min.concen-tration	IAWR 1986/87
Germany river Rhine at Köln	0.07-0.80	1987	dto.	LAWR 1986/87
Germany river Rhine at Düsseldorf	0.07-0.81	1987	dto.	LAWR 1986/87
Germany river Rhine at Lobith	< 0.1-3.6	1987	dto.	LAWR 1986/87
Germany river Rhine at Lobith road.km 862	max.: 8.4	08./09.02. 1987	Detected pollution by cases of damage and illegal introduction origin. a cause unknown	LAWR 1986/87
Germany river Rhine at Lobith	middle: 0.2 north: 0.2	1986	detection limit: 0.1 µg/L extraction with Hexan GC with ECD	LWA NRW1987
Germany river Rhine at Lobith	average: 0.2 max.: 3.6	1987	detection limit at the year: 0.1µg/L number of samples: 47	RIWA 1987/88
Germany river Rhine at Lobith	average: 0.3 max.: 1.3	1988	detection limit at the year: 0.1 µg/L number of samples: 51	RIWA 1987/88

Table C1 continued overleaf

Table C1 continued Surface Water

Location	Concentration [µg/l]	Period	Remark	Reference
Germany river Rhine at Bad Honnef road km 640	1.2	03.03.1988	deceted pollution by cases of damage and illegal introduction origin. a cause unknown	RIWA 1987/88
Germany river Rhine at Bad Honnef. Düsseldorf	max.: 3	12.02.1989	case of damage origin. a cause unknown extraction with Hexan GC with ECD	LWA NRW 1990
Germany river Rhine at Bad Honnef. Düsseldorf	max.: 3 µg/L at Bad Honnef max.: 6 µg/L at Düsseldorf	1.08.1989	the BASF announced the introduction of 400 kg Nitrobenzene methods of analysis: extraction with Hexan. GC with ECD	LWA NRW 1990
Germany river Rhine at Bad Honnef. Düsseldorf	a freight of 1.2 t at Bad Honnef max.: 19 µg/L at Düsseldorf	04.11.1989	the BASF announced the introduction of 650 kg method of analysis: dto.	LWA NRW 1990
Germany river Rhine coordinated application of the measuring chips of Iffezheim to Bimmen	km 641.5: 0.059 km 733.2:0.055 km 745.6:0.047 km 777.8: 0.056 km 800: 0.055 km 869.4: 0.067	1992	the limit determination varies depending upon substance (24 single materials) between 0.01 and 0.1 µg/L	Deutsche Kommission zur Reinhaltung des Rheins 1994
Germany river Rhine (north) tributaries: Sieg, Wupper, Erft, Ruhr, Emscher and Lippe	Rhine north: 1.0 Tributaries < 0.5	1990	detection limit: 0.5 µg/L	LWA NRW 1991
Germany river Rhine (south. middle. north) tributaries: Sieg. Wupper. Erft. Ruhr (east and west) Emscher and Lippe	not detected	1992	detection limit: 0.5 µg/L	LWA NRW 1993
Gemany river Rhine (south. middle. north) tributaries: Sieg. Wupper. Erft. Ruhr (east and west). Emscher and Lippe	not detected	1993	detection limit: 0.5 µg/L	LUA NRW 1997
Germany river Rhine Rhine-km 163.9 pipe-water- with drawal-place IWB	< 0.02 < 0.02 < 0.02 < 0.02 < 0.02	01.03.1994 03.05.1994 30.08.1994 29.11.1994 average	liquidchromatographie with DAD- and fluorescence detection after solid phase-extraction of the sample	Gewässer-schutzamt Basel-Stadt 1994
Germany river Rhine Rhine-km 163.9 pipe-water-withdrawal-place IWB	< 0.02 < 0.02 < 0.02 < 0.02 < 0.02	07.03.1995 06.06.1995 03.10.1995 05.12.1995 average		AWBR 1995

Table C1 continued overleaf

Table C1 continued Surface Water

Location	Concentration [µg/l]	Period	Remark	Reference
Germany river Rhine at Öhningen	average: < 0.05 percentil-90: < 0.05 max.: 0.06	1989	number of samples: 24	Umweltbundes-amt Berlin (UBA)1996
Germany river Rhine at Öhningen	average: < 0.05 percentil-90: < 0.05 max.: < 0.05	1990	number of samples: 26	UBA 1996
Germany river Rhine at Öhningen	average: < 0.05 percentil-90: < 0.05 max: < 0.05	1991	number of samples: 26	UBA 1996
Germany river Rhine at Öhningen	average: < 0.05 percentil-90: < 0.05 max.: < 0.05	1992	number of samples: 13	UBA 1996
Germany river Rhine at Öhningen	average: < 0.05 max.: < 0.05	1993	number of samples: 3	UBA 1996
Germany river Rhine at Dogern	average: < 0.05 percent.-90: <0.05 max: < 0.05	1993	number of samples: 13	UBA 1996
Germany river Rhine at Village-Neuf	average: < 0.05 percentil-90: 0.07 max.: 0.07	1989	number of samples: 14	UBA 1996
Germany river Rhine at Village-Neuf	average: < 0.05 percentil-90: 0.06 max.: 0.10	1990	Number of samples: 25	UBA 1996
Germany river Rhine at Village-Neuf	average: < 0.05 percentil-90: 0.07 max.: 0.08	1991	Number of samples: 22	UBA 1996
Germany river Rhine at Seltz	average: < 0.05 percentil-90: 0.10 max.: 0.13	1989	Number of samples: 21	UBA 1996
Germany river Rhine at Seltz	average: < 0.05 percentil-90: 0.09 max.: 0.15	1990	Number of samples: 25	UBA 1996
Germany river Rhine at Seltz	average: < 0.05 percentil-90: 0.07 max.: 0.07	1991	Number of samples: 15	UBA 1996
Germany river Rhine at Karlsruhe	average:< 0.05 percentil-90: 0.05 max.: 0.05	1993	Number of samples: 14	UBA 1996
Germany river Rhine at Maxau	average: < 0.05 percentil-90: 0.07 max.: 0.12	1989	Number of samples: 26	UBA 1996
Germany river Rhine at Maxau	average: < 0.05 percentil-90:0.08 max.: 0.21	1990	Number of samples: 24	UBA 1996

Table C1 continued overleaf

Table C1 continued Surface Water

Location	Concentration [µg/l]	Period	Remark	Reference
Germany river Rhine at Maxau	average: < 0.05 percentil-90: 0.07 max.: 0.09	1991	number of samples: 25	UBA 1996
Germany river Rhine at Mannheim	average: < 0.05 percentil-90:< 0.05 max.: < 0.05	1990	number of samples: 21	UBA 1996
Germany river Rhine at Mannheim	average: < 0.02 percentil-90: 0.03 max.: 0.08	1991	number of samples: 24	UBA 1996
Germany river Rhine at Mannheim	average: < 0.05 percentil-90:< 0.05 max.: < 0.05	1992	number of samples: 13	UBA 1996
Germany river Rhine at Mainz	average: 0.23 percentil-90: 0.37 max.: 2.70	1989	number of samples: 25	UBA 1996
Germany river Rhine at Mainz	average: < 2 percentil-90:< 2 max.: 22.50	1989	number of samples: 151	UBA 1996
Germany river Rhine at Mainz	average: 0.09 percentil-90: 0.17 max.: 0.20	1990	number of samples: 26	UBA 1996
Germany river Rhine at Mainz	average: 0.07 percentil-90: 0.10 max.: 0.21	1991	number of samples: 25	UBA 1996
Germany river Rhine at Mainz	average: < 0.05 percentil-90: 0.06 max.: 0.11	1992	number of samples: 26	UBA
Germany river Rhine at Mainz	average: < 2 percentil-90: < 2 max.: < 2	1993	number of samples: 25	UBA 1996
Germany river Rhine at Mannheim	average: 0.80 percentil-90: 0.86 max.: 12.80	1989	number of samples: 25	UBA 1996
Germany river Rhine at Mannheim	average: 0.25 percentil-90: 0.79 max.: 1.01	1990	number of samples: 26	UBA 1996
Germany river Rhine at Mannheim	average : 0.14 percentil-90: 0.26 max.: 0.59	1991	number of samples: 26	UBA 1996
Germany river Rhine at Koblenz	average: < 0.05 max.:< 0.05	1993	number of samples: 10	UBA 1996

Table C1 continued overleaf

Table C1 continued Surface Water

Location	Concentration [µg/l]	Period	Remark	Reference
Germany river Rhine at Koblenz	average: 0.14 percentil-90: 0.45 max.: 0.72	1989	number of samples: 26	UBA 1996
Germany river Rhine at Koblenz	average: 0.08 percentil-90: 0.11 max.: 0.60	1990	number of samples: 26	UBA 1996
Germany river Rhine at Koblenz	average: 0.08 percentil-90: 0.12 max.: 0.40	1991	number of samples:26	UBA 1996
Germany river Rhine at Bad Honnef	average: < 0.1 percentil-90: 0.30 max.:0.30	1984	number of samples: 13	UBA 1996
Germany river Rhine at Bad Honnef	average: < 0.1 percentil-90: 0.44 max: 0.70	1985	number of samples: 13	UBA 1996
Germany river Rhine at Bad Honnef	average: < 0.1 percentil-90: < 0.1 max.:< 0.1	1986	number of samples: 13	UBA 1996
Germany river Rhine at Bad Honnef	average: < 0.1 max.: 0.10	1987	number of samples: 7	UBA 1996
Germany river Rhine at Bad Honnef	average: < 0.1 max.: < 0.1 < 0.1	1988	number of samples: 5	UBA 1996
Germany river Rhine at Bad Honnef	average: < 0.1 percentil-90: < 0.1 max: < 0.1	1989	number of samples: 13	UBA 1996
Germany river Rhine at Bad Honnef	average: < 0.5 percentil-90: < 0.5 max.: < 0.5	1990	number of samples: 13	UBA 1996
Germany river Rhine at Bad Honnef	average: < 0.5 percentil-90: < 0.5 max.: < 0.5	1991	number of samples: 35	UBA 1996
Germany river Rhine at Bad Honnef	average: < 0.5 percentil-90: < 0.5 max.: < 0.5	1992	number of samples: 13	UBA 1996
Germany river Rhine at Bad Honnef	average: < 0.5 percentil-90: < 0.5 max.:< 0.5	1993	number of samples: 13	UBA 1996
Germany river Rhine at Düsseldorf	average: 0.28 percentil-90: 1.25 max.: 1.60	1989	number of samples: 25	UBA 1996

Table C1 continued overleaf

Table C1 continued Surface Water

Location	Concentration [µg/l]	Period	Remark	Reference
Germany river Rhine at Düsseldorf	average: 0.06 percentil-90: 0.09 max.: 0.11	1990	number of samples: 25	UBA 1996
Germany river Rhine at Düsseldorf	average: 0.07 percentil-90: 0.13 max.: 0.18	1991	number of samples: 24	UBA 1996
Germany river Rhine at Lobith	average: 0.19 percentil-90: 0.42 max.: 1.20	1989	number of samples: 26	UBA 1996
Germany river Rhine at Lobith	average: 0.06 percentil-90: 0.10 max.: 0.11	1990	number of samples: 26	UBA 1996
Germany river Rhine at Lobith	average: 0.06 percentil-90: 0.12 max.: 0.15	1991	number of samples: 26	UBA 1996
Germany river Rhine at Kleve-Bimmen	average: < 0.1 percentil-90: 0.22 max.: 0.30	1984	number of samples: 13	UBA 1996
Germany river Rhine at Kleve-Bimmen	average: 0.12 percentil-90: 0.56 max: 0.80	1985	number of samples: 12	UBA 1996
Germany river Rhine at Kleve-Bimmen	average: < 0.1 percentil-90: 0.20 max.: 0.20	1986	number of samples: 12	UBA 1996
Germany river Rhine at Kleve-Bimmen	average: 0.14 percentil-90: 0.68 max.: 1.00	1987	number of samples: 13	UBA 1996
Germany river Rhine at Kleve-Bimmen	average: < 0.1 max.: < 0.1	1988	number of samples: 8	UBA 1996
Germany river Rhine at Kleve-Bimmen	average:< 0.1 percentil-90: < 0.1 max.: 0.10	1989	number of samples: 13	UBA 1996
Germany river Rhine at Kleve-Bimmen	average: <0.5 percentil-90: < 0.5 max.: < 0.5	1990	number of samples: 13	UBA 1996
Germany river Rhine at Kleve-Bimmen	average:< 0.5 percentil-90:< 0.5 max.:< 0.5	1991	number of samples: 51	UBA 1996
Germany river Rhine at Kleve-Bimmen	average: < 0.5 percentil-90: < 0.5 max.: < 0.5	1992	number of samples: 13	UBA 1996

Table C1 continued overleaf

Table C1 continued Surface Water

Location	Concentration [µg/l]	Period	Remark	Reference
Germany river Rhine at Kleve-Bimmen	average: < 0.5 percentil-90: < 0.5 max.: < 0.5	1993	number of samples: 13	UBA 1996
Germany river Rhine at Kleve-Bimmen	average: 0.13 percentil-90: 0.29 max.: 0.76	1989	number of samples: 25	UBA 1996
Germany river Rhine at Kleve-Bimmen	average: 0.06 percentil-90: 0.13 max.: 0.17	1990	number of samples: 25	UBA 1996
Germany river Rhine at Kleve-Bimmen	average: 0.06 percentil-90: 0.09 max.: 0.13	1991	number of samples: 26	UBA 1996
Czech Republic river Elbe at Valy	average: 1.8 max.: 5.2 min.: 0.3:	Jan. to Oct. 1994	9 of 11 samples were positive	Medek et al. 1995
Germany river Elbe at Dresden	1.6 µg/L (90-Per-zentil) the total of Nitrobenzene and the three of Nitrotoluenes	1994	freezingdried sample extraction with Toluol/ Aceton or Toluol. GC/MS	HlfU 1997
Germany river Elbe at Zollenspieker river Elbe at Seemannshöft	median: 0.25 percentil-90: 0.53 max.: 0.60 median: 0.196 max.: 0.568	1992/93	unfiltered samples	Freie und Hansestadt Hamburg. Umweltbe-hörde 1995
Germany river Elbe at Zollenspieker	average: 0.35 percentil-90: 0.55 max.: 0.60	1992	number of samples: 12	UBA 1996
Germany river Elbe at Zollenspieker	average: 0.19 max.: 0.53	1993	number of samples: 8	UBA 1996
Germany river Elbe at Seemannshöft	average: 0.28 percentil-90: 0.48 max.: 0.57	1992	number of samples: 12	UBA 1996
Germany river Elbe at Seemannshöft	average: 0.19 max.: 0.50	1993	number of samples: 8	UBA 1996
Germany river Donau at Ulm	average: < 0.05 percentil-90: < 0.05 max.: < 0.05	1990	number of samples: 12	Umwelt-bundesamt Berlin 1996
Germany river Donau at Ulm	average: < 0.02 percentil-90: < 0.02 max.: < 0.02	1991	number of samples: 13	UBA 1996

Table C1 continued overleaf

Table C1 continued Surface Water

Location	Concentration [µg/l]	Period	Remark	Reference
Germany river Donau at Ulm	average: < 0.05 percentil-90: < 0.05 max.: < 0.05	1992	number of samples: 12	UBA 1996
Germany river Donau at Ulm	average: < 0.05 percentil-90: < 0.05 max.: < 0.05	1993	number of samples: 15	UBA 1996
Germany river Main at Bischofsheim	Percentil-90: 0.05	1995. weekly sampling	Freeze-dried sample extraction with Toluol/ Aceton or Toluol (GC/MS)	HLfU 1997
Germany river Main at Bischofsheim	average: 0.13 percentil-90: 0.26 max.: 0.64	1989	number of samples: 25	UBA 1996
Germany river Main at Bischofsheim	average: 0.14 percentil-90: 0.31 max.: 0.50	1990	number of samples: 26	UBA 1996
Germany river Main at Bischofsheim	average: 0.15 percentil-90: 0.20 max.: 0.62	1991	number of samples: 26	UBA 1996
Germany river Main at Bischofsheim	max.: 0.04	1991	number of samples: 1	UBA 1996
Germany river Main at Bischofsheim	max.: 0.06	1992	number of samples: 1	UBA 1996
Germany river Main at Bischofsheim	average: 0.10 percentil-90: 0.22 max.: 0.36	1992	number of samples: 16	UBA 1996
Germany river Main at Bischofsheim	average: 0.08 percentil-90: 0.12 max.: 0.37	1993	number of samples: 53	UBA 1996
Germany river Mosel at Palzem	average: < 2 percentil-90: < 2 max.: < 2	1992	number of samples: 13	UBA 1996
Germany river Mosel at Palzem	average: < 0.4 percentil-90: < 0.4 max.: < 0.4	1993	number of samples: 13	UBA 1996
Germany river Neckar at Poppenweiler	average: < 0.05 percentil-90:< 0.05 max.: < 0.05	1990	Number of samples: 13	UBA 1996

Table C1 continued overleaf

Table C1 continued Surface Water

Location	Concentration [µg/l]	Period	Remark	Reference
Germany river Neckar at Mannheim/Neckar	average: < 0.05 percentil-90:< 0.05 max.: < 0.05	1990	number of samples:23	UBA 1996
Germany river Neckar at Mannheim/Neckar	average: < 0.02 percentil-90: < 0.02 max.: < 0.02	1991	number of samples: 22	UBA 1996
Germany river Neckar at Mannheim/Neckar	average: < 0.05 percentil-90: < 0.05 max.: < 0.05	1992	number of samples: 16	UBA 1996
Germany river Neckar at Mannheim/Neckar	average: < 0.05 percentil-90: < 0.05 max.: < 0.05	1993	number of samples: 13	UBA 1996
Germany river Erft at Neuss	average: < 0.1 max.: < 0.1	1985	number of samples: 5	UBA 1996
Germany river Erft at Neuss	average: < 0.1 max.: < 0.1	1986	number of samples: 7	UBA 1996
Germany river Erft at Neuss	average: < 0.1 max.: < 0.1	1987	number of samples: 7	UBA 1996
Germany river Erft at Neuss	average: < 0.1 max.: < 0.1	1988	number of samples: 7	UBA 1996
Germany river Erft at Neuss	average: < 0.1 percentil-90: < 0.1 max.:< 0.1	1989	number of samples: 13	UBA 1996
Germany river Erft at Neuss	average: < .05 percentil-90: < 0.5 max.: < 0.5	1990	Number of samples: 12	UBA 1996
Germany river Erft at Neuss	average: < 0.5 max.: < 0.5	1991	Number of samples: 8	UBA 1996
Germany river Erft at Neuss	average: < 0.5 max.: < 0.5	1992	number of samples: 3	UBA 1996
Germany river Erft at Neuss	average: < 0.5 percentil-90: < 0.5 max.: < 0.5	1993	Number of samples: 13	UBA 1996
Germany river Fulda at Wahnhausen	max.: < 0.02	1991	Number of samples: 1	UBA 1996
Germany river Fulda at Wahnhausen	max.: < 0.05	1992	Number of samples: 1	UBA 1996
Germany river Lahn at Limburg-Staffel	max.: 0.02	1991	Number of samples: 1	UBA 1996
Germany river Lahn at Limburg-Staffel	max.: < 0.05	1992	Number of samples: 1	UBA 1996

Table C1 continued overleaf

Table C1 continued Surface Water

Location	Concentration [µg/l]	Period	Remark	Reference
Germany river Lippe at Wesel	average.: < 0.1 max.: 0.10	1984	Number of samples: 7	UBA 1996
Germany river Lippe at Wesel	average: < 0.1 max.: < 0.1	1985	Number of samples: 5	UBA 1996
Germany river Lippe at Wesel	average: < 0.1 max.: < 0.1	1986	Number of samples: 7	UBA 1996
Germany river Lippe at Wesel	average: < 0.1 max.: < 0.1	1987	Number of samples: 7	UBA 1996
Germany river Lippe at Wesel	average: < 0.1 max.: < 0.1	1988	Number of samples: 6	UBA 1996
Germany river Lippe at Wesel	average: 0.11 percentil-90: 0.39 max.: 0.43	1989	Number of samples: 13	UBA 1996
Germany river Lippe at Wesel	average: < 0.5 percentil-90: < 0.5 max.: < 0.5	1990	Number of samples: 12	UBA 1996
Germany river Lippe at Wesel	average: < 0.5 percentil-90: < 0.5 max: < 0.5	1991	number of samples: 45	UBA 1996
Germany river Lippe at Wesel	average: < 0.5 percentil-90: < 0.5 max.: < 0.5	1992	number of samples: 13	UBA 1996
Germany river Lippe at Wesel	average: < 0.5 percentil-90: < 0.5 max.: < 0.5	1993	number of samples: 13	UBA 1996
Germany river Nidda at Frankfurt-Nied	max.: 0.02	1991	number of samples: 1	UBA 1996
Germany river Pleiße at Gößnitz	average: < 0.1 max.: < 0.1	1993	number of samples: 4	UBA 1996
Germany river Ruhr at Duisburg-Ruhrort	average: < 0.1 max.: < 0.1	1984	number of samples: 7	UBA 1996
Germany river Ruhr at Duisburg-Ruhrort	average: < 0.1 max.:< 0.1	1985	number of samples: 6	UBA 1996
Germany river Ruhr at Duisburg-Ruhrort	average: < 0.1 max.: < 0.1	1986	number of samples: 7	UBA 1996
Germany river Ruhr at Duisburg-Ruhrort	average: < 0.1 max.: < 0.1	1987	number of samples: 7	UBA 1996
Germany river Ruhr at Duisburg-Ruhrort	average: < 0.1 max.: < 0.1	1988	number of samples: 6	UBA 1996
Germany river Ruhr at Duisburg-Ruhrort	average: < 0.1 max.: < 0.1	1989	number of samples: 10	UBA 1996

Table C1 continued overleaf

Table C1 continued Surface Water

Location	Concentration [µg/l]	Period	Remark	Reference
Germany river Ruhr at Duisburg-Ruhrort	average: < 0.5 percentil-90: < 0.5 max.: < 0.5	1990	number of samples: 13	UBA 1996
Germany river Ruhr at Duisburg-Ruhrort	average: < 0.5 max: 0.66	1991	number of samples: 8	UBA 1996
Germany river Ruhr at Duisburg-Ruhrort	average: < 0.5 max.: < 0.5	1992	number of samples: 3	UBA 1996
Germany river Ruhr at Duisburg-Ruhrort	average: < 0.5 percentil-90: < 0.5 max.: < 0.5	1993	number of samples: 13	UBA 1996
Germany river Saale at Camburg-Stöben	average: < 0.1 max.: < 0.1	1993	number of samples: 4	UBA 1996
Germany river Saar at Kanzem	average: < 2 percentil-90: < 2 max.: < 2	1992	number of samples: 13	UBA 1996
Germany river Saar at Kanzem	average: < 0.4 percentil-90: < 0.4 max.: < 0.4	1993	number of samples: 13	UBA 1996
Germany river Sieg at Bergheim	average: < 0.1 max.: < 0.1	1984	number of samples: 7	UBA 1996
Germany river Sieg at Bergheim	average: < 0.1 max.: < 0.1	1985	number of samples: 6	UBA 1996
Germany river Sieg at Bergheim	average: < 0.1 max.: < 0.1	1986	number of samples: 7	UBA 1996
Germany river Sieg at Bergheim	average: < 0.1 max.: < 0.1	1987	number of samples: 7	UBA 1996
Germany river Sieg at Bergheim	average: < 0.1 max.: < 0.1	1988	number of samples: 6	UBA 1996
Germany river Sieg at Bergheim	average: < 0.1 percentil-90: < 0.1 max.: < 0.1	1989	number of samples: 13	UBA 1996
Germany river Sieg at Bergheim	average: < 0.5 percentil-90: < 0.5 max.: < 0.5	1990	number of samples: 13	UBA 1996
Germany river Sieg at Bergheim	average: < 0.5 percentil-90: < 0.5 max.: < 0.5	1991	number of samples: 13	UBA 1996
Germany river Sieg at Bergheim	average: < 0.5 percentil-90: < 0.5 max.: < 0.5	1992	number of samples: 13	UBA 1996

Table C1 continued overleaf

Table C1 continued Surface Water

Location	Concentration [µg/l]	Period	Remark	Reference
Germany river Sieg at Bergheim	average: < 0.5 percentil-90: < 0.5 max.: < 0.5	1993	number of samples: 13	UBA 1996
Germany river Schwarzbach at Trebur-Astheim	max.: < 0.02	1991	number of samples: 1	UBA 1996
Germany River Unstrut	average: < 0.1 max.: < 0.1	1993	number of samples: 4	UBA 1996
Germany River Weisse Elster	average: < 0.1 max.: < 0.1	1993	number of samples: 4	UBA 1996
Germany River Werra at Gerstungen	average: < 0.1 max.: < 0.1	1993	number of samples: 4	UBA 1996
Germany River Werra at "Letzter Heller"	< 0.02 < 0.05	1991 1992	number of samples: 1 number of samples: 1	UBA 1996
Germany River Wipper at Hachelbich	average: < 0.1 max.: < 0.1	1993	number of samples: 4	UBA 1996
Germany River Pleisse at Gößnitz	average: < 0.1 max.: < 0.1	1993	number of samples: 4	UBA 1996
Germany river Ilm at Niedertrebra	average: < 0.1 max.: < 0.1	1993	Number of samples: 4	UBA 1996
Belgium River Scheldt (estuarine water) between Antwerp and the North See	0.13	1986	number of samples: 1	UBA 1996
Japan not given	0.0001-0.0014 ppm	1976	detection limit: 0.00003-0.0004 ppm	Environment Agency Japan 1985
Japan not given	0.00013-0.0038 ppm	1977	detection limit: 0.0001-0.03 ppm	Environment Agency Japan 1985
Japan southwest and middle Japan Sendai Bay rivers in Hanamaki City, lake Hachiro Tomakomai port mouth of Ishikari	0.1-1.4 ppb	1976	detection limit: 0.003-0.4 ppb	JETOC 1993
Japan dto.	0.13-3.8 ppb	1977	detection limit: 0.1-30 ppb	JETOC 1993
Japan dto.	0.17 ppb	1991	detection limit: 0.15 ppb	JETOC 1993
Japanese river water and seawater	0.16-0.99 ppb		but not quantified in seawater in the Kitakyushi area of Japan	Spectrum Laboratories 08.01.01

Table C1 continued overleaf

Table C1 continued Surface Water

Location	Concentration [µg/l]	Period	Remark	Reference
Netherlands river Waal river Maas river Rhine	[ppb] average: 1.7 max.: 13.8 average: < 0.1 max.: 0.3 0.5			Spectrum Laboratories 08.01.01
USA Buffalo river Cuyahoga river St. Joseph river lake Erie Bansin lake Michigan Bansin	not detected			Spectrum Laboratories 08.01.01

Table C2 Sediment

Location	Concentration	Period	Remark	Reference
Germany river Rhine right bank Rhine-km:	[µg/kg dry substance]		rub the sediment with water-free Natrium-sulfat Soxhlet-extraction with Hexan clean up with TBA-reagent Florisil-säulenchro-matografie	LWA NRW 1990
659.8	< 10	04/1989	with I-Octanol/Toluol (95/5)	
659.8	< 10	09/1989	and Hexan/Diethylether (1/1)	
687.3	< 10	09/1989		
706.9	< 10	07/1989		
706.9	26	09/1989		
743.1	18	09/1989		
814.6	< 10	04/1989		
814.6	< 10	09/1989		
830.0	< 10	04/1989		
830.0	< 10	09/1989		
Germany river Rhine left bank Rhine-km:	[µg/kg dry substance]		dto.	LWA NRW 1990
639.1	< 10	04/1989		
639.1	< 10	09/1989		
773.6	< 10	04/1989		
807.2	< 10	04/1989		
829.5	< 10	04/1989		
829.5	< 10	09/1989		
863.8	< 10	04/1989		
863.8	< 10	09/1989		

Table C3 Waste Water Treatment Plants: Influents and Effluents

Location	Concentration	Period	Remark	Reference
Czech Republic river Elbe at Synthesia a.s.	[µg/l] outflow A average: 84 max.: 210 min.: 13 outflow R average : 1150 max.: 4000 min.: 85	Jan. to Oct. 1994	16 of 18 samples were positive 16 of 16 sample were positive	Medek et al. 1995
Norway 28 samples of industrial effluents polluted fjords	not detected			Spectrum Laboratories 08.01.01
USA mutagenic secondary effluents from industrial plants and publicly-owned treatment works Lockport - petroleum refinery Sauget – heavy chemical plant. although manufactures of alloys and metal tubin	found. however no exact specification available		GC-MS	Ellis et al. 1982
USA following industrial category: leather tanning petroleum refining nonferrous metals organics and plastics inorganic chemicals pulp and paper auto and other laundries pesticides manufactures explosives organic chemical	effluents frequency of ocurrence/median [ppb] 1; 3.7 1; 7.7 1; 47.7 13; 3876.7 3; 1995.3 1; 124.3 1; 40.4 1;16.3 8; 51.7 36; 43.7 100.245 highest effluent conc. in the organics and plastics industry		of the 1245 stations reporting nitrobenzene in industrial effluents in EPA STORET database. 1.8% contanined detectable levels of the chemical	Spectrum Laborates 08.01.01
USA Los Angeles municipal wastewater treatment plants	final effluent 20 ppb < 10 ppb			Spectrum Laboratories 08.01.01

Table C4 Sewage sludge of agricultural Soils

Location	Concentration	Period	Remark	Reference
Canada	[µg/kg dry soil] Ontario maize field: 100 Ontario apple plantation: 150 Quebec corn field: 100		In 3 out of 10 agricultural samples values > detection limit (60 µg/kg dry soil) were found	Webber MD and Wang C 1995

Table C5 Air

Location	Concentration	Period	Remark	Reference
USA New Jersey	[µg/m³] 2 residential areas: average: 0.06 max. 1.6 industrial areas: average: 0.40 max: 3.5	1978	6-20 l per sample, 20 min. sample period. 6 samples/day. 3 days/week 4-9 weeks	Bozelli JW, Kebbukus BB 1982
USA Southern California Long Beach Central LA Azusa Claremont	[ng/m³] Long Beach: 4.45 Central LA: 8.99 Azusa: 16.98 Claremont: 20.74 12-4a.m: 5.41 6-10a.m.: 10.6 12-4p.m.: 18.0 6-10p.m.: 17.0	08.- 09. Sept. 1993	samples at all times aggregated by sampling site samples at all urban sites aggregated by time of day	Fraser et al. 1998

Table C6 Soil and Groundwater

Location	Concentration [µg/l]	Period	Remark	Reference
Germany Leverkusen former ammunition production site	groundwater 17 samples > DL max. 1.0	May 1990	Detection limit (GC-MC) not given	IWS 1994

Appendix D Calculation of Kpsewage and the factor for the adsorption on suspended matter

Calculation of Kp_{sewage} and the factor for the adsorption on suspended matter

Chemical: Nitrobenzene

$a := 0.63 \qquad b := 0.9$

$LOGP_{OW} := 1.86 \qquad P_{OW} := 10^{LOGP_{OW}} \qquad P_{OW} = 7.24436 \cdot 10^{1}$

$K_{OC} := 10^{a \cdot LOGP_{OW} + b} \cdot l \cdot kg^{-1} \qquad K_{OC} = 1.17978 \cdot 10^{2} \ l \cdot kg^{-1}$

$K_{p\_susp} := 0.1 \cdot K_{OC} \qquad c_{susp} := 15 \cdot mg \cdot l^{-1}$

$faktor := \left(1 + K_{p\_susp} \cdot c_{susp}\right)$

$faktor = 1.00018$

Sludge / Sewage

$K_{p\_raw\_sewage} := 0.3 \cdot K_{OC} \qquad K_{p\_activated\_sewage} := 0.37 \cdot K_{OC}$

$K_{p\_raw\_sewage} = 3.53933 \cdot 10^{1} \ l \cdot kg^{-1} \qquad K_{p\_activated\_sewage} = 4.36518 \cdot 10^{1} \ l \cdot kg^{-1}$

Calculation of the Henry coefficient

molecular weight: $MOLW := 0.12311 \cdot kg \cdot mol^{-1}$

vapour pressure: $VP := 2.0 \cdot 10^{1} \cdot Pa$

water solubility: $SOL := 1900 \cdot mg \cdot l^{-1}$

$$HENRY := \frac{VP \cdot MOLW}{SOL}$$

$HENRY = 1.29589 \ Pa \cdot m^{3} \cdot mol^{-1} \qquad \log\left(\frac{HENRY}{Pa \cdot m^{3} \cdot mol^{-1}}\right) = 1.1257 \cdot 10^{-1}$

Simple Treat 3.0 (debugged Version, 07.02.97) $k := 0$

Summary of distribution			
	to air	2.4	
	to water	96.7	
	via primary sludge	0.6	
	via surplus sludge	0.2	
	degraded	0.0	
	total	100.0	%

Appendix E Calculation of PECregional and PECcontinental

SimpleBox2.0a - Berechnung regionaler + kontinentaler PEC's

- Anpassung an TGD (1996) / EUSES 1.00: Michael Feibicke (06/98)

INPUT - Nitrobenzene			
Parameter names acc. SimpleBox20	Unit	Input	Parameter names according Euses
Physicochemical properties			
COMPOUND NAME	[-]	Nitrobenzene	Substance
MOL WEIGHT	[g.mol^{-1}]	123	Molecular weight
MELTING POINT	[° C]	5,26	Melting Point
VAPOR PRESSURE(25)	[Pa]	20	Vapour pressure at 25°C
log Kow	[log10]	1,86	Octanol-water partition coefficient
SOLUBILITY(25)	[mg.l^{-1}]	1900	Water solubility
Distribution - Partition coefficients			
- Solids water partitioning (derived from K_{oc})			
Kp(soil)	[l.kg$_d^{-1}$]	2,36	Solids-water partitioning in soil
Kp(sed)	[l.kg$_d^{-1}$]	5,9	Solids-water partitioning in sediment
Kp(susp)	[l.kg$_d^{-1}$]	11,8	Solids-water partitioning in sudpended matter
- Biota-water			
BCF(fish)	[l.kg$_w^{-1}$]	30,6	Biocentration factor for aquatic biota
Degradation and Transfromation rates			
- Characterisation and STP			
PASSreadytest	[y / n]	n	Characterization of biodegradability
- Environmental Total Degradation			
kdeg(air)	[d^{-1}]	1,06E-02	Rate constant for degradation in air
kdeg(water)	[d^{-1}]	6,93E-07	Rate constant for degradation in bulk surface water
kdeg(soil)	[d^{-1}]	6,93E-07	Rate constant for degradation in bulk soil
kdeg(sed)	[d^{-1}]	6,93E-07	Rate constant for degradation in bulk sediment
Sewage treatment (e.g. calculated by SimpleTreat)			
- Continental			
FR(volatstp) [C]	[-]	2,50E-02	Fraction of emission directed to air (STPcont)
FR(effstp) [C]	[-]	3,90E-02	Fraction of emission directed to water (STPcont)
FR(sludgestp) [C]	[-]	8,00E-03	Fraction of emission directed to sludge (STPcont)
- Regional			
FR(volatstp) [R]	[-]	2,50E-02	Fraction of emission directed to air (STPreg)
FR(effstp) [R]	[-]	3,90E-02	Fraction of emission directed to water (STPreg)
FR(sludgestp) [R]	[-]	8,00E-03	Fraction of emission directed to sludge (STPreg)
Release estimation			
- Continental			
Edirect(air) [C]	[t.y^{-1}]	0,739	Total continental emission to air
STPload [C]	[t.y^{-1}]	12,95	Total continental emission to wastewater
Edirect(water1) [C]	[t.y^{-1}]	5,933	Total continental emission to surface water
Edirect(soil3) [C]	[t.y^{-1}]	0	Total continental emission to industrial soil
Edirect(soil2) [C]	[t.y^{-1}]	0	Total continental emission to agricultural soil
- Regional			
Edirect(air) [R]	[t.y^{-1}]	0,0821	Total continental emission to air
STPload [R]	[t.y^{-1}]	1,44	Total continental emission to wastewater
Edirect(water1) [R]	[t.y^{-1}]	0,66	Total continental emission to surface water
Edirect(soil3) [R]	[t.y^{-1}]	0	Total continental emission to industrial soil
Edirect(soil2) [R]	[t.y^{-1}]	0	Total continental emission to agricultural soil

OUTPUT - Nitrobenzene

Zur **Neuberechnung der Daten**: ->Extras ->Optionen ->Berechnen -> Datei_berechnen -> F9 drücken, sonst keine komplette Neuberechnung aller Bezüge!!

Parameter names acc. SimpleBox20	Unit	Output	Parameter names according Euses
Physicochemical properties			
COMPOUND NAME	[-]	Nitrobenzene	Substance
Output			
- Continental			
PECsurfacewater (total)	[mg.l^{-1}]	2,16E-06	Continental PEC in surface water (total)
PECsurfacewater (dissolved)	[mg.l^{-1}]	2,15E-06	Continental PEC in surface water (dissolved)
PECair	[mg.m^{-3}]	3,02E-08	Continental PEC in air (total)
PECagr.soil	[mg.kg$_{wwt}^{-1}$]	7,31E-07	Continental PEC in agricultural soil (total)
PECporewater agr.soil	[mg.l^{-1}]	3,32E-07	Continental PEC in pore water of agricultural soils
PECnat.soil	[mg.kg$_{wwt}^{-1}$]	2,14E-07	Continental PEC in natural soil (total)
PECind.soil	[mg.kg$_{wwt}^{-1}$]	2,14E-07	Continental PEC in industrial soil (total)
PECsediment	[mg.kg$_{wwt}^{-1}$]	6,80E-06	Continental PEC in sediment (total)
- Regional			
PECsurfacewater (total)	[mg.l^{-1}]	1,29E-05	Regional PEC in surface water (total)
PECsurfacewater (dissolved)	[mg.l^{-1}]	1,29E-05	Regional PEC in surface water (dissolved)
PECair	[mg.m^{-3}]	5,18E-08	Regional PEC in air (total)
PECagr.soil	[mg.kg$_{wwt}^{-1}$]	5,43E-06	Regional PEC in agricultural soil (total)
PECporewater agr.soil	[mg.l^{-1}]	2,47E-06	Regional PEC in pore water of agricultural soils
PECnat.soil	[mg.kg$_{wwt}^{-1}$]	3,66E-07	Regional PEC in natural soil (total)
PECind.soil	[mg.kg$_{wwt}^{-1}$]	3,66E-07	Regional PEC in industrial soil (total)
PECsediment	[mg.kg$_{wwt}^{-1}$]	3,95E-05	Regional PEC in sediment (total)

Appendix F Calculation of the Photolytical degradation in air

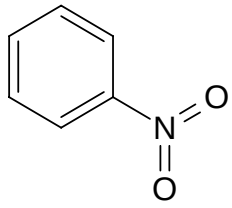
PropertEst :

Estimation of chemical´s properties with QSAR

Endpoint: Photolytical degradation in air

Substance :
Name: Nitrobenzene
Molekular weight : 123,11 g/mol
Sum forumula : C6 H5 N O2
SMILES-Code: c1(ccccc1)N(=O)=O

Strukture



Result:
k : 0.244E-12 cm³/molec/s (AOP v1.9)
$t_{(1/2)}$: 65.8 days

Description of the model:
Photolytical degradation of organic chemicals in the atomosphere is primarily due to their reaction with hydroxyl radicals.
With the programme AOP you can estimate the rate constant (k) for this reaction. Together with the concentration of the hydroxyl radicals (5E5 radicals/cm³ - 24-h mean value) in the atmosphere the half life of the organic chemical in the atmosphere can be calculated.

Source:
Atkinson, R.: "Estimation of Gas-Phase Hydroxyl Radical Rate Constants for organic Chemicals" Environ. Toxicol. Chem. 7, 435-442, 1988

Appendix G Distribution and fate

Distribution and Fate

Substance: Nitrobenzene

melting point:	$MP := 278.41 \cdot K$
vapour pressure:	$VP := 20 \cdot Pa$
water solubility:	$SOL := 1900 \cdot mg \cdot l^{-1}$
part. coefficient octanol/water:	$LOGP_{OW} := 1.86$
molecular weight:	$MOLW := 0.12311 \cdot kg \cdot mol^{-1}$
gas constant:	$R := 8.3143 \cdot J \cdot mol^{-1} \cdot K^{-1}$
temperature:	$T := 293 \cdot K$
conc. of suspended matter in the river:	$SUSP_{water} := 15 \cdot mg \cdot l^{-1}$
density of the solid phase:	$RHO_{solid} := 2500 \cdot kg \cdot m^{-3}$
volume fraction water in susp. matter:	$Fwater_{susp} := 0.9$
volume fraction solids in susp.matter:	$Fsolid_{susp} := 0.1$
volume fraction of water in sediment:	$Fwater_{sed} := 0.8$
volume fraction of solids in sediment:	$Fsolid_{sed} := 0.2$
volume fraction of air in soil:	$Fair_{soil} := 0.2$
volume fraction of water in soil:	$Fwater_{soil} := 0.2$
volume fraction of solids in soil:	$Fsolid_{soil} := 0.6$
aerobic fraction of the sediment comp.:	$Faer_{sed} := 0.1$
product of CONjunge and $SURF_{air}$:	$product := 10^{-4} \cdot Pa$

distribution air/water: Henry-constant

$$HENRY := \frac{VP \cdot MOLW}{SOL} \qquad HENRY = 1.296 \cdot Pa \cdot m^3 \cdot mol^{-1}$$

$$\log\left(\frac{HENRY}{Pa \cdot m^3 \cdot mol^{-1}}\right) = 0.113$$

$$K_{air\_water} := \frac{HENRY}{R \cdot T} \qquad K_{air\_water} = 5.32 \cdot 10^{-4}$$

solid/water-partition ceefficient Kp_{comp} and total compartment/water-partition coefficient K_{comp_water}

$a := 0.63$ (a,b from TGD, p. 539)

$b := 0.90$ $K_{OC} := 10^{a \cdot LOGP_{OW} + b} \cdot l \cdot kg^{-1}$ $K_{OC} = 117.978 \cdot l \cdot kg^{-1}$

Suspended matter

$Kp_{susp} := 0.1 \cdot K_{OC}$ $Kp_{susp} = 11.798 \cdot l \cdot kg^{-1}$

$K_{susp\_water} := Fwater_{susp} + Fsolid_{susp} \cdot Kp_{susp} \cdot RHO_{solid}$ $K_{susp\_water} = 3.849$

factor for the calculation of $Clocal_{water}$:

$faktor := 1 + Kp_{susp} \cdot SUSP_{water}$ $faktor = 1$

Sediment

$Kp_{sed} := 0.05 \cdot K_{OC}$ $Kp_{sed} = 5.899 \cdot l \cdot kg^{-1}$

$K_{sed\_water} := Fwater_{sed} + Fsolid_{sed} \cdot Kp_{sed} \cdot RHO_{solid}$ $K_{sed\_water} = 3.749$

Soil

$Kp_{soil} := 0.02 \cdot K_{OC}$ $Kp_{soil} = 2.36 \cdot l \cdot kg^{-1}$

$K_{soil\_water} := Fair_{soil} \cdot K_{air\_water} + Fwater_{soil} + Fsolid_{soil} \cdot Kp_{soil} \cdot RHO_{solid}$

$K_{soil\_water} = 3.739$

Sludge (activated sludge)

$K_{p\_sludge} := 0.37 \cdot K_{OC}$ $K_{p\_sludge} = 43.652 \cdot l \cdot kg^{-1}$

Raw sewage

$K_{p\_sewage} := 0.30 \cdot K_{OC}$ $K_{p\_sewage} = 35.393 \cdot l \cdot kg^{-1}$

Elimination in STPs

rate constant in STP: k = 0 d^{-1} elimination P = f (k, logpow, logH) =1.4

fraction directed to surface water $Fstp_{water}$ = 96.1

biodegradation in different compartments

surface water

$kbio_{water} := 0 \cdot d^{-1}$

soil

$DT50bio_{soil} := 1000000d$

$kbio_{soil} := \frac{\ln(2)}{DT50bio_{soil}}$ $kbio_{soil} = 6.931 \cdot 10^{-7} \ d^{-1}$

sediment

$kbio_{sed} := \frac{\ln(2)}{DT50bio_{soil}} \cdot Faer_{sed}$ $kbio_{sed} = 6.931 \cdot 10^{-8} \ d^{-1}$

degradation in surface waters

$khydr_{water} := 0 \cdot d^{-1}$

$kphoto_{water} := 5.2 \cdot 10^{-3} \cdot d^{-1}$ (Simmons and Zepp, 1986)

$kdeg_{water} := khydr_{water} + kphoto_{water} + kbio_{water}$

$kdeg_{water} = 5.2 \cdot 10^{-3} \ d^{-1}$

Atmosphere

calculation of CONjunge * SURFaer for the OPS-model

$VPL := \frac{VP}{\exp\left[6.79 \cdot \left(1 - \frac{MP}{285 \cdot K}\right)\right]}$ $VP := wenn(MP > 285 \cdot K, VPL, VP)$ $VP = 20 \ Pa$

$Fass_{aer} := \frac{product}{VP + product}$

$Fass_{aer} = 5 \cdot 10^{-6}$

degradation in the atmosphere

$kdeg_{air}$ = 4.4E-04 h^{-1} =1.06E-02 d^{-1} (see AOP-calculation)

Appendix H Exposure of soil

Exposure of Soil

chemical: Nitrobenzene

Defaults:

mixing depth of soil: $DEPTHsoil_i :=$

0.2·m
0.2·m
0.1·m

bulk density of soil: $RHO_{soil} := 1700 \cdot kg \cdot m^{-3}$

average time for exposure: $T_i :=$

30·d
180·d
180·d

partial mass transfer coefficient at air-side of the air-soil interface: $kasl_{air} := 120 \cdot m \cdot d^{-1}$

partial mass transfer coefficient at soilair-side of the air-soil interface: $kasl_{soilair} := 0.48 \cdot m \cdot d^{-1}$

partial mass transfer coefficient at soilwater-side of the air-soil interface: $kasl_{soilwater} := 4.8 \cdot 10^{-5} \cdot m \cdot d^{-1}$

fraction of rain water that infiltrates into soil: $Finf_{soil} := 0.25$

rate of wet precipitation: $RAINrate := 1.92 \cdot 10^{-3} \cdot m \cdot d^{-1}$

dry sludge application rate: $APPLsludge_i :=$

0.5·kg·m^{-2}·a^{-1}
0.5·kg·m^{-2}·a^{-1}
0.1·kg·m^{-2}·a^{-1}

Input:

annual average total deposition flux: $DEPtotal_{ann} := 8.4 \cdot 10^{-4} \cdot mg \cdot m^{-2} \cdot d^{-1}$

soil-water partitioning coefficient: $K_{soil\_water} := 3.739$

concentration in dry sewage sludge: $C_{sludge} := 0.0 \cdot \mu g \cdot kg^{-1}$

air-water partitioning coefficient: $K_{air\_water} := 5.32 \cdot 10^{-4}$

rate constant for for removal from top soil: $kbio_{soil} := 6.931 \cdot 10^{-7} \cdot d^{-1}$

PECregional: $PECregional_{natural\_soil} := 3.66 \cdot 10^{-7} \cdot mg \cdot kg^{-1}$

Calculation:

aerial deposition flux per kg of soil:

$$D_{air_i} := \frac{DEPtotal_{ann}}{DEPTHsoil_i \cdot RHO_{soil}}$$

rate constant for valatilisation from soil:

$$k_{volat_i} := \left[\left(\frac{1}{kasl_{air} \cdot K_{air\_water}} + \frac{1}{kasl_{soilair} \cdot K_{air\_water} + kasl_{soilwater}} \right) \cdot K_{soil\_water} \cdot DEPTHsoil_i \right]^{-1}$$

rate constant for leaching from soil layer:

$$k_{leach_i} := \frac{Finf_{soil} \cdot RAINrate}{K_{soil\_water} \cdot DEPTHsoil_i}$$

removal from top soil:

$$k_i := k_{volat_i} + k_{leach_i} + kbio_{soil}$$

concentration in soil

concentration in soil due to 10 years of continuous deposition:

$$Cdep_{soil\_10_i} := \frac{D_{air_i}}{k_i} \cdot \left(1 - \exp\left(-365 \cdot d \cdot 10 \cdot k_i\right)\right)$$

concentration just after the first year of sludge application:

$$Csludge_{soil\_1_i} := \frac{C_{sludge} \cdot APPLsludge_i \cdot a}{DEPTHsoil_i \cdot RHO_{soil}}$$

initial concentration in soil after 10 applications of sludge:

$$Csludge_{soil\_10_i} := Csludge_{soil\_1_i} \cdot \left(1 + \sum_{n=1}^{9} \exp\left(-365 \cdot d \cdot n \cdot k_i\right)\right)$$

sum of the concentrations due to both processes:

$$C_{soil\_10_i} := Cdep_{soil\_10_i} + Csludge_{soil\_10_i}$$

average concentration in soil over T days:

$$Clocal_{soil_i} := \frac{D_{air_i}}{k_i} + \frac{1}{k_i \cdot T_i} \cdot \left(C_{soil\_10_i} - \frac{D_{air_i}}{k_i} \right) \cdot \left(1 - \exp\left(-k_i \cdot T_i \right) \right)$$

$$PEClocal_{soil_i} := Clocal_{soil_i} + PECregional_{natural\_soil}$$

	$\frac{Clocal_{soil_i}}{ppt}$
$Clocal_{soil}$ =	$2.3102 \cdot 10^3$
$Clocal_{agr.soil}$ =	$2.314 \cdot 10^3$
$Clocal_{grassland}$ =	$2.361 \cdot 10^3$

	$\frac{PEClocal_{soil_i}}{ppt}$
$PEClocal_{soil}$ =	$2.3107 \cdot 10^3$
$PEClocal_{agr.soil}$ =	$2.3145 \cdot 10^3$
$PEClocal_{grassland}$ =	$2.3615 \cdot 10^3$

Indicating persistency of the substance in soil

initial concentration after 10 years:

$\frac{C_{soil\_10_i}}{ppt}$
$2.3094 \cdot 10^3$
$2.3094 \cdot 10^3$
$2.3608 \cdot 10^3$

initial concentration in steady-state situation:

$$C_{soil\_ss_i} := \frac{D_{air_i}}{k_i} + Csludge_{soil\_1_i} \cdot \left(\frac{1}{1 - \exp\left(-365 \cdot d \cdot k_i \right)} \right)$$

$\frac{C_{soil\_ss_i}}{ppt}$
$2.3612 \cdot 10^3$
$2.3612 \cdot 10^3$
$2.362 \cdot 10^3$

fraction of steady-state in soil achieved:

$$Fst\_st_i := \frac{C_{soil\_10_i}}{C_{soil\_ss_i}}$$

Fst_st_i
0.97805
0.97805
0.99952

calculated k-values

	$\frac{k_{volat_i}}{d^{-1}}$	$\frac{k_{leach_i}}{d^{-1}}$	$\frac{k_i}{d^{-1}}$
soil	$4.0375 \cdot 10^{-4}$	$6.4188 \cdot 10^{-4}$	$1.0463 \cdot 10^{-3}$
agriculture soil	$4.0375 \cdot 10^{-4}$	$6.4188 \cdot 10^{-4}$	$1.0463 \cdot 10^{-3}$
grassland	$8.075 \cdot 10^{-4}$	$1.2838 \cdot 10^{-3}$	$2.092 \cdot 10^{-3}$

concentration in pore water

$$Clocal_{soil\_porew_i} := \frac{Clocal_{soil_i} \cdot RHO_{soil}}{K_{soil\_water}}$$

	$Clocal_{soil\_porew_i}$ / ng·l^{-1}
$Clocal_{soil\_porew}$ =	$1.0504 \cdot 10^3$
$Clocal_{agr.soil\_porew}$ =	$1.0521 \cdot 10^3$
$Clocal_{grassland\_porew}$ =	$1.0735 \cdot 10^3$

$$PEClocal_{soil\_porew_i} := \frac{PEClocal_{soil_i} \cdot RHO_{soil}}{K_{soil\_water}}$$

	$PEClocal_{soil\_porew_i}$ / ng·l^{-1}
$PEClocal_{soil\_porew}$ =	$1.0506 \cdot 10^3$
$PEClocal_{agr.soil\_porew}$ =	$1.0523 \cdot 10^3$
$PEClocal_{grassland\_porew}$ =	$1.0737 \cdot 10^3$

concentration in ground water

$PEClocal_{grw} = PEClocal_{agr\_soil\_porew}$

European Commission

EUR 22480 EN **European Union Risk Assessment Report nitrobenzene, Volume 77**

Editors: S. Pakalin, S.J. Munn, K. Aschberger, O. Cosgrove, A. Paya-Perez, S. Vegro.

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Scientific and Technical Research series - ISSN 1018-5593

The report provides the comprehensive risk assessment of environment part of the substance nitrobenzene. It has been prepared by Germany in the frame of Council Regulation (EEC) No. 793/93 on the evaluation and control of the risks of existing substances, following the principles for assessment of the risks to humans and the environment, laid down in Commission Regulation (EC) No. 1488/94.

Part I – Environment

The evaluation considers the emissions and the resulting exposure to the environment in all life cycle steps. Following the exposure assessment, the environmental risk characterisation for each protection goal in the aquatic, terrestrial and atmospheric compartment has been determined.

The environmental risk assessment for nitrobenzene concludes that risks are not expected.

Part II – Human Health

This part of the evaluation will be published later.

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European Commission – Joint Research Centre
Institute for Health and Consumer Protection
Toxicology and Chemical Substances (TCS)
European Chemicals Bureau (ECB)

European Union Risk Assessment Report

nitrobenzene

Part I - environment

CAS No: 98-95-3 EINECS No: 202-716-0

Series: 3rd Priority List Volume: 77

European Union Risk Assessment Report

NITROBENZENE

CAS No: 98-95-3

EINECS No: 202-95-3

RISK ASSESSMENT

FINAL APPROVED VERSION

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NITROBENZENE

CAS No: 98-95-3

EINECS No: 202-716-0

RISK ASSESSMENT

10 July 2008

Germany

FINAL APPROVED VERSION

Rapporteur for the risk assessment of Nitrobenzene is Germany:

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Review of report by MS Technical Experts finalised:	**[insert month and year]**
Final report:	**[year]**

Foreword

We are pleased to present this Risk Assessment Report which is the result of in-depth work carried out by experts in one Member State, working in co-operation with their counterparts in the other Member States, the Commission Services, Industry and public interest groups.
The Risk Assessment was carried out in accordance with Council Regulation (EEC) 793/93[1] on the evaluation and control of the risks of "existing" substances. "Existing" substances are chemical substances in use within the European Community before September 1981 and listed in the European Inventory of Existing Commercial Chemical Substances. Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of these substances if they are produced or imported into the Community in volumes above 10 tonnes per year.
There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each substance on a priority list, a Member State volunteers to act as "Rapporteur", undertaking the in-depth Risk Assessment and recommending a strategy to limit the risks of exposure to the substance, if necessary.
The methods for carrying out an in-depth Risk Assessment at Community level are laid down in Commission Regulation (EC) 1488/94[2], which is supported by a technical guidance document[3]. Normally, the "Rapporteur" and individual companies producing, importing and/or using the chemicals work closely together to develop a draft Risk Assessment Report, which is then presented at a meeting of Member State technical experts for endorsement. The Risk Assessment Report is then peer-reviewed by the Scientific Committee on Health and Environmental Risks (SCHER) which gives its opinion to the European Commission on the quality of the risk assessment.
If a Risk Assessment Report concludes that measures to reduce the risks of exposure to the substances are needed, beyond any measures which may already be in place, the next step in the process is for the "Rapporteur" to develop a proposal for a strategy to limit those risks.
The Risk Assessment Report is also presented to the Organisation for Economic Co-operation and Development as a contribution to the Chapter 19, Agenda 21 goals for evaluating chemicals, agreed at the United Nations Conference on Environment and Development, held in Rio de Janeiro in 1992 and confirmed in the Johannesburg Declaration on Sustainable Development at the World Summit on Sustainable Development, held in Johannesburg, South Africa in 2002.
This Risk Assessment improves our knowledge about the risks to human health and the environment from exposure to chemicals. We hope you will agree that the results of this in-depth study and intensive co-operation will make a worthwhile contribution to the Community objective of reducing the overall risks from exposure to chemicals.

[1] O.J. No L 084, 05/04/199 p.0001 – 0075

[2] O.J. No L 161, 29/06/1994 p. 0003 – 0011

[3] Technical Guidance Document, Part I – V, ISBN 92-827-801 [1234]

0 OVERALL RESULTS OF THE RISK ASSESSMENT[4]

CAS Number: 98-95-3
EINECS Number: 202-716-0
IUPAC Name: Nitrobenzene

Environment

See separate report.

Human health

Human health (toxicity)

Workers

Conclusion (i) There is a need for further information and/or testing.

For nitrobenzene there is need for further testing regarding skin sensitisation.

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Conclusion (iii) applies to repeated dose toxicity and carcinogenicity. On the background of cancer risks, air concentrations of nitrobenzene at the workplace should be controlled to a level in the range of 0.1 mg/m^3 (critical exposure level for carcinogenicity). Concerning local effects after repeated exposure the critical exposure level with a value of 0.07 mg/m^3 is even lower. If the exposure is reduced to this level, inhalation risks from other endpoints, as systemic repeated dose toxicity would similarly and effectively be mitigated too. Also skin contact with nitrobenzene should be reduced. Based on cancer risk assessment, dermal exposure should be controlled to levels in the range of 0.03 mg/kg/day or 2.1 mg/person/day.

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already

For the other toxicological endpoints the risk orientated conclusions result in no concern with the consequence that risk reduction measures are of low priority

[4] Conclusion (i) There is a need for further information and/or testing.
Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.
Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Consumers

Conclusion (**ii)** There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Humans exposed via the environment

Conclusion (**ii)** There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Combined exposure

Conclusion (**ii)** There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Human health (risks from physico-chemical properties)

Conclusion (**ii)** There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

This conclusion is reached because the risk assessment shows that risks are not expected, and risk reduction measures already being applied are considered sufficient.

CONTENTS

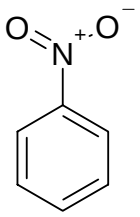
EUSES Calculations can be viewed as part of the report at the website of the European Chemicals Bureau: http://ecb.jrc.it

TABLES

1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS Number: 98-95-3
EINECS Number: 202-716-0
IUPAC Name: Nitrobenzene
Molecular formula: $C_6H_5NO_2$
Structural formula:



Molecular weight: 123 g/mol

Synonyms: Nitrobenzol
Benzene, Nitro-
Essence of Mirbane
Mirbane Oil
Mononitrobenzene

1.1 PURITY/IMPURITIES, ADDITIVES

Purity: > 98.9 %

Impurities: Benzene
o-Nitrotoluene
p-Nitrotoluene
p-Dinitrobenzene
m-Dintirobenzene
o-Dinitrobenzene
Toluene
Water

1.2 PHYSICO-CHEMICAL PROPERTIES

Table 0.1 Summary of physico-chemical properties

Property	Value	Reference
Physical state	liquid	
Melting point	5.26 °C	BASF AG (1986)
Boiling point	210.8 °C	Lide (1991)
Relative density	1.2037	Lide (1991)
Vapour pressure	0.2 hPa at 20 °C 1)	Auer (1988)
Water solubility	1900 mg/l at 20 °C 2)	Bayer AG (1998)
Partition coefficient n-octanol/water (log value)	1.86 at 24.5 °C 3)	BASF AG (1987)
Granulometry		
Conversion factors		
Flash point	88 °C	BAM (1997)
Autoflammability	480 °C (DIN 51794)	BAM (1997)
Flammability	Not extremely flammable Not highly flammable Not flammable 4)	BAM (1997)
Explosive properties	No explosive properties	BAM (1997)
Oxidizing properties	Not applicable (liquid)	
Viscosity		
Henry's constant	1.296 $Pa \cdot m^3 \cdot mol^{-1}$ at 20 °C	calculated
Surface tension	43.9 mN/m at 20 °C (pure substance)	Lide (1991)

1) The vapour pressure of 0.2 hPa at 20 °C was confirmed by entries in safety data sheets of various companies.

2) The flask method was used for the determination of the water solubility. In the safety data sheets of Bayer and Hoechst a water solubility of 2.0 g/l at 20 °C is cited. No information about the purity of the test substance, the test method and the test conditions are available. Therefore the water solubility of 1.9 at 20 °C (pH 6.5) is recommended for the risk assessment.

3) The shaking flask method was used for the determination of the partition coefficient n-octanol/water. The calculation according to Leo Hansch resulted in a logPow of 1.81. For the risk assessment the experimental value is preferred.

4) The tests according to A.12 and A.13 were not conducted. Due to the properties and the handling of the substance it has not to be assumed that flammable gases formate in contact with water or the substance has pyrophoric properties.

1.3 CLASSIFICATION

1.3.1 Current classification

Category 3 carcinogen

Category 3 reprotoxicity

T	toxic
R 23/24/25	toxic by inhalation, in contact with skin and if swallowed
R 40	limited evidence for carcinogenesis
R 48/23/24	toxic: danger of serious damage to health by prolonged exposure through inhalation and in contact with skin.
R 62	possible risk of impaired fertility

In Germany, nitrobenzene is assigned to the MAK pregnancy category "D" (DFG 1997) indicating that current information are not sufficient for a definite assessment whether there is or is not a risk for pregnant female workers from workplace exposures. According to the MAK commission, to decide on this an inhalatory study with an additional, methemoglobin susceptible species would be needed.

1.3.2 Proposed classification

Classification as carcinogenic cat. 3; R 40 is confirmed on the basis of longterm studies in rats and mice. Classification as reprotoxic cat. 3; R 62 is also confirmed on the basis of animal studies. In order to guarantee that the specific hazards posed by a substance causing methemoglobinemia are taken into account appropriately, classification as toxic and labelling with R 23, R 24, R 25 is confirmed.

According to the criteria of the Directive 67/543/EEC, the extension of the labelling to R 48/25 is proposed. Nitrobenzene hematotoxicity was evident at low doses which were about 10-fold lower than the criterial dose for the classification as harmful leading to the proposal for the classification as toxic.

Category 3 carcinogen

Category 3 reprotoxicity

T	toxic

R 40	limited evidence for carcinogenesis
R 23/24/25	toxic by inhalation, in contact with skin and if swallowed
R 48/23/24/25	toxic: danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed
R 62	possible risk of impaired fertility

2 GENERAL INFORMATION ON EXPOSURE

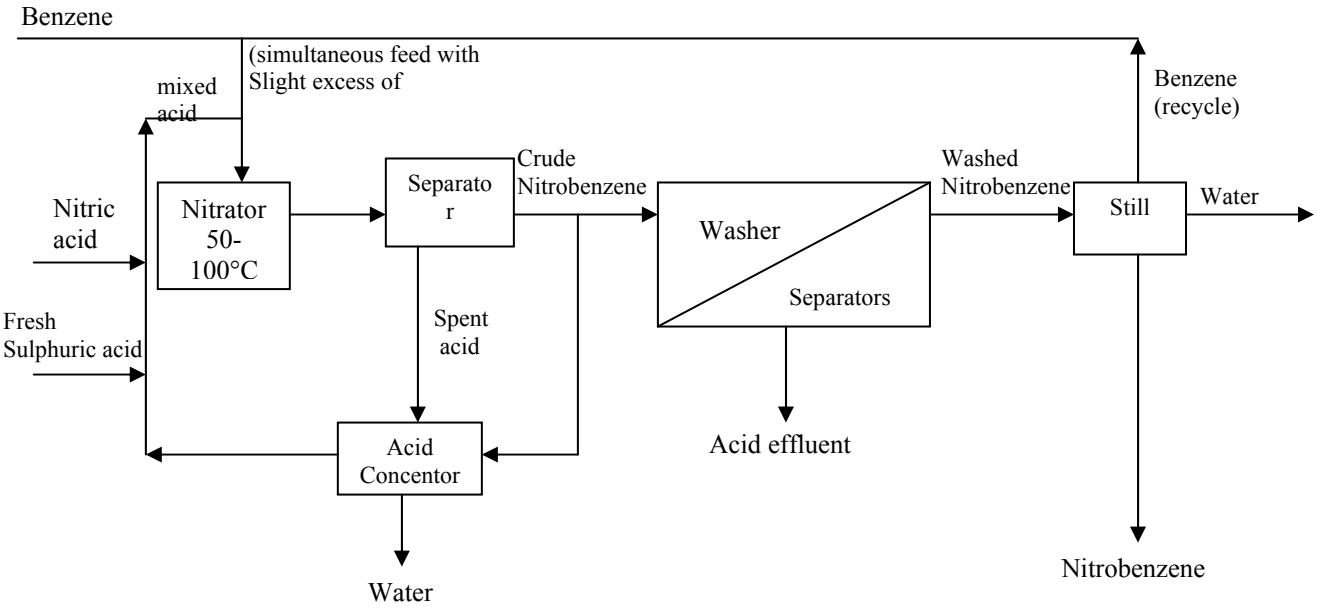
2.1 PRODUCTION

Nitrobenzene does not occur naturally. It is almost exclusively produced by nitration of benzene. Nitrobenzene is mainly used as an intermediate in the manufacture of aniline.

2.1.1 Production processes

Nitrobenzene is produced by nitration of benzene with nitrating acid, which is a mixture of nitric acid, sulphuric acid and water. It is usually performed continuously in stirred-vessel cascades or in loop-type reactors. Clean-up of the reaction mixture takes place in static separators or in centrifuges. The organic phase is washed with water, dilute alkalis and then with water again to free it from acid and by-products containing hydroxyl groups. The washed nitrobenzene is then freed from un-nitrated components, such as benzene, by steam stripping. Finally, it is dried by azeotropic distillation. Spent acid is continuously concentrated and reintroduced to the cycle. Also fresh acid is added.

Figure 1: Production of nitrobenzene – continuous process (Ullmann´s Encyclopedia of Industrial Chemicals, 1995).



2.1.2 Production capacity

According to available data there are 8 production and/or processing sites of nitrobenzene within the EU. The sources of the data are the announcements provided by the individual companies for the year 2000. Taking into account these actual statements, the resultant quantity of nitrobenzene produced in the EU amounts to be 1,179,780 t/year.

2.2 USES

2.2.1 Introduction

According to the producers nitrobenzene is primarily used for the production of aniline and, to a much lesser extent, for the production of pharmaceuticals and various other chemicals. A very small amount is used as a solvent.

Table 2.1: Use pattern

Type of use	Tonnage [t/a]	Appr. % in this application*
Processing to aniline	1,162,900	98.6
Processing to pharmaceuticals	11,880	1.02
Processing to other chemicals	2,285	0.19
Solvent	115	0.01
Total	1,177180	100

* These figures refer to the company information given in this report

There is a difference of 2,600 t/a between production and processing. The destination of this amount of nitrobenzene could not be clarified, nor is it known for which application it is used.

It is not known that any quantities of nitrobenzene are imported from outside of the EU or exported into it.

In Germany nitrobenzene was used for perfuming soaps in the past as the so called Mirbanoil. But in Germany the use of nitrobenzene in cosmetic products has been forbidden since the 1980th (Cosmetic Regulation from 19th June 1985).

The content of nitrobenzene in different products is presented in the Danish Product Register from June 1998. The only product type is solvents.

Table 2.2: Content of nitrobenzene in different products according to Danish Product Register

Content of nitrobenzene in the product	Number of products	Approximate quantitiy [t/a]
0 - 1 %	116	< 1
1 - 5 %		
5 – 10 %		
10 – 20 %		
20 – 50 %		
50 – 80 %		
80 – 100 %		
Total	116	< 1

2.2.2 Scenarios

According to the information given by the companies 98.6 % of the produced nitrobenzene is used for the production of aniline. Another 1.2 % is processed to pharmaceuticals and other chemicals.

Only 0.01 % of the produced nitrobenzene (115 t/a) is used as solvent. No information is given on the type of the solvent and its further use. It is assumed that emissions from solvent formulation are covered by the emission data provided by the corresponding company. Further on it is assumed that this formulation contains 0.5 % nitrobenzene as in the Danish Product Register there are 116 products listed which contain 0-1% nitrobenzene. That leads to 23,000 t/a of formulation which is then expected to be used as solvent in the private sector, as no other information is available.

Table 2.3: Main, industrial and use category according to the TGD

	Main category	Industry category	Use category	Percentage of total use
Production	Non-dispersive use (1b and 3)	Chemical industry: chemicals used in synthesis (3)	Intermediate (33)	100
Processing: Production to aniline, pharmaceuticals and various other chemicals	Non-dispersive use (1b and 3) Non-dispersive use (1c and 3)	Chemical industry: chemicals used in synthesis (3)	Intermediate (33)	> 99
Use: Use of solvents	Wide dispersive use (4)	Paints, lacquers and varnishes industry (14)	Solvents (48)	< 1

2.3 TRENDS

There are no data available.

2.4 LEGISLATIVE CONTROLS

In Germany the use of nitrobenzene in cosmetic products has been forbidden since the 1980th by the Cosmetic Regulation from 19th June 1985.

3 ENVIRONMENT

See separate report.

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 General discussion

According to the producers, nitrobenzene is mainly used as a chemical intermediate in the manufacture of aniline (approx. 99 %) and, to a much lesser extent, for the production of pharmaceuticals and various other chemicals (approx. 1 %). According to new exposure data from industry nitrobenzene is no longer used as a solvent.

The only information on the content of nitrobenzene in different products is given in the Danish Product Register. In 2003 nitrobenzene was present in 23 adhesive or binding products and reprographic agents in a range of 0-2 % with an approximate quantity of less than 1 tonne per year. These products might be used by professionals or consumers.

However, no information on any of these uses in Europe is available at present. It can be assumed that they are of historical relevance only and that they can be neglected. This assumption is supported by the SPIN database where in the year 2001 nitrobenzene was only present in 41 products in Denmark (reprographic agents) but with an amount of 0 tonnes per year.

For detailed information see chapter 2 (general information on exposure).

For workers the inhalation and dermal routes of exposure are likely to occur.

4.1.1.2 Occupational exposure

Industrial activities using nitrobenzene present opportunities for occupational exposure. Exposure ranges depend on the particular operation and the risk reduction measures in use.

The following occupational exposure limits (OEL) and short term exposure levels (STEL) apply in the EU (Ariel, 2008):

Country	OEL	STEL
Ireland (2007), Sweden (2007), Switzerland (2007)	1 mg/m^3	10 mg/m^3
Finland (2007)	1 mg/m^3	5.1 mg/m^3
Austria (2007)	1 mg/m^3	4 mg/m^3
Germany (2007)	1 mg/m^3	2 mg/m^3
EU (2006)	1 mg/m^3	
United Kingdom (2007), Belgium (2007), Denmark (2007), Norway (2007), France (2008), The Netherlands (2007), Italy (2008), Spain (2008)		-

The following scenario is regarded to be relevant for occupational exposure:

Production of nitrobenzene and further processing as an intermediate (4.1.1.2.1)

The exposure assessment generally aims at assessing exposure levels representing the reasonable worst case situation. The reasonable worst case is regarded as the level of exposure which is exceeded in a small percentage of cases over the whole spectrum of likely circumstances of use for a specific scenario.

The assessment of inhalation exposure is mainly based on measured exposure levels from which – if possible – 90th percentiles are derived as representing reasonable worst case situations (see confidential table 1a in the annex). If available, only data measured later than 1990 are used in exposure assessment. Scenarios are clustered as far as possible to make the description transparent. If quantitative exposure data are not available, model estimates are used.

Beside inhalation exposure, dermal exposure is assessed for each scenario. Two terms can be used to describe dermal exposure:

Potential dermal exposure is an estimate of the amount of a substance landing on the outside of work wear and on the exposed skin.

Actual dermal exposure is an estimate of the amount of a substance actually reaching the skin.

Within the framework of existing substances there is an agreement between the EU member states, to assess - as a rule - dermal exposure as exposure to hands and parts of the forearms. In this, the main difference between both terms – potential and actual - is the protection of hands and forearms by work wear and – more important – the protection by gloves. Within this exposure assessment, the exposure reducing effect achievable by gloves is only considered if information is provided indicating that, for a certain scenario, gloves are a widely accepted protective measure and that the gloves are fundamentally suitable for protection against the substance under consideration. As a measure for the latter, tests according to DIN EN 374 are taken as a criterion. For most down stream uses it is commonly known that gloves are not generally worn. In these cases, dermal exposure is assessed as actual dermal exposure for the unprotected worker. Since quantitative information on dermal exposure is often not available, the EASE model is usually used for assessing dermal exposure.

4.1.1.2.1 Production of nitrobenzene and further processing as a chemical intermediate in the large-scale chemical industry

Nitrobenzene is industrially produced on a large scale and primarily manufactured to aniline. In a much lesser extent (approx. 1%) nitrobenzene is used as an intermediate for the production of other various chemicals or pharmaceuticals. According to available data there are 8 production and/or processing sites of nitrobenzene within the EU. The quantity of nitrobenzene produced in the EU amounts to be 1.18 Mio. t/year.

Nitrobenzene is produced by nitration of benzene with nitrating acid, which is a mixture of nitric acid, sulphuric acid and water. It is usually performed continuously in stirred-vessel cascades or in loop-type reactors (see chapter 2.1.1).

The substance is placed on the market in a technical grade of 99.9 % (liquid).

The transfer to user companies occurs by means of tank cars, rail tankers or tank containers. A minor amount is filled into drums. According to information provided by a manufacturer, in-company transfer occurs via closed pipelines using gas balancing system (Quimigal, 1999).

Exposure associated with transporting the chemical would result from loading, unloading, coupling, uncoupling and drumming operations. For the large-scale chemical industry high standards of control at the workplace are assumed to be practiced even if the containment is breached, e.g. during filling, cleaning, maintenance, repair works and taking of samples. Inhalation exposure in other fields is normally minimized by technical equipment (e.g. special designed filling stations, local exhaust ventilation LEV).

Inhalation exposure

Measured data

The manufacturers submitted workplace monitoring data for the production of nitrobenzene and the further processing to aniline. These data are confidential and summarized.

The exposure level of nitrobenzene in 1990 to 1997 were up to 3.5 mg/m³ (177 samples), with 90th percentile of 0.25 mg/m³. For the purpose of measuring nitrobenzene concentration in the workplace air the substance is adsorbed to silica gel /charcoal, desorbed and detected gas-chromatographically (NIOSH 2010). Due to the measurement method and the sampling strategy applied, the measurement results are regarded as valid.

Based on the measurement results an 8 h TWA of 0.25 mg/m³ is regarded as representing a reasonable worst case situation. Short term exposures up to 0.8 mg/m^3 are possible during loading.

According to information provided by one manufacture 60 workers are employed in the area of production of nitrobenzene. Workers normally use personal protection equipment (PPE; gloves, eye glasses) and, during cleaning activities, respiratory protection in addition.

Summary of the exposure level

Inhalation exposure has to be assessed for the production and further processing of nitrobenzene in fields with high levels of protection (large-scale chemical industry).

For the assessment of health risks of daily inhalation exposure to nitrobenzene during the production and further processing an 8 h time weighed average concentration (8 h TWA) of 0.25 mg/m^3 should be taken to represent a reasonable worst case situation.

Short-term exposure up to 0.8 mg/m^3 (sampling time 30 min) is possible during loading.

It is to be assumed that the substance is processed daily. Consequently, the duration and the frequency of exposure to nitrobenzene are assumed to be daily and for the entire length of shift.

Dermal exposure

According to information from industry the plants for production of nitrobenzene and for the production of aniline or other chemical intermediates using nitrobenzene as a source material consist of fully closed components such as reactors, pipes, pumps, etc.. In case of inspection or repair the plant and its components must be cleaned before this is carried out.

Dermal exposure could occur during activities like coupling and decoupling of transfer lines, drumming, sampling, cleaning, maintenance and repair work.

Modelled data

For the unprotected worker, according to the EASE model, potential dermal exposure is assessed as follows (default assumptions for coupling and decoupling of transfer lines, according to Technical Guidance Document - TGD, 2003):

Input parameters:	Non dispersive use, direct handling, incidental
Level of exposure:	0 – 0.1 mg/cm^2/day.

Considering an exposed area of 420 cm^2 (equivalent to one hand) the model yields an exposure level of 0 - 42 mg/person/day.

For assessing actual dermal exposure levels, it has to be considered that the substance is manufactured and further processed primarily in closed systems and that the use of PPE (here gloves and eye protection) is highly accepted in the large-scale chemical industry. The extent of protection by PPE (here gloves) depends inter alia on the suitability of the recommended material with regard to the permeation properties of substance.

According to the information provided by the industry suitable gloves (tested according to EN 374) are used. The gloves are used for one shift only and are then disposed of. Since no measurement results are available, a protection efficiency of 90 % (according to TGD, 2003) is taken as a default value leading to an exposure level of 4.2 mg/person/day. The value is regarded to represent the reasonable worst case situation.

Summary of the exposure level

For assessing the health risks from daily dermal exposure in the area of production and further processing, an exposure level of 4.2 mg/person/day should be taken. This exposure assessment is based on the assumption, that gloves are suitable for the protection against nitrobenzene.

Exposure to the eyes is largely avoided by using eye protection.

4.1.1.2.2 Summary of occupational exposure

Based on the available information, the exposure assessment reveals that handling nitrobenzene during production and further processing is the main source for occupational exposure.

Only very limited and in part contradictory information is available on the direct use of products containing nitrobenzene (down-stream use). The only information on the content of nitrobenzene in different products is given in the Danish Product Register. In 2003 nitrobenzene was present in 23 adhesive or binding products and reprographic agents in a range of 0-2 % with an approximate quantity of less than 1 tonne per year. However, no information on any of these uses in Europe is available at present. It can be assumed that they are of historical relevance only and that they can be neglected. This assumption is supported by the SPIN database where in the year 2001 nitrobenzene was only present in 41 products in Denmark (reprographic agents) but with an amount of 0 tonnes per year. Further information see chapter 2.2.1.

For occupational exposure there is one scenario:

Production of nitrobenzene and further processing as a chemical intermediate
in the large-scale chemical industry

Relevant inhalation and dermal exposure levels are given in table 4.1.

For the large scale chemical industry, it is assumed that the production and further processing of nitrobenzene is mainly performed in closed systems. Exposure occurs if the systems are breached for certain activities, e.g. coupling and decoupling of transfer lines, drumming.

As concerning dermal exposure producers provided information that suitable gloves (tested according to EN 374) are used regularly. This is considered in assessing dermal exposure during production and further processing using the EASE model assuming a protection efficiency of 90 %. The suitability of the used gloves under real conditions is considered in the assumption of 90 % protection (TGD, 2003). However, the protection efficiency of the gloves might be higher.

Table 4.1 Conclusions of the occupational exposure assessment

Scenario	Activity [1]	Frequence Days/year	Duration Hours/day	Inhalation				Dermal			
				Reasonable worst case		Typical concentration		Reasonable worst case		Typical concentration	
				Unit	Method[2]	Unit	Method [2]	Unit	Method [2]	Unit	Method [2]
Production											

Inhalation exposure (RWC)								
Scenario number, Area of production and use	**Form of exposure**	**Activity**	**Duration [h/day]**	**Frequency [days/year]**	**Shift average concentration [mg/m³]**	**Method**	**Short-term concentration [mg/m³]**	**Method**
Production and further processing as an intermediate	vapour (liquid)	charging, drumming	shift length (assumed)	daily	0.25	workplace measurements (90th percentile)	0.8	workplace measurements (Duration: < 1 h)

Dermal exposure (RWC)								
Scenario number, Area of production and use	**Form of exposure**	**Activity**	**Frequency [days/year]**	**Contact level [1)]**	**Level of exposure [mg/cm²/day]**	**Exposed area [cm²]**	**Shift average [mg/person/day]**	**Method (use of gloves)**
Production and further processing as an intermediate	liquid	coupling and decpouling of transfer lines	daily	incidental	0 – 0.1	420	4.2	EASE (90 % protection, suitable gloves)

[1)] Contact level according to the EASE modell

4.1.1.3 Consumer exposure

There is no information for the use of consumer products containing nitrobenzene. In the BfR-product database no product formulations are listed. Also other available European databases do not give evidences that nitrobenzene is used in consumer products. As well the household products database of the American National Institutes of Health contains no products with nitrobenzene as an ingredient. Therefore it is concluded that consumer exposure is not expected to occur.

Smmary/statement of the exposure level

4.1.1.4 Humans exposed via the environment

Indirect exposure via the environment is calculated using data for oral intake via food, drinking water and air (for calculation see Appendix VI-C). One local scenario, site C with the highest PEClocal for surface water, was considered. Following the data for the regional scenario the total daily dose is smaller. The resultant daily doses for the uptake of nitrobenzene are:

DOSEtot = 0.42 µg / kg bodyweight and day (local scenario site C)
DOSEtot = 0.57 ng / kg bodyweight and day (regional background concentrations)

Table 4.2: Results of calculation of the indirect exposure

intake route	% of total intake	
	Local site C	Regional
Drinking water	47.0	64.9
Air	23.7	1.95
Stem	6.31	0.56
Root	2.44	4.23
Meat	< 0.01	< 0.01
Milk	< 0.01	< 0.01
Fish	20.6	28.4

4.1.1.5 Combined exposure

4.1.2 Effects assessment: Hazard identification and dose (concentration) - response (effect) assessment

4.1.2.1 Toxicokinetics, metabolism and distribution

Nitrobenzene is well absorbed via the gastrointestinal tract, the skin and the respiratory tract. Erythrocytes, spleen, liver and testes were main target tissues for nitrobenzene toxicity.
Data on the metabolism of nitrobenzene are largely derived from animal studies but also from human volunteers. Following oral administration in animals (rats, mice and rabbits) the main metabolites are p-nitrophenol, m-nitrophenol, p-hydroxyacetanilide, p-aminophenol and its conjugates. Species-related differences have been observed (see Table 4.1). Following oral poisoning in humans, p-nitrophenol and p-aminophenol (and/or p-hydroxyacetanilide?) were detected as metabolites in the urine. p-Nitrophenol is excreted in the urine following inhalation exposure in humans. However, p-aminophenol was not detected in the urine by the method used. Metabolites were excreted in the urine slowly. The elimination half-life in men was about 60 h.

4.1.2.1.1 Studies in animals

In vivo studies

Inhalation

Five adult Wistar Rats were exposed to nitrobenzene vapour at a concentration of 25 ppm (130 mg/m^3) for 8 hours, urine was collected over three 24-h periods. After acid hydrolysis to cleave conjugates, both p-nitrophenol and p-aminophenol were detected in the urine at the 24h and 48 h collection periods. No quantitative estimation of absorption was made, but in 24 h urine, levels of p-nitrophenol were about 1.7 µmol/24h per rat and levels of p-aminophenol were about 1.1 µmol/24 h per rat. Both metabolites declined to less than one half of these amounts at the 48 h collection (Ikeda and Kita 1964).

Dermal

Data on dermal application of nitrobenzene to animals are not available. However, based on the toxic effects, which could be observed after dermal application of nitrobenzene to different animal species (mouse, rat, dog, guinea pig, rabbit or cat) it can be derived that nitrobenzene is well absorbed after dermal application (Shimkin, 1939; Levin, 1927).

Oral

Rats and mice
Oral absorption of ^{14}C-nitrobenzene (75, 150, 200 or 300 mg/kg bw) resulted in maximum concentrations of ^{14}C bound in erythrocytes (500 nmol ^{14}C-nitrobenzene equivalent/g RBC) and spleen (130 nmol ^{14}C-nitrobenzene equivalent/g tissue) of male Fischer-344 rats. In male B6C3F1 mice, ^{14}C concentrations in erythrocytes and spleen were lower compared to those in rats. (Goldstein and Rickert 1984).

Maples et al. (1989) have studied the formation of haemoglobin thiyl radical adducts in the blood of male Sprague-Dawley rats following administration of either aniline, phenylhydroxylamine, nitrosobenzene, or nitrobenzene (intragastric injection of 0.1 to 2 mmol/kg). Both phenylhydroxylamine and nitrosobenzene reacted directly within the

erythrocytes, whereas the ability of nitrobenzene to induce haemoglobin radical adducts formation in vivo is likely a reflection of its reduction by the gut microflora or possibly by hepatic microsomal nitroreductase.

Morgan et al. (1985) dosed male Fischer F344 rats orally with ^{14}C-nitrobenzene in order to investigate nitrobenzene-induced encephalopathy in rats. Whole-body autoradiography was analysed after administration of 450 mg/kg nitrobenzene to three animals (the substance was diluted to give 100 µCi ^{14}C/animal). For analysis of parent compound and metabolites in cerebellar homogenates, twelve rats were divided into 4 groups (3 animals/group). After oral administartion of 550 mg nitrobenzene/kg bw, animals were killed at 6, 12, 24 or 48 h after dosing, brains were removed and tissue homogenates were prepared and analyzed by HPLC and liquid scintillation. Autoradiographic and analytical studies demonstrated that a very small percentage (0.02%) of the administered dose reached the brain as nitrobenzene. Maximal concentrations of nitrobenzene were measured 12 h after dosing. No detectable metabolites of nitrobenzene were seen at any of the time points. It was discusssed by the authors, that loss of nitrobenzene might have taken place during sample preparation.

Tissue distribution and haemoglobin binding of orally administered ^{14}C-labelled nitrobenzene (0.2 mmol/kg) was investigated in female wistar rats. Tissue concentrations of total radioactivity were determined after 1 and 7 days. Within 24 h, 50 ± 10% of the radioactive dose appeared in the urine and about 4% in the faeces. After one week, 65% of the dose had appeared in the urine and 15.5% in the feaces. Radioactivity in tissues was presented as (pmol/mg)/dose (mmol/kg bw) and the resulting values were 229 ± 48 in blood, 129 ± 9.5 in liver, 204 ± 27 in kidney and 62 ± 14 in lung. At day 7, the tissue levels were 134 ± 19 in blood, 26.5 ± 3.5 in liver, 48 ± 2.4 in kidney and 29 ± 4.1 in lung (n=3). Binding to haemoglobin was also studied. The resulting hemoglobin binding values were 1030 ± 137 and 1024 ± 82 pmol/mg/dose (mg/kg bw) after 1 and after 7 days, respectively (Albrecht and Neumann 1985).

Rickert et al. (1983) have compared the metabolism of nitrobenzene in male Fischer-344 rats (three animals/group; it is not stated in the publication whether also 3 animals/group were used for CD rats and B6C3F1 mice), CD rats and B6C3F1 mice. In both strains of rats the urinary metabolites after a p.o. dose (22.5 or 225 mg/kg 14C-nitrobenzene) were p-hydroxyacetanilide (up to 20%), p-nitrophenol (up to 20%) and m-nitrophenol (up to 10%). Fischer-344 rats excreted the metabolites as sulphate esters, but CD rats excreted them both as sulfate esters (about 22%) and glucuronide (about 4%). In addition to those metabolites Fischer-344 rats excreted one (about 10%), and CD rats two (up to 47%), very polar but unidentified metabolites in the urine. The major metabolites in the urine of B6C3F1 mice after oral administration of 225 mg/kg 14C-nitrobenzene were the sulfate conjugates of p-aminophenol (10%; enzyme hydrolysis of conjugates), p-nitrophenol (6%), m-nitrophenol (6%) and the two as yet unidentified compounds (8%) and p-hydroxyacetanilide (3%) as the glucuronide. In both rat strains and mice urinary excretion of nitrobenzene peaked 12 to 24 h after administration. Similar excretion rates were observed after an i.p. dose of nitrobenzene. Bile was a minor route of excretion in both strains of rats, 2 to 4% of the dose was excreted by this route within 12 h. There were six unidentified metabolites in bile. In all species 12 to 21% of the radioactivity was found in feces and 1 to 2.2% of the radioactivity was found in the expired air. Excretion rates (total excretion in urine, faeces and exhaled air) of 72 to 88% were found in rats and 54% in mice, respectively.

Table 4.1: Urinary excretion of nitrobenzene metabolites in rats and mice given an oral dose of 225 mg/kg bw nitrobenzene (Rickert et al.1983) [a]

	Fischer-344 rats	CD-rats	B6C3F1 mice
Percentage of dose excreted as:			
p-Hydroxyacetanilide			
Free	-	1.3 [b]	0.4[b]
Glucuronide	-	1.8 [b]	3.1[b]
Sulfate	19.0	5.8[b]	0.4[b]
p-Aminophenol			
Free	-	-	0.1
Glucuronide	-	-	0.2
Sulfate	-	-	9.4[b]
p-Nitrophenol			
Free	-	2.2[b]	0.8[b]
Glucuronide	-	0.5[b]	0.1
Sulfate	19.9	10.3[b]	6.3
m-Nitrophenol			
Free	-	1.2[b]	0.1
Glucuronide	-	0.5[b]	-
Sulfate	10.2	6.2[b]	6.1[b]
Unidentified metabolite I	9.8	23.3[b]	4.8[b]
Unidentified metabolite II	-	5.7	2.6[b]

[a] values for three animals over a 72 h period
[b] significantly different from Fischer 344 rats (Student's t-test; $p \leq 0.05$)

Levin and Dent (1982) have studied the urinary metabolites of nitrobenzene in control or antibiotic treated rats after a single oral dose of 225 mg/kg bw. Antibiotic treatment of rats (neomycin, tetracycline or bacitracin) diminished the excretion of the major reduced metabolite, p-hydroxyacetanilide (about 16% of the dose), by 94% (percentages of the total dose excreted after 72 h). The excretion of the metabolites p-nitrophenol (22%) and m-nitrophenol (11%) was unaffected by antibiotic treatment.

Differences in dietary composition of fermentable carbohydrates in cereal-based and purified diets may mediate differences in metabolism of nitrobenzene and in the degree of nitrobenzene-induced methaemoglobinemia (Reddy et al. 1976; Goldstein et al. 1984; Debethizy and Goldstein 1985).

Rabbits

Parke (1956) investigated the metabolism and distribution of ^{14}C-nitrobenzene after oral dosing of rabbits (200, 250 and 400 mg/kg) (one animal per experiment). Within 4-5 days, 70% of the radioactivity was eliminated via the urine (58%), feces (9%) and the respiratory air (1.6%). 38% of the dose was excreted in the urine as aminophenols (31% p-aminophenol;

acid hydrolysis of urine samples), 9% as p-nitrophenol, 4% as m-nitrophenol and up to 3% as o-nitrophenol); 4-nitrocatechol (0.7%), aniline (0.3%), o-nitrophenol (0.1%), nitroquinol (0.1%) and p-nitrophenylmercapturic acid were identified as further urinary metabolites of nitrobenzene. 1.5 days after dosing, about 54% of the administered radioactivity was found in the tissues, particularly in the kidney fat (15.4%), voluntary muscle (12%), intestinal fat (11.6%) and kidneys (3.6%); unchanged nitrobenzene was present in the tissues. Eight days after dosing, 8% of the radioactivity was found in the tissues, the fat showing the highest radioactivity (5.4%). Furthermore, eight days after dosing total excretion via faeces was 11.3 % of the applied dose and the sum of excretion via urine and expired air was 58.8 % of the applied dose. No unchanged nitrobenzene was detected at this time point.

Other routes

Rats

Nitrobenzene injected subcutaneously to rats (5 males/group) stimulated the activity of nitrobenzene reductase in the supernatant of liver cell homogenate when it was applied for three days at 150 mg/kg bw, but no dose-related or significant response was seen after injection of nitrobenzene (no data on purity) at dosages of 5 and 50 mg/kg bw for 30 days (Wisniewska-Knypl et al. 1975). Reddy et al. (1976) compared the nitroreductase activity in the tissue homogenates of liver, kidneys, gut wall, and gut contents of germ-free, germ-free acclimatized and control rats (male Sprague-Dawley rats) after a single intraperitoneal administration of 200 mg/kg of nitrobenzene in sesame oil. There were no marked differences among these animals in nitroreductase activity of tissues beside that nitroreductase activity in the gut contents of germ-free animals was much lower than in the control and germ-free acclimatized animals (aniline formed 0.2, respectively 15.2 and 11.1 nmoles/mg protein/hr).

Rabbits

Salmowa and Piotrowski (1961) administered nitrobenzene intravenously to rabbits at doses of 10 to 100 mg/kg bw 25% of the dose was detected as p-aminophenol in the urine.

Guinea-pigs

In one animal that received 500 mg/kg ^{14}C-labelled nitrobenzene intraperitoneally, about 58% of the applied radioactivity was recovered from the urine. Metabolites identified were p-aminophenol (26% of the dose), m-nitrophenol (10% of the dose), p-aminophenol (7.5%), o-aminophenol (3.6%) and m-nitrophenol (1.2%). Minor metabolites were 4-nitrocatechol, nitroquinol and p-nitrophenylmercapturic acid (Parke 1956).

Cats

After simultaneous intraperitoneal and subcutaneous dosing of 308 mg/kg bw nitrobenzene, the nitrobenzene levels in the blood of 12 cats averaged 51 $\pm$ 9 µg/ml after 40 min; nitrobenzene in blood started to decrease slowly after 5 – 8 hours. Maximum methaemoglobin concentration (70%) was reached after 120 minutes (Uehleke 1964).

In vitro studies

Nitrobenzene was reduced much more rapidly by liver microsomes of rats under anaerobic conditions than under aerobic (0.33 vs 0.022 nmol/min/mg of protein) whereas, in the intestinal flora (anaerobic conditions), the rate of reduction of nitrobenzene was 150 times faster (668 vs 4.4 nmol/min/g cecal contents or liver). Under anaerobic microsomal conditions, aniline was the sole metabolite whereas the metabolites formed under aerobic conditions consisted of 40% of an unidentifiable metabolite and 60% of p-aminophenol, m-nitrophenol and p-nitrophenol. By activity of the intestinal flora (anaerobic conditions), the metabolites formed were nitrosobenzene, phenylhydroxylamine and aniline. An amount of 3 to 5% was metabolized to acetanilide. Liver microsomes (aerobic conditions) of rats pre-treated with phenobarbital metabolized nitrobenzene three times faster than untreated or methylcholanthrene treated rats (Levin and Dent 1982).

After short incubation of nitrobenzene with liver homogenate of rats under anaerobic conditions, Uehlike (1963) detected nitrobenzene, phenylhydroxylamine and aniline. In homogenates of guinea-pigs, the concentration of phenylhydroxylamine and aniline increased more slowly but reached higher values compared to homogenates from rats.

Nitrobenzene reduction by isolated rat hepatocytes, yielding phenylhydroxylamine plus nitrosobenzene in the medium, was stimulated 1.9-fold and 4.3-fold after phenobarbitone pretreatment in vivo for 5 and 10 d, respectively. After reduction of nitrobenzene by isolated hepatocytes, the secretion of N-oxygenated products into the medium was non-linear with time for substrate concentrations higher than 2.5 mmol, probably due to the formation of cytotoxic concentrations of nitrosobenzene (Blaauboer and van Holsteijn 1983).

Green et al. (1956) have studied the nitroreductase activity of tissues of rat, rabbit, and humans with a large number of aromatic nitro compounds. In general, liver, kidney and duodenum were the most active tissues.

Boar spermatozoa (5 x 10^8 cells) were incubated for 120 min at 30 C° with 15 mmol glucose. The metabolite of nitrobenzene (5.0 mmol) was N-phenylhydroxylamine (< 2 nmol/ 2 h) (Yoshioka et al. 1989).

4.1.2.1.2 Studies in humans

In vivo studies

Inhalation

After occupational, chronic exposure of a 47 year old woman, laboratory investigations revealed, in addition to methaemoglobinaemia, the presence of p-aminophenol and p-nitrophenol in the urine (Ikeda and Kita 1964).

Salmova et al. (1963) have studied the absorption of nitrobenzene vapour (5 to 30 µg/l) through lungs of men after single exposures (16 experiments on seven persons have been performed) under conditions where it is ensured, that absorption takes place via lungs only. The subjects remained outside a chamber containing nitrobenzene vapours and inhaled the air from inside through a gas mask connected with the interior of the chamber Exposure time was

six hours. Exhaled nitrobenzene was trapped by using special bubblers and determined by a colometric, previously established method. Urine was collected every few hours for two to five days. Each urine sample was analysed for p-nitrophenol. Urine samples obtained during the first day were also analysed for p-amoniphenol. For the quantification of p-nitrophenol and p-aminophenol in the urine, colorimetric methods were applied. The retention of nitrobenzene vapours averaged 80% in this time and diminished from approximately 87% in the first hour to 73% in the sixth hour. p-Nitrophenol could be identified as a urinary metabolite of nitrobenzene, whereas p-aminophenol could not be determined in the urine, which was most probably due to the low sensitivity of the method applied (limit of colorimetric detection for p-aminophenol was 10 µg/ml). p-Nitrophenol is excreted in the urine very slowly with half-lives of about 5 and > 70 hours. In the investigated dose range an average of 6 to 22% of the dose is excreted in the urine as p-nitrophenol.

Piotrowski (1967) investigated p-nitrophenol excretion after repeated inhalation of nitrobenzene (intended dose: 25 mg/d; mean daily doses were 18.8, 18.2, 24.7 and 19.5 mg/d) for six hours daily, four successive days or for six hours daily for 6 days intermitted by one day and followed by a six hour exposure for a further day, vapour concentration 5 to 30 µg /l). The excretion of p-nitrophenol increased in the first few days and became fairly steady after the third day. Approximately 16% of the quantity of nitrobenzene inhaled daily was excreted as p-nitrophenol. p-Aminophenol, the presence of which was analyzed for in only one person who was exposed for four days, was not detected (limit of colorimetric detection method was 10 µg/ml). From this study, the authors conclude that nitrobenzene vapour is absorbed with 80% efficiency through the lungs (details were not given).

Dermal

Salmowa and Piotrowski (1961) exposed volunteers dermally. 15 mg per square centimeter of nitrobenzene were dropped on the skin by means of a gauze layer. The area of gauze layer was 4 x 9 cm^2. In 16 experiments the contact of nitrobenzene with the skin lasted one hour, in 8 experiments, the contact of nitrobenzene with the skin lasted five hours. The absorbed doses (under non-occlusive exposure conditions where substantial amounts of the compound might have been evapourated) ranged from 12 to 36 mg (2.2 – 6.6% of the applied dose) when based on urinary excretion of p-aminophenol. The absorption rates in the one-hour experiments varied between 0.25 and 3.19 mg/cm^2 x h. In the five-hour experiments the absorption rates ranged from 0.3 to 0.81 mg/cm^2 x h. p-aminophenol was determined in the urines of the exposed persons. Concentrations of p-aminophenol ranged between 3.9 and 13.6 mg/l and were insignificantly higher compared to the urine of non-exposed persons. Thus, the study is of low reliability due to the fact that considerable amounts of the dermally applied nitrobenzene might have been evaporated since the dermal exposure was performed under non-occlusive conditions.

This may be underlined by in vitro findings from Bronaugh and Maibach (1985) (see section in vitro studies).

Piotrowski (1967) investigated the absorption of nitrobenzene after 6 h exposures of dressed and naked humans (7 volunteers) to nitrobenzene vapour (nitrobenzene Concentration: 5 – 30 mg/m^3) (inhalatory exposure could be excluded by the methodology used). The nitrobenzene absorbed through the human skin was estimated from urinary p-nitrophenol determinations. The author calculated that approximately half as much nitrobenzene vapour was absorbed through the skin as through the lungs when volunteers were exposed to 5 – 30 mg/m^3

nitrobenzene..Vapour absorption through the skin was proportional to the concentration of nitrobenzene in the air. Normal working clothes reduced this absorption by 20-30%. In high humidity, skin absorption of vapour was significantly increased.

Feldmann and Maibach (1970) determined the dermally absorbed nitrobenzene in one human volunteer to be 1.5% of the applied dose. Absorption was determined by measuring radioactivity in urine. In view of the fact that only a small amount of nitrobenzene was applied (52 µg) in acetone solvent to an unprotected site, the finding of only limited absorption should be interpreted with caution.

Oral

In autopsies of five cases of nitrobenzene poisoning in humans, the chemical was found in stomach, liver, brain and blood. The highest concentration found in liver was 124 mg/kg tissue, and in brain, 164 mg/kg tissue (Wirtschafter and Wolpaw, 1944).

The excretion of p-nitrophenol after oral uptake of 30 mg nitrobenzene was investigated and compared with the excretion of p-nitrophenol after oral uptake of 5 mg p-nitrophenol (Piotrowski, 1967). In contrast to oral uptake of p-nitrophenol, which leads to a rapid excretion of p-nitrophenol, p-nitrophenol was excreted slowly when nitrobenzene was given. It was concluded, that accumulation and slow excretion of p-nitrophenol were both due to the slow metabolism of nitrobenzene.

A 19 year old girl survived a suicidal oral dose of about 50 ml of nitrobenzene, approximately 11 g of which was absorbed via the gastro-intestinal tract. p-Aminophenol and p-nitrophenol were excreted in the urine. Maximum daily excretion of p-aminophenol was found at the second day, whereas p-nitrophenol showed a maximum at the third day. The ratio of p-nitrophenol: p-aminophenol was 2:1 (molar ratio). The elimination half-life was estimated to be 84 h (Myslak et al. 1971).

Following a case of severe oral poisoning, p-aminophenol peaked in the urine on the third day after intake. Only p-aminophenol was investigated (David et al. 1965).

<u>In vitro studies</u>

Dermal absorption

Bronaugh and Maibach (1985) investigated the in vitro percutaneous absorption of nitrobenzene through the human skin. In experiments using non-occluded skin, 7.8% of the applied dose of nitrobenzene was absorbed percutaneously, whereas under conditions, where evapouration was prevented, percutaneous absorption raised up to 41.1%.The percentages of applied doses of nitrobenzene remaining at the site of application were 75.8, 30.4 and 21.9% after 1 min, 3 h and 24h.

Metabolism

Green et al. (1956) have studied the nitroreductase activity of tissues of rat, rabbit, and humans with a large number of aromatic nitro compounds. In general, liver, kidney and duodenum were the most active tissues.

4.1.2.1.3 Summary of toxicokinetics, metabolism and distribution

Nitrobenzene is readily absorbed by the oral and inhalation route. Based on its physico-chemical properties (water solubility: 1900 mg/l; octanol-water partition coefficient log Pow: 1.89; molecular weight: 123 g/mol and vapour pressure) and based on animal experiments after oral application of nitrobenzene, an absorption percentage up to 100 % can be taken into account for the oral route.
Based on its physico-chemical properties and on experiments with human volunteers, an absorption percentage of 87 % can be assumed for the inhalation route.
Liquid nitrobenzene as well as nitrobenzene vapour can be absorbed through the skin. Absorption rates from exposure to liquid nitrobenzene have been calculated to be higher (up to 2 mg/cm^2/h) compared to those from exposure from nitrobenzene vapour (absorption rate per unit vapour concentration between 0.23 and 0.3 mg/h per mg/m^3).
From in vivo and in vitro experiments it can be derived that up to about 8% nitrobenzene were absorbed from non-occluded skin whereas up to 40% nitrobenzene were absorbed from in vitro experiments with human skin, when evapouration was prevented.
Nitrobenzene was widely distributed into different tissues as e.g. kidney fat, sceletal muscle and intestinal fat in rabbits and stomach, liver, brain and blood in humans. However, there was no evidence of significant retention of nitrobenzene or its metabolites in the body.
Following oral administration in animals (rats, mice and rabbits) the main metabolites are p-nitrophenol, m-nitrophenol, p-hydroxyacetanilide, p-aminophenol and its conjugates. Species-related differences concerning the amounts of excreted metabolites and conjugates of metabolites have been observed. Approximately 60 % of the applied dose was excreted as urinary metabolites in rats, whereas approximately 35 % of the applied dose was excreted as urinary metabolites in mice. Following oral poisoning in humans, p-nitrophenol and p-aminophenol (and/or p-hydroxyacetanilide?) as metabolites were detected in the urine. After inhalation exposure of humans, approximately 13 % of the inhaled concentration of nitrobenzene was excreted as p-nitrophenol, whereas p-aminophenol was not detected in human urine after inhalation exposure by the method used. Metabolites were excreted in the urine slowly. The elimination half-life in men was about 60 h. Exhalation and excretion via feces represent further pathways of nitrobenzene excretion.

4.1.2.2 Acute toxicity

4.1.2.2.1 Studies in animals

<u>In vivo studies</u>

Inhalation

In a LC50 study according to OECD TG 403, groups of 8 week old male rats were exposed, head-only, to atmospheres of nitrobenzene for single 4-hour periods. The LC50 was

determined to be 556 ppm (2847 mg/m^3, 2.847 mg/l). Findings for dose groups, ppm (deaths/exposed) were as follows: 439 (0/10), 514 (0/10), 542 (1/10), 555 (7/10), 578 (8/10), 714 (10/10). Clinical signs observed during exposure included cyanosis, prostration, slight to severe corneal clouding, lacrimation, pallor, tremors, tachypnea, rales, laboured breathing, hyperactive / aggressive behaviour, white foamy mouth and nasal discharge. An 8 - 21% loss of weight was observed 1 to 4 days post-exposure, but normal weight gain was achieved thereafter. The extent to which those clinical signs appeared was generally concentration related. Deaths usually occurred within 1 to 2 days following exposure; time span was shortened with increased concentration (Dupont 1981, unpublished report).
Other inhalation studies are available for the assessment of acute inhalation toxicity but there are no further LC50 tests.
In an inhalation risk test with rats 3/12 animals died after 7 hours of exposure to nitrobenzene vapours saturated at 20°C. The saturated nitrobenzene vapours were generated by conducting 200 l/h of air through undiluted nitrobenzene at 20°C. None of 12 animals exposed for 3 hours died within an 14-days observation period. After exposure for 7 hours, 3/12 animals died demonstrating severe irritation of mucous membranes. At necropsy, dilatation of the heart, brown discoloration of muscles and organs, swelling of the lungs and infarct-like blood status were detected (BASF AG 1977, unpublished report).
In a second study 6 male rats survived an 8-hours inhalation of vapours saturated at 23.1°C. In this study the saturated nitrobenzene vapours were generated by conducting 400 l/h of air through undiluted nitrobenzene at 23.1°C. The animals demonstrated restlessness, hunched posture, lateral position, closed eyes, uncontrolled movements of the head and enhanced respiration during the first hour of exposure. Between 6 and 7 hours after the start of the exposure white discoloration of eyelids, ears and noses and dark discoloration of iris was detected. At the end of the exposure period animals demonstrated lateral position and tumbling movements. All animals survived and recovered within 4 days after exposure. Necropsy at the end of the 14-days observation period revealed no macroscopically visible changes (Hoechst AG 1977, unpublished report).
In a third study 6 female and 6 male rats survived a 7-hours nose-only exposure to vapours saturated at 20°C. Saturated nitrobenzene vapours were generated by conducting 600 l/h of air through undiluted nitrobenzene at 20°C. The animals demonstrated enhanced respiration, paleness of the skin and passivity during the exposure. All rats survived and one hour after exposure all had recovered. Necropsy at the end of the 14-days observation period revealed no macroscopically visible changes (Hoechst AG 1981, unpublished report).
In 1919 „fumigation“ experiments were conducted with dogs, rabbits, guinea pigs, rats, cats, hens, pigeons and certain parasites. The following conclusions were stated: „Apart from a possible disturbance of the digestive functions and a possible asphyxia due to direct action on the blood, most of the symptoms of poisoning by nitrobenzene may be explained on the basis of disturbances of the cerebellum or cerebellar path. Inhalation of nitrobenzene vapours in toxic doses produces chromatolytic degeneration of the Purkinje cells of the cerebellum. Microscopical examinations have shown only the degeneration and morphological changes in the erythrocytes. The size of the lethal dose depends on certain conditions such as the amount and kinds of fat in the blood. These conditions govern the concentration of nitrobenzene in the vicinity of the nerve cells. A latent period clapses between administration of nitrobenzene and the onset of the symptoms of poisoning“ (Chandler 1919).

Dermal

Dermal LD50 values were calculated for rabbits, resulting in 560 < LD50 < 760 mg/kg bw, and for female rats, resulting in 2100 mg/kg bw.

Doses of 560, 760 and 1000 mg/kg bw in ethanol were dermally applied to the clipped skin of 5 rabbits per dose in a well-ventilated area (chemical hoods) to minimize inhalation hazard to both experimenters and animals. Ventilation was maintained throughout the animal exposure period in an effort to keep conflicting inhalation effects at a minimum. The dosage sleeves were secured with extra layers to retard evapouration due to the increased air movement. The animals were immobilized during the exposure period of 24 hours. No mortality occurred after application of 560 mg/kg and 4/5 rabbits died each after application of 760 mg/kg and of 1000 mg/kg. Clinical signs included manifestations of methaemoglobinemia with symptoms evident within less than 20 minutes. Animals that died (deaths within 4 days) exhibited lethargy and collapse as well as loss of motor coordination. Surviving animals demonstrated lethargy and persisting discoloration of skin and eyes. Within a prescreening test, blue discoloration of skin and eyes were observed after dermal application of 330 mg/kg to one rabbit. Data on necropsy are not mentioned (Harton and Rawl 1976).

A dermal LD50 of 2100 mg/kg bw was detected in a percutaneous application study with female rats using undiluted nitrobenzene (no further technical information). Mortalities occurred between 12-72 h and loss of weight and cyanosis were observed as clinical signs. No relationship was observed between dose applied and time of death. At necropsy, hyperemia of the parenchymatous organs was detected. Histology revealed parenchymatous degeneration and fatty degeneration in liver and kidneys. Formation of methaemoglobin was assessed after dermal application of 2100 mg/kg and demonstrated a 16% elevation after half an hour, 25% after 1 hour and 35% after 2 hours.Intensive formation of Heinz bodies was observed after 24 h (Sziza and Magos 1959).

In a Draize test with 6 rabbits a quantity of 0.5 ml of undiluted nitrobenzene was occlusively applied to the skin of each rabbit for an exposure period of 24 hours. Three of the animals died within 2 days exhibiting signs of cyanosis (Hoechst AG 1977, unpublished report). Based on this data, a dermal LD50 < 300 mg/kg bw can be derived.

Oral

Nitrobenzene revealed moderate acute oral toxicity when administered to rats with LD50 values between 588 and 732 mg/kg bw. Nitrobenzene exposed cats demonstrated higher acute oral toxicity by causing pronounced methaemoglobinemia after oral administration of doses as low as 30 mg/kg bw. But despite of this, all cats examined survived a dose of 120 mg/kg nitrobenzene; a valid LD50 value for cats was not determined.

Oral LD50 values of 732 mg/kg bw and of 588 mg/kg bw were determined for male rats: In the first study a LD50 of 732 mg/kg was calculated using doses of 400, 630, 800 and 1000 mg/kg bw administered per gavage to groups of 10 male rats per group (with sesame oil as vehicle). All rats died after administration of 1000 mg/kg, 4 rats died after administration of 800 as well as 630 mg/kg and none of the animals after administration of 400 mg/kg. Mortalities occurred within 3 days, clinical signs included perturbance of equilibrium, hunched posture, closed eyes, lateral position, cyanosis and paralysis of hind legs. Necropsy revealed dark-brown discoloration of blood in the animals that died within the study, surviving animals demonstrated no macroscopically visible changes (Hoechst AG 1977, unpublished report). The second study resulted in an oral LD50 of 588 mg/kg bw (0.49 ml/kg): Doses of 0.3, 0.4, 0.5, 0.6, and 0.7 ml/kg (eqivalent to 360, 480, 600, 720 and 840 mg/kg) undiluted nitrobenzene were administered to 10 male rats per dose. A dose of 0.3 ml/kg did not cause mortalities, but all of the animals demonstrated clinical signs. These clinical signs included perturbance of equilibrium, piloerection, sedation, cyanosis, bloody eyes and poor reflexes. Two rats died after administration of 0.4 ml/kg, 5 rats after 0.5 ml/kg,

8 rats after 0.6 ml/kg and all 10 rats after 0.7 ml/kg. Mortalities occurred on days 2 to 4. Information on necropsy is not given (Bayer AG 1978, unpublished report).
In female rats oral LD50 values ranged within the same order of magnitude: In a first study an oral LD50 of 650 mg/kg bw was calculated after administration of doses of 320, 500, 630 and 800 mg/kg bw administered per gavage to groups of 10 female rats per group using sesame oil as vehicle. All rats died after administration of 800 mg/kg, 5 rats died after 630 mg/kg, 4 rats after 500 mg/kg and none of the animals after administration of 320 mg/kg. Mortalities occurred within 4 days, clinical signs included perturbance of equilibrium, hunched posture, closed eyes, lateral position, cyanosis and loss of reflexes. Necropsy revealed dark-brown discoloration of blood in the animals that died within the study, surviving animals demonstrated no macroscopically visible changes (Hoechst AG 1977, unpublished report). In a second study an oral LD50 of 640 mg/kg bw was calculated after administration of 280 to 2100 mg/kg bw to female rats as 10% gummy arabicum suspensions per gavage: Mortalities occurred within 2 days (no further information given). Clinical signs observed included restlessness and dribbling of urine; discoloration of skin and visible mucous membranes as typical signs of methaemoglobinemia were detected. At necropsy, hyperemia of the parenchymatous organs was detected. Histology revealed parenchymatous degeneration and fatty degeneration in liver and kidneys. Formation of methaemoglobin was assessed after oral administration of 640 mg/kg and demonstrated an 11% elevation after half an hour, 19% after 1 hour and 28% after 2 hours, intensive formation of Heinz bodies was stated (Sziza and Magos 1959).
In a study with cats measurement of methaemoglobin in blood after oral administration is reported: Cyanosis was detected after administration of 30 mg/kg (25 mm^3/kg). After oral administration of 3, 30, 60 and 120 mg/kg nitrobenzene to groups of 2 cats each, all animals survived. The animals of the 3 mg/kg group did not demonstrate significant elevation of methaemoglobin. After administration of 30 mg/kg slight cyanosis was observed with highest methaemoglobin level (21% and 14.5%) at the 6-hour observation time which decreased to values of 5.1% and 1.7% at the end of the fourth day. After administration of 60 mg/kg methaemoglobin levels rose to 47.3% and 34.3% after 6 hours and decreased to 5.8% and 0% after 96 hours; after administration of 120 mg/kg cyanosis, apathy and mydriasis were detected with methaemoglobin levels of 68.9% and 56.0% after 2 hours decreasing to 18.1% and 7.9% after 96 hours (BASF AG 1970, unpublished report).
Male (80-90 day old) Fischer-344 rats weighing approximately 200 g were divided into seven groups of six rats and fasted 16 hours prior to oral administration of 50, 75, 110, 165, 200, 300 or 450 mg nitrobenzene/kg bw. Control rats received the vehicle corn oil. Histopathological changes consistently involved only liver and testes. One rat of the highest dose had cerebellar lesion (bilateral malacic areas and reactive gliosis in the cerebella pedunculus). Hepatotic centrolobular necrosis appeared inconsistently while hepatocellular nuclear enlargement was consistently detected in rats given doses as low as 110 mg/kg. These data suggest that nuclear enlargement was independent of cell death. Testicular lesions were restricted to the seminiferous tubules, and complete destruction of the spermatocytes at days 2 and 3 after 300 and 450 mg/kg was detected. Necrotic debris and decreased numbers of spermatozoa were seen in the epididymidis. No details are given on the effects of the two lowest doses of 50 and 75 mg/kg (Bond et al. 1981).

<u>In vitro studies</u>

no data available

4.1.2.2.2 Studies in humans

In vivo studies

In the literature there are numerous reports on nitrobenzene poisoning mainly dated back for many decades. An attempt is made to cover the specific criteria of nitrobenzene poisoning and exposure-related disturbances. No or only minor attempts are made to cover the aspects of treatment after nitrobenzene poisoning. In general, treatment consisted of oxygen supply, blood transfusions and intravenous injections of methylene blue.
Nitrobenzene (also called oil of mirbane) has the typical odor of bitter almonds that could be detected in the expired air or in the gastric contents.
However, the most prominent clinical symptoms after human exposure to nitrobenzene are among others cyanosis (see Ewer 1920; Mallouh and Sarette 1993).
The most frequently reported side effect is the often life threatening methaemoglobinaemia. In addition, nitrobenzene exposure is mainly associated with the formation of Heinz bodies in erythrocytes, the toxic effects on bone marrow and lymphoid organs, neurotoxic effects and hepatotoxic effects. Large interindividual variations do exist. This is also due to the fact that often the amount of nitrobenzene absorbed is not known. Babies and children appear to be more sensitive to the effects of nitrobenzene (Beauchamp et al. 1982; David et al. 1964; Monnier 1947; Lareng et al. 1974). It should be noted that derivatives of nitrobenzene, especially m-dinitrobenzene, caused similar effects in 8 workers like nitrobenzene (Bresson et al. 1966).
In the following, a short list of case reports (consumers and workers) is documented. This list does not pretend to be complete but it covers the major aspects of nitrobenzene exposure.

A chemical company reported six cases of nitrobenzene poisoning during the years of 1970 to 1976. No data on type and duration of exposure are given. All six patients were admitted to a hospital after having shown the following symptoms: localized cyanosis, breathing problems, and conjunctivitis. No further details are given (BASF AG, unpublished report, 1992).

Inhalation

It is stated that if a worker was exposed all day at a threshold level value of 1 ppm, approximately 25 mg of nitrobenzene would be absorbed, of which about one-third would be by skin absorption, the remainder by inhalation (Piotrowski 1967).
It is reported that 200 ppm (ca. 1 mg/l) is the maximum concentration that can be inhaled for one hour without serious disturbance, and 1 to 5 ppm (ca. 0.005 to 0.025 mg/l) is considered a safe level for daily exposure (Henderson and Haggard 1943).

Dermal

Five babies aged between 16 days and 11 weeks were exposed to a cloth that was marked with a hospital stamp that contained nitrobenzene. The babies exhibited cyanosis, unregular pulse, breathing problems and convulsions. Two of the five babies with skin problems (no further details) showed more severe signs than the other three babies without skin problems. All babies recovered within a few days (Ewer 1920).
A 2-year old boy developed a dirty, grayish blue color of the skin, lips, and nails after he had worn shoes for a few hours that had been dyed with nitrobenzene. While asleep he had wet his shoes and socking. His breathing was shallow and irregular, with short periods of apnea. The

boy was treated by rest in bed and by oxygen inhalation. The next day his color was normal (Levin, 1927).

As recently as 1993, in Saudi Arabia a two-month-old baby developed a chocolate-colored cyanosis but was otherwise healthy-looking with no evidence of pulmonary, cardiac or central nervous symptoms. Methaemoglobin level was 31.5%. The mother admitted that she had rubbed the child with „Oleum Dulcis", a locally available hair oil which is imported from India. This mixture had a strong almond odor and contained 1% of nitrobenzene. As the patient was asymptomatic apart from being cyanosed, he was observed without treatment. The methaemoglobin level dropped during the three day period (Mallouh and Sarette 1993).

A girl received a lice treatment with a nitrobenzene containing oil. After the third treatment the girl had developed a cyanosis and her room had the odor of bitter almonds. The expired air also had the odor of bitter almonds. Urine contained urobilin and urobilinogen. The girl recovered within about 2 days (Bohland 1919).

Oral

Five nursing mothers had eaten a plum cake that must have contained an aromatic ingredient to simulate a bitter almond taste. Instead of natural bitter almonds it may have contained either nitrobenzene or aniline. The mothers did not reveal any symptoms but their babies developed a strong to very strong cyanosis several hours after breast feeding. The children did not show any additional symptoms and the cyanosis disappeared in about 24 hours. The children were not breast fed for up to 2 days. They received large amounts of tea, and if necessary oxygen and heart stabilizing drugs (Dollinger 1949).

A middle-aged white man was brought to the hospital in a coma. He had a marked ashen-gray cyanosis. There was a very strong odor of nitrobenzene (shoe polish) about the patient, especially in his mouth. Gastric lavage revealed the presence of nitrobenzene. Respiration was decreased to about ten a minute. In spite of vigorous stimulation and oxygen supply the patient died within 45 minutes. He did not regain consciousness (Donovan 1920).

A 48-year-old habitual drinker consumed 200 ml of nitrobenzene. He vomited immediately and the contents had an intense odor of bitter almonds. He became cyanotic within a short period of time, had irregular breathing, and increased motor activity. The blood had a chocolate-brown color. Treatment consisted among others of gastric lavage, 600 ml of bleeding, intravenous transfusion of glucose and blood transfusion. The man was in an immovable position for 4 days. Methaemoglobin and haematin was detected in urine. After about 4 weeks the man had recovered (Voll 1936).

A woman (24 years) decided to commit suicide and swallowed a mixture that contained almost 12 ml of nitrobenzene. She was deeply cyanosed after one hour. Treatment consisted among others in a blood transfusion, intravenous treatment with 10% meythylene blue, saline and glucose. The urine contained methaemoglobin and an excess amount various amino acids (e.g. alanine, serine, glutamine). The patient complained of a severe headache, dizziness, and a bad taste in her mouth. She was afebrile and was never jaundiced. The patient made a rapid recovery within approximately four weeks (Parkes and Neill 1953).

A woman (19 years) survived a suicidal oral dose of about 50 ml of nitrobenzene, approximately 11 g of which was reabsorbed from the gastro-intestinal tract. Severe symptoms, including the formation of 82% methaemoglobin, normalized entirely within 24 days due to quick and extensive treatment. Other symptoms present were unconsciousness, cyanosis (persistence for the next 10 days), irregular and shallow breathing, sluggish reaction of the pupils to light. The venous blood had a chocolate-brown color. There was a distinct odor of bitter almonds in the expired air (Myslak et al. 1971).

A severe toxic methaemoglobinaemia was diagnosed at a 19 year- old male chemistry student who had accidentally ingested between 5 and 20 ml of a brown liquid while using a pipette. Analysis of the gastric aspirate revealed the presence of aniline and nitrobenzene (no further details). He became unconscious and his skin and mucous membranes were navy blue to almost black. A strong smell similar of bitter almonds was noted. Methaemoglobin level was in excess of 65% and decreased to normal levels after 3 days. The man underwent intensive treatment (blood transfusions, diuresis among others). He made an uneventful recovery in about 19 days (Harrison 1977).
A 21-year-old man was thought to have taken about 30 to 40 ml of a nitrobenzene-containing dye used in screen printing about 30 min before admission to hospital. He was reported to have peripheral and central cyanosis; pupils were normal size, heartbeat was 160 beats per minute, blood pressure was 80/54 mm Hg and respiration was 28 per minute. Blood samples were dark brown. After 1 h of positive-pressure ventilation, gastric lavage and intravenous fluids, the patient became conscious and well oriented, with a decrease in heart rate and an increase in blood pressure. Serum methaemoglobin was 4.29 g/dl. A slow intravenous infusion of ascorbic acid was started, and methylene blue was injected intravenously; after 35 min, the colour of the patient changed dramatically from brownish-blue to pink. After a second injection of methylene blue and a transfusion of packed red blood cells, methaemoglobin was 0.6 g/dl. A peripheral blood smear revealed evidence of haemolytic anaemia, but there was no evidence of occult blood in the urine. The patient was discharged on the fifth day of admission (Kumar et al. 1990).

In vitro studies

no data available

4.1.2.2.3 Summary of acute toxicity

Based on acute studies in animals the substance is harmful by the inhalation, dermal and oral route. For rats, the inhalation LC50 was determined to be 556 ppm (2847 mg/m^3, 2.847 mg/l). Oral LD50 values between 588 and 732 mg/kg are reported. Dermal LD50 values ranged from 560 < LD50 < 760 mg/kg for rabbits to 2100 mg/kg for rats. Based on an skin irritation study with rabbits (see 4.1.2.3.1, BASF 1977), a dermal LD50 of < 300 mg/kg is calculated.
Other, not mortality related effects, were observed in cats and rats. Cats survived an oral treatment with up to 120 mg nitrobenzene/kg and cyanosis and a significant elevation of methaemoglobin were the most prominent toxic signs. In rats toxic effects were seen, in addition to the increases in methaemoglobin levels, in liver and testes after a single treatment starting with a dose of 110 mg/kg.
For humans many reports are documented in the literature on nitrobenzene poisoning and to some extent on exposure of workers to nitrobenzene. Considerable individual variation exists and no clear-cut relationship can be documented between the absorbed dose of nitrobenzene and the severity of response in man but babies and children appear to be more sensitive to the effects of the substance. Methaemoglobinamia and cyanosis are the most prominent clinical symptoms; others are the formation of Heinz bodies, toxic effects on bone marrow and lymphoid organs, neurotoxic and hepatotoxic effects. The bulk of evidence clearly demonstrate severe toxic effects of nitrobenzene to humans. In order to guarantee that the specific hazards posed by a substance causing methaemoglobinemia are taken into account appropriately, classification as „toxic“ and labelling with R 23,24,25 (toxic by inhalation in contact with skin and if swallowed) is confirmed.

4.1.2.3 Irritation

4.1.2.3.1 Skin

Studies in animals

Nitrobenzene demonstrated only slight local irritant properties in Draize tests with rabbits.

Very slight irritation was detected after 24 hours occlusive exposure of rabbit skin to 20 mg „chemically pure“ nitrobenzene (6 rabbits). At the 24-hours observation time mild irritation grade 1 was detected which had reversed at the 48 hours observation time (Sziza and Magos 1959). In a second Draize test with 6 rabbits a quantity of 0.5 ml of undiluted nitrobenzene was occlusively applied to the skin of each rabbit for an exposure period of 24 hours. Three of the animals died within 2 days exhibiting signs of cyanosis. Slight skin irritation was detected. In a similar test with a 10% dilution of nitrobenzene in sesame oil no mortality occurred, the animals demonstrated mild skin irritation (irritation index 1.2 according to FDA regulations) (Hoechst AG 1977, unpublished report).

Studies in humans

Effects on the skin are not mentioned in the literature and it can be concluded that irritant effects of nitrobenzene are of minor importance in comparison to the systemic effects observed after dermal absorption.

4.1.2.3.2 Eye

In a Draize eye test with 6 rabbits, 0.1 ml nitrobenzene was instilled into the conjunctival sac of each animal. Conjunctival irritation was highest 1 hour after instillation (irritation index of 2 according to FDA regulations). The substance is assessed as „causes no conjunctival irritation“ according to FDA regulations (no further information) (Hoechst AG 1977). Two rabbits were tested in a second Draize eye test using 0.05 ml of „chemically pure“ nitrobenzene each. Slight conjunctival irritation disappeared within 48 hours, no corneal lesions were observed (Sziza and Magos 1959). One rabbit was tested in a third Draize eye test with 0.1 ml of undiluted nitrobenzene. A moderate area of slight corneal opacity was observed at the 1-hour observation time, mild conjunctival redness and slight conjunctival swelling was detected. The eye returned to normal within one day. In a parallel test with one rabbit the eye was washed 20 seconds after instillation of the substance demonstrating less irritation than the unwashed eye (Dupont de Nemours Co. Inc. 1977, unpublished report).

In a study investigating *in vitro* alternatives to the Draize test for eye irritation was concluded that nitrobenzene could be classified as a non-irritant according to the HET-CAM test, a test performed on the chorioallantoic membrane of hen eggs (Spielmann et al. 1991).

Studies in humans

no data

4.1.2.3.3 Respiratory tract

Studies in animals

In an inhalation risk test with rats 3/12 animals died, demonstrating severe irritation of mucous membranes, after 7 hours of exposure to nitrobenzene vapours saturated at 20°C. (BASF AG 1977, unpublished report, refer to 4.1.2.2.1). Since the exposure period was double as long compared to a regular guideline study and no such effects were reported from other acute inhalation studies or human case studies, these findings were not regarded as sufficient to conclude classifcation of nitrobenzene as a respiratory irritant.

Studies in humans

no data available

4.1.2.3.4 Summary of irritation

Very slight to slight skin irritation was observed in rabbits. Three out of six rabbits died after a 24-hour occlusive exposure with 0.5 ml indiluted nitrobenzene after exhibiting signs of cyanosis. Slight eye irritation was observed in rabbits that disappeared within 24 hours. All tests were not conducted according to OECD TG 404/405. Inflammation of the skin (diffuse or focal and of minimal to mild severity) was observed at the site of nitrobenzene application at the two highest doses (400 or 800 mg/kg bw/d) of a dermal 13-weeks study with mice (NTP, 1983b, refer to chapter 4.1.2.6.1). Nevertheless, from the data presented here it can be concluded that a classification and labelling for irritation/ corrosion is not warranted. Data on effects on the skin and eyes of humans are not available, but the data obtained from case reports also do not warrant a classification and labelling for these effects.

4.1.2.4 Corrosivity

From the data presented in the preceding text (4.1.2.3) it is evident that nitrobenzene is not a corrosive substance.

4.1.2.5 Sensitisation

4.1.2.5.1 Studies in animals

Skin

In vivo studies

A Draize test with „chemically pure“ nitrobenzene tested in guinea pigs is mentioned by Sziza and Magos. This study demonstrated no skin sensitisation (no further data) (Sziza and Magos 1959). Since the method to evaluate skin sensitisation in this Draize test is not given, the applicability of this test to detect skin sensitisation is not plausible. An ear-flank test with guinea pigs resulted also in no skin sensitisation: A 10% dilution of nitrobenzene in dimethyl formamide was applied over three days to the ears of 6 guinea pigs; the flanks were

challenged one week later. The erythematous reaction produced 24 hours after challenge was rated and compared with that in unsensitized controls (Stevens 1967). In this comparative study, the method is reported to demonstrate good reproducible results with many classes of chemical compounds. However, the number of tested animals is too low according to international criteria (ECETOC 1999).

In vitro studies

no data available

Respiratory tract

In vivo studies

no data available

In vitro studies

no data available

4.1.2.5.2 Studies in humans

Skin

In vivo studies

In a review paper on allergies caused by aromatic amino- and nitro-chemicals it is mentioned that the potential of nitrobenzene to cause cross-reactivity in patients that were sensitised by p-phenylendiamine or azo-dyes was low. Three weakly positive cases out of 15 patients were reported (Schulz 1962, test concentration: 1%; vehicle not mentioned). Generally, no cases have been reported from Industry.

In vitro studies

no data available

Respiratory tract

In vivo studies

no data available

In vitro studies

no data available

Additional data: QSAR

The existing data are not sufficient to assess the potential of nitrobenzene to cause sensitisation. Hence, a search on structurally related compounds, which are known to cause

sensitisation, was performed. In the paper from Schlede et al. (2003), six substances are listed, which consist of a benzene ring and, among other substituents, contain a nitro group. These structures were categorised as “significant contact allergen” (six structures) or “solid-based indication for a contact allergenic potential” (one structure). Most closely related to nitrobenzene are 2,4-dinitrochlorobenzene and 2,4-dinitrofluorobenzene (both categorised as "significant contact allergen"). Basketter et al. (1996) reported that dichloronitrobenzene, which has one nitro group, shows a reduced potential to cause skin sensitisation compared to 2,4-dinitrochlorobenzene. However, these structural data indicate that also nitrobenzene may bear some sensitising potential. Furthermore, p-aminophenol, an important metabolite of nitrobenzene, is categorised as "significant contact allergen" (Schlede et al. 2003). On the other hand, structurally related 4-nitrololuene was shown not to have skin sensitising properties, based on a negative Single Injection Adjuvant Test (SIAT) and in a Buehler test (OECD 2003).
Searches in publicly available databases did give the following results: there was no hit in the Danish QSAR database. OECD toolbox did not indicate any other experimental data; based on a Multicase model using GPMT data, nitrobenzene was predicted as negative (skin sensitisation).

4.1.2.5.3 Summary of sensitisation

The animal data are insufficient, since the two available studies (Draize test, ear-flank test) were performed with methods that do not meet international guideline requirements and are considered to be too insensitive. In humans, three weakly positive cases out of 15 patients were reported from a study on cross-reactivity. These data are insufficient to exclude a possible skin sensitisation potential of nitrobenzene. There exist several structurally related compounds which are known to cause skin sensitisation. Therefore, in case workers or consumers may be exposed to nitrobenzene, a Local Lymph Node Assay (LLNA) or a Magnusson Kligman Test should be conducted in order to assess the skin sensitisation potential of nitrobenzene appropriately. Considering animal welfare reasons and present international acceptance, a LLNA should be preferred.

4.1.2.6 Repeated dose toxicity

4.1.2.6.1 Studies in animals

<u>In vivo studies</u>

Inhalation

10 male and 10 female F344 rats, Sprague-Dawley (CD) rats and B6C3F1 mice were exposed by inhalation (whole body exposure) to 10, 35 or 125 ppm (50, 175 or 625 mg/m³) nitrobenzene vapours for 6 hours per day on 5 days per week for up to two weeks (Medinsky and Irons 1985). The study design did not follow strictly the OECD TG 412 (no sacrifice at the end of treatment, no data about lung perfusion and levels of nose sections, no data on adrenal weight, clotting parameters, histopathology from selected tissues, incomplete documentation of all summary group or individual results). Despite of the limitations the

results were accepted as valid, because the findings were consistent to the results of other studies. Up to five animals were sacrificed at three or 14 days following the last exposure. Early morbidity among male and female mice exposed to 125 ppm necessitated euthanasia between 2 and 4 days of exposure. Mice of this group were prostated and exhibited slow, labored breathing. Five male and three female CD rats of the 125 ppm groups were found dead after the fourth day of exposure, the remaining animals in the group exhibiting rapid shallow breathing, wheezing and an orange discoloration around the urogenital orifice were sacrificed at the and of the first week of exposure. In contrast, F344 rats exposed to 125 ppm exhibited no adverse clinical signs over the entire two-week period. Concentration-dependent increases of relative weights were seen in F344 rats killed at day 3 of recovery in the liver (all male dose groups, mid and high dose female dose groups), in the kidneys (all male and female dose groups), in the spleen (males and females of the mid and high dose groups) and in the testes of high dose males. In F344 rats of the 14-day recovery group, persistence of increased relative weights were seen in the spleen and testes of males of the 125 ppm group and higher spleen weights were evident in mid and high dose females. Altered organ weights of CD rats were evident in the spleen only in males exposed to 125 ppm and females exposed to 35 or 125 ppm of the 3-day recovery group. The presumptive cause of death of the CD rats exposed to 125 ppm nitrobenzene was bilateral perivascular haemorrhage in the cerebellar peduncle accompanied by edema and malacia. Perivascular hemorrhage was also found in 5 CD rats which were sacrificed after the first week of exposure and in 8 of 19 mice exposed to 125 ppm and sacrificed on day 2-4 of exposure. Species and sex-related differences in liver pathology were observed in animals exposed to 125 ppm nitrobenzene. 125 ppm male mice sacrificed until day 4 exhibited centrilobular necrosis, superimposed on severe central lobular hydropic degeneration. In contrast, no necrosis was observed in livers from female mice at the same concentration. Hepatocytic degeneration was also noted in mice of each sex exposed to 35 ppm and sacrificed on day 3 of recovery. Liver pathology observed in CD rats was similar but not as severe as that described for the mice. Livers from CD rats that died early exhibited centrilobular hydropic degeneration and basophilic hepatocytic degeneration in periportal areas. The remaining rats that were sacrificed after 5 days of exposure had single cell necrosis and basophilic degeneration of periportal hepatocytes. A mild single cell necrosis was found in all male CD rats sacrificed 3 days after final exposure to 35 ppm. Although male and female F344 rats exhibited a concentration-dependent increase of relative liver weights, no significant histological findings was observed in the livers from male and female F344 rats.

Moderate bronchiolar hyperplasia was observed in male and female mice exposed to 125 ppm nitrobenzene; mild hyperplasia was present in animals examined three days after the last exposure to 35 ppm. Perivascular oedema and vascular congestion were found in lungs taken from dead or moribund CD rats after 3 to 5 days of exposure to 125 ppm nitrobenzene. No histopathologic abnormalities were found in the lungs from F344 rats exposed to 125 ppm nitrobenzene. Dead and moribund CD rats also exhibited moderate to severe hydropic degeneration of cortical tubular cells of the kidneys. Minimal degenerative changes of tubules were noted in the kidneys of some mice exposed to 35 ppm. The only renal lesion in F344 rats was a moderate to severe hyaline nephrosis in males exposed to 125 ppm that regressed in animals allowed to recover for 14 days. Splenic lesions were evident in all rats and mice of all groups exposed to nitrobenzene. Lesions consisted of concentration-dependent increased number of haemosiderin-laden macrophages (predominantly in rats), extramedullary haematopoiesis and acute congestion in the red pulp. They were also present in the groups exposed to ≥35 ppm which survived up to day 14 of recovery. The congestion persisted but was diminished in severity in rats allowed to recover 14 days after exposure to 35 ppm. (Details of effects at 10 ppm were not reported for mice.) A capsular hyperplastic lesion was noted in F344 males of both recovery groups exposed to 35 or 125 ppm on day 3 and day 14

of recovery. Testicular lesions were evident in both rat strains and mice exposed at 125 ppm. Lesions persisted throughout the 14-day recovery period of F344 rats. The predominant lesion in F344 rats consisted of an increase in the number of multinucleated giant cells, sertoli cell hyperplasia, and severe dysspermiogenesis. Seminiferous tubules contained few sperm with maturation arrested at the level of primary or secondary spermatocytes. The lumen of the ductus epididymide contained reduced numbers of mature sperm. This lesion persisted throughout the 2-week recovery period; however, sertoli cell hyperplasia and the increased numbers of giant cells were less severe in rats sacrificed at the end of the 14-day recovery period. The dysspermiogenesis in CD rats was of moderate severity. In mice, the predominant feature was testicular degeneration with an absence of spermatozoa in seminiferous tubules and degeneration of tubular epithelial cells as well as maturation arrest at the level of primary and secondary spermatocytes. The lumen of the ductus epididymis contained no mature sperm. A marked elevation of white blood cells (WBC) was observed in CD male rats exposed to 125 ppm nitrobenzene and sacrificed early. Males exposed to 35 ppm exhibited elevated WBC counts and increased numbers of granulocytes and lymphocytes. No significant changes in WBCs were noted in female CD rats, F344 rats or B6C3F1 mice exposed to nitrobenzene. Circulating red blood cells (RBC) were reduced in both male and female F344 rats and CD rats exposed to 125 ppm (with male CD rats with $4.4x10^6$ compared to $7.7x10^6$ in controls, -42%). Less marked suppression was observed in animals exposed to 35 and 10 ppm nitrobenzene sacrificed on day 3 after exposure. No significant reduction in RBCs was observed in animals allowed to recover for 14 days. Haemoglobin (Hb) was reduced in males and female CD rats in the high exposure group, with male rats exposed to 125 ppm exhibiting a mean Hb of 11.8 gm/dl compared to 14.1 gm/dl in controls (-16%). Haematocrit (Hct) levels were reduced in the 125 ppm group, with males exhibiting a Hct of 30 versus 39 in controls (-23%). Similar changes were seen in females (28 versus 42 in controls, -33%). Mean corpuscular volume (MCV) was slightly elevated in CD rats exposed to 125 ppm nitrobenzene but not in other dose groups. (Only few numerical data were reported in the publication.) F344 rats exhibited a dose-dependent reduction in RBCs at day 3 after exposure but recovered by day 14. Figures for males ranged from 8.7 in the controls to 5.8 in the 125 ppm exposure group on day 3 (-33%). Minor shifts were noted in mean Hb and Hcts from male and female F344 rats exposed to 125 ppm. Mice exhibited no abnormalities in RBCs, Hct, or Hgb, however erythrocytes from both males and females exposed to 125 ppm nitrobenzene exhibited greater MCV than controls. A concentration-dependent increase in blood methaemoglobin was noted in F344 rats of all groups (1.9-11.7% in males vs 0% in controls, 4.8-13.4% in females vs. 3.6 in controls), in female CD rats of all groups (6.3-31.3% vs. 4.8% in controls) and in male CD rats of the 35 and 125 ppm group (8.7-14% vs. 6.9% in controls) sacrificed 3 days after exposure, but not in animals sacrificed on day 14 of recovery. Blood methaemoglobin in CD rats and B6C3F1 mice sacrificed early because of morbidity ranged between 13 and 31 percent. A $NOAEC_{sys}$ for systemic effects was not derived for the rat strain, it was 10 ppm in B6C3F1 mice. The $LOAEC_{sys}$ was 10 ppm in F344 rats and CD rats. Considering local effects on the respiratory system, a $NOAEC_{local}$ was 125 ppm in F344 rats and CD rats and 10 ppm in B6C3F1 mice.

Another 14-day inhalation study (report summary without appendices, DuPont 1981) was done on groups of 16 male CD rats which were exposed head-only on 6 hours/day, 5 days/week, for 2 weeks to 0, 12, 39, or 112 ppm (0, 60, 195, or 560 mg/m^3) and maintained during a 14-day recovery period. Thus the study did not fulfill the requirements of OECD TG 412. The findings were consistent to the results of other studies. During and immediately following exposures, rats of the 39 and 112 ppm dose groups were slightly to moderately cyanotic. After the 6th exposure, rats exposed to 112 ppm had significant weight losses and

increasing incidences of mortality (7 premature deaths on days 7 to 9), hind leg ataxia, labored breathing, and semiprostation were observed. Only one rat of this group survived the treatment period and showed recovery of clinical symptoms during the recovery period. Elevations of methaemoglobin content (2-, 6- and 25-fold of control data) and serum cholesterol (+26, 26 and 54%) were observed at all dose levels. Exposures to 39 and 112 ppm dose-dependently decreased haemoglobin content, erythrocyte count (-8 and -14%) and urine osmolarity, and increased platelet count (+16 and 44%), mean cell volume (+5 and 21%), mean cell haemoglobin (+5 and 23%), urine volume and the frequency of rats with elevated urinary urobilinogen. In addition, exposures to 112 ppm increased immature erythrocytes (polychromasia and microcytosis), relative neutrophil counts (+100%) and monocyte counts (+70%), increased total serum proteins (+9%), and decreased relative lymphocyte counts (-30%), serum alkaline phosphatase (-37%) and creatinine levels (-17%). After 14 days recovery, examination of 5 rats each from the 12 and 39 ppm groups and the one survivor from the 112 ppm group revealed the persistence of several of the haematological effects. Methaemoglobin levels at 12 and 39 ppm had returned to control values within 4 days postexposure. Slightly elevated platelet, leucocyte and relative neutrophil counts and decreased serum creatinine persisted in all exposure groups. In addition, haematocrit, haemoglobin, mean cell volume and mean cell haemoglobin were elevated in the 39 and 112 ppm groups with a clear trend in dose-relationship. (No data on total bilirubin.) Gross pathology findings seen at rats of the 112 ppm group were body weight loss, small and soft testes, small thymus and dark-brownish colored blood. A trend towards increasing organ to body weight ratios for spleen (+8-59%), liver (+8-59%), kidney (+4-25%), and heart (+2-52%) was observed after 10 exposures to all levels of nitrobenzene. Dramatically decreased testicular (-50%) and thymus (-40%) weight ratios were also observed in the 112 ppm group. With the exception of testicular weights, all weights were within normal range after recovery. Histopathological examination of 5 rats of each group at the end of treatment revealed that splenic haemosiderin deposition in rats exposed to 39 and 112 ppm was increased. Exposures to 112 ppm also resulted in moderate to severe haemorrhage and vacuolation (edema) within the brain (cerebellar peduncle and medulla oblongata, mid-brain) and cervical spinal cord, as well as germinal cell atrophy and oligospermia within the testes and epidymides. In addition, lymphoid cell atrophy within the thymus and spleen were observed in most of the rats of this group, some of them also showed (haemorrhagic) pulmonary edema and ocular keratitis. Brain lesions were considered to be the primary cause of death in this group. Splenic haemosiderin deposition remained slightly increased in the 39- and 112 ppm rats following 14-day recovery. The testicular and epididymal lesions were present in the one 112 ppm rat still alive at the end of recovery. A $NOAEC_{sys}$ for systemic effects was not derived in this study, the $LOAEC_{sys}$ was 12 ppm. A $N(L)OAEC_{local}$ is not applicable because the upper respiratory tract was not examined.

Subchronic toxicity

In a 90-day inhalation study (similar to OECD TG 413, no details on methaemoglobin methodology, no adrenal weight), 10 male and 10 female F344 rats (Hamm 1984) were exposed to nitrobenzene concentrations of 0, 5, 16, and 50 ppm (0, 25, 80, and 250 mg/m³) for 6 hours daily, five days a week in a whole body chamber. Urine was collected 30 days prior to the final necropsy. In this study, 5 of the 10 animals per sex and group were used for the determination of methaemoglobin. Results of this study were needed to provide the basis for a carcinogenesis bioassay with F344 rats. No premature deaths occured, no treatment-related effect on body growth and clinical observations was seen. Increased spleen and liver weights in the mid and high dose groups of males and females and decreased testicular weights in the

high dose male group were seen. Necropsy revealed dose-related findings of uniformly distributed pattern of small pale brown and red areas on the surface of livers, pale circumscribed foci consistant with necrosis on the cut surface of livers, enlarged spleens in the male and female high dose groups, and small testicles in the high dose males. In male rats, exposure to nitrobenzene caused elevated total bilirubin levels in the mid and high dose groups, elevated calcium in the all dose groups, and lower BUN in the high dose group. Methaemoglobin was significantly elevated (3-10.5% vs. control values 1.2-1.6%) in all male dose groups and in the female mid and high dose groups, the increases were related to the concentrations. Dose-related changes of red blood cell parameters indicating a haemolytic anemia were evident in all dose groups. Significant lower values for erythrocyte numbers (-4%, -11%, -12% for female dose groups, -11% for high dose males), haemoglobin (-4%, -9%, -12% for female dose groups, and -5% for high dose males) and haematocrit was evident in all female dose groups and high dose males. The erythrocytic volume and numbers of nucleated erythrocytes were increased in mid and high dose groups of both sexes. In addition, Howell Jolly bodies were present in all high dose males and in 2/10 females of the mid dose group and 5/10 females of the high dose group. Light microscopic examination revealed treatment-related lesions in the spleen, testes, epididymides, liver, kidneys, bone marrow and presumably treatment-related findings in the adrenal glands, lymph nodes, and lung. Lesions in the spleens of exposed rats consisted of acute sinusoidal congestion, a moderate increase in both extramedullary haematopoiesis and the number of haemosiderin-laden macrophages infiltrating the red pulp. Fibroblastic hyperplasia of the splenic capsule often containing encysted aggregates of lymphoid cells was observed in one 5 ppm female, one female and one male exposed to 16 ppm and in all animals exposed to 50 ppm. In addition, focal proliferation of mesenchymal cells on the serosal surface was seen in 8/10 males and all females of the high dose group. Focal accumulation of lymphocytes and macrophages beneath the capsule, often accompanied by stromal hyperplasia extending into the splenic parenchyma was observed in all males and 6/10 females of the high dose groups. In the kidneys, a dose-related nephrosis consisting of accumulation of hyaline or eosinophilic droplets in the cytoplasm of proximal tubular epithelial cells occurred in all male dose groups and in high dose females. Moderate to severe degeneration of tubular epithelial cells of testes, maturation arrest of spermatocytes, Leydig cell hyperplasia and aspermia in the epididymus were noted in high dose males. The livers showed disorganization of hepatic cord architecture, vascular ectasia and focal centrilobular hepatocyte degeneration in one male and one female of the low dose groups, one mid dose female and 7/10 males and one female of the high dose groups. In 5/10 males and 3/10 females of the high dose groups increased basophilia of the medullary cells of the adrenal glands were noted. Minimal to slight hyperplasia of the bronchial epithelium was noted in some male and female rats in the high exposure group. Erythroid hyperplasia was evident in the bone marrow in the majority of male and female rats exposed to 50 ppm nitrobenzene. There were no data on CNS effects (no tissue list available). The $LOAEC_{sys}$ for systemic effects in this study was 5 ppm (25 mg/m³), for local effects on the respiratory tract a $NOAEC_{local}$ of 16 ppm was derived.

In a second 90-day inhalation study (similar to OECD TG 413, no details on methaemoglobin methodology, no adrenal weight) 10 male and 10 female CD rats (Hamm 1984) were exposed to nitrobenzene concentrations of 0, 5, 16, and 50 ppm (0, 25, 80, and 250 mg/m³) for 6 hours daily, five days a week in a whole body chamber. Urine samples were collected 30 days prior to the final necropsy. In this study, 5 of the 10 animals per sex and group were used for the determination of methaemoglobin. Results of this study were needed to provide the basis for a carcinogenesis bioassay with CD rats. No premature deaths occurred in the mid and high dose groups, but 1/10 males and 1/10 female died during the study without any compound

relationship, no treatment-related effect on body growth and clinical observations was seen. Clinical chemistry revealed that male rats had elevated total bilirubin in the 50 ppm group and elevated methaemoglobin (3.2% and 10.1% vs. 0.6% in control males) in the 16 ppm and 50 ppm groups. 50 ppm females had elevated ALAT activity, total bilirubin, and methaemoglobin levels (9.6% vs. control values 2.1%). Haematology revealed haemolytic anemia consisting of reduced numbers of erythrocytes (-15% and -25% in mid and high dose females, - 7% and 21% in mid and high dose males) and lower haemoglobin level (-12% and – 16% in mid and high dose females and -6% and -11% in mid and high males) in males and females of the mid and high dose groups, lower haematocrit and increased erythrocytes width in high dose males and females of the mid and high dose groups, and increased MCH in high dose males and females. In addition, males exposed to 50 ppm showed increased higher WBC and lymphocyte counts. Polychromatic erythrocytes were observed in male rats of all dose groups and in female rats of the mid and high dose groups. Consistent to increases in erythrocyte cell volumes observed in high dose males and in females of the mid and high dose groups, erythrocytes appeared macrocytic in both sexes of the high dose level. High dose males and females of the mid and high dose levels had Holly Jowell bodies in erythrocytes. Female rats of the mid and high dose groups had increased spleen and liver weights. The only gross findings were prominent lobular markings in the small testes in 50 ppm males and enlarged spleen in both sexes exposed at 50 ppm. Treatment-related microscopic lesions were found in spleen, liver, kidneys, epididymides, bone marrow and nasal turbinates of animals of all dose groups. The splenic lesions consisted of sinusoidal congestion, a moderate increase in extramedullary haematopoiesis and an increase in the number of haemosiderin-laden macrophages infiltrating the red pulp and at the mid and high dose levels they were accompanied by a decreased number of lymphocytes and macrophages in the marginal zone. A focal fibroblastic hyperplasia (increase of thickness) of the splenic capsule was noted in 4/10 males and 3/10 females exposed to 50 ppm. Moderate to severe toxic nephrosis (not further characterised) was evident in the kidneys of 9/10 males and minimal-slight nephrosis in males of the low and mid dose groups and in females of the mid and high dose groups. Additional minor lesions (lipid accumulation in proximal tubules) were seen in 4/10 females exposed to 16 ppm, a slight increase of glomerular cellularity was observed in one mid dose male, 2 males and one female of the high dose groups. Liver lesions were evident in the 16- and 50 ppm groups and included an increase in basophilic cytoplasm and/or vacuolocytic hepatocytes, predominantly in the periportal areas, centrilobular hepatocyte hypertrophy, some cells with enlarged nucleoli, and pigmented Kupffer cells. Males exposed to 50 ppm had bilateral testicular atrophy, interstitial cell hyperplasia, and absence of mature sperm in the epididymides. These testicular lesions were also found in one low dose male and a slight reduction in mature sperm in two mid dose males. There was erythroid hyperplasia in the bone marrow of in half of the male and female rats exposed to 16 ppm and in all high dose animals. Increased basophilia of the medullary cells of the adrenal glands were noted in males of the mid and high dose groups. Lesions of the nasal passages which were confined to males of the 16 ppm and 50 ppm groups and females of the 50 ppm group consisted of rhinitis associated with epithelial hyperplasia on the free margins of the turbinates in the anterior passages and goblet cell hyperplasia on the ventral septum. The $LOAEC_{sys}$ for systemic effects in this study was 5 ppm (25 mg/m³), for local effects on the respiratory tract a $NOAEC_{local}$ of 5 ppm was derived.

In a third 90-day inhalation study (similar to OECD method 413, no details on methaemoglobin methodology, no adrenal weight), 10 male and 10 female B6C3F1 mice (Hamm 1984) were exposed to nitrobenzene concentrations of 0, 5, 16, and 50 ppm (0, 25, 80, and 250 mg/m³) for 6 hours daily, five days a week in a whole body chamber. 5 of the 10

mice per sex and group were used for the determination of methaemoglobin. Results of this study were needed to provide the basis for a carcinogenesis bioassay with B6C3F1 mice. Two of 10 control mice, 2 of 10 5 ppm male mice, 1 of 10 16 ppm male mice, 1/10 50 ppm male mice, 2/10 control female mice, 2/10 16 ppm female mice, 4/10 50 ppm female mice died during the study without any treatment relationship. Body weight development was not influenced by treatment. In the 50 ppm group, males and females had increased spleen weights, enlarged spleens and increased liver weights. At 50 ppm, mean activity of ALAT was elevated in male mice and methaemoglobin was produced at a higher level in males and females (5.8% and 5.1% vs. 0.7% and 1.3%). No signs of anemia were evident in mice. Extramedullary haematopoiesis consisting of an increase in the total number and the proportion of precursor cells of erythroid and myeloid/megakarycyte cell lines was found in the spleen in all dose groups of both sexes of mice. Dose-dependently sinusoidal congestion increased. Haemosiderosis in the red pulp was evident in both sexes at all dose levels, its severity increased dose-related in males and above 16 ppm in females. Reduced cellularity of lymphoid dependent areas was noted in half the animals of both sexes exposed to 50 ppm and in two males exposed to 16 ppm. Livers of male mice (4/9 at 16 ppm, 9/9 at 50 ppm) exhibited centrilobular hyperplasia, basophilic cytoplasm and enlarged hyperchromatic nuclei with multiple nuclei. In 7/9 females exposed to 50 ppm relatively less severe centrilobular hyperplasia and hypertrophy resulting in some disorganization of normal cord architecture were noted. Cellular vacuolization of the zona reticularis of the adrenal was found in female mice at 5 ppm and increased in severity with dose. Mice of both sexes exposed to 50 ppm, had a mild hyperplasia of the bronchial epithelium and a generalized bone marrow hyperplasia. The $LOAEC_{sys}$ for systemic effects in this study was 5 ppm, for local effects on the respiratory tract a $NOAEC_{local}$ of 16 ppm was derived.

Chronic toxicity

A solitary study on chronic toxic effects of nitrobenzene was not yet conducted. In a combined chronic toxicity and carcinogenicity study reported in Section 4.1.2.8 (CIIT 1993) an intermediate group of 10 males and 10 females from two rat strains (F344 and Sprague Dawley (CD)) were exposed to nitrobenzene at concentrations of 0, 1, 5, and 25 ppm (0, 5, 25, and 125 mg/m³) for 6 hours daily, 5 days per week for 15 months. In general, target organ effects were consistent to the findings observed after 2 years. Therefore data were only supplemented in the Table 4.1.2.6. In addition, nonneoplastic findings observed in the final sacrifice groups of the carcinogenicity study after 107 weeks of nitrobenzene exposure were reported in Section 4.1.2.8.
In CD rats, methaemoglobin formation was seen in all dose groups after 15 months of treatment, but only at the high dose level at the end of study.
Information gained from the 15-month studies was not considered for the derivation of a N/LOAEC for risk assessment purposes because the list of tissues examined histopathologically was limited. Provided that the results were extrapolated with respect to the study duration the amount of the $LOAEC_{sys}$ of 1 ppm for systemic effects from the 15-month rat studies correlated well to the $LOAEC_{sys}$ of 5 ppm from the 90-day study of Hamm (1984).

Dermal

Original study reports or reliable publications on studies with repeated dermal nitrobenzene administration were unavailable. For the sake of completeness, the data from the EHC report (2003) on dermal studies were inserted here:

In a range-finding NTP study, nitrobenzene was administered to B6C3F1 mice and Fischer-344 rats (both sexes) by skin painting at doses in the range of 200 to 3200 mg/kg bw/d for 14 days (NTP, 1983b). All rats and mice at the 1600 and 3200 mg/kg bw/d doses died or were sacrificed moribund prior to the end of treatment. Treated animals were inactive, ataxic, prostrate and dyspnoeic. Significant depression of weight gain (>10%) was seen in mice from all dose groups. Histologically, mice and rats showed changes in the brain, liver, spleen and testes, with mice less affected than rats. Reticulocyte counts and methaemoglobin levels were increased in mice and rats (all dosage groups except mice receiving lowest dose, 200 mg/kg bw/d); haemoglobin and red blood cells were decreased in rats. (No quantitative details on blood parameters included in the report.)

In a NTP study, nitrobenzene was administered to B6C3F1 mice (10 per sex per group) by skin painting (in acetone vehicle) at 0, 50, 100, 200, 400 or 800 mg/kg bw/d for 13 weeks (NTP, 1983b). The chemical was applied to a shaved area of the skin in the intercapsular region. Mean final body weights were not significantly affected. Six high-dose males were sacrificed moribund, and three died between weeks 3 and 10; seven high-dose females were sacrificed moribund, and one high-dose female and one female of the 100 mg/kg bw/d group died between weeks 2 and 9. Clinical signs in some animals at the high dose included inactivity, leaning to one side, circling, dyspnoea, prostration and, in one, head tilt, whereas a number of dosed females had extremities cold to the touch. One high-dose female exhibited tremors, and two were insensitive to painful stimuli. Inflammation of the skin (diffuse or focal and of minimal to mild severity) was seen at the site of nitrobenzene application at the two highest doses; inflammatory cells were present in the dermis, with varying degrees of involvement of the subcutaneous tissue. There was acanthosis and hyperkeratosis of the epidermis, with occasional thick crusts of necrotic cells or focal areas of necrosis extending deep into the epidermis. Liver weights in treated male mice from the 400 mg/kg bw/d group and females from the 400 and 800 mg/kg bw/d groups were significantly increased compared with controls. At the high dose, a number of periportal hepatocytes were smaller than those in control livers and in treated mice, and there was a noticeable variation in the size of hepatocyte nuclei, especially in the centrilobular zone. The cytoplasm of hepatocytes in many treated mice had a homogeneous eosinophilic appearance, whereas that in controls had a vacuolated appearance characteristic of glycogen-containing cells. While degeneration of the "X" zone of the adrenal glands (the zone of cells adjacent to the medulla) in female mice was noted, the degree of vacuolation in treated animals was reported to be greater than normally seen in controls. Brain lesions were found in 2 of 10 males and 3 of 10 females at 800 mg/kg bw/d; the lesions appeared to be localized in the brain stem in the area of the vestibular nucleus and/or cerebellar nuclei; one high-dose female had a mild bilateral lesion in a nucleus of the ventrolateral thalamus. Such lesions were probably responsible for the clinical behavioural findings of head tilt, leaning to one side and circling. Brain vascular lesions (as described in the rat dermal study; see below) were not observed in this mouse dermal study.

No clear NOAEL was established in this study, with the following findings (among others) noted at the lowest dose of 50 mg/kg bw/d ($LOAEL_{sys}$): lung congestion, adrenal cortical fatty change and variation in the size of hepatic nuclei, especially the centrilobular zone. For local effects on the skin, the $NOAEL_{local}$ is 200 mg/kg bw/d (corresponding to 1.94 mg/m^2; assuming a mice body weight of 0.035 kg and assuming that 10% of a total body surface area of 36 cm² was exposed).

Nitrobenzene was administered to Fischer-344 rats (10 per sex per group) by skin painting (in acetone vehicle) at 0, 50, 100, 200, 400 or 800 mg/kg bw/d for 13 weeks (NTP, 1983b). The chemical was applied to a shaved area of the skin in the intercapsular region. Mean final body

weights were not significantly affected; the body weights in the high-dose groups were not analysed due to a high incidence of early deaths. Seven high-dose male rats died and 3 of 10 were sacrificed moribund between weeks 4 and 10; five high-dose females died and five were sacrificed between weeks 2 and 12. Clinical signs in high-dose males included ataxia, head tilt, lethargy, trembling, circling, dyspnoea, forelimb paresis, splayed hindlimbs, diminished pain response and reduced righting response. Except for dyspnoea in a few females, the other clinical signs were not noted in females. The extremities of a number of rats (both sexes) were cold to the touch and/or cyanotic. Brain lesions were found in both sexes at 800 mg/kg bw/d; the lesions appeared to be localized in the brain stem to areas of the facial, olivary and vestibular nuclei and to cerebellar nuclei and probably correlate with the clinical behavioural findings. These lesions were characterised by demyelination, loss of neurons, varying degrees of gliosis, haemorrhage, fibrin in and around small vessels and occasional capillary proliferation. The brain vascular lesions were characterised by fibrin in and around vessel walls; red blood cells within macrophages at the site of haemorrhage indicated that the effect was real, not an agonal change or secondary to tissue mishandling at sacrifice. Perivascular haemosiderin-containing macrophages were occasionally observed. Brain vascular lesions as described in this dermal study were not observed in the Fischer-344 rat gavage study or in the B6C3F1 mouse dermal study (see above).

No clear NOAEL was established in this study, with lung congestion and fatty change in the adrenal cortex in addition to the haematological findings noted at the lowest dose of 50 mg/kg bw/d. Since no effects on the skin were reported, the $NOAEL_{local}$ is assumed to be 800 mg/kg bw/d (corresponding to 4.9 mg/cm², assuming a rat body of 0.2 kg and that 10% of a total body surface area of 325 cm² was exposed).

Oral

Subacute toxicity

In a gavage study, 6 male and 6 female F344 rats received an administration of 0, 5, 25 and 125 mg/kg bw/d of nitrobenzene for 28 days (Shimo et al. 1994, Japanese publication, German translation available). Additional two groups of animals exposed to 0 and 125 mg/kg bw/d were used for examinations of subsequent recovery for 2 weeks. One female in the 125 mg/kg bw/d group died on day 27. Decreased movement, pale skin, gait abnormalities and decreases of body weights or their gains were seen in the 125 mg/kg bw/d group. Haematology revealed significant decreases of RBC (-32% and – 46% for mid and high dose males; -10% and -35% in mid and high dose females), Hb (-14% and -16% in mid and high dose males; -11% and -17% in mid and high dose females), haematocrit and increased mean corpuscular volume (MCV) in the 25 and/or 125 mg/kg bw/d groups. Erythrocyte counts were -32% and -45% lower in males of the 25 and 125 mg/kg bw/d groups, females of the 125 mg/kg bw/d group showed decreases of -35%. High dose recovery male and females progressed to show increased MCV, whereas RBC numbers were normalized. The counts of WBC were markedly elevated in males of the mid and high dose groups and in high dose females. Blood biochemistry revealed increases of total cholesterol and albumin and decreases of BUN in the 25 and 125 mg/kg bw/d groups, and increases of A/G ratio in both sexes and ALAT, ALP and total protein in females in the 125 mg/kg bw/d group. In the absolute organ weights, increases of the liver, spleen, kidney weight and decreases of the testis and thymus weight were seen in the 125 mg/kg bw/d group. In addition, higher liver weights were also seen in males receiving 5 mg/kg bw/d, and increased weights of the liver and the spleen were evident in both sexes receiving 25 mg/kg bw/d Weight changes of the

spleen and the testis were still present in high dose animals at the end of the recovery period. Histopathology revealed moderate to severe spongiotic changes and brown pigmentation in the perivascular region of the cerebellum, increased extramedullary haematopoiesis and brown pigmentation of the Kupffer cells in the liver, brown pigmentation of renal tubular epithelium and degeneration/atrophy of seminiferous tubular epithelium in the 125 mg/kg bw/d group, and congestion, increased brown pigmentation in the red pulp and increased extramedullary haematopoiesis of the spleen of all dose groups and increased haematopoiesis of the bone marrow in high dose males and females of all dose groups. Findings mentioned above tended to decrease in severity or incidence until the end of the recovery period, but were still present. There were no data on special stainings to identify the pigmentation in several organs. However, we assume that the pigment in the spleen, liver and kidneys was representative for haemosiderin-laden cells involved in the degradation process of altered erythrocytes. Pigmentation of the perivascular region in the cerebellum may be explainable as consequences of local haemorrhage. Thymic weight loss is interpreted as thymic atrophy, although no microscopic changes were described. The LOAEL from this oral 28-day study on rats was 5 mg/kg bw/d.

In a study to assess the immunotoxic potential of nitrobenzene, female B6C3F1 mice (7-8 animals/dose group of each test, except the host resistance tests) were exposed to 0, 30 100, and 300 mg/kg bw/d of nitrobenzene in corn oil by gavage for 14 consecutive days (Burns et al. 1994). After sacrifice on day 15, material for organ weight determination and histologic examination included the liver, thymus, spleen, lungs, kidney and lymph nodes.

Mice receiving 300 mg/kg bw/d of nitrobenzene showed ataxia, lethargy, and circling. A total of 8.5% of the mice receiving 300 mg died between the first and 14th day of exposure. Mice of the high dose group showed a significant higher body weight than control animals. Both liver and spleen weights were dose-dependently increased at all dose levels (with significance in the mid and high dose groups). Animals of the mid and high dose groups showed hepatomegaly and splenomegaly. At 300 mg/kg bw/d of nitrobenzene, changes in the spleen were severe congestion of the red pulp areas with increased numbers of erythrocytes, reticulocytes, immature-appearing granulocytes and haemosiderin pigmentation. The livers showed very mild hydropic degeneration around central veins. Mice receiving 100 mg/kg bw/d had mild congestion of the splenic red pulp with occasionally increased extramedullary erythropoiesis and slight increase of haemosiderin pigmentation. Serum chemistry profiles of ALAT, ASAT, BUN, albumin, total protein and bilirubin of the high dose animals showed increases in ALAT (non-sign.) and ASAT (sign.) activities, presumably indicating liver toxicity, and increased bilirubin concentrations, presumably related to erythrocytic degradation. Mean levels of blood glucose were decreased and levels of albumin were increased dose dependently with significance in the mid and high dose groups. Haematologic studies (methaemoglobin not measured) showed a dose-dependent decrease in erythrocyte number and a concomitant increase in mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV). A dose-dependent increase in peripheral reticulocytes was also seen. These increases gained significance at 100 mg/kg bw/d of nitrobenzene and higher, the decrease of RBC was significant at the high dose group. DNA synthesis in bone marrow cells monitored by ^{3}H-thymidine incorporation was enhanced dose-dependently and with significance in all dose groups, as was the number of nucleated cells and the number of monocyte/granulocyte stem cells (CFU/GM colonies) in the bone marrow of treated mice. In a haemolytic plaque assay, T-dependent IgM responses to sheep erythrocytes after sensitisation on day 11 of exposure were decreased in animals at the mid and high dose levels. In these dose groups, the phagocytic activity of macrophages determined by the vascular clearance of ^{51}Cr sheep erythrocytes was dose-dependently increased in the liver with a

concomitant decrease in the activities in the spleen and lung, interpreted as a compartment shift. The mobile macrophages of the peritoneal cavity were increased at the 300 mg/kg dose level and a greater percentage of the adherent cells were phagocytic. The mitogenic response of spleen cells to T cell mitogens (PHA and Con A) was suppressed following exposure to 100 and 300 mg/kg of nitrobenzene, but no effects on the response to B cell mitogen LPS were observed. Also, the ability of nitrobenzene-exposed splenic T cells to respond to alloantigens in the mixed lymphocyte response and the function of natural killer cells were depressed in the mid and high dose mice. Other immunological parameters (serum complement levels, delayed hypersensitivity response, host resistance tests) examined were unchanged. No NOAEL could be determined, the LOAEL in this oral 14-day study was 30 mg/kg bw/d.

Unpublished data from two additional studies were taken over from the EHC report (2003):

In a range-finding US National Toxicology Program (NTP) study, nitrobenzene was administered to B6C3F1 mice and Fischer-344 rats (both sexes) by gavage at doses in the range of 38 to 600 mg/kg bw/d for 14 days (NTP, 1983a). All rats and mice at the high dose of 600 mg/kg bw/d and all rats at 300 mg/kg bw/d 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 (>10%) was seen in male mice at 37.5 mg/kg bw/d and in mice of both sexes at 75 mg/kg bw/d. Histologically, mice and rats showed changes in the brain, liver, lung, kidney and spleen.

In a 13 week NTP study, nitrobenzene was administered to B6C3F1 mice (10 per sex per group) by gavage at doses of 0, 19, 38, 75, 150 or 300 mg/kg bw/d (NTP, 1983a). Mean final body weights were not affected. 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. Liver weight in treated mice was increased compared with controls; the increase was statistically significant at the two highest doses in males and at all doses in females. Fatty change was reported in the X-zone (basophilic cells that surround the medulla around 10 days of age, then gradually disappear as mice mature) of the adrenal glands of 8 of 10 high-dose female mice. One high-dose male had acute necrosis in the area of the vestibular nucleus in the brain. No NOAEL could be derived from this study. Based on the limited information from the summary data the LOAEL was 19 mg/kg bw/d.

Nitrobenzene was administered to Fischer-344 rats (10 per sex per group) by gavage (in corn oil) at doses of 0, 9.4, 19, 38, 75 or 150 mg/kg bw/d for 13 weeks (NTP, 1983a). Mean final body weights were not affected. Seven high-dose male rats died, and 2 of 10 were sacrificed moribund during weeks 10, 11 and 13. One high-dose female died and two were sacrificed during weeks 6, 7 and 9. Clinical signs included ataxia, left head tilt, lethargy, trembling, circling and dyspnoea, as well as cyanosis of the extremities in the two highest dose groups in both sexes.

Brain lesions were found in 8 of 10 males and 7 of 10 females at 150 mg/kg bw/d; the lesions appeared to be localized in the brain stem to areas of the facial, olivary and vestibular nuclei and to cerebellar nuclei and probably correlate with the clinical findings of head tilt, ataxia, trembling and circling. These lesions were characterised by demyelination, loss of neurons, varying degrees of gliosis, haemorrhage, occasional neutrophil infiltration and, occasionally, the presence of haemosiderin-containing macrophages. Brain vascular lesions (as described in

the rat dermal study) were not observed in this gavage study. Based on the summary data a NOAEL of 38 mg/kg bw/d could be derived.

Using the OECD Combined Repeat Dose and Reproductive/ Developmental Toxicity Screening (ReproTox) test protocol (OECD TG 422), nitrobenzene was given by gavage to Sprague-Dawley rats (10 per sex per group) at 0, 20, 60 or 100 mg/kg bw/d throughout premating (14 days), mating (14 days), gestation (22 days) and lactation (4 days); females and pups were necropsied at this stage, while surviving males were killed at day 41 or 42 (Mitsumori et al. 1994). Haematology, organ weight and histopathology data were available for treated males. At 100 mg/kg bw/d, animals exhibited piloerection, salivation, emaciation and anaemia from day 13. Two males of the high dose died on Days 21 and 35. Seven and two females in the same dose group died during the gestation period and lactations period, respectively. One female each from the 60 and 20 mg/kg/d groups died during the lactation period. Additionally, some animals exhibited neurological signs (torticollis, circling movement, abnormal gait), with deaths of two males and nine females. High-dose animals showed reduced food consumption during 1-7 days prior to mating, days 0-7 and 14-21 of pregnancy, and body weight gain was significantly depressed during the study. Males (no data on females) from each treated group demonstrated significant decreases in erythrocyte numbers, haemoglobin and haematocrit and, at the mid and high dose level, significant increases in MCV, MCH, reticulocte numbers and erythroblast numbers. A significantly increased leucocyte count was also observed in high dose males. Six mid-dose females showed anaemia, with neurological signs in one and, during the lactation period, reduced food consumption and body weight gain. Blood biochemical changes (increase in total bilirubin and total cholesterol in high dose male) of liver toxicity were reported. Increased absolute and relative organ weights of liver and spleen were seen in treated males; smaller increases were seen in the absolute and relative kidney weights of mid- and high-dose males. Neuronal necrosis and gliosis were observed in certain nuclei in the cerebellar medulla and pons in mid- and high-dose males (respective incidence of 3/10 and 10/10 compared with 0/10 for controls and low-dose rats). All males in the high and mid dose groups and one male in the low dose group showed atrophy of seminiferous tubules, its severity being dose-dependent. In all treated males, centrilobular swelling of hepatocytes, hemsiderin deposition in Kupffer cells and extramedullary haematopoiesis in the liver, increased extramedullary haematopoiesis and haemosiderin deposition in the spleen, increased haematopoiesis in the bone marrow, and haemosiderin deposition in the renal proximal tubular epithelium were also observed (.No data on histopathology on females.)

The LOAELsys was 20 mg/kg bw/d following a total of 41 or 42 treatment days in male rats.

Other information from repeated-dose studies on specific endpoints

Methaemoglobinemia

Nitrobenzene-induced methaemoglobinemia was higher in rats fed with a pectin-rich diet via the influence of the number of anaerobes present in the rat ceca. 50 male F344 rats (no data on exact number of animals/group) were fed either with a purified diet containing 5% cellulose, a diet with 5% pectin replacing the cellulose, or a cereal-based diet containing 8.4% pectin for 28 days (Goldstein et al. 1984). Following this period, 200 mg/kg nitrobenzene (no data on purity) was administered once by gastric intubation, and methaemoglobin concentrations were elevated as early as 1 h, peaked at 4 h and diminished thereafter in rats fed with the diet containing 8.4% pectin. The increase of concentrations was lower in rat fed with the diet

containing 5% pectin, no significant increase was seen in the purified diet containing cellulose. The number of cecal anaerobes was significantly higher in pectin-containing diets, the highest increase was seen in rats fed with the diet containing 8.4% pectin. The maximum concentration of methaemoglobin was 40% in rats receiving 200 mg/kg of nitrobenzene and the 8.4% pectin in the diet, methaemoglobin concentrations increased to levels about 60% and above in animals which were administered to 400 mg/kg or 600 mg/kg of nitrobenzene.

No measurable methaemoglobin production was evident in germ-free or antibiotic pretreated male Sprague-Dawley rats up to seven hours after intraperitoneal administration of 200 mg/kg bw nitrobenzene (no data on purity), whereas normal males showed elevated methaemoglobin concentrations (maximum 30-305, 1-2 hours post injection) (Reddy et al. 1976). As the excretion of p- and m-nitrophenol was unaffected by antibiotic treatment (Levin and Dent 1982, see section 4.1.2.1), in can be assumed that other metabolites than these are responsive for methaemoglobin formation.

Testes toxicity

Koida and coworkers (1985, abstract only) found that nitrobenzene effects on sperm and testis were related to the age at the start of treatment and to the duration of treatment. Male SD rats (5 rats per group) at 6, 8, 10 or 40 week old were treated with nitrobenzene (no data on purity) at 50 mg/kg bw for 2 or 4 weeks (no data on application mode, assumed to be gavage, because of the vehicle used). Control animals were treated with a vehicle (sesame oil). All rats were examined for testis/epididymis weights, morphology, testis histomorphology, activity, and number of sperms obtained from the cauda epididymidis. Rats treated from 6-week old showed depressed sperm activity. Eight-week old rats showed depressed sperm activity and testis weight, and 10-week old rats showed depressed sperm activity, a smaller number of sperm and testis weights. Sperm activity appeared unchanged between different durations of treatment, but number of sperms and testis weight decreased in the longer duration of treatment. Microscopical changes in 6-week old rats were weaker than in 8 or 10-week old rats.

Neurotoxicity

Four Wistar rats of an age of two weeks were exposed to constant nitrobenzene vapour at a concentration of 5.4×10^{-11} mol/l (mol. wt. 123, ≈0.3 ppb) for a period of 5 or 10 weeks (Pinching and Doving 1974). Microscopic examination of the olfactory bulb revealed a degeneration of the mitral cell layer, representing the principal relay neurones, with the most densely degeneration in the ventral region.

In an attempt to characterise the brain lesions F344 rats were administered to a single oral dose of 550 mg/kg bw of nitrobenzene (99%) and sacrificed at 6, 24 and 48 hours later (12 males/group) (Morgan et al. 1985). Petechial haemorrhages in the brain stem and cerebellum, and bilaterally symmetric degeneration (malacia) in the cerebellum and cerebellar peduncles developed within 48 hours after treatment.

In vitro studies

Studies on testes toxicity

Nitrobenzene was directly toxic on testicular cells in vitro (Allenby et al. 1990). In Sertoli cell cultures and Sertoli-germ cell cocultures vacuolation of Sertoli cells (at 10^{-3} M), exfoliation of germ cells ($5x10^{-4}$ M), secretion of lactate and pyrovate by Sertoli cells (>$5x10^{-4}$ M). Inhibin secretion by Sertoli cells as a potential marker for stimulation of FSH hormone release was altered in a biphasic manner, with low (10^{-8} to 10^{-6} M) and high (10^{-4} to 10^{-3} M) doses enhancing inhibin secretion while intermediate (10^{-5} M) doses had no effect.

Further information is presented in section 4.1.2.9.

Cytotoxicity

Nitrobenzene (no data on purity) was toxic to cells of human epidermoid carcinoma (KB) and African green monkey kidney cells (AGMK) (Mochida et al. 1986). Cell viability was reduced to 50% of control cultures during a 72-hour exposure to concentrations of 42 µl/ml, resp. 30 µl/ml medium.

4.1.2.6.2 Studies in humans

No data on repeated dose toxicity of nitrobenzene in humans were available.

4.1.2.6.3 Summary of repeated dose toxicity

The effects on the peripheral blood and histomorphologic lesions on the other target tissues were summarised in Table 4.2

Repeated dose studies on mice and rats demonstrated that prolonged exposure to nitrobenzene caused lesions in several organs or organ systems. Toxicity on the haematopoietic system probably initiated by methemoglobin production was seen as the primary effect and related secondary adverse effects occurred in the peripheral blood, bone marrow, spleen, liver and kidneys. Apart from this, toxic effects were seen in the liver, male reproductive organs, central nervous system, kidneys, adrenals, bronchial and nasal passages. Clinical (cyanosis), haematological (decrease of RBC counts, haematocrit, and haemoglobin) and biochemistry examinations (elevated total bilirubin) indicated that nitrobenzene caused haemolytic anemia. In addition, methaemoglobin concentrations were dose-dependently increased, females were more sensitive than males. Methaemoglobin production increased from 1 ppm (5 mg/m³, 15 months) onwards, haemolytic anaemic was evident at 5 ppm (25 mg/m³, 3 months) and above Secondary responses to the erythrotoxicity were also obvious at ≥5 ppm in the spleen, bone marrow, liver and kidney indicated as increased haematopoiesis and/or intracellular brown pigment accumulation (haemosiderosis). The occurrence of immature erythrocytes and reticulocytes in the peripheral blood confirmed the regenerative capacity of medullary and extramedullary haematopoietic precursor cells. Because of the primary function of the spleen in the degradation process of altered/damaged erythrocytes, haematopoiesis, haemosiderosis and congestion were the most predominant lesions in the spleen. Lymphoid atrophy of the spleen may represent an idiopathic toxic effect on the splenic white pulp, but this finding was only described in a single rat study (DuPont 1981).

While comprehensive data are available for the inhalation route, few studies were available for the oral route. They indicate that the lowest dose causing anaemia is 25 mg/kg (14 day study, Shimo et al., 1994); associated haemosiderosis was already seen at 5 mg/kg. Information on anaemic potential for the oral route could only be derived from study summaries quoted from the EHC report (2003). Due to the sparce information available haemologic changes were reported at ≥50 mg/kg (13 week study).
Premature deaths occurred at high doses of 300 mg of nitrobenzene per kg bw/d in mice between the first and 14th day of exposure (Burns et al. 1984), and in rats (after 4th day of treatment) and mice (between 2d and 4th day of exposure) which inhaled 125 ppm (625 mg/m³) of nitrobenzene vapour (Medinsky and Irons 1985). The cause of death was not estimated in the oral mouse study (Burns et al. 1984) which was focussed on immune effects. The morbidity in the inhalation studies was interpreted to reflect anoxic encephalopathy occurring secondarily to haemolytic anemia. In accordance to this, Morgan et al. (1985) reported haemorrhagic malacia of the cerebellum and cerebellar peduncles, regions which are known to show a high vulnerability to anoxic lesions, already after single exposure to high doses (550 mg/kg). No treatment-related lesions of the central nervous system were observed up to 25 ppm (125 mg/m³) in rats and up to 50 ppm (250 mg/m³) in mice exposed for 2 years (CIIT 1993).
The thymus atrophy may be considered to give some hind on an immunosuppressive effect on T-cells in rats exposed orally or by inhalation (DuPont 1981; Shimo et al. 1994). Further investigations also gave some indications on a T-cell suppressive effect. The T-cell proliferation responses to mitogens were suppressed in mice which received nitrobenzene by gavage administration, and the T-cell dependent immunoglobulin production of B-cells was lower compared to control animals. In addition, unspecific immune responses of the monocytic compartment were stimulated as indicated by increased phagocytic activity and numbers of macrophages (Burns et al. 1984). From this study, increased granulopoietic proliferative activity in the spleen as well as increased number of monocyte/granulocyte stem cell in the bone marrow may be indicative for an activation of unspecific immune responses. A higher demand of leucocytes may also be indicated as some rat studies (Hamm 1984; Shimo et al. 1984) revealed increased numbers of white blood cells.

Irrespective of the species, strain or application route, all studies which examined the male reproductive system consistently demonstrated the toxic effect on the spermatogenesis resulting in hypo/aspermia. Lesions occurred in rats from concentrations of 50 ppm (250 mg/m³, 90-day inhalation) (Hamm 1984) and in an oral 28-day study at 125 mg/kg bw/d (Shimo et al 1994). In mice, degenerative lesions of the testes were evident at 35 ppm (175 mg/m³) nitrobenzene (14-day inhalation) (Medinsky and Irons 1985). The Leydig cell hyperplasia was considered to be a secondary effect to the degeneration of the seminiferous tubules.
Adverse effects seen in the liver were reported to be of degenerative nature at high concentrations of 125 ppm exposed to rats on 14 days (Medinsky and Irons 1985). In addition, degenerations of lower extension/severity were evident at 35 ppm. The prolongation to 90 days or 15 months of nitrobenzene exposure produced liver cell degeneration starting from doses of 5 ppm (25 mg/m³, Hamm 1984; CIIT 1993). However, comparing all repeated dose studies on F344 rats and CD rats and B6C3F1 mice (Table 4.1.2.6) liver lesions were not consistently found in each study.
Mice were less sensitive than rats to the anemic effects and the methaemoglobin formation. Although there was no obvious anemia in mice after 90-day inhalation of nitrobenzene vapour (Hamm 1984) and only single red blood parameters were altered at 125 ppm (625 mg/m³;28-d study) (Medinsky and Irons 1985), increased extramedullary and medullary haematopoiesis

confirmed an increase of regenerative erythropoiesis. It may be hypothesized that minimal aenemic effects cannot be excluded but were so low that the compensation of reinforced erythropoiesis was sufficient. In comparison to the rat increases of methaemoglobin concentrations were of lower extent.

There are some minor differences between different rat strains with respect to the liver effects or the fibroblastic reaction of the spleen (Medinsky and Irons 1985). Unlike the CD rat, the F344 rat did not show liver cell degeneration, cerebellar haemorrhage and had capsular fibrohyperplasia in the spleen as shown in the 14-day inhalation study of Medinsky and Irons (1985). In contrast to their findings, spleen fibrohyperplasia was not confirmed in the F344 rat study of Shimo et al. (1984) and they also recorded cerebellar perivascular pigmentation presumably indicating previous haemorrhagic lesions. Due to high rates of unidentified metabolites in the rat strains, it is not possible to presume any clear relationship to differences in the metabolismus. Overall, the differences on species or sex-specific sensitivity were only minor. Comparing the 28-day and 90-day inhalation studies, the results revealed a good consistency in the identification of the main target organs and target effects. (See section 4.1.2.1).

As the main systemic effects and target organs were similar in studies with inhalation, dermal and oral administration, the exposure route seems not to be of importance for nitrobenzene-induced toxicity.

With respect to local effects on the respiratory tract, no consistency of findings was seen in several subacute and subchronic inhalation studies on rats and mice. Whereas some of them did not show adverse effects on the lower respiratory tract (no histomorphology examination of the tissues of the upper respiratory, DuPont 1981), other studies in mice exposed on 14 days and F344 rats exposed on 90 days reported bronchial hyperplasia (Medinsky and Irons 1985; Hamm 1984). In contrast to this, rats showed rhinitis and hyperplasia of nasal mucosa at concentrations ≥ 5 ppm (25 mg/m³) for 2 years (CIIT, 1993), but no bronchial effects (Hamm 1984). After chronic inhalation of nitrobenzene, rats and mice had nasal inflammatory lesions. In addition, mice demonstrated degeneration of the olfactory epithelium at ≥25 ppm (125 mg/m³) following inhalation exposure for 2 years.

Some rat studies indicated neurotoxic effects in the cerebellum (≥125 mg/kg bw/d for 28 days, Shimo et al. 1994) or brain stem areas at high doses of 150 mg/kg bw/d administered orally during 13 weeks or 800 mg/kg bw/d dermally applied for 13 weeks (NTP, 1983 a,b). Severe symptoms of neurodysfunctions, cyanosis and mortalities were also seen in rats of these dose groups. It could not be ruled out that neurotoxicity is a direct effect, but it might interpreted as a secondary effect to haemolysis-related hypoxia. Haemorhage in the cerebellum and other central nervous regions associated with edema and malacia was observed after repeated inhalation exposure (≥112 ppm, 560 mg/m³)14 days) in rats (DuPont, 1981, Medinsky andIrons, 1985) and mice (Medinsky and Irons, 1985) and may be related to moribundity and premortal extravasation.

Another, less documented rat study on neurotoxicity demonstrated that an extremely low concentration of nitrobenzene induced neuronal degeneration on a specific brain localization, the olfactory bulb (Pinching and Doving 1974). Although the data available are fragmentary and need further confirmation by other studies, a dysfunction of the sense of smell cannot be excluded to be associated to nitrobenzene exposure.

Table 4.2: Summary of target organ effects from repeated-dose studies in rodents*

Study protocol	Blood	Liver	Spleen	Testis	Kidneys	CNS	Other results & N(L)OAEL/C	References
Subacute toxicity:								
Inhalation, 0,10,35,125 ppm, (0, 50, 175, 625 mg/m³) 14 d, 6h/d, 5d/w, m+f F344 rats; (Sacrifice on day 3 and 14 after end of exposure)	RBC↓ , methb↑ ≥10 ppm	weight↑ ≥10 ppm	congestion, haematopoiesis↑, haemosiderosis↑ ≥10 ppm, capsular fibroblastic hyperplasia in m ≥35 ppm weight↑ ≥35 ppm	Germ cell degeneration, phagocytosis & maturation arrest, hypospermia, Sertoli cell hyperplasia at 125 ppm weight↑ ≥125 ppm	hyaline nephrosis at 125 ppm (in 10/10 males and 2/10 females) weight↑ ≥10 ppm	∅	$LOAEC_{sys}$ 10 ppm, $NOAEC_{local}$ 125 ppm	Medinsky and Irons 1985
T, R48/23**: Significant increase in haemosiderosis in the spleen (≥10 ppm) in combination with fibrosis in the spleen (≥35 ppm) and Methb production (≥10 ppm). 10 ppm: 6 (for transformation 14 day to 90 day) = 1.66 ppm ≈ 8.3 mg/l (which is significantly below 250 mg/l for Xn). Uncertainties: No sacrifice at the end of treatment, incomplete data on blood cell counts								
Inhalation, 0,10,35,125 ppm, (0, 50, 175, 625 mg/m³) 14 d, 6h/d, 5d/w, m+f CD rats (Sacrifice on day 3 and 14 after end of exposure)	anemia at 125 ppm; RBC↓ Hb ↓, Hct ↓ ∅ at 10+35 ppm, methb↑ in f ≥10 ppm, in m ≥35 ppm, WBC↑ in m ≥35 ppm	centrilobular or periportal degeneration at 125 ppm, single cell necrosis ≥35 ppm	congestion, haematopoiesis↑, haemosiderosis↑ ≥10 ppm weight↑ ≥35 ppm	Germ cell degeneration, phagocytosis & maturation arrest, hypospermia, at 125 ppm	Hydropic degeneration of cortical tubular cells ≥35 ppm ppm	cerebellar haemorrhage, edema, malacia at 125 ppm	mortality, pulmonary vascular edema & congestion at 125 ppm $LOAEC_{sys}$ 10 ppm, $NOAEC_{local}$ 125 ppm	Medinsky and Irons 1985
T, R48/23: Significant increase in haemosiderosis in the spleen (≥10 ppm) in combination with degenerative effects in the kidney (≥35 ppm) and liver ((≥35 ppm) and methb production (≥10 ppm). 10 ppm: 6 (for transformation 14 day to 90 day) = 1.66 ppm ≈ 8.3 mg/l (which is significantly below 250 mg/l for Xn). Uncertainties: No sacrifice at the end of treatment, incomplete data on blood cell counts								
Inhalation, 0,10,35,125 ppm, (0, 50, 175, 625 mg/m³) 14 d, 6h/d, 5d/w, m+f B6C3F1 mice (Sacrifice on day 3 and 14 after end of exposure)	MCV↑,methb↑at 125 ppm	centrilobular necrosis in m at 125 ppm hydropic degeneration ≥35 ppm	congestion, haematopoiesis↑, (occasionally) haemosiderosis↑ ≥35 ppm	Tubular degeneration, aspermia, germ cell maturation arrest at 125 ppm	tubular degeneration at 35 ppm	cerebellar haemorrhage at 125 ppm	morbidity at 125 ppm, bronchial hyperplasia ≥35 ppm ppm $NOAEC_{sys}$ 10 ppm, $NOAEC_{local}$ 10 ppm	Medinsky and Irons 1985

Study protocol	Blood	Liver	Spleen	Testis	Kidneys	CNS	Other results & N(L)OAEL/C	References
T, R48/23: Degenerative effects in the kidneys and livers (≥35 ppm), bronchial hyperplasia (≥35 ppm) in combination with spleen haemosiderosis (≥35 ppm). 35 ppm: 6 (for transformation 14 day to 90 day) = 5.8 ppm ≈ 29 mg/l (which is significantly below 250 mg/l for Xn). Uncertainties: No sacrifice at the end of treatment, incomplete data on blood cell counts.								
Inhalation, 0,12,39,112 ppm, (0, 60, 195, 560 mg/m³) 14 d (10 exposures), 6h/d, 5d/w, m CD rats	Anemia ↑≥39 ppm, methb↑, ≥12 ppm, abnormal RBCs and neutrophilia at 112 ppm	Weight↑ ≥12 ppm	haemosiderosis↑ ≥39 ppm, lymphoid cell atrophy at 112 ppm Weight↑ ≥12 ppm	Germ cell atrophy, oligospermia at 112 ppm Weight↓ ≥12 ppm	creatinine↑ at 112 ppm Weight↑ ≥12 ppm	cerebellar haemorrhage/edema in cerebellum/mid-brain/cervical spinal cord at 112 ppm	Mortality, bw gain ↓, ataxia, semiprostation, labored breathing, thymus atrophy, pulmonary edema, ocular keratitis at 112 ppm Cyanosis >39 ppm, $LOAEC_{sys}$ 12 ppm, $N(L)OAEC_{local}$ not applicable	Dupont 1981
T, R48/23: Significant increase in haemosiderosis in the spleen (≥39 ppm) in combination with methb production (≥12 ppm), anemia and cyanosis (≥39 ppm). 39 ppm: 6 (for transformation 14 day to 90 day) = 6.5 ppm ≈ 32.5 mg/l (which is significantly below 250 mg/l for Xn).								
Dermal 200-3200 mg/kg, 14 d , (m+f) F344 rats Range finding study	RBC↓, Hb↓, reticulocytes ↑, methb↑ ≥200 mg/kg	liver changes (no details)	spleen changes (no details)	testes changes (no details	no data	changes in brain (no details)	mortalities ≥1600 mg/kg bw/d all animals, treated animals inactive, ataxic, prostate, dyspnoeic	NTP, 1983b cited from EHC 2003
Range finding study, no full report available; argumentation for classification not feasible.								
Dermal 200-3200 mg/kg, 14 d, (m+f) B6C3F1 mice Range finding study	reticulocytes ↑, methb↑ at doses above 200 mg/kg	liver changes (no details)	spleen changes (no details)	testes changes (no details	no data	changes in brain (no details)	mortalities ≥1600 mg/kg bw/d all animals, treated animals inactive, ataxic,	NTP, 1983b cited from EHC 2003

Study protocol	Blood	Liver	Spleen	Testis	Kidneys	CNS	Other results & N(L)OAEL/C	References
							prostate, dyspnoeic; bw gain ↓	
No full study report ; argumentation for classification not feasible.								
Oral, 0,5,25, 125 mg/kg bw/d, 28 d, m+f F344 rats	anemia, RBC↓ Hb ↓, Hct ↓, MCV↑, leucocytosis ≥25 mg/kg (no data on methb or total bilirubin)	extramedullary haematopoiesis↑*, Kupffer cell pigmentation at 125 mg/kg Weight↑ ≥5 mg/kg	pigmentation (haemosiderosis) extramedullary haematopoiesis congestion ≥5 mg/kg Weight↑ ≥25 mg/kg	Tubular degeneration& atrophy, hypospermia at 125 mg/kg Weight↓ 125 mg/kg	brown pigmentation in tubules (haemosiderosis) at 125 mg/kg Weight↑ 125 mg/kg	cerebellar spongiosis & perivascular pigmentation at 125 mg/kg	premature death(1/6 f), lethargy, pale skin, gait abnormalities, bw gain ↓, thymus atrophy at 125 mg/kg LOAEL 5 mg/kg	Shimo et al. 1994
T R48/25: increased in haemosiderosis in spleen (≥5 mg/kg) in combination with anaemia (RBC reduction -32%, Hb reduction -14% at 25 mg/kg). 25 mg/kg : 3 (transformation from 28 day to 90 day duration) = 8.3 mg/kg bw/d (which is significantly lower than 50 mg/kg for Xn, R48).								
Oral,0,30,100,300 mg/kg bw/d,14 d, f B6C3F1 mice	RBC↓ at 300 mg/kg, MCH↑ MCV↑, reticulocytes↑ ≥100 mg/kg (no methb data)	hydropic degeneration, haemosiderin pigmentation at 300 mg/kg	haemosiderin pigmentation, extramedullary haematopoiesis& congestion red pulp ≥100 mg/kg	ND	∅	ND	morbidity at 300 mg/kg, bone marrow: cell counts↑, proliferation rate↑ & number of monocytic/ granulocytic stem cells↑ ≥30 mg/kg altered immune responses ≥100 mg/kg LOAEL 30 mg/kg	Burns et al. 1984
Spleen haemosiderosis at ≥100 mg/kg in combination with liver haemosiderosis and hepatocytic degeneration and anaemia at 300 mg/kg. 300 mg/kg : 3 (transformation from 28 day to 90 day duration) = 100 mg/kg bw/d (would require Xn, R 48, if standing alone)								
Oral, 38-600 mg/kg bw/d, 14 d,	No data	Liver changes (no details)	Spleen changes (no details)	No data	Kidney changes (no details)	Changes in brain	Morbidity at 600 mg/kg,	NTP cited from EHC 2003

Study protocol	Blood	Liver	Spleen	Testis	Kidneys	CNS	Other results & N(L)OAEL/C	References
(no data on sex, no. of animals) B6C3F1 mice (range-finding study)						(no details)	treated animals inactive, ataxic, prostate, cyanotic and dyspnoeic; ≥ 38 mg/kg ↓bw gain, changes (no further detail) in lung,	
Range finding study, no full study report ; argumentation for classification not feasible.								
Oral, 38-600 mg/kg bw/d, 14 d, (no data on sex, no. of animals) F344 rats (range-finding study)	No data	Liver changes (no details)	Spleen changes (no details)	No data	Kidney changes (no details)	Changes in brain (no details)	Morbidity at ≥300 mg/kg, treated animals inactive, ataxic, prostate, cyanotic and dyspnoeic, changes (no further detail) in lung,	NTP cited from EHC 2003
Range finding study, no full study report ; argumentation for classification not feasible.								
Subchronic toxicity:								
Inhalation 0,5,16,50 ppm, 0, 25, 80, 250 mg/m³), 90 d, 6h/d,5d/w, m+f F344 rats	haemolytic anemia ≥5 ppm, methb↑ in m≥5 ppm, f≥16 ppm Howll Jelly bodies in m at 125 ppm total bilirubin ↑ ≥16 ppm	focal centrilobular degeneration, liver cell cord disorganization ≥5 ppm	congestion, haematopoiesis↑, haemosiderosis↑, capsular fibroblastic hyperplasia ≥ 5 ppm stromal hyperplasia at 50 ppm	Germ cell maturation arrest,tubular degeneration Leydig cell hyperplasia at 50 ppm	nephrosis: cytoplasmatic eosinophilic droplets in proximal tubules in m ≥5 ppm, f at 50 ppm	ND	adrenals: medullary basophilia bronchial hyperplasia, bone marrow erythroid hyperplasia at 50 ppm $LOAEC_{sys}$ 5 ppm, $NOAEC_{local}$ 16 ppm	Hamm 1984
T, R48/23: Significant increase in haemosiderosis in spleen (≥ 5 ppm) in combination with fibosis in the spleen (≥ 5 ppm), kidney nephrosis (≥ 5 ppm), liver cell degeneration								

Study protocol	Blood	Liver	Spleen	Testis	Kidneys	CNS	Other results & N(L)OAEL/C	References
(≥ 5 ppm), methb production (≥ 5 ppm) and haemolytic anaemia (≥ 5 ppm). 5 ppm ≈ 25 mg/l (which is significantly below 250 mg/l for Xn)								
Inhalation 0,5,16,50 ppm, 0, 25, 80, 250 mg/m³), 90 d, 6h/d,5d/w, m+f CD rats	haemolytic anemia≥16 ppm, methb↑ in m≥16 ppm, in f at 50 ppm, leucocytosis, immature RBCs at 50 ppm total bilirubin ↑ 50 ppm	hepatocytic basophilia/vacuolation & centrilobular hypertrophy, Kupffer cell pigmentation ≥16 ppm weight ↑ ≥16 ppm	congestion, haematopoiesis↑, haemosiderosis↑, ≥5 ppm capsule thickness↑ 50 ppm weight ↑ ≥16 ppm	Tubular atrophy, Leydig cell hyperplasia, aspermia at 50 ppm, occasionally ≥5 ppm weight ↓ ≥50 ppm	nephrosis at 50 ppm	ND	bone marrow erythroid hyperplasia ≥ 16 ppm, rhinitis, epithelial & goblet cell hyperplasia of nasal turbinates ≥16 ppm $LOAEC_{sys}$ 5 ppm, $NOAEC_{local}$ 5 ppm	Hamm 1984
T, R48/23: Significant increase in haemosiderosis in spleen (≥ 5 ppm) in combination with capsular thickening (surrogate for fibrosis) (≥ 50 ppm), liver cell degeneration and pigmentation (assumed to be hemosiderin deposits) (≥ 16 ppm), methb production (≥ 16 ppm) and haemolytic anaemia (≥ 16 ppm). 16 ppm ≈ 80 mg/l (which is significantly below 250 mg/l for Xn)								
Inhalation 0,5,16,50 ppm, 0, 25, 80, 250 mg/m³), 90 d, 6h/d,5d/w, m+f B6C3F1 mice	methb↑ at 50 ppm	centrilobular hyperplasia/ hypertrophia, m ≥16 ppm f ≥5 ppm weight ↑ 50 ppm	congestion, haematopoiesis↑, haemosiderosis↑ ≥5 ppm weight ↑ 50 ppm	ND	ND	ND	adrenals: cortical vacuolization ≥5 ppm bone marrow hyperplasia, bronchial hyperplasia at 50 ppm $LOAEC_{sys}$ 5 ppm, $NOAEC_{local}$ 16 ppm	Hamm 1984
Spleen haemosiderosis at ≥5 ppm and medullar and extramedullar haematopoeisis pointed to erythrotoxic effects that could be compensated by increased erythropoietic activity. Haemosiderosis at 5 ppm was not accompanied by other organ toxicity, toxic effects at 50 ppm ≈ 250 mg/l (would require Xn, R 48, if standing alone)								
Dermal 0, 50, 100, 200,	No data	Liver weight ↑ ≥400 mg/kg	No data	No data	No data	Brain stem lesions at 800	Mortalities, morbidities at	NTP 1983 b cited from

Study protocol	Blood	Liver	Spleen	Testis	Kidneys	CNS	Other results & N(L)OAEL/C	References
400, 800 mg/kg bw/d; 13 wk (m+f) B6C3F1 mice		centrolobular liver cell heterogenicity ≥50 mg/kg				mg/kg	800 mg/kg Clinical signs at 800 mg/kg: inactivity, leaning to one side, circling, dyspnoea, prostration, head tilt, cold extremities; Fatty change in adrenal cortex, lung congestion ≥400 mg/kg: skin inflammation at application site ≥50 mg/kg: lung congestion, adrenal cortical fatty change; $LOAEL_{sys}$ 50 mg/kg bw/d $NOAEL_{local}$ 200 mg/kg bw/d (based on summary data)	EHC 2003
No full study report ; argumentation for classification not feasible.								
Dermal 0, 50, 100, 200, 400, 800 mg/kg bw/d; 13 wk (m+f) F344 rats	Hematologic changes (no details) ≥50 mg/kg					800 mg/kg: lesions in brain stem: demyelination, loss of neurons, gliosis, hemorrhage, hemosiderin-	Mortalities, morbidities at 800 mg; clinical signs in m: ataxia, head tilt, lethargy, trembling, circling,	NTP 1983 b cited from EHC 2003

Study protocol	Blood	Liver	Spleen	Testis	Kidneys	CNS	Other results & N(L)OAEL/C	References
						laden macrophages, capillary proliferation	dyspnoea, forelimb paresis, splayed hindlimb, pain response↓, righting response ↓, both sexes: cold cyanotic extremities. ≥50 mg/kg : lung congestion, adrenal cortex fatty change $LOAEL_{sys}$ 50 mg/kg bw/d $NOAEL_{local}$ 800 mg/kg bw/d (based on summary data)	
No full study report ; argumentation for classification not feasible.								
Oral 0, 19, 38, 75, 150 or 300 mg/kg bw/d, 90 d, (m+f) B6C3F1 mice	No data	F ≥ 19mg/kg, M ≥ 150 mg/kg: Weight ↑	No data	No data	No data	300 mg/kg: brain stem lesions: necrosis in 1 m	300 mg/kg Mortalities (3/10 m deaths or moribund at wk 4-5) , ≥ 75 mg/kg: ataxia, left head tilt, lethary, trembling, circling, dyspnoe, cyanosis LOAEL 19 mg/kg bw/d (based on	NTP 1983a cited from EHC 2003

Study protocol	Blood	Liver	Spleen	Testis	Kidneys	CNS	Other results & N(L)OAEL/C	References
							summary data)	
No full study report ; argumentation for classification not feasible.								
Oral 0, 9.4, 19, 38, 75 or 150 mg/kg bw/d, 90 d, (m+f) F344 rats	No data	No data	No data	No data	No data	150 mg/kg: brain stem lesions: demyelination, loss of neurons, gliosis, hemorrhage, neutrophil infiltration, hemosiderin-laden macrophages	Mortalities, morbidities at 150 mg/kg ≥ 75 mg/kg: ataxia, left head tilt, lethary, trembling, circling, dyspnoe, cyanosis NOAEL 38 mg/kg bw/d (based on summary data)	NTP 1983a cited from EHC 2003
No full study report ; argumentation for classification not feasible.								
Oral 0, 20, 60 or 100 mg/kg bw/d, 14 d (premating), 14 d (mating), 22 d (gestation) and 4 d (lactation), m killed at d 41/42 OECD 422 (m+f) SD rats (male rats considered only)	Dose-dependent anemia (RBC↓, Hb↓, Htk ↓, Methb ↑ in m ≥20 mg/kg, MCV↑, MCH ↑, Reticulocytes ↑ erythroblasts ↑ in m ≥60 mg/kg, leucocytosis in m at 100 mg/kg	Total bilirubin: Dose-dependent ↑ in m ≥20 mg/kg, Total cholesterol ↑ in m at 100 mg/kg; Liver effects in m≥20 mg/kg: liver weight ↑, centrilobular swelling of hepatocyes , hemosiderin deposition in Kupffer cells	Spleen effects in m≥20 mg/kg: Spleen weight ↑; extramedullary hematopoiesis, hemosiderin deposition	Testes atrophy in and hypospermia in epididymis in m≥60 mg/kg:	Kidney effects in m≥20 mg/kg: Hemosiderin deposition Weight ↑ in m≥60 mg/kg:	M ≥60 mg/kg: cerebellar nuclei and pons: neuronal necrosis, gliosis	100 mg/kg: piloerection, salivation, emaciation from day 13, mortalities, torticollis, circling movement, abnormal gait, food consumption ↓ d 1-7 premating, bw gain↓ from d 21 onwards 60 mg/kg: food consumption ↓,	Mitsumori et al. 1994

Study protocol	Blood	Liver	Spleen	Testis	Kidneys	CNS	Other results & N(L)OAEL/C	References
							bw gain↓ Bone marrow: haematopoiesis ↑ LOAEL 20 mg/kg bw/d	
T, R48/25: Increased haemosiderin deposition in spleen (≥20 mg/kg) in combination with anemia (≥20 mg/kg), hemosiderosis and degeneration in liver (≥20 mg/kg). 20 mg/kg :2 (transformation from 41 days of treatment to 90 day duration) = 10 mg/kg (which is significantly lower than 50 mg/kg for Xn, R 48)								
Chronic toxicity:								
Inhalation 0,1,5,25 ppm, (0, 5, 25, 125 mg/m³) 15 mo, 6h/d,5d/w, m+f F344 rats§	anemia, polychromatic cells, Howell-Jolly bodies, methb↑ at 25 ppm, nucleated RBCs, leucocytosis in f at 25 ppm, bilirubin↑ in m at 25 ppm	cystic degeneration, eosinophilic cell foci, centrilobular hypertrophy in m≥5 ppm	haematopoiesis↑, congestion, haemosiderosis↑ ≥1 ppm	ND	increased severity of chronic nephropathy m≥5 ppm, slight increase in incidence of chronic nephropathy in f at 25 ppm	ND	endometrial polyps f≥1 ppm , pigmentation of olfactory ≥25 ppm , $LOAEL_{sys}$ 1 ppm $LOAEL_{local}$ 5 ppm	CIIT 1993
T, R48/23: Increased haemosiderosis (≥1 ppm) in combination of liver degeneration (≥5 ppm): 5 ppm x 5 (for transformation from 15 month to 3 month duration) = 25 ppm ≈ 125 mg/m³ (which is significantly lower than 250 mg/m³ for Xn). Uncertainties: incomplete data								
Inhalation 0,1,5,25 ppm, (0, 5, 25, 125 mg/m³) 15 mo 6h/d,5d/w, m Sprague-Dawley (CD) rats§	anemia, macrocytes, Howell-Jolly bodies, polychromasia in m at 25 ppm, methb↑ in m≥1 ppm	centrilobular hypertrophy Kupffer cell pigmentation (haemosiderosis) m≥5 ppm	congestion ≥1 ppm, haematopoiesis↑, haemosiderosis↑ at 25 ppm	ND	∅	ND	nasal (resp.) epithelium hyperplasia, pigmentation of olfactory epithelium ≥25 ppm, $LOAEL_{sys}$ 1 ppm, $LOAEL_{local}$ 5 ppm.	CIIT 1993
Liver haemosiderosis (≥5 ppm) in combination with methb production (≥1 ppm) and spleen haemosiderosis (25 ppm) and anaemia (25 ppm). 25 ppm x 5 (for transformation from 15 months to 3 month duration) = 125 ppm ≈ 625 mg/m³. Uncertainties: incomplete data								

ND not determined; ∅ no treatment-related effect
* additional findings in clinical symptoms, clinical chemistry, body/organ weights, and anatomical pathology are described in the full text, effects which were not indicated otherwise were reported for both sexes, ** Classification requirements were checked for each study according to criteria for substances inducing haemoltic anaemia (Muller et al., 2006), d day/s, m males, f females, ND no data, ∅ no histopathologic abnormalities, RBC red blood cell, methb methaemoglobin, MCV mean corpuscular volume, $N/LOAEC_{sys}$ No/Lowest observed adverse effect concentration for systemic effects, $N/LOAEC_{local}$ No/Lowest observed adverse effect concentration for local effects on the respiratory tract; § nonneoplastic lesions observed in the final sacrifice groups (2 y) were reported in Section 4.1.2.8

No/Lowest-observed-effect levels/concentrations

Inhalation route/systemic effects

For the purpose of quantitative risk assessment procedures, the 90-day rat studies of Hamm (1984) were considered to give the most reliable data on the effect levels of systemic toxicity. The method used showed the highest equivalency to the OECD TG 413 in comparison to all other data available. A NOAEC for systemic effects could not be derived for the rat strains. The $LOAEC_{sys}$ was 5 ppm (25 mg/m^3) based on data from these two rat 90-day inhalation studies on F344 and CD rats.
The observed anaemia, methemoglobin formation in combination with spleen effects (especially hemosiderosis as a clearly adverse effect) were critical for the N(L)OAEC in both studies. The 90-day study of Hamm (1984) was preferred since dose-response relationship of hemosiderosis was well documented in summary tables. The CIIT study (1993) reported treatment-related hemosiderosis in all dose groups (≥1 ppm, 5 mg/m³) of the interim sacrifice, but incidences and severity showed variations (which could not be checked since no summary tables were available for interim sacrifices). Increased methaemoglobin levels elevated at 1 ppm in this study could also be considered as the most sensitive effect, but their increases were associated with anaemic effects only at 25 ppm (125 mg/m³). Taking also the incomplete data available for the CIIT study into account, the data from the Hamm study were decided to be taken for N(L)OAEC derivation.

90-d inhalation/F344 rat and CD rat: **$LOAEC_{sys}$ 5 ppm (25 mg/m^3)**

Inhalation route/local effects on the respiratory tract

The combined chronic toxicity and carcinogenicity study revealed irritative effects on the nasal respiratory epithelium in CD rats (CIIT 1994; Section 4.1.2.8).

2-year inhalation/CD rat: **$LOAEC_{local}$ ≅1 ppm (5_mg/m^3)**

Oral administration

At present, none of the oral studies were conducted according to the OECD/EEC guidelines for oral repeated dose studies on rodents. Despite the limitations of the method and documentation of the studies available, LOAELs could be estimated to be:

28-d oral/F344 rat: LOAEL 5 mg kg bw/d (Shimo et al. 1994)
14-d oral/B6C3F1 mice: LOAEL 30 mg/kg bw/d (Burns et al. 1984).

As long as no guideline conform study is available, the most sensitive level of 5 mg/kg bw/d (LOAEL) from the 28-d oral/F344 rat study should be used.

Dermal route

No dermal repeated-dose study with compliance to the OECD/EEC guidelines was available. The non-published NTP studies extracted from EHC report (2003) were considered sufficiently reliable to derive no adverse effect levels.

Following their conclusion based on the NTP studies, no NOAEL could be derived from two range-findings studies in rats and mice with dermal application (skin painting) for 13 weeks. They concluded that the LOAEL in both species was 50 mg/kg bw/d nitrobenzene (in acetone as vehicle).

90-d dermal/F344 rat: **$LOAEL_{sys}$ 50 mg/kg bw/d (NTP, 1983b)**
90-d dermal/B6C3F1 mice: **$LOAEL_{sys}$ 50 mg/kg bw/d (NTP, 1983b)**

In these NTP studies, local inflammatory response of the dermis were seen at doses ≥400 mg/kg bw/d nitrobenzene (in acetone vehicle) in mice, no effects were reported in rats at the maximum dose of 800 mg/kg bw/d.

90-d dermal/ B6C3F1 mice **$LOAEL_{local}$ 200 mg/kg bw/d (**1.94 mg/m²) **(NTP, 1983b)**
90-d dermal/ F344 rat **$NOEL_{local}$ 800 mg/kg bw/d (**4.9 mg/cm²) **(NTP, 1983b)**

Conclusion/Classification

Classification Toxic , T, R 48/23/24/25
Nitrobenzene is already classified and labelled as Toxic, T, R 48/23/24. According to the criteria of the Directive 67/543/EEC, the extension of the labelling to R 48/23/24/25 is proposed.
The original proposal for T, R48/23 is confirmed by adverse effects observed in several inhalation studies at concentrations that were significantly lower than the critical value for Xn (see check for classification in Table 4.2).
For the dermal route no full study report is available from NTP studies (citation from EHC report, 2003) to reconstruct the original proposal for T, R48/24. As far as indicated, the toxic profile of dermal nitrobenzene treatment is in line with effects seen for other routes.
Nitrobenzene orally administered to F344 rats on 28 days induced increased haemosiderosis and haematopoiesis in the spleen at 5 mg/kg bw/d and anemia was evident at 25 mg/kg bw/d (Shimo et al. 1994). This indicated that secondary responses to toxic effects on the peripheral red blood cells may be more sensitive than changes in the peripheral blood. Nitrobenzene haematotoxicity was evident at low doses which were about 10-fold lower than the critical dose for the classification as harmful leading to the proposal for the classification as toxic.

4.1.2.7 Mutagenicity

4.1.2.7.1 Studies in vitro

Bacterial mutation assays

Mutagenicity of nitrobenzene for *Salmonella typhimurium* was tested with the strains TA100, TA1535, TA1537, TA98, TA92 and TA94 in a concentration range from 10 to 3000 µg/plate in the absence and presence of arochlor induced liver S9-mix from rats and hamsters (Haworth et al. 1983, Miyata et al. 1981, article in Japanese, abstract and tables in English). Nitrobenzene did not show mutagenic effects; the highest doses (1000 and 3000 µg/plate) exhibited toxic effects (purity 99.9%, Haworth 1983). Both studies did not completely meet requirements of OECD TG 471 (use of only four strains and three to four analysable concentrations, results only presented as summarised tables) but were considered as sufficiently reliable for risk assessment due to clear negative results in both studies.
Also no mutagenic effect was observed when the arochlor induced rat liver S9-mix was supplemented with flavine mononucleotide to enhance nitroreduction in strains TA98 and TA100 in doses up to 30 µmol/plate (equivalent to 3693.3 µg/plate, purity not given) (Dellarco and Prival 1989). From a validation study for an Ames/*Salmonella* pre-incubation system for detection of vapours in ambient air negative results were reported for nitrobenzene with and without metabolic activation from aroclor-induced hamster an rat liver S9 in strains TA97, TA98 and TA100 (Hughes et al. 1984, only raw data and abstract available).

These negative results in bacterial gene mutation tests are, in principal, supported by others (Chiu et al. 1978; Ho et al. 1981; Nohmi et al. 1984; Shimizu et al. 1983; Vance and Levin 1984) who have either not applied a full battery of tester strains or presented data only in a summerised form to evaluate SAR for nitro-compounds.
In the presence of norharman and rat liver S9 nitrobenzene showed mutagenic effects against TA98 at dosages of 200 and 1000 µg/plate (Suzuki et al. 1983). However, this mutagenicity is diminished in strains lacking bacterial nitro-reductase (Suzuki et al. 1987). The relevance of results obtained with the bacterial co-mutagen norharman to mammalian cells is still unclear. Thus, this study will not be considered for the overall risk characterisation.

Table 4.3: Overview on bacterial genotoxicity tests

Test system	Concentration range		Result	Toxicity	Remarks	Reference
	with S-9 mix	without S-9 mix				
Bacterial gene mutation test	30 - 3000 µg/plate	10 - 3000 µg/plate	negative	at 1000 and 3000 µg/plate	testing of 5 dosages in 6 strains	Miyata et al. 1981
Bacterial gene mutation test	10 - 1000 µg/plate	10 - 1000 µg/plate	negative	at highest dose	testing of 5 dosages in 4 strains	Haworth et al. 1983

Bacterial gene mutation test	36.93 – 3693.3 µg/plate	not tested	negative	at highest dose	Flavine mononucleo-tide supple-mentation	Dellarco and Prival 1989
Bacterial gene mutation test	33 - 3333 µg/plate	33 - 3333 µg/plate	negative	no data	non standard method	Hughes et al. 1984
Bacterial gene mutation test		not tested	positive	no data	positive only in the presence of the comutagen norharman	Suzuki et al. 1983

Mammalian cell gene mutation assays

Mutagenic effects of nitrobenzene in V79 Chinese hamster lung cells were investigated by Kuroda (1986) at concentrations of 0.1 to 1 µg/ml. Data were only presented as a figure. A marginal increase of 8-azaguanine resistant colonies was found at 0.6 µg/ml without S9-mix and a weak increase was seen with S9-mix at 0.1 and 0.2 µg/ml (mutant frequencies of 5.8 and $2.5/10^5$ survivors at 0.1 and 0.2 µg/ml, respectively, compared to lower than $1/10^5$ survivors for solvent controls). These effects may be due to the extremely low values in the negative control cultures. Frequencies of ouabain resistant colonies with and without S9-mix were not considered as increased by the rapporteur (less than $1/10^5$ survivors for nitrobenzene concentratins of 0.1 to 0.9 µg/ml as well as for solvent controls were found). No data on cytotoxicity and plating efficiency were given. The test is of low statistical power since only 2 x 10^5 cells / culture were inoculated for the selection of resistant cells. Since the effects were not dose related and not reproduced (it seems that only one culture/concentration was investigated) the study is of low reliability for the risk assessment of nitrobenzene.

In vitro chromosomal aberration test

The analysis of chromosomal aberrations in Chinese hamster lung-cells after treatment with 0.125, 0.25 and 0.5 mg/ml (up to 4.1mmol/l) nitrobenzene without S9-mix revealed a negative result (data presented in a table in Ishidate 1988). Chromosomes were analyzed 24 h and 48 h after commencing treatment; information on toxicity is not given. It is mentioned that nitrobenzene induced a high proportion of polyploid cells at the highest concentration and an incubation time of 48 h. However, this might well be an unspecific effect.

Huang et al. (1996) report summarised results for the analysis of chromosomal aberrations in human lymphocyte cultures. Five whole blood cultures from one healthy male human donor were incubated with nitrobenzene 48 h after culture initiation. After 24 h treatment, chromosomal aberrations were analysed. No detailed information on nitrobenzene concentrations, toxicity, cell preparation and solvent controls were given. A summarised table states a positive result for 50 mmol/l (6.1 mg/ml) nitrobenzene as lowest effective dose, which is an extremely high concentration (OECD guideline recommends 10 mmol/l as highest dose

to be tested). The result of this test cannot be adequately assessed due to the insufficient study description and was not considered as relevant for risk assessment.

Micronucleus test

In V79 Chinese hamster lung fibroblasts the induction of micronuclei was investigated at 0.001 to 100 µmol/l (corresponding to 0.123 ng/ml to12.31 µg/ml) nitrobenzene in one experiment with 18 h treatment time without S9 mix (Bonacker et al. 2004). Data for micronuclei frequencies were only presented as a figure. The frequency of micronucleated cells seemed to be dose dependently increased at 0.01 to 1 µmol/l and was doubled in nitrobenzene treated cultures compared to control cultures at 1 and 10 µmol/l (1.2 % micronucleated cells in nitrobenzene treated cultures compared to 0.5% in solvent control cultures). No further increase but a slight decrease in micronuclei frequency was seen with higher concentrations of nitrobenzene although no cytotoxic effects were found up to concentrations of 1 mmol/l in a neutral red assay (data not shown). As analysed by CREST staining, mostly micronuclei containing whole chromosomes were induced by nitrobenzene suggesting aneugenic effects (misdistribution of whole chromosomes). It is stated that two parallel cultures were analysed per concentration but the calculation of the standard deviation seems not to be based on two data points. Thus, it is difficult to conclude on the reliability of this study.
In the same publication, weak positive effects of nitrobenzene in cell free systems on tubulin assembly (at 1 mmol/l, higher concentration induced secondary effects) and on functionality of the tubulin-kinesin motor systems investigated in a microtubule gliding assay (at 7.5 to 30 µmol/l) were described.

Unscheduled DNA synthesis (UDS)

At concentrations of 0.01, 0.1 and 1.0 mmol/l (1.23, 12.3, 123 µg/ml) nitrobenzene did not induce UDS in primary human hepatocytes (Butterworth et al. 1989). Cell cultures were obtained from liver tissue sections of five patients. Apparently, healthy tissue from surgeries was used. Additionally, primary rat hepatocytes were investigated. Cells were pre-cultured for 1.5 to 22 h depending on their viability and attachment. Cultures were subsequently incubated with nitrobenzene and ^{3}H-thymidine for 18 to 24 h. No increases in net grain counts or % cells in repair (defined as >= 5 NG) in nitrobenzene treated cultures compared to media control or solvent control cultures were found. No data on toxicity were available.

Further in vitro data from non-routine methods

Robbiano et al. (2004) investigated the induction of micronuclei and DNA-damage (single cell gel electrophoresis e.g. comet assay) of nitrobenzene using non-routine methods in rat and human primary kidney cells. Human kidney cells were obtained from tissue samples of donors that underwent surgery for kidney carcinomas, nephroblastomas or adenomas (Robbiano et al. 2004). Cells from 3 Sprague Dawley rats and 3 human donors were incubated with 0.062, 0.125, 0.25 and 0.5 mmol/l (7.63, 15.39, 30.78, 61.5 µg/ml). There is confusion about the incubation time (20 h or 48 h). A cell viability of lower than 30% was stated for the highest dose tested. No further data on cytotoxicity were given. Data on genotoxic effects were only presented as ratios treated/control cultures in a figure. An increase in the frequency of micronucleated cells was reported for all concentrations tested. Extremely low frequencies were reported for negative control cultures (0.057% for rat kidney cells and 0.079% for human kidney cells). Micronucleus frequencies in nitrobenzene exposed

cultures were all in the range of usual control values (highest micronucleus frequency in rat kidney cells 0.58% at 0.5 mmol/l, in human kidney cells 0.83 at 0.25 mmol/l). For results on the single cell gel electrophoresis increases in tail lengths over controls were reported for concentrations of 0.062 to 0.25 mmol/l nitrobenzene (highest increase in tail length 10.5 µm at 0.25 µmol/l compared to 1.6 µm in negative controls in rat kidney cells). No details were given on the parameters for tail length measurement. However, the tail length of solvent control cells seems extraordinary short. Thus, the results of this study are difficult to interpret.

Mattioli et al. (2006) investigated induction of DNA damage (COMET assay) and DNA repair in primary human thyroid cells. Primary cells were obtained from surgery material from 3 donors and were reported to be 90 % follicular cells and 10 % fibroblasts according to their morphology. Cells were treated with doses of 1.25, 2.5 or 5 mmol/l (153.9, 307.8, 615 µg/ml) for 20 h, which did not lead to cytotoxicity determined by trypan blue exclusion. No details were given regarding the Comet assay procedure. The extent of DNA damage in the cells was evaluated based on mean tail moment and mean tail length. An approximately two-fold increase in both assessment criterions at the highest tested dose compared to control cultures was the maximum effect in this test. The positive control gave an adequate positive response (15-20fold increases). The extent of induction of DNA repair was detected by net nuclear grain count and was weakly elevated in the cells of two of the three donors.

Table 4.4: Overview on *in vitro* genotoxicity tests in mammalian cells

Test system	Concentration range		Result	Toxicity	Remarks	Reference
	with S-9 mix	without S-9 mix				
Gene mutation test in V79 cells	0.1-1 µg/ml	0.1-1 µg/ml	inconclusive	no data	Methodical insufficiencies	Kuroda 1986
Chromosomal aberration test in CHL cells	not done	0.125-500 µg/ml	negative	no data		Ishidate, 1988
Chromosomal aberration test in human lymphocytes	not done	6.1 mg/ml	inconclusive	no data	Inadequate data presentation	Huang et al. 1996
Micronucleus test in CHL cells	not done	0.123 ng/ml -12.31 µg/ml	inconclusive	no toxicity	Insufficient data presentation	Bonacker et al. 2004
UDS test in primary human and rat hepatocytes	not done	1.23-123 µg/ml	negative	no data		Butterworth et al. 1989
Micronucleus test and comet assay in primary rat and human kidney cells	not done	7.63-61.5 µg/ml	inconclusive for both genotoxic endpoints	toxic at least at the highest tested	Methodical insufficiencies	Robbiano et al. 2004

				dose		
DNA damage in primary human thyroid cells and induction of DNA repair	not done	153.9-615 µg/ml	inconclusive for both genotoxic endpoints	no toxicity		Mattioli et al. 2006

CHL, Chinese hamster lung fibroblasts; UDS unscheduled DNA synthesis

4.1.2.7.2 Studies in vivo

Micronucleus test

Nitrobenzene (purity >99.9%) was tested for the induction of micronuclei in bone marrow cells (OECD TG 474) in male and female B6C3F1-mice (5 animals per dose group) after i.p. application of 62.5, 125.0 and 250 mg/kg bw in olive oil (BASF 1996). Sampling times were 24 and 48 h for the solvent control group and the highest dose group. For the low dose, mid dose and positive control group (25 mg/kg cyclophosphamide) only the 24 h sampling time interval was investigated. Animals of the highest dose group showed clinical signs of toxicity (e.g. irregular respiration, apathy, piloerection) in a pretest. Mortality was induced at higher dosages. The test substance did not reveal any effect on the PCE/NCE-ratio up to and including the highest tested dose but bioavailability of the substance can be concluded from the clinical signs and is supported by the toxic effects seen after acute and repeated application. In this test nitrobenzene did not induce micronuclei in bone marrow cells in mice.

Chromosomal aberration test

In a test on induction of chromosomal aberrations and sister chromatid exchanges (SCE), male rats (CDF(F-344)/CrlBR) were exposed for 6h/d, 5 d a week for a total of 21 d during a 29-day period to air concentrations of 5, 16, or 50 ppm (26.2, 81.9, 256 mg/m^3) (Kligerman et al. 1983).

Cultures of lymphocytes from spleen and blood were first incubated in the presence of concanavalin A. Subsequently, bromodeoxyuridine (2 µmol/l) was added. This is not considered as adequate for the investigation of chromosomal aberrations since bromodeoxyuridine may act as a co-mutagen. Colcemid (0.5 µg/ml) was supplemented 3 h before the cells were harvested and analysed for cytogenetic damage. Peripheral blood lymphocytes (PBL) were analysed for the induction of chromosomal aberrations and PBL and splenic lymphocytes were analyzed for the induction of sister chromatid exchanges. Results were only presented in a summarised table. Mitotic indices of cultured PBL from nitrobenzene exposed animals were dose-related decreased compared to PBL from control animals. At the highest nitrobenzene concentration, chromosomal aberrations were three times higher than in controls. However, at this concentration 74 first-division cells were scored from 2 animals (compared to 50 first-division cells/animal for lower concentrations and solvent controls), suggesting that the metaphase preparation at the highest concentration was not adequate. SCE were not induced by nitrobenzene in both of the cell types investigated. Cyclophosphamide (5 mg/kg bw, i.p. application) revealed positive effects on induction of chromosomal aberrations and SCE in both cell types in this test system.

Unscheduled DNA-Synthesis

At doses of 200 and 500 mg/kg bw administered in corn oil by oral gavage to male Fischer-344 rats (3 animals/group) nitrobenzene (purity not indicated) did not induce UDS in hepatocytes, as detected by the *in vivo* UDS-test (Mirsalis et al. 1982). The test performance did not fully meet OECD TG 486 since one single treatment time and only 50 cells/animal were investigated. Hepatocytes were isolated by in situ perfusion 12 h after treatment of the animals. It was stated that the highest dose was near the LD50 but no data on toxicity were presented. Briefly, after ^{3}H-thymidine incorporation (10 µCi/ml ^{3}H-thymidine, 40-50 Ci/mmol) for 4 h and 0.25 mmol/l thymidine for 14-16 h, morphologically unaltered cells were counted autoradiographically. The percentage of cells in repair (%IR) was defined as those exhibiting >= 5 net grains / nucleus. Neither net grain count / nucleus nor the percentage of cells in repair was increased in cultures from nitrobenzene treated animals compared to controls. Positive results were obtained with several compounds in the same study.

DNA-binding

The potential for DNA-binding was investigated in a non-routine, GLP compliant study in rats and mice (Novartis 1997). ^{14}C-nitrobenzene was administered subcutaneously to male and female mice (B6C3F1) and rats (F344) at a dose of 4 mg/kg bw (2 mCi/kg bw). According to the authors the route of application was chosen to simulate a complete absoption by the skin. 24 h after application the animals were killed and DNA from rat liver and kidney and mouse liver and lung was isolated and bound radioactivity was determined. By analysis with HPLC the majority of radioactivity was identified as DNA-adducts. DNA-bound radioactivity between 4.1-5.9 dpm/mg DNA (rat liver), 3.3-6.1 dpm/mg DNA (rat kidney), 7.3-9.8 dpm/mg DNA (mouse liver) and 11.9-38.9 dpm/mg DNA (mouse lung) were determined. The measured radioactivity accounted for approx. 3 adducts per 10^{9} to 2 adducts per 10^{8} nucleotides. This was interpreted as relatively low DNA-binding effect. Data on toxicity were not given.

Male Kunming mice were treated i.p. with 0.1, 0.5, 2.0, 5.0, 20 and 100 µg and 10 mg/kg bw ^{14}C-nitrobenzene. The specific activities for the three lowest dose levels were 30 mCi/mmol (Li 2003). For nitrobenzene doses above 2.0 µg/kg bw the specific activities were adjusted by adding unlabeled nitrobenzene (12, 3, 0.6, 0.006 mCi/mmol at 5.0, 20 and 100 µg and 10 mg /kg bw). After 2 h animals were sacrificed and DNA- or haemoglobin-binding was analysed in liver and blood. For a time course study mice were treated with 4.1 µg/kg bw and DNA-binding was analysed 4, 12, 24 h and 3, 7, 14, 21 d later. ^{14}C-Nitrobenzene bound to purified hepatic DNA or haemoglobin was analysed by accelerator mass spectrometry (AMS). Over the whole dose range tested a linear dose response was found for DNA- as well as for haemoglobin binding of ^{14}C-nitrobenzene. Approximately 22 pg ^{14}C-nitrobenzene equivalents/g DNA (approx. 5 adducts per 10^{11} nucleotides) were detected at the lowest dose level and 5 x10^{5} pg ^{14}C-nitrobenzene equivalents/g DNA (1 adduct per 10^{6} nucleotides) at the highest dose level. ^{14}C-Nitrobenzene equivalents found in haemoglobin increased parallel to equivalents bound to DNA and were at all doses tested twofold lower than the DNA-bound ^{14}C-nitrobenzene equivalents. The time course study revealed that DNA-adduct levels reached a peak before 4 h, then dropped rapidly within 24 h and subsequently dropped down more slowly. This was interpreted as evidence for several nitrobenzene-metabolites forming DNA-adducts of divers stability.

Further in vivo data from non-routine methods

Using a non-routine method the genotoxic potential of nitrobenzene to rat kidney cells was investigated *in vivo* (Robbiano et al. 2004). 3 male Sprague Dawley rats were subjected to unilateral nephrectomy. 24 h later 250 mg/kg folic acid were injected i.v. Nitrobenzene was administered as a single p. o. gavage of 300 mg/kg bw 48 h after folic acid injection. No indications on the treatment of control animals were provided. The dosage was indicated as corresponding to 1/2 LD50. There is confusion about the time point when rats were sacrificed and kidney cells were prepared for the investigation of micronucleus induction and DNA-damage. Results were presented in a figure as ratio treated/control (one single data point for each genotoxic endpoint). Weak positive effects were reported for the induction of micronuclei (mean frequencies of micronucleated cells recalculated from presented data were 0.25% for treated animals compared to 0.02% for controls). For the investigation of the DNA-damaging potential no details were given on the measurement of the tail length. Positive results were reported (data recalculated from the figure are tail lengths of 7.3 µm for nitrobenzoene treated animals compared to 1.88 +/- 0.62 µm for control animals). The study was judged as methodically inadequate by the rapporteur due to the strong pretreatment of the animals (possible cell cycle disturbance by forced proliferation), the rather low values in negative controls, the insufficient description of cytotoxicity, sampling times and parameters for measurement of the tail length and the very limited data presented.

Mattioli et al. (2006) investigated the potential of nitrobenzene to induce DNA damage in rat thyroid, liver and kidney. Three male animals received 310 mg/kg bw nitrobenzene in DMSO by gastric intubation and were sacrificed after 16 h. Thyroid, liver and kidney were sampled and prepared to receive single cell suspensions for the detection of DNA fragmentation by Comet assay. Cell viability was reported to be 80 %. As assessment criteria tail length and tail moment were used. An 10-15fold increase in DNA fragmentation compared to solvent control was observed in all cell samples as well as in a pooled cell sample for both criteria.

Table 4.5: Overview on *in vivo* genotoxicity tests in rodents

Test system	Doses	Expos. regimen	Sampl. times	Result	toxicity	Remark	Reference
Micro-nucleus test mice bone marrow	62.5-250 mg/kg bw	1 x i.p.	24, 48 h	negative	at highest dose tested	OECD TG 474	BASF 1996

Chromo-somal aberrations & SCE rat spleen and blood lympho-cytes	5, 16, or 50 ppm	inhalation 6h/day, 5 days/week for 21 days	beginning of primary cell cultures less than 1 h after termination of exposure, cytogenetic analysis after 54 h (blood lympho-cytes) or 72 h (spleen lympho-cytes)	negative for both endpoints	mitotic index decreased in blood lympho-cytes, cell cycle delay		Kligerman et al. 1983
UDS test rat hepatocytes	200, 500 mg/kg bw	1 x p.o.	12 h	negative	no data		Mirsalis et al. 1982
DNA-binding rat liver & kidney, mouse liver & lung	4 mg/kg bw	1 x sc	24 h	weak positive	no data	non-routine method	Novartis 1997
DNA-binding rat liver	0.1 µg - 10 mg/kg bw 4.1 µg/kg bw	1 x i.p. 1 x i.p.	2 h 4, 12, 24 h, 3, 7, 14, 21 d	positive	no data	non-routine method	Li 2003
DNA damage & Micro-nuclei rat kidney	300 mg/kg bw	1 x p.o.	unclear	inconclusive for both endpoints	no data	methodi-cally inadequate	Robbiano et al. 2004
DNA damage rat thyroid, liver, kidney	310 mg/kg bw	1 x p.o.	16 h	positive			Mattioli et al., 2006

4.1.2.7.3 Summary of mutagenicity

Nitrobenzene was negative in several bacterial tests with a number of *Salmonella typhimurium* strains. For genotoxicity of nitrobenzene in mammalian cells *in vitro* no test according to current guidelines was available. Inconclusive results were obtained in a mammalian cell gene mutation test, a chromosomal aberration test in primary human lymphocytes, a micronucleus test in Chinese hamster lung cells and further non-routine tests. These studies were either methodically inadequate or insufficiently described and were not considered as relevant for risk assessment. The two most reliable tests, a chromosomal aberration test in Chinese hamster lung cells and a test on unscheduled DNA synthesis in human hepatocytes revealed negative results.

In vivo a DNA-binding capacity was detected in two studies after subcutaneous or i.p. application in liver and lung of mice and in liver and kidney of rats (Novartis 1997 and Li 2003). Considering the routes of application (s.c. and i.p.) which limit the relevance of those studies and the low adduct counts it can be concluded that nitrobenzene has a low DNA-binding capacity the biological relevance of which is unclear. In rats, no UDS was induced in rat liver after a single high oral dose (Mirsalis et al. 1982). In a test on chromosomal aberrations and SCE in lymphocytes from peripheral blood and spleen from subacute exposed rats via inhalation (Kligerman et al. 1983) no mutagenic effect was detected. A bone marrow micronucleus test in mice was negative up to the maximum tolerable dose (OECD TG 474) (BASF 1996).

From the available *in vitro* and *in vivo* data a weak genotoxic potential can be concluded. Therefore, it is unlikely that carcinogenicity is caused by a genotoxic mode of action. From the available negative data for micronuclei formation, chromosomal aberrations, SCE and UDS in rodents *in vivo*, it can be concluded, that nitrobenzene is not suspected to exert mutagenic effects on germ cells. Nitrobenzene should not be classified as a mutagen.

4.1.2.8 Carcinogenicity

4.1.2.8.1 Studies in animals

<u>In vivo studies</u>

Inhalation

Neoplastic lesions

No other long-term studies than the inhalation studies on combined chronic toxicity and carcinogenicity (conducted in accordance to OECD TG 453) are available. Male and female B6C3F1 mice and male and female F344 rats and male Sprague-Dawley (CD) rats were exposed via inhalation (whole body inhalation chambers) to nitrobenzene (99.8%) (CIIT 1993; Cattley et al. 1994): Treatment-induced neoplastic and non-neoplastic lesions of the CIIT-study (1993) were summarised in Tables 4.1.2.8 A - D (cited from Cattley et al. 1994).

70 female and male B6C3F1 mice were exposed to nitrobenzene at concentrations of 0, 5, 25, and 50 ppm for 6 hours daily, 5 days per week for 107 weeks. 60 female and male F344 rats and 60 male CD (Sprague-Dawley) rats were exposed to nitrobenzene at concentrations of 0, 1, 5, and 25 ppm (0, 5, 25, and 125 mg/m³) for 6 hours daily, 5 days per week for 107 weeks. Male CD rats were selected to evaluate chronic testicular effects, since male F344 rats have a high spontaneous rate of testicular neoplasms. An interim necropsy was conducted at 15 months following the initiation of exposure for the F344 and CD rats but not for the mice. Chronic inhalation exposure of mice and rats resulted in some decreases in body weight, these decreases were inconsistent and never exceeded 10% of control group means. No consistent nitrobenzene exposure related changes in incidences of clinical signs were observed in rats or mice. Survival was no adversely affected by nitrobenzene inhalation.

Neoplastic lesions in the CIIT study (1993)
Summarising the neoplastic effects of this CIIT-study, it was demonstrated that nitrobenzene by inhalation was carcinogenic in male and female B6C3F1 mice, male and female F344 rats, and male CD rats. In male B6C3F1 mice, the incidences of alveolar/bronchiolar adenoma, alveolar/bronchiolar carcinoma, and thyroid adenoma were increased. In female B6C3F1 mice, the incidence of mammary gland adenocarcinoma was increased. In addition, female B6C3F1 mice exposed to nitrobenzene had a marginally increased incidence of hepatocellular adenomas. In male F344 rats, the incidences of hepatocellular adenoma, hepatocellular adenoma or carcinoma, and renal tubular adenoma were increased. Male F344 rats had a marginally increased incidence of thyroid follicular neoplasia (adenoma or adenocarcinoma). In female F344 rats, the incidence of endometrial stromal polyps was increased. In male CD rats, the incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma were increased, but the incidence of hepatocellular carcinoma was not affected by nitrobenzene exposure.
In rats, the tumor rates at multiple target organs increased with dose-dependency (see Table 4.6). The increases in tumor incidences reached significance at 25 ppm in rats. Although there may be some increase in tumor rates, 5 ppm appear as the dose without significant tumor response.
In mice, increases in tumor incidences were also evident in multiple target organs and significant increase was seen at the lowest dose tested (5 ppm) and above (Table 4.8).

TABLE 4.6

Incidence of Selected Neoplastic Lesions in F344 and CD Rats Following Nitrobenzene Exposure

		Female F344 incidence (%)				Male F344 incidence (%)				Male CD incidence (%)		
Tissue	Diagnosis	0 ppm	1 ppm	5 ppm	25 ppm	0 ppm	1 ppm	5 ppm	25 ppm	0 ppm	1 ppm	5ppm
Liver	Hepatocellular adenoma	0/70 (0)	2/66 (3)	0/66 (0)	3/70 (4)	1/69 (1) T	3/69 (4)	3/70 (4)	15/70 (21) P	1/63 (2) T	1/67 (1)	2/70 (3)
	Hepatocellu-lar carcinoma	0/70 (0) T	0/66 (0)	0/66 (0)	2/70 (3)	0/69 (0) T	1/69 (1)	2/70 (3)	4/70 (6)	2/63 (3)	0/67 (0)	2/70 (3)
	Hepatocellu-lar adenoma or carcinoma	0/70 (0) T	2/66 (3)	0/66 (0)	4/70 (6)	1/69 (1) T	4/69 (6)	5/70 (7)	16/70 (23) P	2/63 (3) T	1/67 (1)	4/70 (6)
Kidney	Tubular adenoma	0/70 (0)	0/66 (0)	0/66 (0)	0/70 (0)	0/69 (0) T	0/68 (0)	0/70 (0)	5/70 (7) P	2/63 (3)	0/67 (0)	2/70 (3)
	Tubular carcinoma	0/70 (0)	0/66 (0)	0/66 (0)	0/70 (0)	0/69 (0)	0/68 (0)	0/70 (0)	1/70 (1)	0/63 (0)	1/67 (1)	0/70 (0)
	Tubular ademona or carcinoma	0/70 (0)	0/66 (0)	0/66 (0)	0/70 (0)	0/69 (0) T	0/68 (0)	0/70 (0)	6/70 (9) P	2/63 (3)	1/67 (1)	2/70 (3)
Thyroid	Follicular cell adenoma	0/69 (0)	—	—	2/68 (3)	0/69 (0)	0/69 (0)	2/70 (3)	2/70 (3)	2/63 (3)	4/64 (6)	2/68 (3)
	Follicular cell adeno-carcinoma	0/69 (0)	—	—	1/68 (1)	2/69 (3) T	1/69 (1)	3/70 (4)	6/70 (9)	4/63 (6)	1/64 (2)	1/68 (1)
	Follicular cell adenoma or adeno-carcinoma	0/69 (0)	—	—	3/68 (4)	2/69 (3) T	1/69 (1)	5/70 (7)	8/70 (11)	5/63 (8)	5/64 (8)	3/68 (4)
Uterus	Endometrial stromal polyp	11/69 (16) T	17/65 (26)	15/65 (23)	25/69 (36) P	—	—	—	—	—	—	—
Testes	Interstitial cell tumor	—	—	—	—	61/69 (88)	52/56 (93)	58/61 (95)	65/70 (93)	3/62 (5)	6/66 (9)	7/70 (10)
Multiple	Mononuclear cell leukemia	11/70 (16)	5/66 (8)	4/66 (6)	0/70 (0)	12/69 (17)	5/69 (7)	4/70 (6)	0/70 (0)	1/63 (2)	4/67 (6)	0/70 (0)

Note: T, significantly positive nitrobenzene exposure-related trend in incidence determined by Cochran-Armitage trend test, $p < 0.05$. P, significantly different from incidence in 0-ppm control group determined by Fisher Exact Test, $p < 0.05$.

TABLE 4.7
Nonneoplastic Lesions-Rats

		Female F344 incidence (%)				Male F344 incidence (%)				Male CD incidence (%)			
Tissue	Diagnosis	0 ppm	1 ppm	5 ppm	25 ppm	0 ppm	1 ppm	5 ppm	25 ppm	0 ppm	1 ppm	5ppm	
Liver	Eosinophilic foci	6/70 (9) T	9/66 (14)	13/66 (20)	16/70 (23) P	26/69 (42) T	25/69 (36)	44/70 (63) P	57/70 (81) P	11/63 (17) T	3/67 (4)	8/70 (11)	
	Centrilobular hepatocytomegaly	0/70 (0)	0/66 (0)	0/66 (0)	0/70 (0)	0/69 (0) T	0/69 (0)	8/70 (11) P	57/70 (81) P	3/63 (5) T	1/67 (1)	14/70 (20) P	3
	Spongiosis hepatis	0/70 (0) T	0/66 (0)	0/66 (0)	6/70 (9) P	25/69 (36) T	24/69 (35)	33/70 (47)	58/70 (83) P	25/63 (40) T	25/67 (37)	25/70 (36)	3
Kidney	Chronic nephropathy	58/70 (83)	51/66 (77)	60/66 (91)	67/70 (96)	69/69 (100)	64/68 (94)	70/70 (100)	70/70 (100)	54/63 (86)	60/67 (90)	63/70 (90)	
	Tubular hyperplasia	0/70 (0)	0/66 (0)	2/66 (3)	2/70 (3)	2/69 (3) T	2/68 (3)	2/70 (3)	13/70 (19) P	3/63 (5)	1/67 (1)	5/70 (7)	
Thyroid	Follicular cell hyperplasia	1/69 (1)	—	—	0/68 (0)	0/69 (0) T	1/69 (1)	2/70 (3)	4/70 (6)	2/63 (3)	2/64 (3)	1/68 (1)	
Nose[a]	Pigment deposition olfactory epith.	37/67 (55) T	54/65 (83) P	60/65 (92) P	66/66 (100) P	40/67 (60) T	53/67 (79) P	67/70 (96) P	68/69 (99) P	42/63 (67) T	49/64 (77)	60/66 (91) P	5
Testes	Atrophy, bilateral	—	—	—	—	61/69 (88)	50/56 (89)	59/61 (97)	61/70 (87)	11/62 (18) T	17/66 (26)	22/70 (31)	3
Epididymis	Hypospermia, bilateral	—	—	—	—	15/69 (22)	21/54 (39)	12/59 (20)	12/70 (17)	8/60 (13) T	13/65 (20)	15/67 (22)	3
Spleen	Extramedullary haemato-	60/69 (87)	62/66 (94)	60/66 (91)	65/69 (94)	53/69 (77)	62/69 (90) P	65/70 (93) P	61/70 (87)	58/63 (92)	56/67 (84)	61/69 (8)	
	Pigmentation	62/69 (90) T	61/66 (92)	60/66 (91)	68/69 (99) P	55/69 (91) P	63/69 (91) P	64/70 (91) P	70/70 (100) P	59/63 (94) T	58/67 (87)	67/69 (97)	6

Note: T, significantly positive nitrobenzene exposure-related trend in incidence determined by Cochran-Armitage trend test, $p < 0.05$. P, significantly different from incidence in 0-ppm control group determined by Fisher Exact Test, $p < 0.05$.

[a] Level 3

TABLE 4.8

Incidence of Selected neoplastic Lesions in B6C3F1 Mice Following Nitrobenzene Exposure

		Male incidence (%)				Female incidence (%)			
Tissue	Diagnosis	0 ppm	5 ppm	25 ppm	50 ppm	0 ppm	5 ppm	25 ppm	50 ppm
Lung	A/B adenoma	7/68 (10) T	12/67 (18)	15/65 (23) P	18/66 (27) P	4/53 (8)	11/60 (18)	3/64 (5)	2/62 (3)
	A/B carcinoma	4/68 (6)	10/67 (15)	8/65 (12)	8/66 (12)	2/53 (4)	0/60 (0)	4/64 (6)	4/62 (6)
	A/B adenoma or carcinoma	9/68 (13)T	21/67 (31) P	21/65 (32) P	23/66 (35) P	6/53 (11)	11/60 (18)	6/64 (9)	6/62 (10)
Thyroid	Follicular cell adenoma	0/65 (0) T	4/65 (6)	1/65 (2)	7/64 (11) P	2/49 (4)	0/59 (0)	3/61 (5)	2/61 (3)
Mammary gland	Adeno-carcinoma	—	—	—	—	0/48 (0)	—	—	5/60 (8) P
Liver	Hepatocellular adenoma	14/68 (21)	18/65 (28)	15/65 (23)	14/64 (22)	6/51 (12) T	5/61 (8)	5/64 (8)	13/62 (21)
	Hepatocellular carcino-ma	12/68 (18)	13/65 (20)	12/65 (18)	8/64 (13)	1/51 (2)	2/61 (3)	3/64 (5)	1/62 (2)
	Hepatocellular adenoma or carcinoma	25/68 (37)	30/65 (46)	22/65 (34)	21/64 (33)	7/51 (14)	7/61 (11)	7/64 (11)	14/62 (23)

Note: T, significantly positive nitrobenzene exposure-related trend in incidence determined by Cochran-Armitage trend test, $p < 0.05$. P, significantly different from incidence in 0-ppm control group determined by Fisher Exact Test, $p < 0.05$.

TABLE 4.9

Incidence of Selected Nonneoplastic Lesions in B6C3F1 Mice Following Nitrobenzene Exposure

		Male incidence (%)				Female incidence (%)			
Tissue	Diagnosis	0 ppm	5 ppm	25 ppm	50 ppm	0 ppm	5 ppm	25 ppm	50 ppm
Lung	A/B hyperplasia	1/68 (1) T	2/67 (3)	8/65 (12) P	13/66 (20) P	0/53 (0)	2/60 (3)	5/64 (8) P	1/62 (2)
	Bronchializatio	0/68 (0) T	58/67 (87) P	58/65 (89) P	62/66 (94) P	0/53 (0) T	55/60 (92) P	63/64 (98) P	62/62
Thyroid	Follicular cell hyperplasia	1/65 (2) T	4/65 (6)	7/65 (11) P	12/64 (19) P	2/49 (4) T	1/59 (2)	1/61 (2)	8/61 (13)
Liver	Centrilobular hepatocytomeg	1/68 (1) T	15/65 (23) P	44/65 (68) P	57/64 (89) P	0/51 (0) T	0/61 (0)	0/64 (0)	7/62 (11) P
	Multinucleated hepatocytes	2/68 (3) T	14/65 (22) P	45/65 (69) P	56/64 (88) P	0/51 (0)	0/61 (0)	0/64 (0)	2/62 (3)
Nose[a]	Glandularizatio n of respiratory	10/67 (15) T	0/66 (0)	0/65 (0)	27/66 (41) P	0/52 (0) T	0/60 (0)	0/63 (0)	7/61 (11) P
	Increased secretory product. respiratory epith.	0/67 (0) T	0/66 (0)	3/65 (5)	6/66 (9) P	2/52 (4) T	7/60 (12)	19/63 (30) P	32/61 (52) P
	Degeneration/lo ss. olfactory epith.	1/67 (1) T	1/66 (2)	32/65 (49) P	41/66 (62) P	0/52 (0) T	19/60 (32) P	47/63 (75) P	42/61 (69) P
	Pigment deposition. olfactory epith.	0/67 (0) T	7/66 (11) P	46/65 (71) P	49/66 (74) P	0/52 (0) T	6/60 (10) P	37/63 (59) P	29/61 (48) P
Testes	Diffuse atrophy	1/68 (1)	—	—	6/66 (9)	—	—	—	—
Epididymis	Hypospermia	3/68 (4)	—	—	11/66 (17) P	—	—	—	—
Bone marrow. Femur	Hypercellula-rity	3/68 (4) T	10/67 (15) P	4/64 (6)	13/66 (20) P	4/52 (8)	—	—	9/62 (15)
Thymus	Involution	10/48 (21)	—	—	10/44 (23)	7/41 (17)	—	—	22/57 (39) P
Kidney	Cyst	2/68 (3)	—	—	12/65 (18) P	0/51 (0)	—	—	0/62 (0)
Pancreas	Mononuclear cell infiltrate	3/65 (5)	—	—	3/64 (5)	1/46 (2)	—	—	8/62 (13) P

Note: T, significantly positive nitrobenzene exposure-related trend in incidence determined by Cochran-Armitage trend test, $p < 0.05$. P, significantly different from incidence in 0-ppm control group determined by Fisher Exact Test, $p < 0.05$.

[a] Level 3

Nonneoplastic lesions in the CIIT study (1993)
At final sacrifice, nitrobenzene exposure was associated with various chronic toxic effects in mice and rats (summarised in Table 4.7 and 4.9):

B6C3F1 mice
At 2 year sacrifice, RBC counts and haematocrit were lower for 50 ppm males and 5 and 25 ppm females, females of these groups also had decreases in haemoglobin. The MCH and MCHC were higher for males and females of the 50 ppm groups. Males of the 50 ppm exposure group and female 25 and 50 ppm exposure groups had increases in methaemoglobin. Clinical chemistry revealed higher activities of ALAT in males of the 25 and 50 ppm groups. In 50 ppm exposed female mice, absolute and relative organ weights of the liver and kidney were increased, 25 ppm exposed male mice also showed increased relative liver weights.
In addition to the non-neoplastic lesions of organs with treatment-related tumors already reported above, non-neoplastic lesions were also observed in other organs. Nasal inflammatory lesions consisting of increased incidence of secretion of respiratory epithelial cells (in female mice of all dose groups, and 50 ppm exposed males), glandularization of respiratory epithelium (50 ppm exposed males and females), were evident. In addition, increased incidences of degeneration and loss of olfactory epithelium (females of all dose groups, 25 ppm and 50 ppm exposed males) along with dilation of submucosal glands and accumulation of submucosal brown pigment-containing macrophages (all nitrobenzene exposure groups) was observed. Nitrobenzene inhalation also resulted in increased incidence of lymphoid hyperplasia of the spleen (50 ppm exposed females), bone marrow hypercellularity (5 ppm and 50 ppm exposed males), increased incidence of adrenal cortical vacuolization (25 ppm and 50 ppm females), increased incidence of thymic involution (50 ppm females), increased incidence of testicular atrophy (50 ppm males), bilateral hypospermia of the epididymis (50 ppm males), increased incidence of renal cysts (50 ppm males), and mononuclear cell infiltration in pancreas (50 ppm females). Based on the degenerative-inflammatory lesions in the nasal mucosa, the $LOAEC_{local}$ for local effects on the respiratory tract was 5 ppm.

F344 rats
Nitrobenzene exposure was associated to increased relative and absolute organ weights of the liver and kidneys in the 25 ppm exposed rats of both sexes. Higher incidences of rough granular cortical surfaces noted in the rats of the final sacrifice groups (males ≥5 ppm and 25 ppm females) were considered indicative of chronic progressive nephrosis. Exposure-related anemic effects were seen in 25 ppm males and females. Red blood cell counts, haematocrit and haemoglobin levels were depressed, methaemoglobin concentrations were elevated at 25 ppm nitrobenzene. An increase of MCV, nucleated RBCs, polychromatic cells, macrocytes, the presence of Howell-Jolly bodies and leucocytosis were noted in one or both sexes of this dose group. An elevation of GGT (males) and elevated bilirubin (both sexes) were noted in 25 ppm exposed groups. In general, the incidence and severity of microscopic lesions at the final sacrifice were greater in males than in females. Several lesions represent a progression from that which was observed at the interim sacrifice. In addition to the above reported non-neoplastic lesions at tumor sites, there was an increased incidence of extramedullary haematopoiesis of the spleen (1 and 5 ppm males), pigment-laden macrophages (25 ppm exposed females and males of all dose groups), an increased incidence and severity of sinusoidal congestion of the red pulp (all dose groups). Stromal hyperplasia of the spleen was found in two 5 ppm and two 25 ppm males and one 5 ppm and one 25 ppm females versus none in the control groups. An increased severity of chronic nephropathy and an increase in

the number of convoluted tubules containing intracytoplasmatic and intraluminal eosinophilic droplets, an increase in the amount of yellowish-brown pigment, and an increase in suppurative tubular inflammation was noted in exposed males and females (significancy at exposure ≥5 ppm). Hyperplasia and inflammation of the submucosal glands in the anterior portion of the nose (level 2 + 3) lined by respiratory epithelium were present in 25 ppm males and females (data not included in Table 4.7). The incidences of pigment deposition of the olfactory mucosa were increased in all dose groups of male and female rats ≥1 ppm. Because of high incidences of spontaneous lesions of the testis no treatment-related effect could be identified in F344 males. Regarding the uncertainty of the toxicological significance that is attributable to the pigmentation of the olfactory epithelium due to its high spontaneous occurrence, the $NOAEC_{local}$ for local effects on the respiratory tract was 5 ppm.

CD rats
Except renal changes associated with chronic progressive nephropathy, no other macroscopic finding was found in CD males at the end of the study. An increased incidence of sinusoidal congestion was evident at all nitrobenzene concentrations. A minor exposure-related increase in the severity of splenic extramedullary haematopoiesis and degree of pigmentation was also noted. Testes atrophy was present both in control and nitrobenzene exposed males. There was a positive trend in exposed rats, in that increased incidences of this lesion were present in 25 ppm exposed and 5 ppm exposed rats. An increased incidence of bilateral hypospermia in the epididymides was observed in the 25 ppm exposed males. Chronic nephropathy was noted in both the control and nitrobenzene-exposed males, with only a slight increase in severity of the change noted in the 25 ppm exposure group. In addition, the secondary lesions associated with severe chronic nephropathy (parathyroid hyperplasia, fibrous osteodystrophy, soft tissue mineralization) were increased in these animals. Nasal changes consisted of inflammation in the anterior nasal passages. Increased incidences and slightly increased severity of suppurative exudate, subacute inflammation, and mucosal epithelial hyperplasia were seen in males of all dose groups ≥1ppm (5 mg^3) (data not included in Table 4.7). Brown pigment in the submucosa of the olfactory epithelium was evident in a slightly increased amount in males of the all dose groups. However, slight degrees of olfactory pigmentation and subacute inflammation were also commonly seen in the control animals. Based on inflammatory exsudate and mucosal epithelial hyperplasia, the $LOAEC_{local}$ for effects on the respiratory tract was 1 ppm.

Dermal

No data available.

Oral

No data available.

Other
Additional data of concern:

Splenic carcinoma was not induced by nitrobenzene exposure, although previous studies documented an increase of this lesion in F344 rats following oral exposure to aniline (see EU RAR on Aniline, 2005), which is prospected to have common intermediaty with nitrobenzene. In the carcinogenicity study on F344 rats (CIIT 1993), stromal hyperplasia, a preneoplastic lesio in aniline carcinogenesis, was also observed in a few males and females exposed to 5

and 25 ppm nitrobenzene for 2 years. Stromal hyperplasia was also seen in subacute and subchronic inhalation studies on F344 rats, but not in CD rats (Medinsky and Irons 1985; Hamm 1984). Although haemolytic anemia and methaemoglobinemia were caused by nitrobenzene as well as by aniline, a possible relationship of nitrobenzene effects to effects associated with aniline remains unclear.

In vitro studies

No data available.

4.1.2.8.2 Studies in humans

In vivo studies

Inhalation

Dermal

Oral

No data available.

In vitro studies

Nitrobenzene, tested in the range from 0.08 µg/ml to 250 µg ml, did not transform human lung cells WI-38 at the LC50; further details are not given (Purchase et al. 1978).

4.1.2.8.3 Summary of carcinogenicity

Following long-term inhalation of nitrobenzene tumor incidences at six organ sites were significantly increased. (Tables 4.6 and 4.8 this section).

Liver tumors
Hepatocellular neoplasms (adenoma and adenoma or carcinoma) were induced by nitrobenzene in male F344 rats and in male CD rats but not in F344 females. Female CD rats had not been tested. An increased incidence of eosinophilic foci was seen in mid and high dose male F344 rats and female F344 rats of the high dose group. Spongiosis hepatis (used as a synonym to focal cystic degeneration) was present in all high dose groups of both strains and sexes. In mid and high dose males of both rat strains, a dose-related increase of centrilobular hepatomegaly (syn. centrilobular hypertrophy) was observed. Spongiosis hepatis and eosinophilic foci did not show a coincidental occurrence with the liver tumor rates. The only lesion which may be considered as a possible critical event preceding the tumor development in nitrobenzene exposed mammals was the occurrence of centrilobular hypertrophy. This hypothesis is supported by that solely males of both rat strains showed hypertrophy and liver cell tumors. Assumed that hypertrophy represents the precursor lesion in liver cell tumor development, the absence of the hypertrophy may be indicative for the prevention of tumor growth in the low dose group. Whereas liver tumors were only seen at the high concentration, hypertrophy was evident in the mid and high dose groups. - Hypertrophy of centrilobular hepatocytes in rodents is commonly associated with a metabolic activation of microsomal enzymes. Up to now, there is no evidence that nitrobenzene activates liver cell enzymes. Due to the differences in the occurrence, hypertrophy was obviously not associated with other hepatotoxic effects. Degenerative lesions consisting of spongiotic hepatis was also described in female F344 rats, but none of them showed hypertrophy - In B6C3F1 mice, the incidences of centrilobular hypertrophy were increased significantly in all male dose groups and in high dose females, but no significant increase of liver cell tumors was seen. Exclusively female mice showed a non-significant higher rate of liver adenomas at the high

dose group. Another non-neoplastic lesion described in male B6C3F1 mice of all treatment groups was the occurrence of multinucleated hepatocytes which did not show any association to tumor development in the liver.
In summary, liver cell tumors in males of two rat strains appeared to be linked to nitrobenzene. Looking for nonneoplastic liver effects as possible underlying toxic events in the tumor development, none of the toxic effects observed supported enough evidence to explain the tumor development. There was no coincidence of toxic lesions and neoplasia and no concurrent dose-response relationships except an apparent coherence of centrilobular hypertrophy and liver cell tumors.

Kidney tumors
Nitrobenzene exposure resulted in higher rates of tubular adenomas and of combined incidence of tubular adenomas or carcinomas in high dose males of F344 rats. In this dose group, the incidence of tubular hyperplasia considered to represent a preneoplastic lesion was also increased significantly. Chronic nephropathy observed at rates of 77-96% in female (control and dose) groups and at 94-100% in male (control and dose) groups of F344 rats was was not correlated to the tumor response in high dose male F344 rats only. Intratubular eosinophilic (hyaline) droplet inclusions were observed in F344 rats (10/10 males, 2/10 females at 125 ppm) following 14 day-inhalation and in males at 5 ppm and above and in females at 25 ppm following a 90 day period of exposure. This finding may indicate a degenerative effect in renal tubular cells of rats of this strain with males more sensitive than females which could be considered as a toxic effect preceeding tumor growth.

Thyroid tumors
Chronic nitrobenzene exposure was associated with significantly increased incidence of thyroid follicular cell adenomas in male B6C3F1 mice. The observed tumor rates (none in control group, 6%, 2% and 11% in low, mid and high dose groups) were not dose-related. Tumor rates in high dose female mice were lower (3%) than in the control group (4%). The incidence of thyroid follicular cell adenocarcinoma was increased in nitrobenzene exposed male F344 rats (3% in controls, 1%, 4%, or 9% in low, mid and high dose groups), but this effect was considered marginal since only the Trend test was positive, and the higher incidences in the mid and high dose groups were not significantly different from control values. No treatment-related effect was observed in female F344 rats or male CD rats. In both male mice and male F344 rats, the increased incidence of follicular cell neoplasms was associated with an increased incidence of follicular cell hyperplasia (significant only for male mice). In mice, it has been suggested that hyperplasia of the thyroid follicular epithelium represents a preneoplastic change (McConnell 1992).
No other toxic effect on the thyroid was observed in studies with repeated administration of nitrobenzene.
Thyroid carcinogenesis in rodents may occur as a secondary response to microsomal enzyme induction in hepatocytes, which elevates glucuronidation and excretion of thyroid hormones. This causes a continuously stimulated TSH production and chronic activation of thyroid. The observed hepatocytic hypertrophy in rats and mice could be interpreted as indicative for enzyme induction and could give hint on a rodent-specific mechanism. However, hypertrophy of hepatocytes was also significantly increased in male CD rats which did not develop thyroid tumors. Therefore at least in the rat strains tested the occurrence of hepatocyte hypertrophy is not consistent to the development of thyroid tumors as a secondary mechanism. Also, no data on enzyme induction, no proof of altered serum levels of thyroid hormones and TSH and no data on biliary excretion is available to support this mode of action. Life-long metabolic activation is also known to induce liver tumors. In opposite to other hepatic enzyme inducers

where the treated rat is much more sensitive towards thyroid effects than the mouse, the nitrobenzene-treated mice developed increased rates of follicular cell hyperplasia and thyroid tumors but no liver tumors and males of both rat strains had liver tumors, while only marginal increases in follicular cell hyperplasia were found in both strains and marginal increase in thyroid adenomacarcinomas were only observed in the F344 male rats.
While for the rat the induction of UDP-glucuronosyltransferase is often supposed to contribute to this mechanism, an induction of UDP-glucuronosyltransferase is unknown for the mouse. The knowledge of species differences among the rats' and the humans' regulation of thyroid homeostasis (such as a higher turnover of thyroid hormones, higher TSH serum levels, lack of thyroxine binding globulin(TBG) in rats than in humans) could not simply be applied on the mouse thyroid status (e.g. the mouse TBG is similar to humans).
In principle, UDP-glucuronosyltransferases are inducible in humans through a number of substances (Griem et al., 2002). However, such an inductive mechanism is not known for nitroaromates.
Finally, a rodent-specific mode of thyroid carcinogenesis could not completely be ruled out, but at present no sufficient evidence is available to postulate a likely mode of action.

Uterus tumors
High dose F344 females exposed to nitrobenzene had an increased incidence of endometrial stromal polyps, a relatively common spontaneous lesion of the uterus in this strain. The overall incidences of endometrial stromal polyp in all exposure groups (23-36% vs. 16% in control females) were within the range of historical data (up to 37%, Leininger and Jokinen 1990). Toxic nitrobenzene-related effects on the uterus were not observed in this study or any other repeated dose study. Because of the high spontaneous rate in the F344 females and that the tumor rate of high dose females was within historical control values, the association of these benign uterus tumors to nitrobenzene exposure was considered as equivocal..

Lung tumors
The incidence of alveolar/bronchiolar adenomas in male mice increased related to the dosage, but gained significance only at 50 ppm nitrobenzene. A higher rate of adenocarcinomas was seen in all dose groups, but their incidences did not reach significancy and were not dose-related. The spontaneous incidences in control groups were 6 and 10% for lung alveolar/bronchiolar adenomas, respectively adenocarcinomas for males and 4 and 8% for females. The combined incidence of lung adenomas and carcinomas in male B6C3F1 mice did not exceed the 2-year historical control ranges (up to 42%, Rittinghausen et al., 1996). Consistently, the incidences of alveolar/bronchiolar hyperplasia considered as a preneoplastic lesion were increased in males of the 25 and 50 ppm groups. Another nonneoplastic lesion, the alveolar bronchialization was evident with dose-related higher incidences in all male and female groups of B6C3F1 mice. Other repeated dose inhalation studies revealed the hyperplasia of the bronchial epithelium in male and female B6C3F1 mice (Medinsky and Irons 1985). Females did not show lung tumors after nitrobenzene treatment, but alveolar bronchialization, respectively bronchial hyperplasia was evident.
The moderate spontaneous lung tumor rates, the lack of dose-relationship (for combined adenomas and adenocarcinomas and for adenocarcinomas alone) and the fact that increased tumor rates are still in the historical control range are uncertainties to consider lung tumors as nitrobenzene-related.

Mammary tumors
Increased incidence of mammary gland adenocarcinomas was seen in female mice exposed to 50 ppm nitrobenzene. No other adverse effect was seen in this or other repeated dose study.

Conclusion and rationale for classification

Nitrobenzene was classified with Carc. Cat. 3, R40 in 1994 and introduced in Annex I of 67/548/EEC with the 22nd ATP. With respect to carcinogenicity, there are no new relevant data available.

Chronic inhalation of nitrobenzene induced increased incidence of tumors of the lung and thyroid in male B6C3F1 mice, and higher tumor rates of the mammary gland in the female mice. No clear causal relationship of nitrobenzene to the murine lung tumors could be recognized. Altough a clear dose-response-relationship was not present for the low and mid dose groups, the thyroid tumors (only adenomas) at the high dose must be considered as nitrobenzene-induced since no rodent-specific mechanism could be applied. Due to the absence of tumors in untreated controls the mammary tumors were also contributed to the nitrobenzene treatment.

The tumor sites observed in nitrobenzene exposed mice did not clearly show coincidence with the tumor sites in the rat strains. In male F344 rats exposed to nitrobenzene higher rates of liver and kidney tumors were seen and female F344 rats had higher incidences of uterine neoplasms. Although not gaining significance, it could not be excluded that increased rates of thyroid adenocarcinomas in F344 rats were associated to nitrobenzene.A single tumor site was related to nitrobenzene treatment in CD rats, males of this strain had liver cell adenomas and adenocarcinomas similar to F344. The treatment relationship of the uterine tumors appears unequivocal due to the high spontaneous rates while the liver tumors in two rat strains and the kidney tumors in one rat strain have to be considered as caused by nitrobenzene treatment.

Table 4.10: Incidences of the significantly increased tumors after inhalation of nitrobenzene

	F344 rats f	F344 rats m	CD rats m	B6C3F1 mice f	B6C3F1 mice m
Lung					
Adenoma or carcinoma*					13% (0 ppm) [T], 31% (5 ppm) [I], 32% (25 ppm), 35% (50 ppm)
Liver					
Hepatocellular adenoma or carcinoma*		1% (0 ppm) [T], 6% (1 ppm), 7% (5 ppm), 23% (25 ppm)	3% (0 ppm), 1% (1 ppm), 6% (5 ppm), 14% (25 ppm)		
Kidney					
Tubular Adenoma or carcinoma*		0% (0 ppm) [T], 0% (1 ppm), 0% (5 ppm), 9% (25 ppm)			
Thyroid					
Follicular cell adenoma or adenocarcinoma*		3% (0 ppm) [T] 1% (1 ppm) 7% (7 ppm) 11% (25 ppm)			0% (0 ppm) [T], 6% (5 ppm), 2% (25 ppm), 11% (50 ppm)
Uterus					
Endometrial stromal polyp	16% (0 ppm) [T], 26% (1 ppm), 23% (5 ppm), 36% (25 ppm)				
Mammary gland					
Adenocarcinoma				0% (0 ppm), 8% (50 ppm)	

[I] underlined values: significantly different from incidence in 0-ppm control group determined by Fisher Exact Test, $p<0.05$.

[T] only significantlx positive exporsure-related trend in incidence determined in Cochran-Armita Trend test

*combined incidences

In summary, nitrobenzene is carcinogenic in two species, mice and rats, and in two rat strains. For the kidney tumors a cytotoxic mode of action might be acceptable as the likely mode, but for the other the tumors oberved, a toxic effect possibly preceding the tumor development was not clearly identified. Other target sites with marked toxicity such as haematopoetic system (erythrocytes and spleen), nose or testes did not show a tumor response.

From a conservative view, the findings – tumors in multiple organs and multiple species and strains - support the classification into carcinogen of category 2. Supportive arguments for category 2 may be given by a general concern for carcinogenicity of nitroaromatic compounds. A number of substances with structural similarities to nitrobenzene were already classified as carcinogens, category 2 such as 2-nitrololuene (CAS 88-72-2), 2,4-dinitrotoluene (CAS 121-14-2), 2,6-dinitrotoluene (CAS606-20-2), 2,3-dinitrotoluene (602-01-7), 3,4-dintrotoluene (CAS 618-85-9), 3,5-dinitrotoluene (CAS 618-85-9), 2,5-dinitrotoluene (CAS 619-15-8), 4-nitrobiphenyl (CAS 92-93-3), 2-nitroanisole (CAS 91-23-6), 5-nitroacenapthalene (CAS 602-87-9), and 2-nitronapthalene (CAS 581-89-2).

Weighing the evidence for the distinction between category 2 and 3, there are also arguments to propose a classification as category 3 carcinogen:

- The genotoxicity data available did not give a substantial concern that nitrobenzene is mutagenic. Testing in vitro (bacterial tests, chromosomal aberrations test, UDS in human hepatocytes) and in vivo (MN test in the mouse, tests on chromosomal aberrations and SCE on rat lymphocytes, UDS in rat hepatocytes) resulted were negative. Although DNA binding for rat liver and kidney and for mouse liver and lung could in principle indicate a mutagenic effect, relatively low DNA binding activities were estimated in the study of Novartis (1997). Also, the DNA binding activities did not reflect the distribution of tumors among sexes since no sex-specific distribution of activities was found in the rat liver, the rat kidneys and the mouse liver. Covalent binding indices were equally low in both sexes for the rat liver and the mouse liver, however liver tumors were only observed in the male rat liver. The weak positive DNA binding alone was interpreted as a insufficient argument for a genotoxic mode of action. At present, nitrobenzenes' carcinogenicity is thought to be mediated by an albeit non-identified, non-genotoxic mechanism.

- Nitrobenzene is readily metabolized in humans and animals via all exposure routes to a number of nitroaromatic compounds. A nitroreductive enzyme activity in organs or intestinal nitroreduction produces aniline probably via nitrosobenzene, and phenylhydroxylyamine. Aniline is classified as a carcinogen, category 3 and a mutagenic, category 3. The sparce data available for nitrosobenzene and phenylhydroxylamine do not allow a conclusion about their genotoxic potential (Bomhard and Herbold, 2005), no data are available to conclude on their carcinogenic potential. Aniline might be applied for comparative evaluation sharing with nitrobenzene the same metabolites (nitrosobenzene and phenylhydroxylamine), and its classification as carcinogen, category 3 would support the same category for nitrobenzene. But this comparison is limited by differences in the observed tumor spectrum: Associated to the hemolytic toxicity - the spleen was the only tumors site for aniline. The comparison is hindered by differences in databases – positive cancer data on aniline are from a feeding study and the lack of a suitable biomarker for comparison.

- Although there is supportive evidence from category 2-classified nitroaromates with structural similarities, it must be considered that the spectrum of metabolites from which one or multiple metabolites should be suspected to be active as the ultimate carcinogen is quite different to those of nitrobenzene. Multiple tumor sites appeared to be common for representatives of the compound group, however the spectrum of target tumor sites could differ considerably. Liver tumors were also observed 2-4-dinitrotoluene and 2-nitroanisole, but other tumor types or tumor sites were not consistent to those of nitrobenzene.

- A consistency of tumor findings does only exist for liver tumors found in one sex of two rat strains. Other target organs did not show consistency across rodent species, sex and across strains. The observed diversity of tumor sites among rats and mice may be explained by differences in the metabolic pattern. For example, F344 rats are known to form p-hydroxyanilide at a higher rate than CD rats or B6C3F1 mice (ratio 19:9:3.6, see Table 4.1 in 4.1.2.1.1). In rats no excretion of p-aminophenol was found and a higher percentage of p-nitrophenol and n-hydroxyacetanilide was seen (see Table 4.1 in Section 4.1.2.1.1). Differences in enteral nitroreduction and its contribution to the generation and absorption of metabolites could also exist between species, strains and sexes. Although the exact mechanisms of tumor production remains unknown for nitrobenzene, the absence of consistency for tumor responses among species and sexes weakens the evidence for category 2.

Based on the evidence from the present database it is proposed to confirm the classification and labelling is confirmed: Carcinogen, Category 3, Harmful, Xn, R 40, Limited evidence of a carcinogenic effect.

The tumors in the liver and the kidneys were selected as the most sensitive tumor effect for the derivation of a dose without tumor response. Due to the uncertainties of the nitrobenzene-related causality to the lung tumors these were disregarded.
The lowest concentration with significantly elevated tumor rates in the liver and kidneys from rat studies was 25 ppm. A concentration without tumors of 5 ppm (dose without tumor response) is recommended as a starting point for risk assessment.

4.1.2.9 Toxicity for reproduction

4.1.2.9.1 Effects on fertility

Further information is presented in Table 4.2 and section 4.1.2.6.

Studies in animals

Inhalation
Possible effects of nitrobenzene to reproductive performance and capability including reproductive organ impairment were investigated in a two-generation study with rats by the route of inhalatory exposure (Dodd et al. 1985, 1987).

Male and female Sprague-Dawley rats were randomly assigned to three test groups and one control group (30 animals/sex/group) and exposed to nitrobenzene vapours at target concentrations of 0 (air), 1, 10, and 40 ppm (5.1, 51.2 and 204.8 mg/m^3) for 6 hr/day, 5 days/week (whole chamber administration). After a 10 week premating period treatment continued on an exposure regimen of 6 hr/day, 7 days/week and the animals were mated on a one-to-one basis during an additional period of two weeks. At the end of the mating period, the F_0 males were sacrificed and necropsied. The F_0 females positive for appearance of copulatory plugs were further continuously exposed through g.d. 19. After delivery from p.n. day 5 on, the dams (without their litters) recurred to the exposure regimen for nitrobenzene until p.n. day 21, when they were necropsied. Offspring of the F_0 rats was selected on p.n. day 21 to yield the F_1 animals. At least one male and one female were randomly picked from each litter to form groups with 30 animals/sex (remaining F_1 weanlings were removed from the study). F_1 animals were allowed a 2-week growth period without nitrobenzene exposure. The mating procedure and the exposure regimen for the F_1 animals were identical with those for the F_0 rats, however, some F_1 males, which were not sacrificed after mating were used for recovery studies. Pregnant F_1 rats were exposed in the same way as pregnant F_0 rats. F_2 pups were never exposed to nitrobenzene by inhalation and were sacrificed on p.n. day 21. For the recovery study, F_1 males of the high-dose and control groups were allowed a 9-week (one spermatogenesis cycle) nonexposure period after the 2-week mating period. At the end of the recovery period, they were mated to nonexposed virgin females on a one-to-one basis. The

procedures for premating, mating, gestation, and lactation periods were the same as for the F_0 and F_1 generations with the exception that there were no nitrobenzene exposures.

All animals were observed for overt signs of toxicity and weighed weekly. F_1 and F_2 litters were examined as soon as possible after parturition for number of pups, sex, stillbirths, and the presence of external anomalies and weighed on a per litter basis. On p.n. day 4, litters were culled and randomly adjusted to 4 males and 4 females per litter. Thereafter pups were weighed individually. Recovery group F_1 males were weighed every 2 weeks during the 9-week recovery period. After sacrifice of F_0 and adult F_1 rats a complete gross necropsy of all body cavities and organs was performed and organ weights of testes and epididymides from F_0 and F_1 males were taken separately. Reproductive tissues from the high-dose and control rats of both the F_0 and F_1 generations were examined microscopically. In addition, the testes from the intermediate- and low-dose males from both generations were examined.

There were no treatment-related clinical signs of abnormality in the F_0 and F_1 rats during the entire exposure period and during the recovery phase of male F_1 rats. Also, there was no mortality during all phases of the study for the F_0, F_1 (21 days and older), and recovery group rats. For the F_0 and F_1 (21 days and older) rats, there were no biologically significant alterations in absolute body weights or body weight gains due to nitrobenzene exposure. During gestation, the F_0 and F_1 female rats exposed to 40 ppm had lower body weight gains when compared to control F_0 and F_1 females, however, this finding was attributed to the decreased number of pregnant rats. Differences in female body weight during gestation in the recovery phase of the study were also attributed to the lack of pregnancies in the females mated with the F_1 males formerly exposed to 40 ppm nitrobenzene. The fertility index (number of pregnancies/number of females mated) was clearly decreased in the 40-ppm groups of the F_0, F_1 and recovery generations (16/30, 3/30, and 14/30, respectively). No statistically significant alterations in fertility were observed in the 1- or 10-ppm groups. In the F_1 females of the 40-ppm group also the gestation index (numbers of pregnancies with live litters/number of pregnancies) and the number of implantations were decreased: of the three pregnant F_1 females only one delivered and in two of the three uteri examined a decreased number of implantations was observed. No biologically significant differences in gestation index, number of implantations, number of resorptions, resorption index (number resorptions/number of implantations) or duration of gestation were observed in the other groups. There were no biologically significant differences in litter size at birth, number of viable pups, sex ratio, and survival indices on p.n. day 1, 4, or 21 of any generation. For the F_1 offspring body weights of the male and female pups of the 40 ppm group were approximately 12% lower than respective control values on p.n. day 21. There were no body weight differences between control and 40-ppm pups of the recovery generation.

An obvious reduction in the size of the testes and statistically significant reductions in both absolute and relative weights of testes and epididymides were observed in F_0 males of the 40-ppm group after 12 weeks of exposure. Similar observations occurred in the F_1 males of the 40-ppm group after 9 weeks of recovery. Mean weights of testes and epididymides for the 1- and 10-ppm groups of F_0 and F_1 generations were similar to control values. Biologically significant histopathological findings were limited to the testes and epididymides of F_0 and F_1 rats exposed to 40 ppm nitrobenzene. There were no microscopic changes in the reproductive organs of the female rats that could be attributed to nitrobenzene exposure. Specifically, the testes of the F_0 generation males of the 40-ppm group had seminiferous tubule atrophy and spermatocyte degeneration. The degree and distribution of the atrophy were marked to severe and multifocal or diffuse, respectively, in 14 of 30 animals. In addition, there were giant

syncytial spermatocytes observed in the seminiferous tubules of 22 of 30 animals. The epididymides of these males had degenerated spermatocytes in the tubular lumina and decreased numbers of spermatids. The microscopic findings for the testes of the F_1 generation males exposed to 40 ppm for 12 weeks followed by a 9-week recovery period, were similar to those for lesions of 40 ppm F_0 males. Marked or severe atrophy of seminiferous tubules persisted in 21 of 30 animals. Yet, giant syncytial spermatocytes were nearly absent and the active stages of spermatocyte degeneration in the seminiferous tubules were much less frequent. As with the F_0 males, the epididymides of these F_1 males contained degenerated spermatocytes and reduced numbers of spermatids.

In terms of fertility, male reproductive organ toxicity and spermatogenesis a NOAEC of 10 ppm can be derived from this study. This NOAEC is recommended to be used for risk assessment for the inhalatory route of administration. No signs of systemic toxicity were observed in this study up to and including the highest concentration tested (40 ppm equivalent to 205 mg/m^3).

Similar adverse effects on the male reproductive organs had been further revealed from several repeated dose toxicity studies with rats and mice in the male sex (4.1.2.6).

Persistent testicular and epididymal lesions as well as severe spermatotoxic effects were reported from histopathological examination of the gonads from two 14-day inhalation studies with Sprague Dawley and F344 rats as well as with B6C3F1 mice at high concentration levels of 112 ppm (573 mg/m^3) (DuPont 1981) and 125 ppm (640 mg/m^3) (Medinsky and Irons 1985). Considering testicular toxicity, respectively dysspermatogenesis, a NOAEC in the range of 35 to 39 ppm (179 to 200 mg/m^3) might be derived from these subacute toxicity studies.

Likewise, in a 90-day inhalation study with F344 and CD rats as well as with B6C3F1 mice (Hamm 1984, cited from the study summary, original data not available) moderate to severe degeneration of tubular epithelial cells of the testes, Leydig cell hyperplasia and aspermia in the epididymis were found at concentration levels of 50 ppm (256 mg/m^3) for the male rats but not for male mice. The NOAEC for testicular and spermatotoxic effects in this study for rats was 16 ppm (82 mg/m^3).

Oral

Nitrobenzene was investigated in a combined repeated dose and reproductive/developmental toxicity study according to OECD TG 422 (Mitsumori et al. 1994). The agent was given daily by gavage to groups of 10 male and female Sprague-Dawley rats at doses of 0, 20, 60, and 100 mg/kg bw as a 10% stock solution dissolved in sesame oil. Male and female animals were continuously treated for 14 days prior to mating, and during the mating (up to 14 days) and gestation (22 days) periods to day 3 of lactation. The general appearance of the animals was checked daily. Body weights and food consumption were recorded once weekly. Each litter was examined as soon as possible after delivery to establish the number of pups, stillbirths, live births, and the presence of gross abnormalities. Haematological and serum biochemical examinations were performed only on male rats at the study termination. Full autopsy was performed on all animals after sacrifice including organ weight determinations and the counting of corpora lutea and implantation sites in the females. Brain, heart, liver, kidney, spleen, ovaries testes and epididymides and bone marrow were taken for histopathological examinations. Some of the high dose animals exhibited neurological signs, and 2 males and 9

females (7 during pregnancy, 2 during lactation) died during the study. Food consumption and body weight gain was also reduced in this group. Haemolytic anaemia due to methaemoglobin formation was evident in treated males. There were significant increases in absolute and relative organ weights of the liver and spleen in treated males along with significant decreases in testis and epididymidis weights (60 and 100 mg/kg bw dose groups). Toxic changes were observed in the liver, kidney, spleen, bone marrow and brain. Histopathologically, all males in the high and middle dose groups and one male in the low dose group (20 mg/kg bw) showed atrophy of seminiferous tubules, the severity being dose-dependent. In addition, Leydig cell hyperplasia and decreased numbers of cells with round nuclei per seminiferous tubule in the testes and loss of intraluminal sperm in the epididymidis were observed. With respect to reproduction, there were no evident effects on copulation, fertility, and implantation indices in treated dam, although the survival index of the dams was dramatically decreased in the high dose group. There were no abnormalities in the gestation period and in delivery conditions in remaining treated females and controls. One dam died in the 20 and 60 mg/kg bw groups as well as the remaining two dams of the 100 mg/kg bw group during day 1 and 3 of lactation. The number of pups alive on day 0 of lactation and the live birth index were significantly decreased in the high dose group and no pubs were alive on day 4 of lactation. The viability index was significantly decreased at that day also in the 60 mg/kg bw dose group. The pup body weights were decreased in the middle and high dose group on day 0 and on day 4 in male pups of all treatment groups and in the females of the middle dose group. No pups showed any external or visceral malformations. A LOAEL systemic toxicity of 20 mg/kg bw/d was derived from this study based on changes in haematological parameters in males from each treated group. No dosage without adverse effect on male reproductive system (LOAEL 20 mg/kg bw/d, atrophy of seminiferous tubules) was investigated in this study.

Based on the results of this latter study and since nitrobenzene has been known to inhibit male fertility from the inhalatory studies, a further experiment was conducted to determine which spermatogenic endpoints were affected by nitrobenzene, how changes were related to male fertility and how long a treatment period is needed before damage can be detected (Kawashima et al. 1995). An experimental group (n=70) of male Sprague-Dawley rats was given nitrobenzene via gavage for up to 70 consecutive days at a dosage of 60 mg/kg bw/d. 70 control male rats received 1 ml/kg sesame oil. Groups of treated and control males were mated to normal proestrus females on day 7, 14, 21, 28, 42, 56, or 70 of treatment. Male rats were sacrificed on the day after mating, and testes and epididymides weights, sperm count and sperm morphology, sperm motility, progressive motility of sperm, as well as copulation and fertility indices were examined. No change in testicular and epididymal weight was observed in the 7-day treatment group. Significant and pronounced organ weight decreases however were observed in all groups sacrificed thereafter. Histopathological observations of the testes revealed a decrease in elongated spermatids and the appearance of multi-nuclear giant cells in the day 14 group. No change in sperm count was observed in the 7-day group. The sperm count of the 14-day group was significantly reduced to 34% of the control value. Sperm counts of the 21-day group and all groups thereafter were dramatically decreased mostly to less than 10% of the control values. Sperm motility was decreased beginning on day 14 of treatment as was progressive motility, and no progressive motility was observed beginning on day 21. Sperm viabilities of the 7-day and 14-day treatment groups were comparable to control values, whereas it was significantly decreased to 20% at 21 days. Thereafter sperm viability was less than 10%. Abnormal sperm rate increased from treatment day 21 on to about 40 to 50% in the later treatment groups. Copulation indices were comparable in the control and all treatment groups. In the control group all females were fertilized. Fertility indices of the 7- and 14-day treatment group were unaffected. A significant

decrease in fertility index was observed in the 21-day treatment group. No more pregnant animals were obtained from groups in which rats were treated for 28 days or longer. Data from this study thus demonstrated, that the fertility index due to oral nitrobenzene exposure was not affected until sperm count was depressed at or below 10%.

Furthermore, data for nitrobenzene had been included in the evaluation of a screening system for reproductive toxicants, where the results of a total of 24 chemicals had been compiled (Morrissey et al. 1988). It is reported that data had been extracted from 13 week oral (gavage) studies on mice and rats. Only summarising data and no dose-related quantitative results were presented. For mice as well as for rats reduced organ weights for testes and epididymides as well as a decrease in sperm density and sperm motility and an increase in percentage of abnormal sperm were indicated for dosages of 18.75, 75, and 300 (mice) or 9.4, 37.5, and 75.0 (rats) mg/kg bw/d (testing of lower dosages was not indicated). For the female sex no data were given, since monitoring of vaginal cytology was revealed not to be a suitable screening parameter. For the oral route of administration the dosage of 9.4 mg/kg bw/d investigated in this study in rats was the lowest dose tested. Although no original data on the studies summarised by Morrissey et al. were available, a LOAEL of 9.4 mg/kg bw/d is recommended to be used for risk assessment for the oral route of administration.

Testicular toxicity is further reported from a series of single dose studies mostly applying high doses of 300 mg/kg bw. These studies were considered as further informations.

In an oral study with F344 rats (Bond et al. 1981) groups of six rats each received single dosages of 50, 75, 110, 165, 200, 300, or 450 mg nitrobenzene/kg bw in corn oil for dose response evaluations. Three rats at each dosage were sacrificed 2 and 5 days after administration. For time-response evaluations groups of three rats each were orally dosed with 300 mg nitrobenzene/kg bw in corn oil and sacrificed at 1, 2, 3, 4, 7, and 10 days after administration. Liver, testes, and epididymides were histologically examined. Testicular lesions were restricted to the seminiferous tubules in this study. The early lesion consisted of enlarged, pale staining cytoplasm of the primary and secondary spermatocytes. Progressive necrosis of these layers was seen with complete destruction of the spermatocytes at days two and three after 300 and 450 mg nitrobenzene/kg bw. Within three days after administration, multinucleated giant cells within the seminiferous tubules were detected. In addition, necrotic debris and decreased number of spermatozoa were noted in the epididymis as early as three days and as late as ten days after nitrobenzene administration. No apparent effect on the epididymal epithelium was reported from this study.

To investigate the possible regeneration of the seminiferous epithelium after single dose administration, in a further study (Levin et al. 1988) sperm production had been continuously monitored in F344 rats, the vas deferentia of which had been anastomosed with the urinary bladder to allow chronic monitoring of sperm output by microscopically counting the number of sperm in collected urine. Six weeks after surgery rats were dosed p.o. with a single dose of 300 mg nitrobenzene/kg bw in corn oil and followed up to for up to 100 days. Degenerative changes in the seminiferous tubules were observed histologically as early as 3 days after dosing. Pachytene spermatocytes and step 1-2 spermatids were shown the most susceptible stages and were observed forming giant cell stages as early as three days after treatment. A 17-day period of aspermia resulted: sperm were not detected in the urine of treated rats between 32 and 48 days after treatment. By days 76 - 100, the rate of sperm output recovered and reached 78% of the control group. By day 100 after treatment, an approximately 90% regeneration of the seminiferous epithelium could be observed.

Nitrobenzene was further investigated within in a short duration test design evaluated for screening of reproductive responses (Linder et al. 1992). Groups of six male Sprague-Dawley rats each were orally treated once with a single dose of 300 mg nitrobenzene/kg bw, sacrificed after 2, respectively 14 days, and investigated for organ weight and histopathology of testes and epididymides, sperm count and sperm morphology. No quantitative data are available from this study. It is reported that fourteen days after treatment testes and epididymides weights had decreased as well as had epididymal sperm count and an increase in abnormal sperm morphology was observed. Histopathology revealed degeneration of spermatocytes as soon as two days after treatment.

Nitrobenzene was further tested within a comparative *in vivo*/*in vitro* test design using modulation of the Sertoli cell immunoreactive inhibin secretion as an indicator for early detection of adverse effects of chemicals on spermatogenesis (Allenby et al. 1991). Groups of six male Sprague-Dawley rats were gavaged with doses of 300 mg nitrobenzene/kg bw in corn oil and sacrificed 1 and 3 days after treatment for collection of testicular interstitial fluid. Testicular weight was significantly reduced at 3 days post-treatment, and there was a significant increase in the levels of immunoreactive inhibin in testicular fluid at both 1 and 3 days after treatment. Also in cultures of isolated seminiferous tubules or Sertoli cells of untreated adult males the incubation with 0.01 or 1 mM nitrobenzene for 1-3 days induced a dose-related increase in both basal and stimulated secretion of immunoreactive inhibin.

Nitrobenzene was further evaluated within an *in vitro*/*ex vivo* test design where male Wistar rats received a single oral dose of 300 mg nitrobenzene/kg bw or the vehicle (McLaren et al. 1993). Seminiferous tubules were isolated 1 or 3 days after treatment at different stages of the spermatocytic cycle and cultured in the presence of radiolabelled methionine for 24 hr. The culture medium was then analyzed for secreted proteins containing radiolabelled methionine. Testicular weight was significantly reduced after 1 and 3 days post-treatment. Incorporation of methionine into the secreted proteins was significantly decreased in treated groups and dependent on the stage of the spermatogenic cycle at which the tissues had been isolated. A similar effect was noted, when tissues from control rats were incubated in vitro with 0.1 mM nitrobenzene for 24 or 72 hr. The relative abundance of several potential marker proteins secreted by seminiferous tubules was changed dramatically upon treatment.

Dermal route of exposure

Data for investigations on nitrobenzene with the dermal route of exposure had been included in the evaluation of a screening system for reproductive toxicants, where the results of a total of 24 chemicals had been compiled (Morrissey et al. 1988). It is reported that data had been extracted from 13 week NTP studies on mice and rats. Only summarising data and no dose-related quantitative results were presented. For male rats reduced organ weights for testes and epididymides as well as a decrease in sperm density and sperm motility and an increase in percentage of abnormal sperm were indicated for dosages of 0.05, 0.2 and 0.4 g/kg bw/d. For male mice decreased testicular weight and sperm motility as well as increased percentage of abnormal sperm were indicated for dosages of 0.05, 0.2 and 0.4 g/kg bw/d. For the female sex no data were given, since monitoring of vaginal cytology was revealed not to be a suitable screening parameter. Although no original data on the studies summerised by Morrissey et al. were available, a LOAEL of 0.05 g/kg bw/d is recommended to be used for risk assessment for the dermal route of administration.

Original study reports publications on studies with repeated dermal nitrobenzene administration were unavailable. For the sake of compleness, the information from the EHC report (2003) on dermal studies is cited here:

In a range-finding NTP study, nitrobenzene was administered to B6C3F1 mice and Fischer-344 rats (both sexes) by skin painting at doses in the range of 200 to 3200 mg/kg bw/d for 14 days (NTP, 1983b). All rats and mice at the 1600 and 3200 mg/kg bw/d doses died or were sacrificed moribund prior to the end of treatment. Treated animals were inactive, ataxic, prostrate and dyspnoeic. Significant depression of weight gain (>10%) was seen in mice from all dose groups. Histologically, mice and rats showed changes in the brain, liver, spleen and testes, with mice less affected than rats.

In an NTP study, nitrobenzene was administered to B6C3F1 mice (10 per sex per group) by skin painting (in acetone vehicle) at 0, 50, 100, 200, 400 or 800 mg/kg bw/d for 13 weeks (NTP, 1983b). The chemical was applied to a shaved area of the skin in the intercapsular region. Mean final body weights were not significantly affected. Six high-dose males were sacrificed moribund, and three died between weeks 3 and 10; seven high-dose females were sacrificed moribund, and one high-dose female and one female of the 100 mg/kg bw/d group died between weeks 2 and 9. Clinical signs in some animals at the high dose included inactivity, leaning to one side, circling, dyspnoea, prostration and, in one, head tilt, whereas a number of dosed females had extremities cold to the touch. One high-dose female exhibited tremors, and two were insensitive to painful stimuli. Inflammation of the skin (diffuse or focal and of minimal to mild severity) was seen at the site of nitrobenzene application at the two highest doses; inflammatory cells were present in the dermis, with varying degrees of involvement of the subcutaneous tissue. There was acanthosis and hyperkeratosis of the epidermis, with occasional thick crusts of necrotic cells or focal areas of necrosis extending deep into the epidermis. Liver weights in treated male mice from the 400 mg/kg bw/d group and females from the 400 and 800 mg/kg bw/d groups were significantly increased compared with controls. At the high dose, a number of periportal hepatocytes were smaller than those in control livers and in treated mice, and there was a noticeable variation in the size of hepatocyte nuclei, especially in the centrilobular zone. The cytoplasm of hepatocytes in many treated mice had a homogeneous eosinophilic appearance, whereas that in controls had a vacuolated appearance characteristic of glycogen-containing cells. While degeneration of the "X" zone of the adrenal glands (the zone of cells adjacent to the medulla) in female mice was noted, the degree of vacuolation in treated animals was reported to be greater than normally seen in controls. Brain lesions were found in 2 of 10 males and 3 of 10 females at 800 mg/kg bw/d; the lesions appeared to be localized in the brain stem in the area of the vestibular nucleus and/or cerebellar nuclei; one high-dose female had a mild bilateral lesion in a nucleus of the ventrolateral thalamus. Such lesions were probably responsible for the clinical behavioural findings of head tilt, leaning to one side and circling. Brain vascular lesions (as described in the rat dermal study; see below) were not observed in this mouse dermal study.

Studies in humans

There are presently no data available.

4.1.2.9.2 Developmental toxicity

Studies in animals

Nitrobenzene had been investigated for developmental toxicity in two guideline-according studies with rats and rabbits with the inhalatory route of administration.

In the study with CD rats (Tyl 1984; Tyl et al. 1987) groups of 26 plug-positive females were exposed to nitrobenzene vapours (the test material used remained at least 99.94% nitrobenzene) at 0, 10, and 40 ppm (5.1, 51.2 and 204.8 mg/m^3) on g.d. 6 to 15 for 6 hr/day (whole chamber administration). The animals were observed daily for clinical signs throughout the study (g. d. 0 to 21), and maternal body weights were taken on g.d. 0. 6, 9, 12, 15, 18, and 21. After sacrifice on g.d. 21 maternal liver, spleen, kidney, and uteri weights were taken and the ovarian corpora lutea of pregnancy were counted. All live and dead fetuses as well as late and early resorption sites were noted and recorded. All live fetuses were weighed and sexed and examined for external malformations including cleft palate. One-half of the fetuses in each litter was examined for thoracic and abdominal visceral abnormalities including craniofacial structures, the other half was examined for skeletal alterations. As a result of this study there were no maternal deaths, early deliveries, or abortions. The pregnancy rate was high and equivalent for the control and all treatment groups. There were no exposure-related or concentration-related clinical signs of toxicity reported. In the 40 ppm group maternal weight gain was transiently reduced during the treatment period, however, at sacrifice maternal body weight was equivalent across all groups. Spleen weights (absolute and relative) were statistically significantly increased at 10 and 40 ppm with a clear-cut exposure-related response. Absolute and relative liver weights were also increased at 40 ppm but the differences were not statistically significant. Histological examination of maternal organs and measurement of methaemoglobin levels were not performed. Gestational parameters were unaffected by treatment. The control and treatment groups did not differ in number of corpora lutea per dam, in number of resorptions, dead and live fetuses per litter, in percentage pre- or postimplantation loss, in sex ratio or in fetal body weight per litter. Fetal evaluations revealed that there was no significant increase in the number of litters with one or more affected fetuses at any exposure concentration relative to controls for individual and total external, visceral, or skeletal malformations. There was a significant increase in the incidence of total malformations at 1 ppm but not at 10 or 40 ppm relative to that of controls. In the absence of an increased incidence of any specific malformation and in the absence of any concentration response, this finding was not considered treatment related. The incidences of variations did not indicate fetal toxicity, likewise there were no indications of reduced fetal body weights or any other signs of fetal toxicity. Thus, no effects on development were seen in this study. A NOAEC of $\geq$ 40 ppm (205 mg/m^3) can be derived from this study. In terms of maternal toxicity a LOAEC of 10 ppm (51 mg/m^3) can be derived.

In the study with New Zealand White rabbits (Bio/dynamics Inc. 1984a) groups of 22 pregnant females were exposed to nitrobenzene at target concentration levels of 10, 40, and 100 ppm (mean nominal levels were 13, 59, and 129 ppm, equivalent to 67, 302 and 660 mg/m^3) on g.d. 7 to 19 for 6 h/d (whole chamber administration). Animals were weighed and given detailed physical evaluations at regular intervals during gestation. At sacrifice on g.d. 30 each female was given a gross post-mortem evaluation and the livers as well as a blood sample were taken for analysis of haemoglobin and methaemoglobin levels. Corpora lutea and uterine implantation data were also recorded. Fetuses were measured for body weight and crown-rump length. After gross external examination all fetuses were evaluated for visceral

and skeletal malformations or variations in ossification. No adverse effect of treatment was evident from maternal mortality data. Mean body weight data during gestation were comparable between the control and treated groups. No adverse effect of treatment was evident from physical in-life evaluations or from gross post-mortem evaluations. Mean liver weight (absolute and relative) were increased in the mid-dose (relative liver weight 2.81 +/- 0.56 at 40 ppm compared to 2.52 +/- 0.6 for controls) and high-dose group animals (relative liver weight 2.82+/- 0.53 at 100 ppm). While haemoglobin values at sacrifice were comparable between control and treated groups mean methaemoglobin values were significantly higher than controls (40 and 60% increase) at the mid-dose and high-dose group. No adverse effect of treatment was evident from pregnancy rate data, premature delivery or abortion data. Corpora lutea and uterine implantation data were comparable between the control, the 10 and the 40 ppm group. In the high-dose group, the mean number of resorption sites, the mean percentage of resorptions to implants and the incidence of females with resorptions were slightly higher than control; however, these differences from control data were not statistically significant. No adverse effect of treatment was evident from fetal weight or crown-rump distance data or fetal sex distribution data. External, visceral and skeletal evaluation of fetuses from treated females did not reveal an increase in malformation rate nor an increase in the incidence of external, visceral or ossification variations.
In terms of developmental toxicity, a NOAEC of 40 ppm (205 mg/m^3) can be derived from this study based on increased resorption data suggesting some embryotoxicity. No teratogenicity was evident. For maternal toxicity a NOAEC of 10 ppm (51 mg/m^3) based on increased methaemoglobin levels and increased liver weights was derived.
Supplementary data are available from a range-finding inhalatory study performed on rats (Bio/dynamics Inc. 1984b), in which twenty-two pregnant CD rats were exposed to nitrobenzene at target exposure levels of 10, 40, and 80 ppm (51, 205 and 410 mg/m^3) during g.d. 6 to 15 (6 hr/day, whole chamber administration). As one component of the study five animals per group were sacrificed and bleeded each on days 10 and 15 of gestation to collect blood samples for methaemoglobin determinations and to evaluate uterine implantation data. The remaining dams served for a pilot teratology evaluation and were sacrificed on g.d. 16. During physical in-life evaluations, all high-dose animals were noted with dark eyes and bluish extremities at least once during the treatment period. Similar types of observation were seen at low incidence in mid-dose females. While no mortality occurred in the control, low- or mid-dose groups for either the bleeding study or pilot teratology study animals, in the high-dose (80 ppm) group all but two of the animals died or were killed in moribund condition before the end of the study. In the pilot teratology study mean body weight gain during g.d. 6 to 16 was unaffected for the low-dose group but was significantly lower than control data for the 40 ppm group. Mean kidney weight data were comparable between the control and the 10 and 40 ppm groups, however, mean liver weight data and mean liver to body weight ratios were significantly higher than control in each of the low- and mid-dose groups. Corpora lutea and uterine implantation data for the animals in the pilot teratology study were comparable between the control, low- and mid-dose groups as was for the dams from the bleeding study. For these latter ones, methaemoglobin values on days 10 and 15 were considerably higher in each treated group and statistically significantly different from controls at 40 and 80 ppm.

Delay in the development of the embryo and production of malformations and other disturbances of organogenesis were reported from the translation of a Russian study in which pregnant rats had received daily subcutaneous injections of nitrobenzene in a dose of 125 mg/kg bw starting from day 4 to 6 or 9 to 12 of pregnancy (Kazanina 1968). Due to the poor documentation the study it is not further considered for hazard evaluation.

Nitrobenzene was investigated in a combined repeated dose and reproductive/developmental toxicity study according to OECD TG 422 (Mitsumori et al. 1994, c.f. 4.1.2.9.1). With respect to reproduction, there were no evident effects on copulation, fertility, and implantation indices in treated dam, although the survival index of the dams was dramatically decreased in the high dose (100 mg/kg bw) group. There were no abnormalities in the gestation period and in delivery conditions in remaining treated females and controls. One dam died in the 20 and 60 mg/kg bw groups as well as the remaining two dams of the 100 mg/kg bw group during day 1 and 3 of lactation. The number of pups alive on day 0 of lactation and the live birth index were significantly decreased in the high dose group and no pubs were alive on day 4 of lactation. The viability index was significantly decreased at that day also in the 60 mg/kg bw dose group. The pup body weights were decreased in the middle and high dose group on day 0 and on day 4 in male pups of all treatment groups and in the females of the middle dose group. Pups did not show any external or visceral malformations. The study design, however, is not considered appropriate for derivation of an appropriate L/NOAEL for prenatal developmental toxicity.

Studies in humans

There are presently no data available.

4.1.2.9.3 Summary of toxicity for reproduction

Numerous studies with rats and mice revealed nitrobenzene to persistently adversely affect male reproductive organs (atrophy of the germ epithelium) and spermatogenesis independently from the route of administration (inhalation, oral, dermal). As a consequence of this, also reduced fertility in terms of reduced number of pregnancies and offspring was demonstrated in a rat two-generation inhalation study. A NOAEC (fertility) of 10 ppm (51 mg/m^3) was derived from the study of Dodd et al. (1987). LOAELs (fertility) of 9.4 mg/kg bw/d for the oral route of administration to rats and of 0.05 g/kg bw/d for the dermal route of application (rats and mice) were derived from the study of Morissey et al. (1988).

It should be recognised that haematotoxicity is the most sensitive effect after treatment with nitrobenzene and that this effect was also observed in the available reproduction toxicity studies with nitrobenzene. It is further recognised that humans in comparison to the rat species are much more sensitive to the induction of methaemoglobinaemia and that the rat as an experimental model rather may underestimate the significance of methaemoglobin-induced haematotoxicity of nitrobenzene. Also, as far as both haematological as well as reproduction parameters had been evaluated in the studies available with nitrobenzene, haematotoxicity was induced at dose levels below those inducing testes toxicity (Table 4.11). Therefore, nitrobenzene is not considered to represent a specific reproductive hazard. Based on the evaluation of the available animal data, the current classification and labelling as Reprotox. Cat. 3, R 62 is confirmed.

Table 4.11

	hematotoxicity		testes toxicity		species
inhalation, rat					
	NOAEC	LOAEC	NOAEC	LOAEC	

	(ppm)	(ppm)	(ppm)	(ppm)	
Mendinsky & Irons, 1985	lower concentrations not tested	10	35	125	F-344 rat, 14 d
Mendinsky & Irons, 1985	lower concentrations not tested	10	35	125	CD rat, 14 d
DuPont, 1981	lower concentrations not tested	12	39	112	CD rat, 28 d
Hamm, 1984	lower concentrations not tested	5	16	50	F-344 rat, 90 d
Hamm, 1984	lower concentrations not tested	5		50 (occas-ionally 5)	CD rat, 90 d
Dodd et al., 1985, 1987	not determined	not determined	10	40	CD rat, 2-gen study
inhalation, mouse					
Mendinsky & Irons, 1985	10	35	35	125	B6C3F1 mouse, 14 d
Hamm, 1984	lower concentrations not tested	5	50		B6C3F1 mouse, 90 d
oral, rat					
	NOAEL (mg/kg bw)	LOAEL (mg/kg bw)	NOAEL (mg/kg bw)	LOAEL (mg/kg bw)	
Shimo et. al. 1994	5	25	25	125	F-344 rat, 28 d
Mitsumori et al., 1994	lower doses not tested	20	lower doses not tested	20	SD rat, 54 d
Morrissey et al. 1988; NTP, 1983a	no data	no data	lower doses not tested	9,4	F-344 rat, 13 weeks

Investigations in rats and rabbits with the inhalatory route of application did not reveal any developmental toxicity (including teratogenicity) associated with the exposure to nitrobenzene during organogenesis at concentration levels that produced no observable maternal toxicity or produced some slight maternal toxicity. A NOAEC (developmental toxicity) of $\geq$ 40 ppm (205 mg/m^3) was derived from the study of Tyl et al. (1987). A slight fetotoxic effect (reduced pup body weight on p.n. day 21) was observed in the F_1 offspring of rats of a generation-study at concentration levels (40 ppm) that resulted in reduced body weight gain for the dams during gestation and in significantly reduced fertility index due to testes toxicity in the parental males. Developmental toxicity studies with other routes of application are not available.

4.1.3 Risk characterisation [5]

4.1.3.1 General aspects

Nitrobenzene is readily taken up by inhalation and skin penetration of the vapour and by digestion and dermal absorption of the liquid. As urinary metabolites of nitrobenzene o- and m-nitrophenol were identified. Nitrobenzene is metabolized to methaemoglobin-forming metabolites. Nitrobenzene is distributed to a variety of tissues, follwed by a slow excretion of nitrobenzene metabolites.
Nitrobenzene is readily absorbed by the oral and inhalation route. Based on its physico-chemical properties (water solubility: 1900 mg/l; octanol-water partition coefficient log Pow: 1.89; molecular weight: 123 g/mol and vapour pressure) and based on animal experiments after oral application of nitrobenzene, an absorption percentage up to 100 % can be assumed for oral risk characterisation purposes. Based on its physico-chemical properties and on experiments with human volunteers, an absorption percentage of 87 % can be assumed for the inhalation route.

Liquid nitrobenzene as well as nitrobenzene vapour can be absorbed through the skin. Absorption rates from exposure to liquid nitrobenzene have been calculated to be higher (up to 2 mg/cm^2/h) compared to those from exposure from nitrobenzene vapour (absorption rate per unit vapour concentration between 0.23 and 0.3 mg/h per mg/m^3). From in vivo and in vitro experiments it can be derived that up to about 8% nitrobenzene were absorbed from non-occluded skin whereas up to 40% nitrobenzene were absorbed from in vitro experiments with human skin, when evapouration was prevented.
Based on acute studies in animals the substance is harmful by the inhalation, dermal and oral route. For rats, the inhalation LC50 was determined to be 556 ppm (2847 mg/m^3, 2.847 mg/l). Oral LD50 values between 588 and 732 mg/kg bw are reported. Dermal LD50 values ranged from about 300 mg/ kg bw and 560 < LD50 < 760 mg/kg bw for rabbits and to 2100 mg/kg bw for rats. Other not mortality related effects were observed in cats and rats. Cats survived an oral treatment with up to 120 mg/kg bw nitrobenzene and cyanosis and a significant elevation of methaemoglobin were the most prominent toxic signs. In rats toxic effects were seen, in addition to the increases in methaemoglobin levels, in liver and testes after a single treatment starting with a dose of 110 mg/kg bw.
For humans many reports are documented in the literature on nitrobenzene poisoning and to some extent on exposure of workers to nitrobenzene. Considerable individual variation exists and no clear-cut relationship can be documented between the absorbed dose of nitrobenzene and the severity of response in man; babies and children appear to be more sensitive to the effects of the substance compared to adults. Methaemoglobinamia and cyanosis are the most prominent clinical symptoms; others are the formation of Heinz bodies, toxic effects on bone marrow and lymphoid organs, neurotoxic and hepatotoxic effects. The bulk of evidence clearly demonstrate severe toxic effects of nitrobenzene to humans.
Slight skin irritation was observed in rabbits. Three out of six rabbits died after a 24-hour occlusive exposure with 0.5 ml indiluted nitrobenzene after exhibiting signs of cyanosis. In a dermal 13-week toxicity study with mice, mild inflammation occurred at the site of

[5] Conclusion (i) There is a need for further information and/or testing.
Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.
Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

application in the two highest dose groups of 400 and 800 mg/kg bw/d. Slight eye irritation was observed in rabbits that disappeared within 24 hours.
The existing data on skin sensitisation testing in guinea pigs are insufficient to possible skin sensitizing properties of nitrobenzene. In humans, three weak positive cases out of 15 cases are reported. These data are insufficient to evaluate a possible skin sensitisation potential.
In repeated dose studies in rodents oral or inhalation administration of nitrobenzene caused haemolytic anemia and in response to that increased regenerative medullary and extramedullary haematopoiesis and splenic haemosiderosis, which was also observed in the liver and kidneys. Other treatment-related lesions consisted of testis atrophy, degeneration and hypertrophy of liver cells, renal tubular degeneration or nephrosis and haemorrhagic malacia of the cerebellum. Occasionally, there were seen thymus involution, adrenal cortical vacuolation or medullary basophilia, endometrial polyps of the uterus and only after inhalation nasal respiratory epithelium inflammation/hyperplasia and increased pigmentation. Only in mice after inhalation degeneration of the olfactory epithelium of the nasal passages, bronchial hyperplasia, pulmonary edema and ocular keratitis were seen. The LOAEC for systemic effects was derived to be 5 ppm (25 mg/m³) from 90-day inhalation studies on two rat strains. The NOAEC for local effects on the respiratory tract was considered to be 1 ppm (5 mg/m^3) derived from the 2-year studies on rats and mice.

Nitrobenzene was negative in several bacterial tests with numerous *Salmonella typhimurium* strains. For genotoxicity of nitrobenzene in mammalian cells *in vitro* no test according to current guidelines was available. Inconclusive results were reported in tests in mammalian cells *in vitro* which were either methodically inadequate or insufficiently described. These studies were not considered as relevant for risk assessment. The two most reliable tests revealed negative results.
In vivo a DNA-binding capacity was detected in two studies after subcutaneous or i.p. application in liver and lung of mice and in liver and kidney of rats (Novartis 1997 and Li 2003). Considering the routes of application (s.c. and i.p.) which limit the relevance of those studies and the low adduct counts it can be concluded that nitrobenzene has a low DNA-binding capacity the biological relevance of which is unclear. In rats, no UDS was induced in rat liver after a single high oral dose (Mirsalis et al. 1982). In a test on chromosomal aberrations and SCE in lymphocytes from peripheral blood and spleen from subacute exposed rats via inhalation (Kligerman et al. 1983) no mutagenic effect was detected. A bone marrow micronucleus test in mice was negative up to the maximum tolerable dose (OECD TG 474) (BASF 1996). From the available *in vitro* and *in vivo* data a weak genotoxic potential of nitrobenzene can be concluded. Therefore, it is unlikely that carcinogenicity is caused by a genotoxic mode of action. From the available negative data for micronuclei formation, chromosomal aberrations, SCE and UDS in rodents *in vivo*, it can be concluded, that nitrobenzene is not suspected to exert mutagenic effects on germ cells. Nitrobenzene should not be classified as a mutagen.

Nitrobenzene is carcinogenic in B6C3F1 mice, F344 rats and CD rats. There were tumors in several organs, and tumor sites were different across species, sex, and strain of rodent. In male B6C3F1 mice, the incidences of alveolar/bronchiolar adenoma, alveolar/bronchiolar carcinoma, and thyroid adenoma were increased. In female B6C3F1 mice, the incidence of mammary gland adenocarcinoma was increased. In addition, female B6C3F1 mice exposed to nitrobenzene had a marginally increased incidence of hepatocellular adenomas. In male F344 rats, the incidences of hepatocellular adenoma, hepatocellular adenoma or carcinoma, and renal tubular adenoma were increased. Male F344 rats exposed to nitrobenzene had a

marginally increased incidence of thyroid follicular neoplasia (adenoma or adenocarcinoma). In female F344 rats, the incidence of endometrial stromal polyp was increased. In male CD rats, the incidences of hepatocellular adenoma and of hepatocellular adenoma or carcinoma were increased.

Based on the positive cancer studies in two rodent species, nitrobenzene is considered to be a carcinogen. The mutagenicity data suggest that other mechanisms than genotoxicity are likely to be involved in the tumor development, althoug the possible mode of action was not yet identified. Actually there are no data to exclude that carcinogenicity of nitrobenzene may have relevance for humans.

There are no human data on reproductive toxicity. Animal studies revealed nitrobenzene to adversely affect male reproductive organs (atrophy of the germ epithelium) and spermatogenesis independent from the route of administration (inhalation, oral, dermal) and persisting for longer periods (NOAEC (fertility) derived from the study of Dodd et al. (1987) 10 ppm, equivalent to 51 mg/m^3). Also reduced fertility in terms of reduced number of pregnancies and offspring was demonstrated in a rat two-generation inhalation study. LOAELs (fertility) of 9.4 mg/kg bw/d for the oral route of administration to rats and of 0.05 g/kg bw/d for the dermal route of application (rats and mice) were derived from the study of Morrissey et al. (1988). Investigations in rats and rabbits with the inhalatory route of application did not reveal any developmental toxicity (including teratogenicity) associated with the exposure to nitrobenzene during organogenesis at concentration levels that produced no observable maternal toxicity or produced some slight maternal toxicity (NOAEC developmental toxicity derived from the study of Tyl et al. (1987) 40 ppm, equivalent to 205 mg/m^3). Developmental toxicity studies with other routes of application are not available.

Table 4.12 Summary of effect

Substance name	Inhalation	Dermal	Oral
Acute toxicity	LC50 (rat) 2.847 mg/l	560 < LD50 (rabbit) 760 mg/kg bw LD50 (rat) 2100 mg/kg bw	LD50 (rat) 588-732 mg/kg bw
Irritation / corrositivity	skin: no hazard identified (very slight effects in animal studies, no evidence for a irritating potential from human case reports) eye: no hazard identified (animal studies) respiratory tract: no data		
Sensitisation	skin: concern due to cross reactivity caused in humans, concern due to skin sensitising potency of structurally related compounds; proposal for further testing respiratory tract: no data		
Repeated dose toxicity (local)	$LOAEC_{local}$ 1 ppm	$LOAEL_{local}$ 100 mg/kg bw/d	LOAEL 5 mg/kg bw/ d
Repeated dose toxicity (systemic)	$LOAEC_{sys}$ 5 ppm	$LOAEL_{sys}$ 50 mg/kg bw/d	
Mutagenicity	no relevant evidence for germ cell mutagenicity from a number of studies genotoxic mechanism for carcinogenesis cannot be excluded		
Carcinogenicity	Evidence of a carcinogenic effect – most likely by a non-genotoxic effect Lowest concentration with a	no data	no data

	significant increase in tumor response 25 ppm (NOAEC 5 ppm)		
Fertility impairment	$NOAEC_{fertility}$ 10 ppm $NOAEC_{sys}$ 40 ppm	LOAEL 50 mg/kg bw/d	LOAEL 9.4 mg/kg bw/d
Developmental toxicity	$NOAEC_{dev.\ tox.}$ 40 ppm $LOAEC_{mat.\ tox}$ 10 ppm	no data	no data

4.1.3.2 Workers

Introductory remarks

Human exposure of nitrobenzene occurs nearly solely during production and further processing of the substance. For occupational risk assessment the MOS approach as outlined in the TGD (Human Health Risk Characterisation, Final Draft) is applied. This occupational risk assessment is based upon the toxicological profile of nitrobenzene (chapter 4.1.2) and the occupational exposure assessment (chapter 4.1.1.2). The threshold levels identified in the hazard assessment are taken forward to this occupational risk assessment.

Systemic availability for different routes of exposure

Experimental data from humans and animals for nitrobenzene show different absorption percentages for the different routes of exposure: According to the chapter 4.1.2.1 on toxicokinetics, metabolism and distribution an adsorption percentage up to 100% is taken for the oral route and 87% is assumed for the inhalation route. 40% absorption percentage is taken for the dermal route. For most of the assessed endpoints there are inhalation studies and dermal studies available. Therefore considerations about the different absorption percentages are minor important.

Occupational exposure and internal body burden

In table 4.13 the exposure levels of table 4.1 are summarised and the route-specific and total internal body burdens are identified. Risk assessment for combined exposure requires the calculation of a total internal body burden; to this end the derived route-specific percentages for absorption are used (87% for inhalation and 40% for dermal exposure).

Table 4.13: Occupational exposure levels and internal body burden (nitrobenzene)

Exposure scenario	Inhalation shift average	Dermal contact shift average		Internal body burden of workers after repeated exposure		
				Inhalation(1)	Dermal(2)	Combined
	mg/m³	mg/p/day	mg/kg/day	mg/kg/day		
1. Production and further processing as an intermediate	0.25	4.2 (3)	0.06	0.03	0.024	0.054

(1) based on the assumption of 87% inhalative absorption; breathing volume of 10 m³ per shift
(2) based on the assumption of 40% systemic availability of nitrobenzene after dermal contact
(3) EASE (90 % protection by suitable gloves)

MOS Approach

The MOS approach for human risk characterisation is described in detail in the TGD (Human Health Risk Characterisation, Final Draft). The following chapter contains a short introduction to the MOS approach used. The basic principle of the MOS approach is a comparison of scenario-specific MOS values (the relationship between the experimental NOAEL respectively the adjusted starting point and the exposure level) with a reference MOS (product of various assessment factors).

MOS calculation and the adequate starting point

Basically, MOS values are calculated as quotient of a relevant NOAEL from experimental animal testing or human studies and actual workplace exposure levels. In specific situations, the MOS approach requires to convert the original NOAEL into an adequate starting point or corrected NOAEL previously to MOS calculation in order to be directly comparable to the exposure assessment. If the route of application in animal or human studies is different from the actual occupational exposure, the dose units of the experimental data should be converted to the dose unit of the exposure data. Additionally, possible differences in bioavailability between routes, as well as possible differences in bioavailability between animals and humans should be accounted for the calculation of the corrected NOAEL. If route-specific information on oral and inhalation absorption is not available, the TGD recomments to assume 50% oral absorption and 100% inhalation absorption. For nitrobenzene 87% absorption after inhalation, 40% absorption after dermal contact and 100% absorption after oral exposure are assumed (experimental values).

For occupational risk assessment, the corrected inhalation NOAEC accounts for the difference of the standard respiratory volume (6.7 m³) and the respiratory volume for light activity (10 m³).

MOS values are calculated for different routes of exposure and for different toxicological endpoints. The routes of exposure specifically considered in occupational risk assessment are exposure by inhalation and dermal contact.

In addition, for risk assessment of combined exposure (exposure by inhalation and dermal contact) an adequate NOAEL is derived from external NOAELs and specific information on route-specific absorption. For MOS calculation, the adjusted internal starting point is divided by the internal body burden. Depending on route-specific exposure and absorption, inhalation exposure and/or dermal exposure may contribute to the internal body burden. With respect to the possible outcome of an assessment for combined risks, interest focuses on scenarios with

conclusion ii at both exposure routes. Based on theoretical considerations, combined exposure will not increase the most critical route-specific risk component more than twice.

Reference MOS

The MOS values calculated have to be compared with a reference MOS. The reference MOS is an overall assessment factor, which is obtained by multiplication of individual assessment factors. The Technical Guidance Document emphasises several aspects which are involved in the extrapolation of experimental data to the human situation. For these assessment factors, default values are recommended. It is important to point out that any relevant substance-specific data and information may overrule the defined default values.

Interspecies extrapolation on the one hand is based on allometric scaling (factor 4 for rats, factor 7 for mice, and factor 2 for rabbits). For remaining interspecies differences the TGD proposes an additional factor of 2.5.

For workers, an adjustment factor for intraspecies differences of 5 is recommended. Based on an evaluation of empirical data by Schneider et al. (2004) it is anticipated that a factor of 5 will be sufficient to protect the major part of the worker population (about 95%).

For chemical substances it is usually expected that the experimental NOAEL will decrease with increasing duration of application. Furthermore, other and more serious adverse effects may appear with prolonged exposure duration. For duration adjustment, a default factor of 6 is proposed for extrapolation from a subacute to chronic exposure. The duration adjustment factor is lower (a factor of 2) for the transition from subchronic experimental exposure to chronic exposure. For nitrobenzene the factor of 2 for an adaptation from subchronic to chronic exposure is used.

The TGD defines two further adjustment factors (uncertainty in route-to-route extrapolation and dose-response relationship including severity of effect). In specific cases these factors may be different from one. For nitrobenzene uncertainties remain at the endpoint of carcinogenicity because the LOAEC describes an increase of several tumors at different organs. The uncertainties about the slope of the dose-response relationship are described with an additional factor of 10.

Comparison of MOS and reference MOS

The MOS values for different toxicological endpoints and different exposure scenarios are compared with the substance- and endpoint-specific reference MOS. MOS values clearly above the reference MOS do not lead to concern, whereas MOS values that are clearly below the reference MOS are cause for concern. There may be various risk-related aspects which are not covered by default assessment factors. These additional qualitative aspects should be carefully considered when performing a risk assessment and should have adequate influence on finding of conclusions.

Critical Exposure Levels

In a parallel procedure, which gives identical but more direct results, the adjusted toxicological starting point is directly divided by the reference MOS. As a result, an exposure level (in mg/m³ or mg/kg/d) is identified, which may serve as a direct trigger for decisions when compared with the occupational exposure levels. In the context of this risk assessment

report this trigger value is called “critical exposure level”. Concern will be expressed for scenarios with occupational exposure levels higher than the relevant “critical exposure level”.

4.1.3.2.1 Acute toxicity

For humans many reports are documented in the literature on nitrobenzene poisoning. Methaemoglobinamia and cyanosis are the most prominent clinical symptoms; others are the formation of Heinz bodies, toxic effects on bone marrow and lymphoid organs, neurotoxic and hepatotoxic effects. Because no clear dose relationship after inhalation or dermal contact of nitrobenzene can be drawn from the case reports by humans, risk assessment for acute toxicity is done on the basis of animal studies.

Inhalation exposure

A LC50 of 556 ppm (2847 mg/m^3) for rats is described after inhalation of atmospheres of nitrobenzene. For risk assessment of acute inhalation toxicity (8-hour exposure) data on nitrobenzene-induced lethality are considered less relevant than the results from a 12 day developmental rabbit study (Bio/dynamics Inc. 1984a). 22 pregnant rabbits were treated with nitrobenzene at dosages of 10, 40, and 100 ppm (51, 205 und 410 mg/m^3) on gestation day 7 to 19 for 6 h/d. At dosages of 205 and 410 mg/kg/day liver weights were increased and methaemoglobin values were significantly higher than controls (40% increase at the mid-dose and 60% increase at the high-dose). The corresponding NOAEC for maternal toxicity was 51 mg/m^3.

This NOAEC is taken as starting point for acute inhalation toxicity, taking the following considerations into account: The conversion from a NOAEC resulting from a 12-days study to a value which corresponds to a situation of one day would lead to an increase of the NOAEC. On the other hand the NOAEC would be reduced, if experimental inhalation duration from 6 to 8 hours or activity-driven differences of respiratory volumes from experimental animals and workers would be included. To avoid these calculations, which would be partly rather speculative, the NOAEC is taken as starting point for MOS calculation without further adaptation.

For the identification of the reference MOS, (1) an adjustment factor of 2.5 for interspecies differences (the factor for allometric scaling is already implicitly applied) and (2) intraspecies differences for workers (factor of 5) are applied. Multiplying the different adjustment factors the reference MOS calculates to 12.5 (2.5 • 5). The critical inhalation exposure at the workplace is identified as 4 mg/m^3 (51 / 12.5).

There is no concern for scenario 1 (see table 4.1.3.2.B). Also short term exposures with values up to 0.8 mg/m^3 are around a factor of 5 lower than the critical exposure level of 4 mg/m^3.

Conclusion: ii

Dermal contact

In rats, a dermal LD50 of approximately 2100 mg/kg resulted when undiluted nitrobenzene was tested. In rabbits the dermal LD50 was found to be 760 mg/kg when nitrobenzene in ethanol was applied.

For assessing the acute dermal toxicity of nitrobenzene a range-finding study (application by skin painting for 14 days) with mice and rats is used (for more details see chapter 4.1.2.6.1).

At the lowest administered dose of 200 mg/kg/day significant depression of weight gain (> 10%) was seen in mice. Histologically, mice and rats showed changes in the brain, liver, spleen and testes, with mice less affected than rats. Additionally rats showed at this dose an increase in reticolocyte counts and methaemoglobin levels and a decrease in haemoglobin and red blood cells. The LOAEL of 200 mg/kg/day based on the histological and blood changes in the rats is taken for risk assessment of acute dermal toxicity and serves as starting point.

To calculate the reference MOS (1) an interspecies factor of 4 • 2.5 (rat) and (2) an intraspecies factor of 5 is used. (3) To extrapolate from the LOAEL to a possible NOAEL a factor of 3 is used. Alltogether the reference MOS calculates to 150 (4 • 2.5 • 5 • 3) the corresponding critical exposure level calculates to 1.3 mg/kg/day (200 / 150).

For acute toxicity the MOS approach indicates no concern for the dermal exposure scenario, see table 4.14.

Conclusion: ii

Combined exposure

The LOAEL of 200 mg/kg/day from the 14-day dermal rat study is taken for the risk assessment of combined toxicity after acute exposure.

The absorption after dermal uptake of nitrobenzene is calculated with the default of 40%, thus the internal starting point corresponds to a value of 80 mg/kg/day (200 mg/kg/day / 0.4).

The reference MOS calculates to 150 (the same derivation than for the assessment of acute dermal contact, see above) the corresponding internal critical exposure level calculates to 0.53 mg/kg/day (80 / 150), see table 4.14. No concern is given for combined exposure.

Conclusion: ii

Table 4.14: Acute toxicity, systemic effects

	Inhalation			Dermal			Combined		
Starting point for MOS calculation	51 mg/m^3			200 mg/kg/day			80 mg/kg/day		
Reference MOS	12.5			150			150		
Critical exposure level	4 mg/m^3			1.3 mg/kg/day			0.53 mg/kg/day		
	Exposure (mg/m^3)	MOS	Conclusion	Exposure (mg/kg/d)	MOS	Conclusion	Internal body burden (mg/kg/d)	MOS	Conclusion
1. Production and further processing as an intermediate	0.25	204	ii	0.06	3333	ii	0.054	1480	ii

4.1.3.2.2 Irritation and corrosivity

Skin, Eye

Effects on the skin and on eyes are not mentioned in the literature. In Draize tests with rabbits the substance did cause only slight signs of irritation to the skin and only mild reversible irritation to the eyes of rabbits. The observed effects are not considered sufficient for classification. There is no concern for dermal or eye irritation at the workplace.

Conclusion: ii

Respiratory tract

In a study with humans (seven volunteers were exposed 6 h with nitrobenzene vapours in the range 5 to 30 mg/m^3) no local effects on the respiratory tract were described (Salmova et al., 1963). Also in several subacute and subchronic studies no local effects were seen. However, in some animal studies (mice exposed on 14 days with 625 mg/m^3 nitrobenzene and F344 rats exposed on 14 days with 560 mg/m^3 nitrobenzene) slight bronchiolar hyperplasia was reported. Also after chronic inhalation of nitrobenzene, rats and mice had nasal inflammatory lesions.

Thus, with respect to acute local effects on the respiratory tract, severe airway damage is not anticipated and no concern is expressed. For the assessment of local effects after repeated contact see the chapter below.

Conclusion: ii

4.1.3.2.3 Sensitisation

Skin

Available data on skin sensitisation (Draize test, ear-flank test) were performed with methods that do not meet international guideline requirements and are considered to be too insensitive. In humans, three weakly positive cases out of 15 patients were reported from a study on cross-reactivity. These data are insufficient to exclude a possible skin sensitisation potential of nitrobenzene. Further testing is considered necessary (see chapter 4.1.2.5).

Conclusion: i

Respiratory tract

No information on the sensitising potential of the substance at the respiratory tract is available. For the time being a valid study to investigate respiratory sensitisation in experimental animals cannot be recommended. However, nitrobenzene is not suspected to be a potent respiratory sensitiser in humans according to the fact that during all the years of use no notice of specific case reports has been given. There is no concern with respect to respiratory sensitisation at the workplace.

Conclusion: ii

4.1.3.2.4 Repeated dose toxicity

Local effects

Inhalation exposure

With respect to local effects on the respiratory tract, no consistency with respect of nature and location of the effects were seen in several subacute and subchronic inhalation studies on rats and mice. Whereas in a 90-day study with rats no local effects were described (Hamm, 1984) the same author found a mild hyperplasia of the bronchial epithelium in mice after a 90-day exposure of nitrobenzene. In contrast to this, rats showed rhinitis and hyperplasia of nasal mucosa at concentrations ≥ 5 ppm for 2 years (CIIT, 1993), but no bronchial effects (Hamm 1984). In addition, mice demonstrated degeneration of the olfactory epithelium at ≥ 25 ppm following inhalation exposure for 2 years. For the assessment of local effects after inhalation, the 2 year inhalation study of rats and mice is taken (CIIT, 1993).

CD-rats were exposed to nitrobenzene concentrations of 1, 5, and 25 mg/m^3, B6C3F1-mice inhaled 5, 25, and 50 mg/m^3 for 6 hours per day, 5 days per week for a time period of 107 weeks. Both species showed irritative effects on the nasal respiratory epithelium. Based on nasal inflammation and increased incidences and severity of suppurative exudate, subacute inflammation and mucosal epithelial hyperplasia, seen at the lowest applied concentration of male CD-rats, the $LOAEC_{local}$ was derived as 1 ppm ≈ 5 mg/m^3.

The experimental $LOAEC_{local}$ of 5 mg/m^3 is (1) adapted by a factor of 6/8 to account for differences between the experimental inhalation duration of 6 hours per day and the average working day of 8 hours per day, and (2) is multiplied by a factor of 6.7/10 for activity-driven differences of respiratory volumes in workers. This results in an adjusted inhalation starting point of 2.5 mg/m^3 (5 • 6/8 • 6.7/10)

For the identification of the reference MOS, (1) interspecies differences the default factor is 2.5, (2) an adjustment factor of 5 for intraspecies differences (workers) and (3) a factor of 3 to extrapolate from the LOAEL to a possible NOAEL are applied. Multiplying the different adjustment factors the reference MOS calculates to 37.5 (2.5 • 5 • 3). The critical inhalation exposure at the workplace is identified as 0.0.7 mg/m^3 (2.5 / 37.5).

There results concern for scenario 1 (see table 4.15) with respect to local effects after repeated inhalation.

Conclusion: iii

Dermal contact

A 90-day mice study is taken to assess the local effects after repeated dermal contact. Nitrobenzene was administered on B6C3F1 mice by skin painting (10% of the the skin was painted, Nitobenzene was diluted in acetone) at 0, 50, 100, 200, 400 or 800 mg/kg. Inflammation of the skin (minimal to mild severity) was seen at the site of nitrobenzene application at the two highest doses. The $NOAEL_{local}$ of 200 mg/kg corresponding to 1.94 mg/cm^2 (assuming a mice body weight of 0.035 kg and assuming that 10% of a total body surface area of 36 cm² was exposed), is taken as starting point for the risk assessment.

To calculate the reference MOS (1) an intraspecies factor of 5 and (2) an interspecies factor 2.5 for remaining differences is used. Since local effects are independent of the basal metabolic rate, allometric scaling is not applied (allometric scaling factor of 1). Alltogether

the reference MOS calculates to 12.5 (5 • 2.5) the corresponding critical exposure level calculates to 0.16 mg/cm^2 (1.94 / 12.5).

There is no concern for scenario 1 (see table 4.1.3.2.C) with respect to local effects after repeated dermal contact.

Conclusion: ii

Table 4.15: Repeated dose toxicity, local effects

	Inhalation			Dermal		
Starting point for MOS calculation	2.5 mg/m^3			1.94 mg/cm^2		
Reference MOS	37.5			12.5		
Critical exposure level	0.07 mg/m^3			0.16 mg/cm^2		
	Exposure (mg/m^3)	MOS	Conclusions	Exposure (mg/cm^2)	MOS	Conclusions
1. Production and further processing as an intermediate	0.25	10	iii	0.01	194	ii

Systemic effects

No information on the effects in humans after repeated exposure to nitrobenzene is available.

Repeated dose studies on mice and rats demonstrated that prolonged exposure to nitrobenzene caused lesions in several organs or organ systems. Toxicity on the haematopoetic system was seen as primary and secondary adverse effects in the peripheral blood, bone marrow, spleen, liver, testes and kidneys. Independent from the route of exposure (inhalation, dermal application, oral route) the main systemic effects and target organs were similar. The occurred effects seen at the animals are thought to be relevant for man.

Inhalation exposure

Data from several subchronic inhalation studies and one 2-year carcinogenicity inhalation study with mice and rats are available. The 90-day rat studies of Hamm (1984) were considered to give the most reliable data with respect to the assessment of systemic toxicity. 10 male and 10 female F344 and CD-rats were exposed to nitrobenzene concentrations of 0, 5, 16, and 50 ppm 6 h/day, 5 days/week, which corresponds to about 0, 1, 25, 80, and 250 mg/m^3. Dose-related changes of red blood cell parameters indicating a haemolytic anemia were evident in all dose groups. Based on changes of several blood parameters and microscopic lesions of different organs (spleen, liver, kidneys) the LOAEC for systemic effects in this study was 5 ppm (25 mg/m^3).

The experimental LOAEC of 25 mg/m^3 is (1) adapted by a factor of 6/8 to account for differences between the experimental inhalation duration of 6 hours per day and the average

working day of 8 hours per day, and (2) is multiplied by a factor of 6.7/10 for activity-driven differences of respiratory volumes in workers. This results in an adjusted inhalation starting point of 12.5 mg/m^3 (25 • 6/8 • 6.7/10).

The following adjustment factors are applied for the identification of the reference MOS. For (1) interspecies differences the default factor is 2.5 (the factor for allometric scaling is already implicitly applied), for (2) intraspecies differences (workers) the default factor is 5, for (3) duration adjustment a factor of 2 is used and (4) a factor of 3 to extrapolate from the LOAEL to a possible NOAEL is applied. Thus the reference MOS calculates to 75 (2.5 • 5 • 2 • 3). The critical inhalation exposure level at the workplace is identified as 0.17 mg/m^3 (12.5 / 75).

The shift average value for inhalation is reported as 0.25 mg/m^3 for production and further processing of nitrobenzene. The exposure level in this occupational scenario is higher than the critical inhalation exposure of 0.17 mg/m³. Concern is expressed for scenario 1. For corresponding MOS values see table 4.16.

Conclusion: iii

Dermal contact

For assessing the toxicity of nitrobenzene after repeated dermal contact a 90-day skin painting study is used (NTP, 1983a). The following doses were applied on the skin of F344 rats: 0, 50, 100, 200, 400 or 800 mg/kg/day nitrobenzene for 13 weeks. No clear NOAEL was established in this study. A LOAEL of 50 mg/kg/day was derived, based on lung congestion and fatty change in the adrenal cortex in addition to haematological findings.

Starting point for the assessment of dermal toxicity after repeated exposure is the LOAEL of 50 mg/kg/day.

For the calculation of the reference MOS the following assessment factors are taken: (1) a factor of 3 to extrapolate from the LOAEL to a possible NAEL, (2) a factor of 4 x 2.5 (rat) for interspecies and (3) a factor of 5 for intraspecies differences. Additionally (4) a duration factor of 2 is used. Alltogether the reference MOS calculates to 300 (3 • 4 • 2.5 • 5 • 2) the corresponding critical exposure level calculates to 0. 17 mg/kg/day (50 / 300).

The calculated exposure value for dermal contact is reported as 0.06 mg/kg /day and is nearly three times lower than the critical dermal exposure level of 0.17 mg/kg /day. There is no concernfor this scenario. For corresponding MOS values see table 4.16.

Conclusion: ii

Combined exposure

For the exposure scenario of nitrobenzene, there is already concern for inhalation exposure and thus for combined exposure as well.

Conclusion: iii

Table 4.16: Repeated dose toxicity, systemic effects

	Inhalation			Dermal			Combined		
Starting point for MOS calculation	12.5 mg/m^3			50 mg/kg/day			no specific calculation (see text)		
Reference MOS	75			300			-		
Critical exposure level	0.17 mg/m^3			0.17 mg/kg/day			-		
	Exposure (mg/m^3)	MOS	Conclusion	Exposure (mg/kg/d)	MOS	Conclusion	Internal body burden (mg/kg/d)	MOS	Conclusion
1. Production and further processing as an intermediate	0.25	50	iii	0.06	833	ii	0.054	-	iii[(1)]

[(1)]conclusion iii already results from inhalation exposure

4.1.3.2.5 Mutagenicity

Nitrobenzene was negative in several bacterial tests. For genotoxicity of nitrobenzene in mammalian cells *in vitro* no test according to current guidelines was available. The two most reliable tests - a chromosomal aberration test in Chinese hamster lung cells and a test on unscheduled DNA synthesis in human hepatocytes - revealed negative results.

In vivo a DNA-binding capacity was detected in two studies after subcutaneous or i.p. application in liver and lung of mice and in liver and kidney of rats. Considering the routes of application (s.c. and i.p.) which limit the relevance of those studies and the measured adduct counts it can be concluded that nitrobenzene has a low DNA-binding capacity. In rats, no UDS was induced in rat liver after a single high oral dose. In a test on chromosomal aberrations and SCE in lymphocytes from peripheral blood and spleen from subacute exposed rats via inhalation no mutagenic effect was detected. A bone marrow micronucleus test in mice was negative up to the maximum tolerable dose.

Although due to the DNA-binding capacity of nitrobenzene a tissue specific genotoxic potential of nitrobenzene responsible for a genotoxic mechanism or carcinogenesis cannot be excluded, mutagenic effects of nitrobenzene on germ cells are not suspected.

Conclusion: ii

4.1.3.2.6 Carcinogenicity

No studies are known regarding long-term toxicity or carcinogenic effects in animals after oral or dermal exposure to nitrobenzene. Occupational risk assessment relies on the results of the 2-year inhalation studies with B6C3F1 mice, F344 rats and CD rats from CIIT (1993) and Cattley et al. (1994, for tables and details see chapter 4.1.2.8). 70 female and male B6C3F1 mice were exposed to nitrobenzene at concentrations of 0, 5, 25, and 50 ppm for 6 hours daily, 5 days per week for 107 weeks. 60 female and male F344 rats and 60 male CD

(Sprague-Dawley) rats were exposed to nitrobenzene at concentrations of 0, 1, 5, and 25 ppm (0, 5, 25, and 125 mg/m^3) for 6 hours daily, 5 days per week for 107 weeks. In rats, the tumor rates increased with dose-dependency in liver, kidney and uterus. The increases in tumor incidences reached significance at 25 ppm. In mice, increases in tumor incidences were also evident in multiple target organs (lung, thyroid and mammary glands). Here a significant increase of lung tumors was seen at the lowest dose tested (5 ppm) and above, whereas the tumor rate in thyroid and mammary glands increased significantly at 50 ppm.

The lung adenoma and carcinoma of the male mice show the strongest increase at the lowest air concentration of 1 ppm (5 mg/m^3) compared with the tumor incidences of other locations. However, a dose dependent increase of lung tumors at higher air concentrations is completely missing. Due to the uncertainties of the nitrobenzene-related causality to the lung tumors these were disregarded. Thus it is preferred to look at the other tumor incidences. The tumor rate of liver, kidney and uterus increased significantly at a dose of 125 mg/m^3 in F344 and CD rats (see table 4.10). At the low and mid dose no significant increase of tumors was found by the rats.

With reference to chap. 4.1.2.7 there is no evidence of a direct genotoxic mode of action. However, there is DNA binding for rat liver and kidney. The indistinct mutagenicity data and the missing of a clear association to toxic effects as causal factors in nitrobenzene tumorigenicity, lead to the conclusion that actually no clear mode of action for carcinogenicity can be identified.

The available data on nitrobenzene do not allow for a scientifically-based identification of a threshold level for nitrobenzene carcinogenicity. Basically, there is no valid information on the specific thresholded mode-of-action that results in secondary formation of tumors (see discussion in chapter 4.1.2.8). But, however, there is no evidence of a direct genotoxic mode of action and a significant increase of the different tumors is only found on doses of 250 mg/m^3 (if the lung tumors of the male mice are not considered). Thus the risk assessment is based on the assumption that the mode-of-action of nitrobenzene follows a threshold mechanism. The NOAEC of 5 ppm (25 mg/m^3) is taken as starting point for the risk assessment.

The experimental NOAEC of 25 mg/m^3 is (1) adapted by a factor of 6/8 to account for differences between the experimental inhalation duration of 6 hours per day and the average working day of 8 hours per day, and (2) is multiplied by a factor of 6.7/10 for activity-driven differences of respiratory volumes in workers. This results in an adjusted inhalation starting point of 12.5 mg/m^3 (25 • 6/8 • 6.7/10).

The following adjustment factors are applied for the identification of the reference MOS. For (1) interspecies differences the default factor is 2.5 (the factor for allometric scaling is already implicitly applied), for (2) intraspecies differences (workers) the default factor is 5, and for (3) the remaining uncertainties of the slope of the dose-response relationship (at the LOAEC an increase of several tumors at different organs is described), and the mechanism of the effect an additional factor of 10 is applied. Thus the reference MOS calculates to 125 (2.5 • 5 • 10). The critical inhalation exposure level at the workplace is identified as 0.1 mg/m^3 (12.5 / 125, see also table 4.17).

Expressed as (external) dose the value of 0.1 mg/m^3 corresponds to 0.014 mg/kg/day (0.1 µg/m^3 • 10 m^3 / 70 kg). With an adsorption percentage of 87% after inhalation the internal critical exposure level corresponds to 0.012 mg/kg/day (0.014 mg/kg/day • 0.87). To get the (external) critical exposure level for dermal contact the dermal absoption percentage of 40%

has to be included. Thus the internal value has to be multiplied with a factor of 2.5. This results in a value of 0.03 mg/kg/day (0.012 mg/kg/day • 2.5).

There is concern for all assessed exposure ways for nitrobenzene related carcinogenicity. The corresponding critical exposure level for carcinogenicity are about 2.5 times smaller than the occupational exposure with respect of inhalation (inhalation exposure of 0.25 mg/m^3 vs a cel of 0.1 mg/m^3) and about two times smaller with respect of dermal contact (dermal exposure of 0.06 mg/kg/day vs a dermal cel of 0.03 mg/kg/day). To reduce the risks resulting from carcinogenicity, technical and organisational risk reduction measures have to be performed.

Conclusion: iii

Table 4.17: MOS values for cancer risks of nitrobenzene

	Inhalation			Dermal			Combined		
Starting point for MOS calculation	12.5 mg/m^3			no specific calculation (see text)			no specific calculation (see text)		
Reference MOE	125			-			-		
Critical exposure level	0.1 mg/m^3			0.03 mg/kg/day (external value)			0.012 mg/kg/day (internal value)		
	Exposure (mg/m^3)	MOS	Conclusion	Exposure (mg/kg/d)	MOS	Conclusion	Internal body burden (mg/kg/d)	MOS	Conclusion
1. Production and further processing as an intermediate	0.25	50	iii	0.06	-	iii	0.054	-	iii[(1)]

[(1)]conclusion iii already results from inhalation and dermal exposure

4.1.3.2.7 Toxicity for reproduction

Effects on fertility

Inhalation exposure

Effects of nitrobenzene to reproductive performance and capability were investigated in a two-generation study with rats.

Male and female Sprague-Dawley rats were exposed to nitrobenzene vapours at target concentrations of 0, 1, 10, and 40 ppm (0, 5.1, 51.2, and 204.8 mg/m^3) for 6 hours/day, 5 days/week. Besides of significant decreases of the fertility index (number of pregnancies / number of females mated) in the 40 ppm groups, also significant histopathological changes of the testes and epididymides were described for the F_0 and F_1 generations. No statistically

significant alterations in fertility and histopathology of the reproductive organs were observed in the 1- or 10 ppm groups. A NOAEC of 10 ppm (51.2 mg/m^3) is derived from this study.

The experimental NOAEC of 51.2 mg/m^3 is (1) adapted by a factor of 6/8 to account for differences between the experimental inhalation duration of 6 hours per day and the average working day of 8 hours per day, and (2) is multiplied by a factor of 6.7/10 for activity-driven differences of respiratory volumes in workers. This yields to an adjusted inhalation starting point of 25.7 mg/m^3 (51.2 • 6/8 • 6.7/10).

The following adjustment factors are applied for the identification of the reference MOS. For (1) interspecies differences the default factor is 2.5 (the factor for allometric scaling is already implicitly applied) and for (2) intraspecies differences (workers) the default factor is 5. This gives a reference MOS of 12.5 (2.5 • 5). The critical inhalation exposure level at the workplace is identified as 2 mg/m^3 (25.7 / 12.5).

The shift average value for inhalation is reported as 0.25 mg/m^3 for production and further processing of nitrobenzene. There is no concern for scenario 1 (see table 4.18).

Conclusion: ii

Dermal contact and combined exposure

The subchronic rat study from NTP, which was used for the risk assessment of repeated dose toxicity, (NTP, 1983) did not describe any adverse effects on the testes. Thus summarized data from a 90-day study (Morrissey et al., 1988) are used to assess the effects regarding the fertility of nitrobenzene afer dermal contact. For male rats reduced organ weights for testes and epipidymides as well as a decrease in sperm densitiy and sperm motility and an increase in percentage of abnormal sperm were found at dosages of 50, 200, and 400 mg/kg/day. Based on this findings, a LOAEL of 50 mg/kg/day was derived. This LOAEL of 50 mg/kg/day is used as starting point for the assessment of fertility effects after dermal contact. The external value of 50 mg/kg/day corresponds to an internal value of 20 mg/kg/day taking a 40% dermal absorption into account.

For the calculation of the reference MOS the following assessment factors are taken: (1) a factor of 3 to extrapolate from the LOAEL to a possible NAEL, (2) a factor of 4 x 2.5 (rat) for interspecies and (3) a factor of 5 for intraspecies differences. Alltogether the reference MOS calculates to 150 (3 • 4 • 2.5 • 5) the corresponding external dermal critical exposure level calculates to 0.33 mg/kg/day (50 / 150). The internal critical exposure level results in 0.13 mg/kg/day (20 / 150).

The calculated exposure value for dermal contact in scenario 1 is reported as 0.06 mg/kg/day. Compared to the critical exposure level of 0.33 mg/kg/day this scenario is out of concern. Also for combined exposure the internal critical exposure level of 0.13 mg/kg/day is about two times higher than the internal exposure value of 0.054 mg/kg/day (see table 4.18). No concern is expressed.

Conclusion: ii

Table 4.18: Fertility, systemic effects

	Inhalation			Dermal			Combined		
Starting point for MOS calculation	25.7 mg/m^3			50 mg/kg/day (external value)			20 mg/kg/day (internal value)		
Reference MOS	12.5			150			150		
Critical exposure level	2 mg/m^3			0.33 mg/kg/day			0.13 mg/kg/day		
	Exposure (mg/m^3)	MOS	Conclusion	Exposure (mg/kg/d)	MOS	Conclusion	Internal body burden (mg/kg/d)	MOS	Conclusion
1. Production and further processing as an intermediate	0.25	100	ii	0.06	833	ii	0.054	370	ii

Developmental toxicity

Nitrobenzene is not classified with respect to developmental toxicity. Investigations in rats and rabbits with the inhalatory route of application did not reveal any developmental toxicity (including teratogenity) associated with the exposure to nitrobenzene during organogenesis at concentration levels that produced no or some slight maternal toxicity ($NOAEC_{dev. tox.}$ of ≥ 40 ppm for rats).

Compared to the relatively low LOAEL for repeated dose toxicity of 5 ppm and against the background of the carcinogenic potential of the substance experimental testing for reproductive toxicity is considered to be sufficiently adequate. Based on the available data, there seems to be no specific risk for developmental toxicity.

Conclusion: ii

4.1.3.2.8 Summary of risk characterisation for workers

As result of occupational risk assessment nitrobenzene, concern is raised and risk reduction measurs have to be initiated. The most important toxicological endpoints are carcinogenicity and repeated dose toxicity. Table 4.19 indicates the toxicological endpoints of concern for nitrobenzene. There is concern for carcinogenicity, repeated dose toxicity (local and systemic) and fertility. For the endpoints acute toxicity, irritation, respiratory sensitisation mutagenicity and developmental toxicity no concern is expressed.

Further information and/or testing is needed for the endpoint skin sensitisation.

Table 4.19: Endpoint-specific overall conclusions for the occupational risk assessment of nitrobenzene

Toxicological endpoints		concern
Acute toxicity	inhalation	ii
	dermal	ii

	combined	ii
Irritation/ Corrosivity	dermal	ii
	eye	ii
	acute respiratory tract	ii
Sensitisation	skin	i
	respiratory	ii
Repeated dose toxicity	local, inhalation	iii
	local, dermal	ii
	systemic, inhalation	iii
	systemic, dermal	ii
	systemic, combined	iii (1)
Mutagenicity		ii
Carcinogenicity	inhalation	iii
	dermal	iii
	combined	iii(1)
Fertility impairment	inhalation	ii
	dermal	ii
	combined	ii
Developmental toxicity	inhalation	ii
	dermal	ii
	combined	ii

(1) conclusion iii already results from dermal exposure and/or inhalation, therefore no specific concern for the combined exposure scenario is indicated

Risk estimation is mainly based on animal inhalation studies and dermal studies. Based on experimental data an adsorption percentage up to 87% is taken for the inhalation route and 40% is assumed for the dermal route.

The most important toxicological endpoints are repeated dose toxicity and carcinogenicity of nitrobenzol. On the background of the exposure assessment and the proposed critical exposure levels, the according health risks have to be reduced. This evaluation of risk is based on the assumption that a non-thresholded mode-of-action might be possible for nitrobenzene tumor induction.

Tables 4.20 (inhalation) and 4.21 (dermal contact) try to visualize the risk profile of nitrobenzene. According to the tables you will find the relatively high risks on the left, the relatively low risks on the right side of the tables.

On the background of cancer risks, air concentrations of nitrobenzene at the workplace should be controlled to a level in the range of 0.07 mg/m^3 (critical exposure level for local effects after repeated exposure). If the exposure is reduced to this level, inhalative risks from other endpoints, as carcinogenicity and systemic repeated dose toxicity would similarly and effectively be mitigated too. Also skin contact with nitrobenzene should be reduced (even if dermal absorption proved to be 40%). Based on cancer risk assessment, dermal exposure should be controlled to levels in the range of 0.03 mg/kg/day or 2.1 mg/person/day.

Table 4.20: Ranking of health risks for workers (inhalation)

Exposure scenario	Exposure level in mg/m³	Repeated dose toxicity, local	Carcinogenicity	Repeated dose toxicity, systemic	Fertility	Acute toxicity, systemic
		Critical exposure level in mg/m^3				
		0.07	0.1	0.17	2	4
1. Production and further processing as an intermediate	0.25	iii	iii	iii		

(1) blank fields: conclusion ii

Table 4.21: Ranking of health risks for workers (dermal contact)

Exposure scenario	Exposure level		Carcinogenicity	Repeated dose toxicity, systemic	Fertility	Acute toxicity, systemic	Repeated dose toxicity, local
	mg/kg/d	mg/cm^2	Critical exposure level in mg/kg/day				mg/cm^2
			0.03	0.17	0.33	1.3	0.16
1. Production and further processing as an intermediate	0.06	0.01	iii	ii	ii		

(1)blank fields: conclusion ii

4.1.3.3 Consumers

Exposure of the consumers is not assumed to exist. Therefore, the conclusion (ii) is reached.

Conclusion (ii): There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

4.1.3.4 Humans exposed via the environment

4.1.3.4.1 Exposure via food and water

The human exposure estimations via the environment for the local scenario (site C) and regional scenario are summarised in section 4.1.1.4 (Table 4.2). The daily doses are 4.2×10^{-4} mg/kg bw/d for the local scenario (site C) and 5.7×10^{-7} mg/kg bw/d for regional scenario. The main contributions are the $DOSE_{drw}$ and $DOSE_{fish}$ with fractions of 47% and 21%, respectively, for the oral route (local scenario).

Comparison of exposure and effects

When considering possible risks to human health arising from indirect exposure to nitrobenzene via the environment the key areas of concerns are for repeated dose toxicity, mutagenicity, carcinogenicity, and reproductive toxicity.

Repeated dose toxicity

Repeated dose studies on mice and rats demonstrated that prolonged exposure to nitrobenzene caused lesions in several organs or organ systems. The prime systemic toxic effect is haemolytic anaemia resulting from metHB formation. Apart from this, toxic effects were seen in the liver, male reproductive organs, central nervous system, kidneys, adrenals, bronchial and nasal passages. Clinical, haematological, and biochemical examinations indicated that nitrobenzene caused haemolytic anemia. Secondary responses to the erythrotoxicity were obvious in the spleen, bone marrow, liver and kidney indicated as increased haematopoiesis and/or intracellular brown pigment accumulation (haemosiderosis).

Animal studies with oral administration

In a gavage study, male and female F344 rats received 0, 5, 25 and 125 mg/kg bw/d of nitrobenzene for 28 days (Shimo et al. 1994; cf. 4.1.2.6). Haematology revealed significant decreases of RBC, Hb, haematocrit and increased mean corpuscular volume (MCV) in the 25 and/or 125 mg/kg bw/d groups. Erythrocyte counts were -32% and -45% lower in males of the 25 and 125 mg/kg bw/d groups. Female rats of the 125 mg/kg bw/d group showed a decrease of -35%. The LOAEL from this oral 28-day study on rats was 5 mg/kg bw/d.

In a study to assess the immunotoxic potential of nitrobenzene, female B6C3F1 mice were exposed to 0, 30, 100, and 300 mg/kg bw/d of nitrobenzene in corn oil by gavage for 14 consecutive days (Burns et al. 1994; cf. 4.1.2.6). A total of 8.5% of the mice receiving 300 mg died between the first and 14th day of exposure. Both liver and spleen weights were dose-dependently increased at all dose levels (with significance in the mid and high dose groups). Animals of the mid and high dose groups showed hepatomegaly and splenomegaly. The LOAEL in this oral 14-day study was 30 mg/kg bw/d.

In a 13-week study (unpublished data from the EHC report, 2003), nitrobenzene was administered to B6C3F1 mice by gavage at doses of 0, 19, 38, 75, 150 or 300 mg/kg bw/d (NTP, 1983a; cf. 4.1.2.6). No NOAEL could be derived from this study.

Using the OECD TG 422 test protocol nitrobenzene was given by gavage to Sprague-Dawley rats at 0, 20, 60 or 100 mg/kg bw/d (Mitsumori et al. 1994; cf. 4.1.2.6). All males in the high and mid dose groups and one male in the low dose group showed atrophy of seminerferous tubules with dose-dependent severity. In all treated males, centrilobular swelling of hepatocytes, hemsiderin deposition in Kupffer cells and extramedullary haematopoiesis in the liver, increased extramedullary haematopoiesis and haemosiderin deposition in the spleen, increased haematopoiesis in the bone marrow, and haemosiderin deposition in the renal proximal tubular epithelium were also observed. Thus, the LOAEL was 20 mg/kg bw/d following a total of 54 treatment days in male rats.

The LOAEL of 5 mg/kg bw/d derived from the 28-d oral study with rats is used as starting point for the risk characterisation for repeated dose toxicity.

In the following text the data base on repeated dose toxicity of nitrobenzene is considered to explain the conclusion about the appropriateness of the MOS for this endpoint.

- Overall confidence in the database

The data taken into account for performing the risk characterisation have been evaluated with regard to their reliability, relevance and completeness according to section 3.2 of the TGD. The data were published in peer reviewed journals or submitted to the Competent Authority in private reports being adequately detailed and in accordance with internationally recognised guidelines and to GLP.

The findings of all studies are not contradictory so that the judgement can be based on the database (cf. sections 4.1.2.6 and 4.1.2.8).

There are no reasons to assume limited confidence.

- Uncertainty arising from the variability in the experimental data

The studies cited above allow to conclude on the LOAEL of severe toxicity from on rats and mice. The main findings of the studies which are not in full compliance to current test guidelines showed good consistency.

There are no reasons to assume a special extent of uncertainty which have to be taken into account.

- Intra- and interspecies variation

Data on kinetics of the substance do not allow to calculate the intraspecies and interspecies variability by applying modern approaches. However, the available data give no hint on a particular high variability in kinetics. The variability of the data on the toxicodynamics has

been described (cf. section 4.1.2.6). For establishing the MOS, the LOAEL of the most sensitive animal study (rats) has been used.

- Dose response relationship

There is no reason to assume a special concern.

- Nature and severity of the effect

Nitrobenzene orally administered to F344 rats on 28 days induced increased haemosiderosis and haematopoiesis in the spleen at 5 mg/kg bw/d and anemia was evident at 25 mg/kg bw/d (Shimo et al. 1994). This indicated that secondary responses to toxic effects on the peripheral red blood cells may be more sensitive than changes in the peripheral blood. These effects have to be considered to be severe health effects. Nitrobenzene is classified and labelled as Toxic, T, R 48/23/24. Comparing the effect levels no clear sex preference seems to exist. There are no reasons to assume that the non-neoplastic effects shown in the animal experiments are limited to the species tested, thus being not of relevance for humans.

The carcinogenic action of nitrobenzene in rats and mice is proven. However, there are no data on carcinogenicity in humans.

- Differences in exposure (route, duration, frequency and pattern)

The estimated total daily intake (with an assumed absorption of 100%) is compared with an oral LOAEL from a 28-day study.

There are no reasons to assume that special concern can be derived from this procedure.

- The human population to which the quantitative and/or qualitative information on exposure applies

Following the exposure scenario there is no reason to assume a special risk for elderly or children.

- Other factors

There are no other factors known requiring a peculiar high margin of safety.

MOS for the local exposure scenario

The daily intake was calculated to be 0.00042 mg/kg bw/d (local scenario). The margin of safety between the

exposure level of 0.00042 mg/kg bw/d

and the

oral LOAEL of 5 mg/kg bw/d

is judged to be sufficient, even if special considerations on intra- and interspecies variation, nature and severity of the effects and the use of a LOAEL for risk characterisation are taken into consideration. Thus, with respect to non-neoplastic effects the substance is considered to be of no concern in relation to local indirect exposure via the environment (conclusion (**ii**)).

MOS for the regional exposure scenario

Given the considerably lower daily intake of 5.7×10^{-7} mg/kg bw/d for the regional scenario there will be no concern in relation to regional indirect exposure via the environment, too (MOS of about 10^7, conclusion (**ii**)).

ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Mutagenicity

Nitrobenzene was negative in several bacterial tests with numerous *Salmonella typhimurium* strains. For genotoxicity of nitrobenzene in mammalian cells *in vitro* no test according to current guidelines was available. Inconclusive results were reported in tests in mammalian cells *in vitro* which were either methodically inadequate or insufficiently described. These studies were not considered as relevant for risk assessment. The two most reliable tests a chromosomal aberration test in Chinese hamster lung cells and a test on unschedulded DNA synthesis in human hepatocytes revealed negative results.
In vivo a DNA-binding capacity was detected in two studies after subcutaneous or i.p. application in liver and lung of mice and in liver and kidney of rats. Considering the routes of application (s.c. and i.p.) which limit the relevance of the those studies and the measured low adduct counts it can be concluded that nitrobenzene has a low DNA-binding capacity the biological relevance of which is unclear. No effects were detected after UDS-induction in rats (single high oral dose), in a test on chromosomal aberrations and SCE in rats after subacute inhalative exposure, or in a bone marrow micronucleus test in mice up to the maximum tolerable dose (OECD TG 474).
From the available *in vitro* and *in vivo* data a weak genotoxic potential can be concluded. Therefore, it is unlikely that carcinogenicity is caused by a genotoxic mode of action. From the available negative data for micronuclei formation, chromosomal aberrations, SCE and UDS in rodents *in vivo*, it can be concluded, that nitrobenzene is not suspected to exert mutagenic effects on germ cells (conclusion (**ii**)).

ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Carcinogenicity

No carcinogenicity study with oral application of nitrobenzene is available. Chronic inhalation of nitrobenzene induced increased incidence of tumors of the lung and thyroid in male B6C3F1 mice at the lowest dose tested (5 ppm, LOAEC) and above and higher tumor rates of the mammary gland in female mice (at 50 ppm). The tumor sites observed in nitrobenzene exposed mice did not clearly show coincidence with the tumor sites in different rat strains. In male F344 rats exposed to nitrobenzene higher rates of liver and kidney tumors

were seen. Female F344 rats had higher incidences of uterine neoplasms than mice. The increases in tumor incidences in rats reached significance at 25 ppm (LOAEC). Although there may be some increase in tumor rates, the concentration of 5 ppm appears as the dose without significant tumor response in rats (NOAEC).

In summary, nitrobenzene is carcinogenic in the species mice and rats. It is generally accepted that multiple organ tumorgenicity is associated to a higher carcinogenic potential of a test substance. However, the lack of target organ consistency between sexes and to a lower extent between species did not justify a classification as category 2 carcinogen. The mutagenicity data suggest that other mechanisms than genotoxicity are likely to be involved in the tumour development. However, a clear causal association between specific toxic effects of nitrobenzene and its tumorigenicity was not evident, and no clear mode of action for carcinogenicity could be identified (classification as a category 3 carcinogen, R 40).

For risk characterisation purposes the LOAEC of 5 ppm (25 mg/m^3) based on data from the 107-week inhalation study on B6C3F1 mice will be used (CIIT 1993, exposure conditions cf. 4.1.2.8). The concentration in air is converted as follows to the inhaled amount of the substance using the respiratory minute volume of 1.3 l/min/kg, an exposure duration of 360 min/d and an inhalation rate of 80% (cf. 4.1.2.1):

0.025 mg/l x 1.3 l/min/kg x 360 min/d x 0.8 = 9.36 mg/kg bw/d (LOAEL)

The margin of safety between the converted LOAEL of 9.36 mg/kg bw/d and the daily intake of 0.00042 mg/kg bw/d is judged to be sufficient to conclude on no concern for tumor formation in relation to local indirect exposure via the environment (MOS of about 22300, conclusion (**ii**)).

Given the considerably lower intake of 5.7 x 10^{-7} mg/kg bw/d for the regional scenario there will also be no concern for tumor formation in relation to regional indirect exposure via the environment (MOS of about 10^7, conclusion (**ii**)).

ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Toxicity for reproduction

Fertility

Numerous studies with rats and mice revealed nitrobenzene to persistently adversely affect male reproductive organs (atrophy of the germ epithelium) and spermatogenesis independently from the route of administration (inhalation, oral, dermal). As a consequence of this, also reduced fertility in terms of reduced number of pregnancies and offspring was demonstrated in a rat two-generation inhalation study (cf. 4.1.2.9). LOAELs (fertility) of 9.4 mg/kg bw/d for the oral route of administration to rats and of 50 mg/kg bw/d for the dermal route of administration (rats and mice) were derived from the study of Morrissey et al. (1988). A NOAEC (fertility) of 10 ppm (51 mg/m^3) was derived from the study of Dodd et al. (1987).

Developmental toxicity

Since no adverse effects had been identified in developmental studies there is no need for performing MOS calculations.

Margin of safety (MOS)

For the decision on the MOS, the following aspects have been considered and taken into account:

- Uncertainty arising from the variability in the experimental data

There are no reasons to assume an important uncertainty which has to be taken into account. The findings of all studies are not contradictory.

- Overall confidence in the database

There are no reasons to assume no confidence.

- Intra- and interspecies variation

Data on kinetics of the substance do not allow to calculate the intra- and interspecies variability by applying modern approaches.

- Dose response relationship

There is no reason to assume a special concern.

- Nature and severity of the effects

The effects are considered to be severe health effects.

There are no reasons to assume that the effects shown in the animal experiments are limited to the species tested, thus being not of relevance for humans.

- Differences in exposure (route, duration, frequency and pattern)

Fertility

The estimated total daily intake with an assumed absorption rate of 100% is compared with an oral LOAEL (fertility) of 9.4 mg/kg bw/d.

- The human population to which the quantitative and/or qualitative information on exposure applies

There is no reason to assume a special risk for children.

- Other factors

There are no other factors known requiring a peculiar margin of safety.

Fertility

MOS for the local exposure scenario

The calculated intake is 0.00042 mg/kg bw/d (local scenario). The margin of safety between the

exposure level of 0.00042 mg/kg bw/d

and the

oral LOAEL of 9.4 mg/kg bw/d

is judged to be sufficient, even taking into consideration the use of a LOAEL for risk characterisation. Thus, with respect to fertility effects the substance is considered to be of no concern in relation to local indirect exposure via the environment (conclusion (**ii**)).

MOS for the regional exposure scenario

Given the considerably lower intake of 5.7×10^{-7} mg/kg bw/d for the regional scenario there will be no concern in relation to regional indirect exposure via the environment, too (MOS of $> 10^7$, conclusion (**ii**)).

ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

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4.1.3.4.2 Summary of risk characterisation for exposure via the environment

When considering the risks to human health arising from indirect exposure to nitrobenzene via the environment no concern is derived for non-neoplastic effects, mutagenicity, carcinogenicity, and toxicity to reproduction.

ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

4.1.3.5 Combined exposure

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

4.2.1 Exposure assessment

4.2.1.1 Workers

See Section 4.1.1.2

4.2.1.2 Consumers

Nitrobenzene has not been detected in consumer products

4.2.1.3 Humans exposed via the environment

Not applicable

4.2.2 Effects assessment: Hazard identification

4.2.2.1 Explosivity

Nitrobenzene is not explosive

4.2.2.2 Flammability

Nitrobenzene is not highly flammable

4.2.2.3 Oxidizing potential

Due to its chemical structure, nitrobenzene is not expected to possess oxidizing properties.

4.2.3 Risk characterisation

4.2.3.1 Workers

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

4.2.3.2 Consumers

Not aplicable

4.2.3.3 Humans exposed via the environment

5 RESULTS [6]

5.1 INTRODUCTION

5.2 ENVIRONMENT

See separate report.

5.3 HUMAN HEALTH

5.3.1 Human health (toxicity)

5.3.1.1 Workers

Conclusion (i) There is a need for further information and/or testing.

For nitrobenzene there is need for further testing regarding skin sensitisation.

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Conclusion (iii) applies to repeated dose toxicity and carcinogenicity. On the background of cancer risks, air concentrations of nitrobenzene at the workplace should be controlled to a level in the range of 0.1 mg/m^3 (critical exposure level for carcinogenicity). Concerning local effects after repeated exposure the critical exposure level with a value of 0.07 mg/m^3 is even lower. If the exposure is reduced to this level, inhalation risks from other endpoints, as systemic repeated dose toxicity would similarly and effectively be mitigated too. Also skin contact with nitrobenzene should be reduced. Based on cancer risk assessment, dermal exposure should be controlled to levels in the range of 0.03 mg/kg/day or 2.1 mg/person/day.

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already

For the other toxicological endpoints the risk orientated conclusions result in no concern with the consequence that risk reduction measures are of low priority

[6] Conclusion (i) There is a need for further information and/or testing.
Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.
Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

5.3.1.2 Consumers

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

5.3.1.3 Humans exposed via the environment

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

5.3.1.4 Combined exposure

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

5.3.2 Human health (risks from physico-chemical properties)

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

This conclusion is reached because the risk assessment shows that risks are not expected, and risk reduction measures already being applied are considered sufficient.

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ABBREVIATIONS

ADI	Acceptable Daily Intake
AF	Assessment Factor
ASTM	American Society for Testing and Materials
ATP	Adaptation to Technical Progress
AUC	Area Under The Curve
B	Bioaccumulation
BBA	Biologische Bundesanstalt für Land- und Forstwirtschaft
BCF	Bioconcentration Factor
BMC	Benchmark Concentration
BMD	Benchmark Dose
BMF	Biomagnification Factor
bw	body weight / *Bw*, *b.w.*
C	Corrosive (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
CA	Chromosome Aberration
CA	Competent Authority
CAS	Chemical Abstract Services
CEC	Commission of the European Communities
CEN	European Standards Organisation / European Committee for Normalisation
CMR	Carcinogenic, Mutagenic and toxic to Reproduction
CNS	Central Nervous System
COD	Chemical Oxygen Demand
CSTEE	Scientific Committee for Toxicity, Ecotoxicity and the Environment (DG SANCO)
CT_{50}	Clearance Time, elimination or depuration expressed as half-life
d.wt	dry weight / dw
dfi	daily food intake
DG	Directorate General
DIN	Deutsche Industrie Norm (German norm)
DNA	DeoxyriboNucleic Acid
DOC	Dissolved Organic Carbon
DT50	Degradation half-life or period required for 50 percent dissipation / degradation
DT90	Period required for 50 percent dissipation / degradation
E	Explosive (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
EASE	Estimation and Assessment of Substance Exposure Physico-chemical properties [Model]
EbC50	Effect Concentration measured as 50% reduction in biomass growth in algae tests

EC	European Communities
EC10	Effect Concentration measured as 10% effect
EC50	median Effect Concentration
ECB	European Chemicals Bureau
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECVAM	European Centre for the Validation of Alternative Methods
EDC	Endocrine Disrupting Chemical
EEC	European Economic Communities
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EN	European Norm
EPA	Environmental Protection Agency (USA)
ErC50	Effect Concentration measured as 50% reduction in growth rate in algae tests
ESD	Emission Scenario Document
EU	European Union
EUSES	European Union System for the Evaluation of Substances [software tool in support of the Technical Guidance Document on risk assessment]
F(+)	(Highly) flammable (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
FAO	Food and Agriculture Organisation of the United Nations
FELS	Fish Early Life Stage
GLP	Good Laboratory Practice
HEDSET	EC/OECD Harmonised Electronic Data Set (for data collection of existing substances)
HELCOM	Helsinki Commission -Baltic Marine Environment Protection Commission
HPLC	High Pressure Liquid Chromatography
HPVC	High Production Volume Chemical (> 1000 t/a)
IARC	International Agency for Research on Cancer
IC	Industrial Category
IC50	median Immobilisation Concentration or median Inhibitory Concentration
ILO	International Labour Organisation
IPCS	International Programme on Chemical Safety
ISO	International Organisation for Standardisation
IUCLID	International Uniform Chemical Information Database (existing substances)
IUPAC	International Union for Pure and Applied Chemistry
JEFCA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
Koc	organic carbon normalised distribution coefficient
Kow	octanol/water partition coefficient

Kp	solids-water partition coefficient
L(E)C50	median Lethal (Effect) Concentration
LAEL	Lowest Adverse Effect Level
LC50	median Lethal Concentration
LD50	median Lethal Dose
LEV	Local Exhaust Ventilation
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
LOED	Lowest Observed Effect Dose
LOEL	Lowest Observed Effect Level
MAC	Maximum Allowable Concentration
MATC	Maximum Acceptable Toxic Concentration
MC	Main Category
MITI	Ministry of International Trade and Industry, Japan
MOE	Margin of Exposure
MOS	Margin of Safety
MW	Molecular Weight
N	Dangerous for the environment (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC
NAEL	No Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NOEC	No Observed Effect Concentration
NTP	National Toxicology Program (USA)
O	Oxidizing (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational Exposure Limit
OJ	Official Journal
OSPAR	Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic
P	Persistent
PBT	Persistent, Bioaccumulative and Toxic
PBPK	Physiologically Based PharmacoKinetic modelling
PBTK	Physiologically Based ToxicoKinetic modelling
PEC	Predicted Environmental Concentration
pH	logarithm (to the base 10) (of the hydrogen ion concentration $\{H^+\}$

pKa	logarithm (to the base 10) of the acid dissociation constant
pKb	logarithm (to the base 10) of the base dissociation constant
PNEC	Predicted No Effect Concentration
POP	Persistent Organic Pollutant
PPE	Personal Protective Equipment
QSAR	(Quantitative) Structure-Activity Relationship
R phrases	Risk phrases according to Annex III of Directive 67/548/EEC
RAR	Risk Assessment Report
RC	Risk Characterisation
RfC	Reference Concentration
RfD	Reference Dose
RNA	RiboNucleic Acid
RPE	Respiratory Protective Equipment
RWC	Reasonable Worst Case
S phrases	Safety phrases according to Annex III of Directive 67/548/EEC
SAR	Structure-Activity Relationships
SBR	Standardised birth ratio
SCE	Sister Chromatic Exchange
SDS	Safety Data Sheet
SETAC	Society of Environmental Toxicology And Chemistry
SNIF	Summary Notification Interchange Format (new substances)
SSD	Species Sensitivity Distribution
STP	Sewage Treatment Plant
T(+)	(Very) Toxic (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
TDI	Tolerable Daily Intake
TG	Test Guideline
TGD	Technical Guidance Document
TNsG	Technical Notes for Guidance (for Biocides)
TNO	The Netherlands Organisation for Applied Scientific Research
UC	Use Category
UDS	Unscheduled DNA Synthesis
UN	United Nations
UNEP	United Nations Environment Programme
US EPA	Environmental Protection Agency, USA
UV	Ultraviolet Region of Spectrum
UVCB	Unknown or Variable composition, Complex reaction products of Biological material
vB	very Bioaccumulative

vP	very Persistent
vPvB	very Persistent and very Bioaccumulative
v/v	volume per volume ratio
w/w	weight per weight ratio
WHO	World Health Organization
WWTP	Waste Water Treatment Plant
Xn	Harmful (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
Xi	Irritant (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)

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The report provides the comprehensive risk assessment of the substance Nitrobenzene. It has been prepared by Germany in the frame of Council Regulation (EEC) No. 793/93 on the evaluation and control of the risks of existing substances, following the principles for assessment of the risks to man and the environment, laid down in Commission Regulation (EC) No. 1488/94.

This part of the evaluation considers the emissions and the resulting exposure to human populations in all life cycle steps. The scenarios for occupational exposure, consumer exposure and humans exposed via the environment have been examined and the possible risks have been identified.

The human health risk assessment concludes that there is concern for workers with regard to local and systemic repeated inhalation exposure and with regard to carcinogencity for dermal and inhalation exposure. .
There is a need for further information and for testing on sensitisation of the skin.
For consumers, humans exposed via the environment and for human health (physico-chemical properties) there is no concern.