CLH Report

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name: **Nitrobenzene**

EC Number: **202-716-0**

CAS Number: **98-95-3**

Submitted by: **Germany**

Date: **November 2009**

CONTENTS

TABLES

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name:	Nitrobenzene
EC Number:	202-716-0
CAS number:	98-95-3
Registration number (s):	
Purity:	> 99.3 %
Impurities:	< 0.3 % benzene < 0.1 % dinitrobenzene < 0.1 % dinitrophenol < 0.5 % water < 0.1 % picric acid

Proposed classification based on Directive 67/548/EEC criteria, impurities of less than 0.1% each

Carcinogen Category 3, R40 limited evidence for carcinogenesis
T toxic, R23/24/25 toxic by inhalation, in contact with skin and if swallowed
R48/23/24/25 toxic: danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed
Reproductive toxicant Category 3, R62 possible risk of impaired fertility
R64 may cause harm to breast-fed babies
R65 may cause lung damage if swallowed
R52-53 Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

Proposed classification based on GHS criteria: (EC Reg. No 1272/2008*), impurities of less than 0.1% each

H351 Suspected human carcinogen, Carc. Cat. 2
H361f Suspected human reproductive toxicant, Repr. Cat. 2
H362 May cause harm to breast-fed children, Reproductive toxicant
H301/311/331 Acute toxicity (oral, dermal, inhalation)
H372 STOT Rep. 1, causes damage to organs through prolonged or repeated oral, dermal or inhalation exposure.
H304 May be fatal if swallowed and enters airways.
H412 Harmful to aquatic life with long lasting effects

* Hereafter in short referred to as *CLP*

Proposed classification based on Directive 67/548/EEC criteria, containing benzene as an impurity of equal or more than 0.1%

Carcinogen Category 1, R45 may cause cancer
Mutagen Category 2, R46 may cause heritable genetic damage
T toxic, R23/24/25 toxic by inhalation, in contact with skin and if swallowed
R48/23/24/25 toxic: danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed
Reproductive toxicant Category 3, R62 possible risk of impaired fertility
R64 may cause harm to breast-fed babies
R65 may cause lung damage if swallowed
R52-53 Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

Proposed classification based on GHS criteria: (EC Reg. No 1272/2008[†]), containing benzene as an impurity of equal or more than 0.1%
H350 known human carcinogen, carcinogen category 1A
H340 known human mutagen, mutagen category 1B
H361f suspected human reproductive toxicant, repr. cat. 2
H362 may cause harm to breast-fed children, reproductive toxicant
H301/311/331 acute toxicity (oral, dermal, inhalation)
H372 STOT Rep. 1, causes damage to organs through prolonged or repeated oral, dermal or inhalation exposure.
H304 May be fatal if swallowed and enters airways.
H412 Harmful to aquatic life with long lasting effects

It is proposed to change the current classification to the above mentioned. The risk assessment committee is asked to review and confirm this.

Proposed labelling:

Table 1: Entry of nitrobenzene in Table 3.2 of Annex VI of EC Regulation No 67/548, extended by the proposed classifications R48/25, R64 and R65 and classifications due to impurities as well as the reclassification of R51/53 to R52/53.

Index No	International Chemical Identification	EC No	CAS No	Classification	Labelling
609-003-00-7	nitrobenzene <0.1% benzene	202-716-0	98-95-3	Carc.Cat.3,R40 Repr.Cat.3,R62,R64 T; R23/24/25-48/23/24/25 Xn; R65 R52-53	T R: 23/24/25-40-48/23/24/25-52/53-62-64-65 S: (1/2-)28-36/37-45-61
609-003-00-7	nitrobenzene ≥0.1%	202-716-0	98-95-3	Carc.Cat.1,R45 Muta.Cat.2,R46	T R: 23/24/25-45-46-48/

[†] Hereafter in short referred to as *CLP*

	benzene			Repr.Cat.3,R62,R64 T; R23/24/25-48/23/24/25 Xn; R65 R52-53	23/24/25-52/53-62-64-65 S: (1/2-)28-36/37-53-45-61

Table 2: Entry of nitrobenzene in Table 3.1 of Annex VI of EC Regulation No 1272/2008 (CLP), extended by the proposed classifications H362 and H304 and classifications due to impurities as well as the reclassification of H411 to H412.

Index No	International Chemical Identification	EC No	CAS No	Classification (1272/2008)		Labelling	
				Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)
609-003-00-7	nitrobenzene <0.1% benzene	202-716-0	98-95-3	Carc. 2 Repr. 2 +lactation Acute Tox. 3‡ Acute Tox. 3‡ Acute Tox. 3‡ STOT RE 1 Asp. Tox. 1 Aquatic Chronic 3	H351 H361f§ H362 H331 H311 H301 H372** H304 H412	GHS06 GHS08 Dgr	H351 H361f§ H362 H331 H311 H301 H372** H304 H412
609-003-00-7	nitrobenzene ≥0.1% benzene	202-716-0	98-95-3	Carc. 1A Muta. 1B Repr. 2 +lactation Acute Tox. 3‡ Acute Tox. 3‡ Acute Tox. 3‡ STOT RE 1 Asp. Tox. 1 Aquatic Chronic 3	H350 H340 H361f§ H362 H331 H311 H301 H372** H304 H412	GHS06 GHS08 Dgr	H350 H340 H361f§ H362 H331 H311 H301 H372** H304 H412

Proposed specific concentration limits (if any):

Proposed notes (if any):

Benzene can be an impurity of up to 0.3%. Therefore, the classification is given twice: once for nitrobenzene with impurities of less than 0.1% (except water); and once for Nitrobenzene with impurities of benzene of up to 0.3%.

This dossier reviewed the carcinogenicity, mutagenicity and reproductive toxicity endpoints, as well as acute and repeated-dose toxicity. Corrosivity and irritation data show no effects, and respiratory sensitisation data are insufficient for a strict classification.

‡ Minimum classification according to Reg. (EC) No 1272/2008, 1.2.1 (p. 338)

§ Hazard statement for reproductive toxicity acc. to Reg. (EC) No 1272/2008, 1.2.3 (p. 338)

** Route of exposure cannot be excluded acc. to Reg. (EC) No 1272/2008, 1.2.2 (p. 338)

The data presented in the Risk Assessment Report (RAR 2007) do not support the current classification as N R51/53. According to this data the current classification should be changed from N R51/53 (H411) to R52/53 (H412).

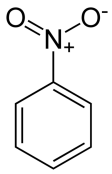
JUSTIFICATION

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

Chemical Name:	Nitrobenzene
EC Name:	Nitrobenzene
CAS-Name:	Benzene, nitro-
CAS Number:	98-95-3
IUPAC Name:	Nitrobenzene

1.2 Composition of the substance

Chemical Name:	Nitrobenzene
EC Number:	202-716-0
CAS Number:	98-95-3
IUPAC Name:	Nitrobenzene
Molecular Formula:	$C_6 H_5 N O_2$
Structural Formula:	
Molecular Weight:	123 g/mol
Typical concentration (% w/w):	99.7
Concentration range (% w/w):	99 - 100

1.3 Physico-chemical properties

Table 3: Summary of physico- chemical properties

REACH ref Annex, §	Property	IUCLID section	Value	[enter comment/reference or delete column]
VII, 7.1	Physical state at 20°C and 101.3 KPa	3.1	liquid	
VII, 7.2	Melting/freezing point	3.2	5.26 °C	BASF AG (1986)
VII, 7.3	Boiling point	3.3	210.8 °C	Lide (1991)
VII, 7.4	Relative density	3.4 density	1.2037	Lide (1991)
VII, 7.5	Vapour pressure	3.6	0.2 hPa at 20 °C[1)] 32.6 Pa at 25 °C[1)]	Auer (1988) Daubert and Danner, 1989
VII, 7.6	Surface tension	3.10	43.9 mN/m at 20 °C (pure substance)	Lide (1991)
VII, 7.7	Water solubility	3.8	1900 mg/l at 20 °C	Bayer AG (1998)
VII, 7.8	Partition coefficient n-octanol/water (log value)	3.7 partition coefficient	1.86 at 24.5 °C	BASF AG (1987)
VII, 7.9	Flash point	3.11	88 °C	BAM (1997)
VII, 7.10	Flammability	3.13	Not extremely flammable Not highly flammable Not flammable	BAM (1997)
VII, 7.11	Explosive properties	3.14	No explosive properties	BAM (1997)
VII, 7.13	Oxidising properties	3.15	Not applicable (liquid)	
	Auto flammability	3.12	480 °C (DIN 51794)	BAM (1997)

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 (1989) present an experimental vapour pressure as 0.245 mm Hg equivalent to 32.6 Pa at 25 C.

2 MANUFACTURE AND USES

2.1 Manufacture

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 (RAR 2007).

According to available data there are 13 production and/or processing sites of nitrobenzene within the EU. The data are provided via the European Chemicals Bureau website ESIS (http://ecb.jrc.ec.europa.eu). The resultant quantity of nitrobenzene produced in the EU amounts to be 1'175'000 t/year (2000).

2.2 Identified uses

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 (RAR 2007).

Type of use	Tonnage [t/a]	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

There is a difference of about 5,000 t/a 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. It is not known that any quantities of nitrobenzene are imported from outside the EU.

In Germany nitrobenzene was used for perfuming soaps in the past as the so called *Oil of Mirbane*. 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 EU countries other than Germany and whether this possible use may have become discontinued.

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 per 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 can be neglected in the future. 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. In Sweden, Norway or Finland no nitrobenzene containing products were listed (RAR 2007).

3 CLASSIFICATION AND LABELLING

3.1 Classification in Annex I of Directive 67/548/EEC

Nitrobenzene is covered by the following entries in Annex VI of EC Regulation No 1272/2008 (CLP).

Table 4: Entry of nitrobenzene in Table 3.2 of Annex VI of EC Regulation No 67/548.

Index No	International Chemical Identification	EC No	CAS No	Classification	Labelling
609-003-00-7	nitrobenzene	202-716-0	98-95-3	Carc. Cat. 3; R40 Repr. Cat. 3; R62 T; R23/24/ 25-48/23/24 N; R51-53	T; N R: 23/24/25-40-48/ 23/24-51/53-62 S: (1/2-)28-36/37-45-61

Table 5: Entry of nitrobenzene in Table 3.1 of Annex VI of EC Regulation No 1272/2008 (CLP) as amended by the 22nd ATP.

Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling	
				Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)
609-003-00-7	nitrobenzene	202-716-0	98-95-3	Carc. 2 Repr. 2 Acute Tox. 3†† Acute Tox. 3†† Acute Tox. 3†† STOT RE 1 Aquatic Chronic 2	H351 H361f‡‡ H331 H311 H301 H372§§ H411	GHS06 GHS08 GHS09 Dgr	H351 H361f‡‡ H331 H311 H301 H372§§ H411

†† Minimum classification according to Reg. (EC) No 1272/2008, 1.2.1 (p. 338)

‡‡ Hazard statement for reproductive toxicity acc. to Reg. (EC) No 1272/2008, 1.2.3 (p. 338)

§§ Route of exposure cannot be excluded acc. to Reg. (EC) No 1272/2008, 1.2.2 (p. 338)

4 ENVIRONMENTAL FATE PROPERTIES

4.1 Degradation

4.1.1 Stability

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.

4.1.2 Biodegradation

4.1.2.1 Biodegradation estimation

4.1.2.2 Screening tests

Table 6 summarised the results of screening tests of ready biodegradability for nitrobenzene.

Table 6: Tests of ready biodegradability

Test	Degradation	Conditions	Result	Reference
OECD 301C	3.3 % BOD	initial concentration: 100mg/l incubation of 14 d	not readily biodegradable	(CITI 1992)
OECD 301E	100 % DOC 88 % DOC	initial concentration: 38.5 mg/l after incubation of 21d physico-chemical batch	Evaporation of nitrobenzene – test system is not appropriate	(BASF AG 1989a)
similar to OECD 301F	48 % BOD 0-16 % BOD	initial concentration: 60 mg/l (after incubation of 32 d and a lag phase of 25 d 100 or 120 mg/l	not readily biodegradable	(BASF AG 1989b)
Warburg respirometry test system	33 % BOD 30 % BOD 0 % BOD	initial concentration: 100 mg/l (after incubation of 14 d and a lag phase of 90 h) 300 mg/l (after incubation of 10 d) 1400mg/l	microorganisms are inhibited when c > 1000 mg/l	(Gomólka and Gomólka 1979)
Electrolytic respirometer system similar to MITI	10 % BOD	initial concentration: 100 mg/l incubation of 10 d	not readily biodegradable (but incubation <28 d)	(Urano and Kato 1986)

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, 1989b) 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 32 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 1400 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 1000 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.

4.1.2.3 Simulation tests

Not relevant for this type of dossier.

4.1.3 Summary and discussion of persistence

It can be stated that nitrobenzene is not biodegradable with unadapted inoculum. In the screening tests of ready biodegradability nitrobenzene did not achieved the pass level (70% DOC or 60% ThOD, ThCO2).

4.2 Environmental distribution

Not relevant for this type of dossier.

4.3 Bioaccumulation

4.3.1 Aquatic bioaccumulation

4.3.1.1 Bioaccumulation estimation

4.3.1.2 Measured bioaccumulation data

The following table gives an overview of different bioaccumulation studies.

Table 7: BCF of Nitobenzene based on different bioaccumulation studies

Species	BCF	Reference
Cyprinus carpio	1.7 – 7.7	(CITI 1992)
Poecilia reticulata	22.4-38.9 (related to fat weight)	(Deneer et al 1987)
Leuciscus idus melanotus	< 10 (related to wet weight)	(Freitag et al. 1982)
Chlorella fusca	24	(Freitag et al. 1982)
Chlorella fusca var. vacuolata	24	(Geyer et al. 1984)

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.

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.

The bioaccumulation of nitrobenzene in fish and algae 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 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 was 24.

4.3.2 Terrestrial bioaccumulation

No data available.

4.3.3 Summary and discussion of bioaccumulation

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

4.4 Secondary poisoning

Not relevant for this dossier.

5 HUMAN HEALTH HAZARD ASSESSMENT

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

Nitrobenzene is a volatile liquid that can readily gain access to the body by inhalation and skin penetration of the vapour, as well as by ingestion and dermal absorption of the liquid. Nitrobenzene activation in rats to methaemoglobin-forming metabolites appears to be mediated to a significant degree by intestinal microflora. In test animals, the major part of nitrobenzene (about 80% of the dose) is metabolized and eliminated within 3 days. The remainder is eliminated only slowly. The slow compartment is likely due to erythrocyte recycling of nitrobenzene redox forms and glutathione conjugates. Covalent binding, presumably to sulfhydryl groups of haemoglobin, was demonstrated.

In rodents and rabbits, *p*-nitrophenol and *p*-aminophenol are major urinary metabolites. In humans, part of the absorbed dose is excreted into the urine; 10–20% of the dose is excreted as *p*-nitrophenol (which thus may be used for biological monitoring). The half-times of elimination for *p*-nitrophenol are estimated to be about 5 h (initial phase) and >20 h (late phase). The urinary metabolite *p*-aminophenol is significant only at higher doses (EHC 2003).

5.2 Acute toxicity

Numerous reports on nitrobenzene poisoning in the literature are 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 odour of bitter almonds that could be detected in the expired air or in the gastric contents.

Nitrobenzene is rapidly absorbed by the oral, inhalation and dermal route as demonstrated in the following case reports. For example, dermal absorption is in the range 1.54% after application of four micrograms of nitrobenzene to the forearm. This absorption rate is low in comparison to dinitrobenzene (53%) and to benzoic acid (43%; Feldmann and Maibach 1970) and it seems to contradict the clinical effects seen after dermal (and probably also inhalation) exposure of nitrobenzene. Case reports also demonstrate an efficient dermal absorption. However, the most prominent clinical symptoms 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).

5.2.1 Acute toxicity: oral

5.2.1.1 Human data

As residents of the maternity ward after parturition, five mothers had eaten a cake that had contained an ingredient to simulate a bitter almond taste in autumn 1944. Lacking a comprehensive chemical analysis for the causative agent, instead of natural bitter almonds and almond paste it may have contained either nitrobenzene and/ or other substances like aniline, benzaldehyde or benzonitrile. The mothers did not reveal any clinical symptoms but on the next morning (approx. 15 hours after ingestion), their breast-fed babies had developed a strong to very strong cyanosis. The children did not show any additional symptoms and the cyanosis receded largely in the next 24 hours. The children were not breast fed for 1.5 to 2 days. They received large amounts of tea, and if necessary oxygen and heart stabilizing drugs (Dollinger 1949). (see 5.9.3 et seqq.)

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 odour 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 odour 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 colour. 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% methylene blue, saline and glucose. The urine contained methaemoglobin and an excess amount various amino acids (e.g. alanine, serine, and 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). (calculated: 14g/60kg = ca. 230 mg/kg)

A woman (19 years) survived a suicidal oral dose of about 50 ml of nitrobenzene, approximately 11 g of which was absorbed 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, and sluggish reaction of the pupils to light. The venous blood had a chocolate-brown colour. There was a distinct odour of bitter almonds in the expired air (Myslak et al. 1971). (calculated: 11g/60kg = ca. 180 mg/kg)

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). (calculated: max. 24g/60kg = 400 mg/kg)

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. There was no evidence of occult blood in the urine. The patient was discharged on the fifth day of admission (Kumar et al. 1990).

5.2.1.2 Animal data

Species	LD_{50} (mg/kg)	Observations and remarks
Rat (m)	732	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 discolouration of blood in the animals that died within the study, surviving animals demonstrated no macroscopically visible changes. (Hoechst AG 1977, unpublished report)

Species	LD_{50} (mg/kg)	Observations and remarks
Rat (m)	588	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 (equivalent 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)
Rat (f)	650	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 discolouration of blood in the animals that died within the study, surviving animals demonstrated no macroscopically visible changes. (Hoechst AG 1977, unpublished report)
Rat (f)	640	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; discolouration of skin and visible mucous membranes as typical signs of methaemoglobinaemia were detected. At necropsy, hyperaemia 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)

Species	LD_{50} (mg/kg)	Observations and remarks
Rat (m) (Fischer-344)	>450	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 lesions (bilateral malacic areas and reactive gliosis in the cerebella pedunculus). Hepatocytic 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 epididymides. No details are given on the effects of the two lowest doses of 50 and 75 mg/kg. (Bond et al. 1981)
cat	>120	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)
Conclusion: Classification as "T" and labelling with R25 is confirmed (see Summary and discussion).		

5.2.2 Acute toxicity: inhalation

5.2.2.1 Human data

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).

Seven volunteers were exposed for six hours with nitrobenzene vapours in the range of 0.005 to 0.03 mg/l. The exposure was a nose-only-exposure. Retention of nitrobenzene diminished from 87% to 73% during the 6-hour exposure, indicating a low rate of conversion of nitrobenzene in the body that leads to blood saturation. In urine, the metabolite p-nitrophenol was present at about 13%

of the inhaled concentration of nitrobenzene. The metabolite p-aminophenol could not be detected in urine. The conversion of nitrobenzene to p-nitrophenol was in the range of 16% (Salmowa et al. 1963).

5.2.2.2 Animal data

Species	LC_{50} (mg/l)	Exposure time (h/day)	Observations and remarks
Rat (m)	2.847	4h	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 / $^{mg}/_{L}$ (deaths/exposed) were as follows: 439 / 2.24 (0/10), 514 / 2.63 (0/10), 542 / 2.78 (1/10), 555 / 2.84 (7/10), 578 / 2.96 (8/10), 714 / 3.70 (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)
Rat		3h; 7h	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 a 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 discolouration of muscles and organs, swelling of the lungs and infarct-like blood status were detected. (BASF AG 1977, unpublished report)

Species	LC_{50} (mg/l)	Exposure time (h/day)	Observations and remarks
Rat (m)		8h	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 discolouration of eyelids, ears and noses and dark discolouration 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)
Rat (m/f)		7h	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)
dog; rabbit; guinea pig; rat; cat; hen; pigeon; certain parasites			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. Microscopic 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)
Conclusion: Classification as "T" and labelling with R23 is confirmed (see Summary and discussion).			

5.2.3 Acute toxicity: dermal

5.2.3.1 Human data

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, irregular 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, greyish blue colour 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 apnoea. The boy was treated by rest in bed and by oxygen inhalation. The next day his colour was normal (Levin 1927).

As recently as 1993, in Saudi Arabia a two-month-old baby developed a chocolate-coloured 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 odour 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 odour of bitter almonds. The expired air also had the odour of bitter almonds. Urine contained urobilin and urobilinogen. The girl recovered within about 2 days (Bohland 1919).

5.2.3.2 Animal data

Species	LD_{50} (mg/kg)	Observations and remarks
rabbit	760	Dermal LD50 values were calculated for rabbits resulting in 760 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 evaporation 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 methaemoglobinaemia 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 discolouration of skin and eyes. Within a pre-screening test, blue discolouration 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)

rat	2100	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, hyperaemia 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)
Conclusion: Classification as "T" and labelling with R24 is confirmed (see Summary and discussion).		

5.2.4 Acute toxicity: other routes

5.2.5 Summary and discussion of acute toxicity

Taking into account exclusively the effects observed in acute studies in animals for classification the substance would have to be classified 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 760 mg/kg for rabbits to 2100 mg/kg for rats.

Yet moreover, many reports are documented in the literature on nitrobenzene poisoning in adults and children and to some extent on exposure of workers to nitrobenzene. Considerable individual variations exist and no clear-cut relationship could be documented between the absorbed dose of nitrobenzene and the severity of a response in humans, but babies and children appear to be more sensitive to the effects of the substance. Methaemoglobinaemia 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 demonstrates severe toxic effects of nitrobenzene to humans. In order to guarantee that the specific hazards posed by a substance causing methaemoglobinaemia 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. (CLP: H301, H311, H331, Acute toxicity (oral, dermal or inhalation))

Oral

Nitrobenzene revealed moderate acute oral toxicity when administered to rats with LD50 values between 588 and 732 mg/kg bw (CLP: Category 4). Nitrobenzene exposed cats demonstrated higher acute oral toxicity by causing pronounced methaemoglobinaemia after oral administration of doses as low as 30 mg/kg bw. Despite of this, all cats examined survived a dose of 120 mg/kg nitrobenzene; a valid LD50 value for cats was not determined in this study. But depending on human case reports and in order to cover individual differences in sensitivity appropriately, the existing classification as "toxic" and labelling with R 25 (CLP: H301) is confirmed.

Inhalation

For rats, the inhalation LC50 was determined to be 556 ppm (2847 mg/m^3, 2.847 mg/l; CLP: Cat. 3). Depending on human case reports and in order to cover individual differences in sensitivity appropriately, the existing classification as "toxic" and labelling with R 23 (CLP: H311) is confirmed.

Dermal

Dermal LD50 values ranged from 760 mg/kg for rabbits to 2100 mg/kg for rats (CLP: Cat. 3). Depending on human case reports and in order to cover individual differences in sensitivity appropriately, the existing classification as "toxic" and labelling with R 24 (CLP: H331) is confirmed.

5.3 Irritation

5.3.1 Skin

Species	No. of animals	Exposure time (h/day)	Conc. (w/w)	Dressing: (occlusive, semi-occlusive, open)	Observations and remarks (specify degree and nature of irritation and reversibility)
rabbit	6			occlusive	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 0.05 ml (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)
Rabbit	6	24		occlusive	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)
Conclusion: R-phrase none (see Summary and discussion).					

5.3.2 Eye

Species	No. of animals	Exposure time (hours)	Conc. (w/w)	Observations and remarks (specify degree and nature if irritation, any serious lesions, reversibility)
rabbit	6			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)
rabbit	2			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)
Rabbit	1			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 vitro: In a study investigating *in vitro* alternatives to the Draize test for eye irritation was concluded that nitrobenzene could be considered as a non-irritant according to the HET-CAM test, a test performed on the chorioallantoic membrane of hen eggs (Spielmann et al. 1991).
Conclusion: R-phrase none (see Summary and discussion).				

5.3.3 Respiratory tract

No data available

5.3.4 Summary and discussion of irritation

Nitrobenzene is not a corrosive substance. 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 undiluted nitrobenzene after exhibiting signs of cyanosis. Slight eye irritation was observed in rabbits which disappeared within 24 hours. None of the tests were conducted according to OECD TG 404/405. 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 data obtained from the case reports do not warrant a classification and labelling for these effects either.

5.4 Corrosivity

See 5.3.4

5.5 Sensitisation

5.5.1 Skin

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-phenylenediamine or azo-dyes was low. Three weakly positive cases out of 15 patients were reported (Schulz 1962, test concentration: 1%; vehicle not mentioned).

Species	Type of test	No. of animals	Incidence of reactions observed
guinea pig	ear-flank test	6	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. 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). (Stevens 1967)
			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)
Conclusion: insufficient data; R-phrase none (see Summary and discussion).			

5.5.2 Respiratory system

No data available

5.5.3 Summary and discussion of sensitisation

Skin

The animal data are insufficient to assess the intrinsic property of nitrobenzene to cause sensitisation, since the available studies (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 conclude on classification.

This lack of knowledge is paired with a concern from several structurally related compounds which are known to cause skin sensitisation. In case that workers or consumers may be exposed to nitrobenzene, the conduction of a Local Lymph Node Assay (LLNA) or a Magnusson Kligman Test should be considered within a substance evaluation procedure to appropriately assess the skin sensitisation potential of nitrobenzene.

Respiratory

No data available.

5.6 Repeated dose toxicity

5.6.1 Repeated dose toxicity: oral

Species/ strain, group size	Dose (mg/kg bw, mg/kg diet)	Duration of treatment	Observations and remarks (effects of major toxicological significance)
rats F344 (6m+6f)	0, 5, 25, 125 (gavage , mg/kg bw/day)	28 d (plus 2 weeks of recovery for control and high dose groups)	Blood: anaemia (RBC↓ haemoglobin↓, haematocrit ↓, MCV↑, reticulocytes↑, leucocytosis ≥25 mg/kg (no methb data) Liver: extramedullary haematopoiesis↑, Kupffer cell pigmentation at 125 mg/kg, liver weight ↑ ≥5 mg/kg Spleen: pigmentation (haemosiderosis) extramedullary haematopoiesis congestion ≥5 mg/kg, spleen weight↑ ≥25 mg/kg Testis: Tubular degeneration& atrophy, hypospermia at 125 mg/kg Kidneys: brown pigmentation in tubules (haemosiderosis) at 125 mg/kg CNS: cerebellar spongiosis and perivascular pigmentation at 125 mg/kg Other results: premature death(1/6 f), decreased movement, pale skin, gait abnormalities, reduced body weight & bw gain & thymus atrophy at 125 mg/kg LOAEL 5 mg/kg (Shimo et al. 1994)

Mouse B6C3F1 (7-8 f)	0,30,100, 300 (gavage , mg/kg bw/day)	14 d	Blood: RBC↓ at 300 mg/kg, MCH↑ MCV↑, reticulocytes↑ ≥100 mg/kg (no methb data) Liver: hydropic degeneration, haemosiderin pigmentation at 300 mg/kg Spleen: haemosiderin pigmentation, extramedullary haematopoiesis& congestion red pulp ≥100 mg/kg Testis: ND Kidneys: ∅ CNS: ND Other results: 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. 1994)
Mouse B6C3F1; Rat Fischer-344 (m+f)	38, 300, 600 mg/kg bw/day (gavage)	14 d	Range finding study: Other results: mortalities or sacrificed in a moribund status at 600 mg/kg (rats & mice) and at 300 mg/kg (rats). Treated animals were inactive, ataxic, prostate, cyanotic and dyspnoeic. Reduced weight gain in mice at ≥37.5 mg/kg. Liver, spleen, lung, kidney, brain: significant histological changes in both species (no further details). (NTP, 1983a, cited from EHC Report 2003)
Mouse B6C3F1 (10m+10f)	0, 19, 38, 75, 150, 300 mg/kg bw/day (gavage)	13 weeks	Liver: weight increase significant in all female dose groups and in two highest groups of male mice. Brain: acute necrosis in vestibular nucleus in 1 male at 300 mg/kd Other results: mortalities in week 4 and 5. Clinical signs included ataxia, lethargy, dyspnoea, convulsions, irritability and rapid head-bobbing movements. (NTP, 1983a, cited from EHC Report 2003)
Rat F-344 (10m+10f)	0, 9.4, 19, 38, 75, 150 mg/kg bw/day (gavage)	13 weeks	Brain: lesions in brain stem areas (facial, olivary & vestibular nuclei), cerebellar nuclei consisting of demyelination, loss of neurons, varying degrees of gliosis, haemorrhage, occasional neutrophil infiltration and occasionally haemosiderin-laden macrophages. Other results: mortalities (7 males, 1 female) and sacrification due to moribundity in week 6-9 (2 females) and in week 10-13 (2 males) at 150 mg/kg. Clinical signs: ataxia, left head tilt, lethargy, trembling, circling, dyspnoea, cyanosis at ≥75 mg/kg. (NTP, 1983a, cited from EHC Report 2003)

Rat Sprague-Dawley (10m+10f)	0, 20, 60, 100 mg/kg bw/day (gavage)	54 d (females: throughout premating (14d), mating(14d), gestation (22d) and lactation (4d), males sacrificed on Day 41 or 42)	OECD Combined Repeat Dose and Reproductive/ Developmental Toxicity Screening test protocol, TG 422: Effects in surviving males sacrificed on day 41 or 42: Blood: RBC↓, haemoglobin↓, haematocrit ↓ at 100 mg/kg, MCH↑ MCV↑, reticulocytes↑, erythroblasts↑, leucocyte no.↑ ≥60 mg/kg Liver: serum cholesterol ↑ at 100 mg/kd, liver weight ↑ (all dose groups),centrilobular swelling of hepatocytes, haemosiderin deposition in Kupffer cells, extramedullary haematopoiesis Spleen: weight ↑ (all dose groups), extramedullary haematopoiesis and haemosiderin deposition (also in the renal tubular epithelium and bone marrow) Brain: neuronal necrosis and gliosis in nuclei areas of cerebellar medulla and pons at 60 mg/kg (3/10 males) and at 100 mg/kd (10/10 males) Testes: atrophy of seminiferous in 10/10 males at ≥60 mg/kg and in 1 male at 20 mg/kg Other results: mortalities (2 males) at 100 mg/kg on Day 21 and 35 LOAEL: 20 mg/kg (Mitsumori et al. 1994, cited from EHC Report 2003)
Conclusion: R-phrase 48/25 is proposed (see Summary and discussion).			

5.6.2 Repeated dose toxicity: inhalation

Species, group size	conc. (mg/l)	Exposure time	Duration of treatment	Observations and remarks (effects of major toxicological significance)
rats F-344 (m+f)	0,10,35, 125 ppm (0, 0.05, 0.18, 0.64 mg/L)	6h/d, 5d/w	14 d	**Subacute toxicity**: Blood: RBC↓, methb↑ ≥10 ppm Liver: ∅ Spleen: congestion, haematopoiesis↑, haemosiderosis↑ ≥10 ppm, capsular fibroblastic hyperplasia in m ≥35 ppm Testis: Germ cell degeneration, phagocytosis & maturation arrest, hypospermia, Sertoli cell hyperplasia at 125 ppm Kidneys: hyaline nephrosis at 125 ppm (in 10/10 males and 2/10 females) CNS: ∅ Other results: $LOAEC_{sys}$ 10 ppm, $NOAEC_{local}$ 125 ppm (Medinsky and Irons 1985)

rats CD (m+f)	0,10,35, 125 ppm (0, 0.05, 0.18, 0.64 mg/L)	6h/d, 5d/w	14 d	Blood: anaemia at 125 ppm; RBC↓ & other red cell parameters ∅ at 10+35 ppm, methb↑ in f ≥10 ppm, in m ≥35 ppm, WBC↑ in m ≥35 ppm Liver: centrilobular or periportal degeneration at 125 ppm, single cell necrosis >35 ppm Spleen: congestion, haematopoiesis↑, haemosiderosis↑ ≥10 ppm Testis: Germ cell degeneration, phagocytosis & maturation arrest, hypospermia, at 125 ppm Kidneys: degeneration of cortical tubular cells at 125 ppm CNS: cerebellar haemorrhage, edema, malacia at 125 ppm Other results: morbidity, pulmonary vascular edema & congestion at 125 ppm $LOAEC_{sys}$ 10 ppm, $NOAEC_{local}$ 125 ppm (Medinsky and Irons 1985)
rats CD (m)	0,12,39, 112 ppm (0, 0.06, 0.20, 0.57 mg/L)	6h/d, 5d/w	14 d	Blood: anaemia, methb↑, ≥12 ppm, immature RBCs and neutrophilia at 112 ppm Liver: ∅ Spleen: haemosiderosis↑≥39 ppm, lymphoid cell atrophy at 112 ppm Testis: Germ cell atrophy, oligospermia at 112 ppm Kidneys: creatinine↑ at 112 ppm CNS: cerebellar haemorrhage/edema in cerebellum/mid-brain/cervical spinal cord at 112 ppm Other results: morbidity thymus atrophy, pulmonary edema, ocular keratitis at 112 ppm $LOAEC_{sys}$ 12 ppm, no N(L)OAEC $_{local}$ (DuPont 1981)
mice B6C3F1 (m+f)	0,10,35, 125 ppm (0, 0.06, 0.20, 0.57 mg/L)	6h/d, 5d/w	14 d	Blood: MCV↑,methb↑at 125 ppm Liver: centrilobular necrosis in m at 125 ppm hydropic degeneration ≥35 ppm Spleen: congestion, haematopoiesis↑, (occasionally) haemosiderosis↑ ≥35 ppm Testis: Tubular degeneration, aspermia, germ cell maturation arrest at 125 ppm Kidneys: tubular degeneration at 35 ppm CNS: cerebellar haemorrhage at 125 ppm Other results: morbidity at 125 ppm, bronchial hyperplasia >35 ppm ppm NOAECsys10 ppm, NOAEClocal10 ppm (Medinsky and Irons 1985)

Rats F-344 (m+f)	0,5,16, 50 ppm (0, 0.025, 0.08, 0.26 mg/L)	6h/d,5d/w	90 d	**Subchronic toxicity**: Blood: haemolytic anaemia ≥5 ppm, methb↑ in m≥5 ppm, f≥16 ppm Howell-Jolly bodies in m at 125 ppm Liver: focal centrilobular degeneration, liver cell cord disorganization ≥5 ppm Spleen: congestion, haematopoiesis↑, haemosiderosis↑, capsular fibroblastic hyperplasia ≥ 5 ppm stromal hyperplasia at 50 ppm Testis: Germ cell maturation arrest, tubular degeneration Leydig cell hyperplasia at 50 ppm Kidneys: nephrosis: cytoplasmatic eosinophilic droplets in proximal tubules in m ≥5 ppm, f at 50 ppm CNS: ND Other results: adrenals: medullary basophilia bronchial hyperplasia, bone marrow erythroid hyperplasia at 50 ppm $LOAEC_{sys}$ 5 ppm, $NOAEC_{local}$ 16 ppm (Hamm 1984)
Rats CD (m+f)	0,5,16, 50 ppm (0, 0.025, 0.08, 0.26 mg/L)	6h/d,5d/w	90 d	Blood: haemolytic anaemia ≥16 ppm, methb↑ in m≥16 ppm, in f at 50 ppm, leucocytosis, immature RBCs at 50 ppm Liver: hepatocytic basophilia/vacuolation & centrilobular hypertrophy, Kupffer cell pigmentation ≥16 ppm Spleen: congestion, haematopoiesis↑, haemosiderosis↑, capsule thickness↑ ≥5 ppm Testis: Tubular atrophy, Leydig cell hyperplasia, aspermia at 50 ppm, occasionally ≥5 ppm Kidneys: nephrosis at 50 ppm CNS: ND Other results: 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)

Mice B6C3F1 (m+f)	0,5,16, 50 ppm (0, 0.025, 0.08, 0.26 mg/L)	6h/d,5d/w	90 d	Blood: methb↑ at 50 ppm Liver: centrilobular hyperplasia/ hypertrophia, m ≥16 ppm, f ≥5 ppm Spleen: congestion, haematopoiesis↑, haemosiderosis↑ ≥5 ppm Testis: ND Kidneys: ND CNS: ND Other results: adrenals: cortical vacuolization ≥5 ppm bone marrow hyperplasia, bronchial hyperplasia at 50 ppm $LOAEC_{sys}$5 ppm, $NOAEC_{local}$16 ppm (Hamm 1984)
Rat F-344 (10m+10f) §	0,1,5,25 ppm (0, 0.005, 0.025, 0.13 mg/L)	6h/d,5d/w	15 mo	**Chronic toxicity**: Interim sacrifice groups of the CIIT cancer study: Blood: anaemia, polychromatic cells, Howell-Jolly bodies, methb↑ at 25 ppm, nucleated RBCs, leucocytosis in f at 25 ppm Liver: bilirubin↑ in m at 25 ppm cystic degeneration, eosinophilic cell foci, centrilobular hypertrophy in m≥5 ppm Spleen: haematopoiesis↑, congestion, haemosiderosis↑ ≥1 ppm Testis: ND Kidneys: increased severity or incidence of chronic nephropathy m≥5 ppm, slight increase in incidence of chronic nephropathy in f at 25 ppm CNS: ND Other results: endometrial polyps f≥1 ppm, pigmentation of olfactory ≥25 ppm, $LOAEL_{sys}$ 1 ppm $LOAEL_{local}$ 25 ppm (CIIT 1993)
rat Sprague-Dawley (CD) § (10m)	0,1,5,25 ppm (0, 0.005, 0.025, 0.13 mg/L)	6h/d,5d/w	15 mo	Interim sacrifice groups of the CIIT cancer study: Blood: anaemia, macrocytes, Howell-Jolly bodies, polychromasia in m at 25 ppm, methb↑ in m≥1 ppm Liver: centrilobular hypertrophy Kupffer cell pigmentation (haemosiderosis) m≥5 ppm Spleen: congestion ≥1 ppm, haematopoiesis↑, haemosiderosis↑ at 25 ppm Testis: ND Kidneys: ∅ CNS: ND Other results: nasal (resp.) epithelium hyperplasia, pigmentation of olfactory epithelium ≥25 ppm, $LOAEL_{sys}$ 1 ppm, $LOAEL_{local}$ 25 ppm. (CIIT 1993)

Abbreviations:
ALAT Alanin-Aminotransferase, d day/s, m males, f females, ND no data, ∅ no histopathological abnormalities, RBC red blood cell, methb methaemoglobin, MCH mean corpuscular haemoglobin, MCHC mean corpuscular haemoglobin concentration, 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 5.8 Carcinogenicity

Conclusion: R-phrase 48/23 is confirmed (see Summary and discussion).

5.6.3 Repeated dose toxicity: dermal

Species group size	Dose mg/kg	Exposure time	Duration of treatment	Observations and remarks (effects of major toxicological significance)
Mouse B6C3F1; Rat F-344 (m+f)	200 1600 3200		14 d	Range finding study: Nitrobenzene was administered to B6C3F$_1$ mice and Fischer-344 rats (both sexes) by skin painting at doses in the range 200–3200 mg/kg of body weight per day for 14 days. 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 RBC were decreased in rats (no details were given in the report). (NTP, 1983b, cited from EHC Report 2003)
Mouse B6C3F1 (10m+10f)	0, 50, 100, 200, 400 or 800, daily, on shaved skin in the interscapular area		13 weeks	Nitrobenzene was administered to B6C3F$_1$ mice (10 per sex per group) by skin painting (in acetone vehicle) at 0, 50, 100, 200, 400 or 800 mg/kg of body weight per day for 13 weeks (NTP, 1983b); the chemical was applied to a shaved area of the skin in the intercapsular region.

Species group size	Dose mg/kg	Exposure time	Duration of treatment	Observations and remarks (effects of major toxicological significance)
				(contd.) No effect on mean final body weights. 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.

Species group size	Dose mg/kg	Exposure time	Duration of treatment	Observations and remarks (effects of major toxicological significance)
				(cont.) 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: lung congestion, adrenal cortical fatty change and variation in the size of hepatic nuclei, especially the centrilobular zone. (NTP, 1983b, cited from EHC Report 2003)
Rat F-344 (10m+10f)	0, 50, 100, 200, 400 or 800, daily		13 weeks	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 of body weight per day 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 hind limbs, 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. (NTP, 1983b, cited from EHC Report 2003)

Species group size	Dose mg/kg	Exposure time	Duration of treatment	Observations and remarks (effects of major toxicological significance)
Conclusion: R-phrase 48/24 is confirmed (see Summary and discussion).				

5.6.4 Other relevant information

Species group size	Dose mg/kg	Exposure time	Duration of treatment	Observations and remarks (effects of major toxicological significance)
Rat F-344 (≈ 50m total)	200,400,600 (gavage)		Single application following a 28 d feeding, groups with different diets (with/ without 5-8% pectin)	Methaemoglobinaemia: Elevated Methb levels up to 60%, concentration raised at 1 h, maximum at 4 h (Goldstein et al. 1984)
Rat SD (5m/group)	50 study start at an age of 6, 8, 10 or 40 weeks		2 or 4 weeks	Dysspermatogenesis: Reduced sperm numbers and testes weight (aggravation with duration), depressed sperm activity (no effect of duration), (Koida and coworkers, 1985, abstract only)
Rat Wistar			5 or 10 weeks	Neurotoxicity: 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. (Pinching and Doving 1974)
Rat F-344 (12 m/group)	550 (oral)		Single treatment and sacrifice after 6, 24, or 48 h	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. (Morgan et al. 1985)
				In vitro studies: Dysspermatogenesis: Nitrobenzene was directly toxic on testicular cells in vitro. 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. (Allenby et al. 1990)
Conclusion: R-phrase (see Summary and discussion**).**				

5.6.5 Summary and discussion of repeated dose toxicity:

Repeated dose studies on mice and rats demonstrated that prolonged exposure to nitrobenzene caused lesions in several organs or organ systems, irrespective of the type of exposure, warranting classification R 48/23/24/25. Toxicity on the haematopoietic system was seen as primary and secondary adverse effects 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 anaemia. In addition, methaemoglobin concentrations were dose-dependently increased; females were more sensitive than males. 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). 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).

Premature deaths occurred at high oral doses of 300 mg of nitrobenzene per kg bw/d in mice between the first and 14th day of exposure (Burns et al. 1994), and in rats (after 4th day of treatment) and mice (between 2d and 4th day of exposure) which inhaled 125 ppm (0.64 mg/L) of nitrobenzene vapour (Medinsky and Irons 1985). The cause of death was not estimated in the oral mouse study (Burns et al. 1994) which was focussed on immune effects. The morbidity in the inhalation studies was interpreted to reflect anoxic encephalopathy occurring secondarily to haemolytic anaemia. 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 (0.13 mg/L) in rats and up to 50 ppm (0.26 mg/L) 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. 1994). 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 leukocytes may also be indicated as some rat studies (Hamm 1984; Shimo et al. 1994) 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-/a-spermia. Therefore, classification R 62 is supported by findings in repeated dose studies.

Lesions occurred in rats from concentrations of 50 ppm (0.26 mg/L) (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 (0.18 mg/L) 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 (0.64 mg/L) exposed to rats on 14 days (Medinsky and Irons 1985). In addition, degenerations of lower extension/severity were evident at 35 ppm (0.18 mg/L). The prolongation to 90 days or 15 months of nitrobenzene exposure produced liver cell degeneration starting from doses of 5 ppm (0.026 mg/L) (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 anaemic effects and the methaemoglobin formation. Although there was no obvious anaemia in mice after 90-day inhalation of nitrobenzene vapour (Hamm 1984) and only single red blood parameters were altered at 125 ppm (0.13 mg/L) (28-d study) (Medinsky and Irons 1985), increased extramedullary and medullary haematopoiesis confirmed an increase of regenerative erythropoiesis. It may be hypothesized that minimal anaemic effects can not 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. (1994) 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 metabolisms. 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.

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 (0.026 mg/L) 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 (0.026 mg/L) 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 be interpreted as a secondary effect to haemolysis-related hypoxia. Haemorrhage in the cerebellum and other central nervous regions

associated with edema and malacia were observed after repeated inhalation exposure (≥112 ppm (0.57 mg/L), 14 days) in rats (DuPont 1981, Medinsky and Irons 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.

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.

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. According to the Directive 1272/2008 labelling to H372 STOT-RE 1 (inhalative; haematopoietic system, liver, testis, CNS, kidneys, adrenals, bronchial/nasal passages) is proposed. "causes damage to organs through prolonged or repeated exposure”

Nitrobenzene orally administered to F344 rats on 28 days induced increased haemosiderosis and haematopoiesis in the spleen at 5 mg/kg bw/d and anaemia 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. Therefore, nitrobenzene haematotoxicity corresponds to criteria in the guidance to Regulation (EC) No 1272/2008 in section 3.9.2.5.2 and subsection g) therein and was evident at low doses. These are below the guidance value of 30 mg/kg bw/day (28 day study, Cat. 1, listed in Table 3.9.2.2 of the guidance) and are about 10-fold lower than the critical dose for the classification as harmful under Directive 67/548/EC, leading to the proposal for the classification as toxic. Therefore, classification as STOT-RE (R48/23/24/25) is warranted (cp. sections 3.9.2.5.2 of the guidance to Regulation (EC) No 1272/2008 and section 3.2.4 (1c) of Commission Directive 2001/59/EC).

5.7 Mutagenicity

5.7.1 In vitro data

Test	Cell type	Conc. range	Observations and remarks
Bacterial gene mutation test	S. typhimurium, TA92, TA1535, TA100, TA94, TA98	30 - 3000 µg/plate with S-9 mix; 10 - 3000 µg/plate without S-9 mix	Result: negative Toxicity: at 1000 and 3000 µg/plate study does 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 is considered as sufficiently reliable for risk assessment due to clear negative results (Miyata et al. 1981)
Bacterial gene mutation test	S. typhimurium TA1535, TA1537, TA100 and	10 - 1000 µg/plate with and without S-9 mix	Result: negative Toxicity: at highest dose study does not completely meet requirements of OECD TG 471 (use of only four strains and three to four analysable concentrations, results only presented as summarised

Test	Cell type	Conc. range	Observations and remarks
	TA98		tables) but is considered as sufficiently reliable for risk assessment due to clear negative results (Haworth et al. 1983)
Bacterial gene mutation test	S. typhimurium TA 98 and TA100	36.93 - 3693,3 µg/plate with S-9 mix; not tested without S-9 mix	Result: negative Toxicity: at highest dose Remarks: Flavine mononucleotide supplementation (Dellarco and Prival 1989)
Bacterial gene mutation test	S. typhimurium TA97, TA98 and TA100	33 - 3333 33, 100, 333, 1000 and 3333 µg/plate with and without S-9 mix	Result: negative Toxicity: no data Remarks: non standard method only raw data and abstract available (Hughes et al. 1984)
Bacterial gene mutation test	S. typhimurium TA98 and TA100	200, 1000 µg/plate with S-9 mix; not tested without S-9 mix	Result: positive Toxicity: no data Remarks: positive only in the presence of the comutagen norharman (Suzuki et al. 1983)
Mammalian cell gene mutation test	Chinese hamster lung fibroblasts (V79)	0.1 - 1 µg/ml with and without S-9 mix	Result: inconclusive Toxicity: no data Remarks: methodical insufficiencies (no data on cytotoxicity and plating efficiency; low statistical power of test since only 2 x 10^5 cells / culture were inoculated for the selection of resistant cells; effects are not dose related and not reproduced) (Kuroda 1986)
mammalian cell chromosomal aberration test	Chinese hamster lung fibroblasts (V79)	0.125 - 500 µg/ml with S-9 mix; not done without S-9 mix	Result: negative Toxicity: no data (Ishidate, 1988)
Mammalian cell chromosomal aberration test	human lymphocytes	6.1 mg/ml with S-9 mix; not done without S-9 mix	Result: inconclusive Toxicity: no data Remarks: insufficient study description (no detailed information on nitrobenzene concentrations, toxicity, cell preparation and solvent controls; results only as summarised table) (Huang et al. 1996)
mammalian cell micronucleus test	Chinese hamster lung fibroblasts (V79)	0.123 to 12.31 µg/ml with S-9 mix; not done without S-9 mix	Result: weakly positive Toxicity: no toxicity Remarks: insufficient data presentation (data for micronuclei frequencies were only presented as a figure.) (Bonacker et al. 2004)
mammalian cell UDS test	human and rat hepatocytes	1.23 to 123 µg/ml with S-9 mix;	Result: negative Toxicity: no data

Test	Cell type	Conc. range	Observations and remarks
		not done without S-9 mix	(Butterworth et al. 1989)
mammalian cell micronucleus test and comet assay	rat and human kidney cells	7.63 to 61.5 µg/ml with S-9 mix; not done without S-9 mix	Result: inconclusive for both genotoxic endpoints Toxicity: toxic at least at the highest tested dose Remarks: methodical insufficiencies (non-routine method; confusion about the incubation time of 20 h or 48 h; data on genotoxic effects were only presented as ratios treated/control cultures in a figure) (Robbiano et al. 2004)

5.7.2 In vivo data

Test	Species (tissue)	Dose groups	Harvest time	Observations and remarks (include route of administration)
Micro-nucleus test	mice bone marrow	62.5-250 mg/kg bw 1 x i.p.	24, 48 h	Result: negative Toxicity: at highest dose tested Remark: OECD TG 474 (BASF AG 1996)
Chromo-somal aberrations & SCE	rat spleen and blood lympho-cytes	5, 16, or 50 ppm (0.025, 0.082, 0.25 mg/L) 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)	Result: negative for both endpoints Toxicity: mitotic index decreased in blood lymphocytes, cell cycle delay (Kligerman et al. 1983)
UDS test	rat hepatoc ytes	200, 500 mg/kg bw 1 x p.o.	12 h	Result: negative Toxicity: no data (Mirsalis et al. 1982)
DNA-binding	rat liver & kidney, mouse liver & lung	4 mg/kg bw 1 x sc	24 h	Result: weak positive Toxicity: no data Remark: non-routine method (Novartis 1997)
DNA-binding	rat liver	0.1 µg - 10 mg/kg bw 1 x i.p. 4.1 µg/kg bw 1 x i.p.	2 h 4, 12, 24 h, 3, 7, 14, 21 d	Result: positive Toxicity: no data Remark: non-routine method (Li et al. 2003)
DNA damage & Micronuclei	rat kidney	300 mg/kg bw 1 x p.o.	unclear	Result: inconclusive for both endpoints Toxicity: no data Remark: methodically inadequate

Test	Species (tissue)	Dose groups	Harvest time	Observations and remarks (include route of administration)
				(Robbiano et al. 2004)
Conclusion: no classification.				

5.7.3 Human data

5.7.4 Other relevant information

5.7.5 Summary and discussion 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. 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. Inconclusive results were obtained in a mammalian cell gene mutation test, a chromosomal aberration test in primary human lymphocytes and further non-routine tests and a weak positive result were reported from micronucleus test in Chinese hamster lung cells. These studies were either methodically inadequate or insufficiently described and were not considered as relevant for risk assessment.

In vivo no mutagenic effect was detected in a bone marrow micronucleus test in mice (OECD TG 474) (BASF AG 1996) and 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). In rats, no UDS was induced in rat liver after single high oral doses (Mirsalis et al. 1982). However, a DNA-binding capacity was detected in vivo 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 et al. 2003).

Due to the DNA-binding capacity a tissue specific genotoxic potential of nitrobenzene responsible for a genotoxic mechanism of carcinogenesis cannot be excluded, but due to the low binding capacity alone a genotoxic mode of carcinogenicity is rather unlikely. 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.

5.8 Carcinogenicity

5.8.1 Carcinogenicity: oral

Species/strain group size	Dose (mg/kg bw, mg/kg diet)	Duration of treatment	Observations and remarks (effects of major toxicological significance)

Species/strain group size	Dose (mg/kg bw, mg/kg diet)	Duration of treatment	Observations and remarks (effects of major toxicological significance)
Conclusion: R-phrase (see Summary and discussion). No data available.			

5.8.2 Carcinogenicity: inhalation

Species/strain group size	conc. mg/l	Exposure time	Duration of treatment	Observations and remarks (effects of major toxicological significance)

Rat F-344 (m/f) **(60 animals/ sex/ group, plus 10 animals/ sex/group for interim sacrifice at 6 months)**	**0, 1, 5, 25 ppm** (0, 0.005, 0.025, 0.13 mg/L)	**6h/d** **5d/week**	**107 weeks**	**F-344 rats (Table 6, Table 9)** In male F-344 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. The tumor rates at multiple target organs increased with dose-dependency (see Table 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. 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 anaemic 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 what 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 (significance at exposure ≥5 ppm). Hyperplasia and inflammation of the submucosal glands in the anterior portion of the nose (level 1 + 2) lined by respiratory epithelium were present in 25 ppm males and females. Because of high incidences of spontaneous lesions of the testis no treatment-related effect could be identified in F-344 males. (CIIT 1993; Cattley et al. 1994)
Rat Sprague-Dawley (CD) **(m) (60 animals/sex/ group, plus 10 animals/sex/ group for interim sacrifice at 6 month)**	**0, 1, 5, 25 ppm** (0, 0.005, 0.025, 0.13 mg/L)	**6h/d** **5d/week**	**107 weeks**	**CD rats (Table 8, Table 9:)** In male CD rats, the incidences of hepatocellular adenoma and the combined incidences of hepatocellular adenoma and carcinoma were increased; the incidence of hepatocellular carcinoma alone was not affected by nitrobenzene exposure. 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 severity of suppurative exudate, subacute inflammation, and mucosal epithelial hyperplasia were seen in males of all dose groups. Brown pigment in the submucosa of the olfactory epithelium, which was commonly found in control and exposed animals, was evident in a slightly increased amount in males of the 5 and 25 ppm groups. (CIIT 1993; Cattley et al. 1994)
Mouse B6C3F1 **(70m/70f)**	**0, 5, 25, 50 ppm** (0, 0.025, 0.13, 0.26 mg/L)	**6h/d** **5d/week**	**107 weeks**	**B6C3F1 mouse (Table 10, Table 11)** In male B6C3F1 mouse, the incidences of alveolar/bronchiolar adenoma, alveolar/ bronchiolar carcinoma, and thyroid adenoma were increased. In female B6C3F1 mouse, the incidence of mammary gland adenocarcinoma was increased.

				(contd.) In addition, female B6C3F1 mice exposed to nitrobenzene had a marginally increased incidence of hepatocellular adenomas. In mouse, 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. 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), and 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). (CIIT 1993; Cattley et al. 1994)
Conclusion: Classification as Carcinogen Category 3 and R-phrase R 40 is confirmed (see Summary and discussion).				

Table 8: Incidence of selected neoplastic lesions in F-344 and Sprague-Dawley CD rats following nitrobenzene exposure

		Female F-344 incidence (%)				Male F-344 incidence (%)				Male CD incidence (%)			
Tissue	Diagnosis	0 ppm 0 mg/L	1 ppm 0.005 mg/L	5 ppm 0.026 mg/L	25 ppm 0.128 mg/L	0 ppm 0 mg/L	1 ppm 0.005 mg/L	5 ppm 0.026 mg/L	25 ppm 0.128 mg/L	0 ppm 0 mg/L	1 ppm 0.005 mg/L	5 ppm 0.026 mg/L	25 ppm 0.128 mg/L
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)	7/65 (11) P
	Hepatocellular 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)	2/65 (3)
	Hepatocellular 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)	9/65 (14) P
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)	0/65 (0)
	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)	0/65 (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)	0/65 (0)
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)	3/64 (5)
	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)	2/64 (3)
	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)	5/64 (8)
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)	4/61 (7)
Multiple	Mononuclear cell leukaemia	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)	0/65 (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 9: Incidence of nonneoplastic lesions in rats

Tissue	Diagnosis	Female F344 incidence (%)				Male F344 incidence (%)				Male CD incidence (%)			
		0 ppm 0 mg/L	1 ppm 0.005 mg/L	5 ppm 0.026 mg/L	25 ppm 0.128 mg/L	0 ppm 0 mg/L	1 ppm 0.005 mg/L	5 ppm 0.026 mg/L	25 ppm 0.128 mg/L	0 ppm 0 mg/L	1 ppm 0.005 mg/L	5 ppm 0.026 mg/L	25 ppm 0.128 mg/L
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)	19/65 (29)
	Centrilobular hepato-cytomegaly	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	39/65 (60) P
	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)	37/65 (57) P
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)	59/65 (91)
	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)	6/65 (9)
Thyroid	Follicular cell hyper-plasia	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)	4/64 (6)
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	58/61 (95) P
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)	35/61 (57) P
Epidi-dymis	Hyposper-mia, bilateral	—	—	—	—	15/69 (22)	21/54 (39)	12/59 (20)	12/70 (17)	8/60 (13) T	13/65 (20)	15/67 (22)	32/59 (54) P
Spleen	Extramedul-lary haemato-poiesis	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)	60/65 (92)
	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)	65/65 (100)

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 10: Incidence of selected neoplastic lesions in B6C3F1 mice following nitrobenzene exposure

Tissue	Diagnosis	Male incidence (%)				Female incidence (%)			
		0 ppm 0 mg/L	5 ppm 0.026 mg/L	25 ppm 0.128 mg/L	50 ppm 0. 256 mg/L	0 ppm 0 mg/L	5 ppm 0.026 mg/L	25 ppm 0.128 mg/L	50 ppm 0. 256 mg/L
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 carcinoma	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 11: Incidence of selected nonneoplastic lesions in B6C3F1 mice following nitrobenzene exposure

		Male incidence (%)				Female incidence (%)			
Tissue	Diagnosis	0 ppm 0 mg/L	5 ppm 0.026 mg/L	25 ppm 0.128 mg/L	50 ppm 0. 256 mg/L	0 ppm 0 mg/L	5 ppm 0.026 mg/L	25 ppm 0.128 mg/L	50 ppm 0. 256 mg/L
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 hepatocytomegaly	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]	Glandularization 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/loss. 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	Hypercellularity	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

5.8.3 Carcinogenicity: dermal

5.8.4 Carcinogenicity: human data

5.8.5 Other relevant information

5.8.6 Summary and discussion of carcinogenicity

Following long-term inhalation of nitrobenzene tumor incidences at six organ sites were significantly increased. (see Table 12)

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. Increased incidences of eosinophilic foci were 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 solely males of both rat strains showing hypertrophy and liver cell tumors. Assuming 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 were 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. Female mice exclusively 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 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 preceding 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 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 are 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 adenocarcinomas 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 nitroaromatic compounds.

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 it gained significance only at 50 ppm nitrobenzene. A higher rate of adenocarcinomas was seen in all dose groups, but their incidences did not reach significance 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 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 22. ATP, corresponding to Carc. Cat. 2, H351 under EC Regulation No 1272/2008 (CLP). 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. Although 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 12: 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)[(1)], 32% (25 ppm), 35% (50 ppm)
Liver: Hepatocellular adenoma or carcinoma*		1% (0 ppm) [(T)], 4% (1 ppm), 4% (5 ppm), 21% (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 adeno-carcinoma*		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: Adeno-carcinoma				0% (0 ppm), 8% (50 ppm)	

[(1)]underlined values: significantly different from incidence in 0-ppm control group determined by Fisher Exact Test, $p<0.05$.
[(T)] only significantly positive exposure-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 tumors observed, a toxic effect possibly preceding the tumor development was not clearly identified. Other target sites with marked toxicity such as hematopoietic system (erythrocytes and spleen), nose or testes did not show a tumor response.

From a conservative view, the findings support the classification as a carcinogen of category 2 (CLP: 1B) although no mutagenic potential has been identified. Supportive arguments for category 2 (CLP: 1B) 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 (CLP: 1B) such as 2-nitrotoluene (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-dinitrotoluene (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 (CLP: 1B) and 3 (CLP: 2), there are also arguments to propose a classification as category 3 (CLP: 2) 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) 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 an insufficient argument for a genotoxic mode of action. At present, nitrobenzenes' carcinogenicity is thought to be mediated by a non-identified, non-genotoxic mechanism.

- Nitrobenzene is readily metabolized in humans and animals via all exposure routes to a number of nitroaromatic compounds. A nitro-reductive enzyme activity in organs or intestinal nitro-reduction produces aniline probably via nitrosobenzene, and phenylhydroxylamine. Aniline is classified as a carcinogen, category 3 (CLP: 2) and a mutagen, category 3 (CLP: 2). The sparse 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 (CLP: 2) would support the same category for nitrobenzene. But this comparison is limited by differences in the observed tumor spectrum: associated to the haemolytic toxicity - the spleen was the only tumors site for aniline.

- Although there is supportive evidence from category 2 (CLP: 1B)-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 from 2-4-dinitrotoluene and 2-nitroanisole, but 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 RAR 2007, 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 RAR 2007, Table 4.1 in Section 4.1.2.1.1). Differences in intestinal nitro-reduction 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 remain unknown for

nitrobenzene, the absence of consistency for tumor responses among species and sexes weakens the evidence for category 2 (CLP: 3).

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.

(CLP: Carcinogen category 2, H351 Suspected human carcinogen)

5.9 Toxicity for reproduction

5.9.1 Effects on fertility

Species	Route Dose	Exposure time	Number of gen. exposed	Observations and remarks
Rat Sprague-Dawley (m/f) (30 animals/ sex/ group)	Inhalation 0 (air), 1, 10, and 40 ppm (0.005, 0.051 and 0.204 mg/L)	Premating (10 wks): 6 hr/day, 5 days/week mating and gestation: 6 hr/day, 7 days/week dams exposed until g.d. 19 after delivery from p.n. d. 5 on dams only (without litters) returned to exposure until p.n. day 21 F_1 animals were allowed a 2-week growth period without nitro-benzene exposure.		Mating procedure and exposure regimen for the F1 animals identical with those for the F0 rats. F2 pups were never exposed to nitrobenzene by inhalation. F1 males not sacrificed after mating were used for recovery studies: high-dose and control group males 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. No mortalities and no treatment-related clinical signs of abnormality in the F0 and F1 rats during the entire exposure period and during recovery. 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 controls, 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) clearly decreased in the 40-ppm group in F_0, F_1 and the recovery groups (16/30, 3/30, and 14/30, respectively). No statistically significant alterations in fertility were observed in the 1- or 10-ppm groups, Also, in F_1 females of the 40-ppm group 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 were 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 1- or 10-ppm groups, 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.

				(cont.) 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 differences in body weight between control and 40-ppm pups of the recovery generation Reductions in the size of the testes occurred, and stat. sign. reductions in both abs. and rel. weights of testes and epididymides in F_0 males of the 40-ppm group after 12 weeks of exposure. There were similar observations 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. There were no microscopic changes in the reproductive organs of the female rats that could be attributed to nitrobenzene exposure. Biologically significant histopathological findings were limited to the testes and epididymides of F_0 and F_1 rats exposed to 40 ppm nitrobenzene; specifically, the testes of the F_0 generation males of the 40-ppm group had seminiferous tubule atrophy and spermatocyte degeneration. Degree and distribution of the atrophy were marked to severe and multifocal or diffuse, respectively, in 14/30 animals. In addition, there were giant syncytial spermatocytes observed in the seminiferous tubules of 22/30 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 21/30 animals. However, 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. With regard to male reproductive organ toxicity and spermatogenesis a NOAEC of 10 ppm (equivalent to 51 mg/m^3) is derived from this study. No signs of systemic toxicity were observed in this study up to and including the highest tested concentration of 40 ppm (equivalent to 205 mg/m^3). (Dodd et al. 1985, 1987)

Rat (CD, 16/ group); Sprague Dawley and F344; Mouse B6C3F1 (10/ sex/ group)	Inhalation 0.573 mg/L 0.640 mg/L	14 days		see also 5.6.2: 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) and 125 ppm (640 mg/m^3). Considering testicular toxicity, respectively dysspermatogenesis, a NOAEC in the range of 35 to 39 ppm (179 to 200 mg/m^3) can be derived from these subacute toxicity studies. (DuPont 1981; Medinsky and Irons 1985)
Rat F344 and CD; Mouse B6C3F1	Inhalation 0.256 mg/L	90 days		see also 5.6.2: Likewise, in a 90-day inhalation study with F344 and CD rats as well as with B6C3F1 mice 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). (Hamm 1984)

Rat Sprague-Dawley (10m/10f)	Oral 0, 20, 60, and 100 mg/kg		Study according to OECD TG 422	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. (Mitsumori et al. 1994)

Rat Sprague-Dawley (70 males/group)	Oral, gavage 60 mg/kg bw/d controls received 1 ml/kg sesame oil	Up to 70 days	"ReproTox-Protocol" (OECD-proposed OECD 1990)	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. An experimental group (n=70) of male Sprague-Dawley rats was given nitrobenzene via gavage (10% in sesame oil) each morning 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%. (Kawashima et al. 1995)

Rat Mouse	Oral 18.75, 75, and 300 (mouse) or 9.4, 37.5, and 75.0 (rats) mg/kg bw/d	13 weeks		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 (LOAEL) investigated in this study in rats was the lowest dose tested. (Morrissey et al. 1988)
Rat F344	Oral 50, 75, 110, 165, 200, 300, or 450 mg/kg bw in corn oil	One single treatment		In an oral study with F344 rats 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. (Bond et al. 1981)

Rat F-344	Oral 300 mg/kg bw in corn oil	100 days		To investigate the possible regeneration of the seminiferous epithelium after single dose administration, in a further study 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. (Levin et al. 1988)
Rat Sprague-Dawley (6m)	Oral 300 mg nitrobenzene/kg bw			Nitrobenzene was further investigated within in a short duration test design evaluated for screening of reproductive responses. 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. (Linder et al. 1992)

Rat Sprague-Dawley (6m)	Oral 300 mg/kg bw in corn oil			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. 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. (Allenby et al. 1991)
Rat Wistar (m)	Oral 300mg/kg bw			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. 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. (McLaren et al. 1993)
Rat/ Mouse	Dermal male rats: 0.05, 0.2 and 0.4 mg/kg bw/d male mouse: 0.05, 0.2 and 0.4 mg/kg bw/d	13 weeks		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 mg/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 mg/kg bw/d. (Morrissey et al. 1988)
Conclusion: Repr. Cat 3; R62 confirmed (see Summary and discussion).				

5.9.2 Developmental toxicity

Species/Strain	Dose (mg/kg)	Exposure	Observations and remarks
Rat CD (26 f)	0.005, 0.051 and 0.205 mg/L	g.d. 6 to 15 for 6 hr/day (whole chamber administra- tion)	Female rats were exposed to nitrobenzene vapours at 0, 10, and 40 ppm (5.1, 51.2 and 204.8 mg/m^3) 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 foetuses as well as late and early resorption sites were noted and recorded. All live foetuses were weighed and sexed and examined for external malformations including cleft palate. One-half of the foetuses in each litter were examined for thoracic and abdominal visceral abnormalities including craniofacial structures, the other half was examined for skeletal alterations. 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 foetuses per litter, in percentage pre- or postimplantation loss, in sex ratio or in foetal body weight per litter. Foetal evaluations revealed that there was no significant increase in the number of litters with one or more affected foetuses 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 foetal toxicity, likewise there were no indications of reduced foetal body weights or any other signs of foetal toxicity. In terms of developmental toxicity, a NOAEC of 40 ppm (205 mg/m^3) can be derived from this study. In terms of maternal toxicity a NOAEC of 10 ppm (51 mg/m^3) can be derived. (Tyl 1984; Tyl et al. 1987)

Species/Strain	Dose (mg/kg)	Exposure	Observations and remarks
New Zealand White rabbits	10, 40, and 100 ppm (0.051, 0.205, 0.513 mg/L)	g.d. 7 to 19 for 6 h/d (whole chamber administra-tion)	Groups of 22 pregnant females were exposed to nitrobenzene at target concentration levels of 10, 40, and 100 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. Foetuses were measured for body weight and crown-rump length. After gross external examination all foetuses 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 foetal weight or crown-rump distance data or foetal sex distribution data. External, visceral and skeletal evaluation of foetuses 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. For maternal toxicity a NOAEC of 10 ppm (51 mg/m^3) based on increased methaemoglobin levels and increased liver weights is derived. (Bio/dynamics Inc. 1984)
Conclusion: no classification			

5.9.3 Human data

As residents of the maternity ward after parturition, five mothers had eaten a cake that had contained an ingredient to simulate a bitter almond taste in autumn 1944. Lacking a comprehensive chemical analysis for the causative agent, instead of natural bitter almonds and almond paste it may have contained either nitrobenzene and/ or other substances like aniline, benzaldehyde or benzonitrile. The mothers did not reveal any clinical symptoms but on the next morning (approx. 15 hours after ingestion), their breast-fed babies had developed a strong to very strong cyanosis. The children did not show any additional symptoms and the cyanosis receded largely in the next 24 hours. The children were not breast fed for 1,5 to 2 days. They received large amounts of tea, and if necessary oxygen and heart stabilizing drugs (Dollinger 1949).

5.9.4 Other relevant information

5.9.5 Summary and discussion of reproductive toxicity

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. It is recognised, however, that haematotoxicity is the predominating toxic effect after treatment with nitrobenzene and that these latter effects were 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 consistently induced at dose levels clearly below those inducing testes toxicity. Therefore, nitrobenzene is not considered to represent a specific reproductive toxicant. Based on the evaluation of the available animal data, classification and labelling as

Reprotox. Cat. 3, R 62 is proposed.

(CLP: Reprotox Cat. 2, H 361f suspected human reproductive toxicant)

Developmental toxicity

Investigations in rats and rabbits with the inhalation 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.

Effects on or via lactation

Very young children are more susceptible to methaemoglobinaemia, the main cause of toxicity of nitrobenzene in man and animals (Beauchamp et al. 1982). This is due to newborns still having foetal Hb[***], which is more susceptible to metHb formation than adult Hb (Goldstein et al. 1969). Also, the activity of NADH-cytochrome b5 reductase, the enzyme required for the conversion of

[***] More fetal Hb than adult Hb until about 10 months of age. Fetal Hb is present until about 2 years of age.

ferric iron to ferrous iron in Hb, is not fully developed in infants and very young children (Wentworth et al. 1999) and neither is G6PD activity, an enzyme required to replenish NADPH (Goldstein et al. 1969). Lastly, the neonates' clearance capacity is estimated to be about half of an adults' in the first four weeks post parture (Begg 2000).

Based on its low molecular weight of 123 $^{g}/_{mol}$ and its lipophilicity with a partition coefficient logP of 1.85 at 30°C, a good permeability into the lactiferous glands after systemic distribution is reasonable. Nitrobenzenes water solubility is sufficient for toxic doses, notwithstanding the lipophilic components of milk. There is a high likelihood of accumulation and milk concentrations in excess of the maternal plasma concentration. Even substances with a comparable molecular weight and lower lipophilicity such as p-acetylaminophenol (Paracetamol, MW 151.17 $^{g}/_{mol}$, logP 0.49) and α-methylphenethylamine (Amphetamine, MW 135.21 $^{g}/_{mol}$, logP 1,80) exhibit milk/plasma-ratios in ranges of 1.0 to 3.0 and half-lives in mammary tissue extended by 50% (Berlin et al. 1980, Findlay et al. 1981, Steiner et al. 1984).

While a study in Sprague-Dawley rats (Mitsumori et al. 1994, largely along OECD TG 422, which is insufficient for postnatal effects) mentions no toxic effects exclusively through lactation, the case of breast-fed cyanosed babies whose mothers had eaten an aromatized cake (Dollinger 1949) is an indication of a nitrobenzene-associated effect, since nitrobenzene was frequently found in solutions of false bitter-almond-oil (Zeitoun 1959) and other substances used to substitute natural ingredients even in Europe during and right after the war (Högl 1952). Other substances with bitter almond smell such as benzaldehyde (LD_{oral} 1.3g/kg, rat; 1.0g/kg, guinea pig) or benzonitrile ($LD50_{oral}$ 971mg/kg; mouse) are less toxic, or do not induce clinical symptoms of cyanosis such as hydrogen cyanide (HCN).

With a cue from the above mentioned case, and the physico-chemical properties of nitrobenzene, it is suggested to classify and label nitrobenzene as

Reproductive toxicant R64, may cause harm to breast-fed babies.

(CLP: Reproductive toxicant H362, may cause harm to breast-fed children.)

Later on, to confirm the concern, it might be considered to conduct cross-fostering studies in the substance evaluation procedure within REACH to distinguish between toxic effects from in utero or lactational exposure, although the milk composition of humans and animals differs considerably (Welch and Findlay 1981).

5.10 Other effects

5.10.1 Aspiration toxicity hazard:

Aspiration toxicity hazard category 1 is warranted, if the substance is a hydrocarbon and has a kinematic viscosity ν of 20.5 mm^2/s or less, measured at 40°C (EC Regulation No 1272/2008, section 3.10.2). The data on the viscosity of nitrobenzene varies with temperature, but allows the generation of two data points on either side of 40°C relevant for classification.

The kinematic viscosity ν relates to the dynamic viscosity η via the density ρ:

$$\nu = \frac{\eta}{\rho}$$

$\nu = \eta/\rho$

With density ρ = 1.2037 g·cm^{-3} (Lide, DR 1991) and dynamic viscosities η of 1.863 mP s at 25°C and 1.262 mP s at 50°C (Lide, DR 2009), the respective kinematic viscosities obtained are 1.55 mm^2/s at 25°C and 1.05 mm^2/s at 50°C.

Conclusion: Based on physico-chemical properties it is concluded to propose classication of nitrobenzene as

Xn; R65 Harmful: may cause lung damage if swallowed

Aspiration toxicity category 1 (Asp. Tox. 1) H304 May be fatal if swallowed and enters airways.

5.11 Derivation of DNEL(s) or other quantitative or qualitative measure for dose response

Not relevant for this type of dossier.

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES

6.1 Explosivity

Including C&L

6.2 Flammability

Including C&L

6.3 Oxidising potential

Including C&L

7 ENVIRONMENTAL HAZARD ASSESSMENT

7.1 Aquatic compartment (including sediment)

7.1.1 Toxicity test results

7.1.1.1 Fish

Short-term toxicity to fish

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 13: Acute toxicity data to fish

Species	Endpoint	Effect concentrations [mg/l]	Test system	Reference
Brachydanio rerio	96 hours LC_{50}	92 (mc)	Flow-through (OECD Guideline)	(Roederer 1990)
Lepomis macrochirus	96 hours LC_{50}	43 (nc)	Static (method: US-EPA)	(Buccafasso et al. 1981)
Pimephales promelas	96 hours LC_{50}	119 (mc)	Flow-through (no standard method)	(Geiger et al. 1985)
Pimephales promelas	96 hours LC_{50}	44 (nc)	Flow-through larval test (method: US-EPA)	(Marchini et al. 1992)
Cyprinodon variegatus (saltwater)	96 hours LC_{50}	59 (nc)	Static (method: US-EPA)	(Heitmuller et al. 1981)

(mc) - measured concentration
(nc) - nominal concentration

In acute toxicity tests to fresh (and one salt) water species values in the range from 43 mg/l to 119 mg/l were obtained.

Heitmuller et al (1981) tested the acute toxicity of *Cyprinodon variegatus*. The method was described in "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians" (U.S. EPA 1975). The tests were performed in natural seawater (saltwater) and without aeration. After 96 hours a LC_{50} value of 59 mg/l was achieved.

The same test method was used by Buccafasso et al. (1981) without seawater but deionized water. To control volatilization, the jars with high volatile substances were capped. For substances which appeared to be soluble in water (like nitrobenzene) a concentrated stock solution was prepared or the appropriate amount of the compound was added directly in the deionized water in the jars. For *Lepomis macrochirus* an LC_{50} (96 hours) of 43 mg/l was observed.

At the fathead minnow (*Pimephales promelas*) larval survival and growth test the fish larvae were exposed to nitrobenzene (Marchini et al. 1992). This test was chosen because the larval stage is the most sensitive stage and consequently the heaviest toxic effects are frequently exhibited in early larval development. Marchini et al. compared the results of the larvae stage with values from the literature at the juvenile stage (28-33 days old). For larvae a 96 hours LC_{50} of 44 mg/l was detected.

Compared to literature data for juveniles 96 hours LC_{50} of 119 mg/l was reported (Geiger et al. 1985).

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

Long-term toxicity to fish

Only one fish early life stage test for nitrobenzene exists. But this study cannot be considered valid. 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. It was not possible to confirm the low effect values for the other substances. Hence it can be assumed that the values for nitrobenzene (e.g. 27 days NOEC < 1 µg/l) are not representative as well. Because of the doubt about valididity of the results, the values were also not used in the risk assessment (RAR 2007, WHO 2003).

7.1.1.2 Aquatic invertebrates

Short-term toxicity to aquatic invertebrates

Table 14 shows the available test results for nitrobenzene obtained in short-term tests with aquatic invertebrates.

Table 14: Acute toxicity data to aquatic invertebrates

Species	Endpoint	Effect concentrations [mg/l]	Test system	Reference
Daphnia magna	48 hours EC_{50}	35 (nc)	Semistatic (OECD proposal 1979) Endpoint: behaviour	(Canton et al. 1985)
Daphnia magna	24 hours EC_{50}	50 (nc)	Static (German DIN method) Endpoint: immobilisation	(Bringmann and Kühn 1982)
Daphnia magna	48 hours LC_{50}	27 (nc)	Static (method: U.S. EPA) Endpoint: mortality	(LeBlanc 1980)
Ceriodaphnia dubia	24 hours LC_{50}	54 (mc)	Static (method: U.S. EPA) Endpoint: mortality	(Marchini et al. 1993)

(mc) - measured concentration
(nc) - nominal concentration

Only the EC_{50} 48 hours is a criterion for C&L. But the other result supports an EC_{50} between 10mg/l and 100mg/l in 48 hours.

The test of short-term toxicity (OECD proposal 1979) was a semisatic test (Canton et al. 1985). The test solution was daily refreshed. A stability test in water showed no loss within 1 day. For *Daphnia magna* an EC_{50} (48 hours) of 35 mg/l was measured.

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

Long-term toxicity to aquatic invertebrates

The lowest long-term effect value for *Daphnia magna* was a 21 days NOEC of 1.9 mg/l (measured) with the endpoint reproduction rate (Canton et al., 1985). No information about test conditions was given in this article, but for the performance of the standard tests the authors referred to their former publications (Canton and Slooff, 1982). 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.

7.1.1.3 Algae and aquatic plants

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

Table 15: Toxicity data to algae

Species	Endpoint	Effect concentrations [mg/l]	Test system	Reference
Chlorella pyrenoidosa	96 hours EC_{50}	18*)	Static (OCED-Guideline 201) Endpoint: growth inhibition	(Maas-Diepeveen and van Leeuwen 1986)
Selenastrum capricornutum	96 hours EC_{50}	23.8*)	Static (US-standard test) Endpoint: growth inhibition	(Bollmann et al. 1989)
Chlorella pyrenoidosa	72 hours EC_{10} EC_{50}	8.5 (mc) 28 (mc)	Static (no standard method) Endpoint: growth inhibition	(Ramos et al. 1999)
Scenedesmus obliquus	48 hours EC_{50}	67.7 (nc)	Static (OCED-Guideline 201) Endpoint: growth inhibition	(Liu and Lang 1995)

(mc) - measured concentration
(nc) - nominal concentration
*) no data whether mc or nc

EC_{50}-values for different algal species are in the range from 18 mg/l to 28 mg/l (96 or 72 hours). The lowest effect value from a test with a standardized exposure time of 96 hours was found by Maas-Diepeveen and van Leeuwen (1986) with *Chlorella pyrenoidosa* with a 96 hours EC50 of 18 mg/l.

The effects of nitrobenzene on the same algal species have been studied in 72 hours growth inhibition tests (Ramos et al. 1999). The EC_{10} and EC_{50} were established by fitting the relative growth rate as a function of the test concentration using the Weibull function. An EC_{10} of 8.5 mg/l and an EC_{50} of 28 mg/l was estimated.

The several results from Table 15 fulfilled the criterion of Aquatic chronic 3 (10 mg/l < EC_{50} 96 hours/72 hours ≤ 100mg/l).

7.1.1.4 Sediment organisms

Not relevant for this type of dossier.

7.1.1.5 Other aquatic organisms

Not relevant for this type of dossier.

7.1.2 Calculation of Predicted No Effect Concentration (PNEC)

Not relevant for this type of dossier.

7.2 Terrestrial compartment

Not relevant for this type of dossier

7.3 Atmospheric compartment

Not relevant for this type of dossier

7.4 Microbiological activity in sewage treatment systems

Not relevant for this type of dossier

7.5 Calculation of Predicted No Effect Concentration for secondary poisoning (PNEC_oral)

Not relevant for this type of dossier.

7.6 Conclusion on the environmental classification and labelling

Nitrobenzene did not fulfill the pass level of ready biodegradability. EC_{50} for fish, invertebrates and alga were observed between 10mg/l and 100mg/l. These results were conform to the criteria of classification for R52/53 (based on Directive 67/548/EEC) respectively H412 (EC Regulation No 1272/2008).

JUSTIFICATION THAT ACTION IS REQUIRED ON A COMMUNITY-WIDE BASIS

All endpoints have been addressed within this C&L proposal, since nitrobenzene was a priority substance in the existing chemicals program (EEC) 793/93. The proposal to add R48/25 was already submitted to TC C&L and was agreed on in September 2007. In addition to the previously agreed classification, R64 and R65 are now suggested. Furthermore, the Risk Assessment Report (RAR 2007) did support the reclassification from N R51/53(H411) to R 52/53(H412)

Additionally, nitrobenzene is a substance with very high production volumes and widespread use in EU countries, with production volumes exceeding one million metric tonnes per year. Although its main use is in the production of aniline, several thousand tonnes per year are unaccounted for in their use. It is therefore proposed to extend and amend the current harmonized classification listed in Table 3.2 of Annex VI of EC Regulation No 67/548 and Table 3.1 of Annex VI of EC Regulation No 1272/2008 (CLP) by those reasoned for in this dossier.

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