

European Union Risk Assessment Report

2-NITROTOLUENE

CAS No: 88-72-2

EINECS No: 201-853-3

RISK ASSESSMENT

FINAL APPROVED VERSION

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RISK ASSESSMENT

Final report, 2008

SPAIN

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Foreword

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

The Risk Assessment was carried out in accordance with Council Regulation (EEC) 793/93¹ on the evaluation and control of the risks of “existing” substances. “Existing” substances are chemical substances in use within the European Community before September 1981 and listed in the European Inventory of Existing Commercial Chemical Substances. Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of these substances if they are produced or imported into the Community in volumes above 10 tonnes per year.

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

The methods for carrying out an in-depth Risk Assessment at Community level are laid down in Commission Regulation (EC) 1488/94², which is supported by a technical guidance document³. Normally, the “Rapporteur” and individual companies producing, importing and/or using the chemicals work closely together to develop a draft Risk Assessment Report, which is then presented at a meeting of Member State technical experts for endorsement. The Risk Assessment Report is then peer-reviewed by the Scientific Committee on Health and Environmental Risks (SCHER) which gives its opinion to the European Commission on the quality of the risk assessment.

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

The Risk Assessment Report is also presented to the Organisation for Economic Co-operation and Development as a contribution to the Chapter 19, Agenda 21 goals for evaluating chemicals, agreed at the United Nations Conference on Environment and Development, held in Rio de Janeiro in 1992 and confirmed in the Johannesburg Declaration on Sustainable Development at the World Summit on Sustainable Development, held in Johannesburg, South Africa in 2002. This Risk Assessment improves our knowledge about the risks to human health and the environment from exposure to chemicals. We hope you will agree that the results of this in-depth study and intensive co-operation will make a worthwhile contribution to the Community objective of reducing the overall risks from exposure to chemicals.

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

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

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

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OVERALL RESULTS OF THE RISK ASSESSMENT⁴

CAS Number: 88-72-2
EINECS Number: 201-853-3
IUPAC Name: 2-nitrotoluene

Environment

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

No conclusion (i) has been assigned.

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

Conclusion (ii) applies to the aquatic compartment (marine and freshwater), including sediments.

Conclusion (ii) applies to the terrestrial compartment.

Conclusion (ii) applies to the atmospheric compartment.

Conclusion (ii) is also applied to secondary poisoning according to the low bioaccumulation potential and the rapid elimination of this compound in fish and mammals. In this sense, no secondary poisoning potential is expected from this substance.

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

No conclusion (iii) has been assigned.

Human health

Human health (toxicity)

Workers

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

This conclusion is reached because of:

- concerns for carcinogenicity and mutagenicity as a consequence of inhalation and dermal exposure.
- concerns for repeated dose toxicity and toxicity for reproduction (fertility and development) as a consequence of dermal exposure.

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

Conclusion (i) “on hold” There is need for further information and/or testing

This conclusion is proposed for skin sensitization, assuming that the knowledge that the substance be a skin sensitizer would not lead to stricter control measures than need to be applied for a genotoxic carcinogen

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

This conclusion is reached for acute toxicity by inhalation and dermal routes; irritation/corrosivity to skin, eye or the respiratory tract; repeated dose toxicity by inhalation; and toxicity for reproduction (fertility and development) by inhalation, because these endpoints are of no concern.

Consumers

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

This conclusion is reached because exposure of consumers is not assumed to exist.

Humans exposed via the environment

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

This conclusion is reached because of:

- concerns for carcinogenicity and mutagenicity as a consequence of inhalation and oral exposure arising from the local site C.

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

This conclusion is reached for repeated dose toxicity and toxicity for reproduction (fertility and development) because the calculated MOS for total exposure (oral and inhalation routes) of man via the environment in both local and regional scales are judged to be enough for these endpoints.

Combined exposure

The risk to human health under conditions of combined exposure is dominated by occupational exposure.

Human health (physico-chemical properties)

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

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

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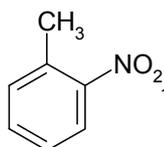
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EUSES Calculations can be viewed as part of the report at the website of the European Chemicals Bureau:
<http://ecb.jrc.it>

1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS Number: 88-72-2
EINECS Number: 201-853-3
IUPAC Name: 2-nitrotoluene
Molecular formula: C₇H₇NO₂



Structural formula:

Molecular weight: 137.14
Synonyms: 1-methyl-2-nitrobenzene
2-methyl-1-nitrobenzene
2-methylnitrobenzene
2-methylnitrobenzol
2-nitro-1-methylbenzol
benzene, 1-methyl-2-nitro
o-methylnitrobenzene
o-nitrotoluene
o-mononitrotoluene
o-nitrotoluol
toluene, o-nitro

1.2 PURITY/IMPURITIES, ADDITIVES

Purity: ≥ 99.5%
Impurity: 0.2% 3-nitrotoluene
0.01% 4-nitrotoluene

1.3 PHYSICO-CHEMICAL PROPERTIES

Melting point

Different values are reported in IUCLID: Verschueren, 1996 (Handbook of Environmental Data on Organic Chemicals) indicates – 10.6 °C and – 4.1 °C, and the Safety Data Sheets of Bayer and Hoechst point out – 9 °C and – 9.1 °C respectively. According to Kirk-Othmer, 1996 (Encyclopedia of Chemical Technology), – 9.55 °C has been selected as melting point.

Boiling point

Measured values of 221.9 °C and 222 °C at 1013 hPa are reported by Bayer AG, 1987 and 2001, respectively. The value of 221.7 °C indicated in Kirk-Othmer, 1996 (Encyclopedia of Chemical Technology), has been selected.

Relative density

Verschueren, 1996, (Handbook of Environmental Data on Organic Chemicals), as well as the Data Sheet of Hoechst, give a value of 1.16 g/cm³ at 20 °C and it will be considered in calculations.

Vapour pressure

The vapour pressure of 2-nitrotoluene has been quoted in IUCLID as 0.016 kPa at 20 °C and 0.13 kPa at 50 °C. The value of 0.028 kPa at 25 °C (Hine and Mookerjee, 1975) has been considered in the calculations, as vapour pressure at 25 °C is required by EUSES.

Water solubility

Patty Ind. (1981) and Sax (1985) give a value of 652 mg/l at 25 °C. The IUCLID Data Set quotes values of 537 mg/l and 437 mg/l at 20 °C (Safety Data Sheets of Hoechst and Bayer, respectively). This last value will be used for the modelling processes.

Partition coefficient n-octanol/water (log value)

The value measured by Hansch & Leo (1985) of 2.3 will be taken into account. Other calculated values are indicated in IUCLID, like 2 (Hansch & Leo, 1979) and 2.358 (Knowin, 1993).

Granulometry

Not applicable.

Conversion factors

No data have been supplied in the IUCLID Data Set.

Flash point

Hommel Handbuch der gefährlichen Gueter and Safety Data Sheets of Bayer and Hoechst point out a measured value of 95 °C, therefore this will be considered in the risk assessment. Besides, all the references in the IUCLID Data Set indicate this value.

Autoflammability

The ignition temperature measured with DIN 51794 for 2-nitrotoluene is 420 °C, as indicated in Safety Data Sheet of Bayer.

Flammability

No data appear in the IUCLID Data Set for this property.

Explosive properties

The explosion limits quoted in the IUCLID Data Set are 1.47% in volume as lower limit and 8.8% in volume as upper limit.

Oxidizing properties

2-nitrotoluene is not an oxidising agent on the basis of its chemical structure.

Viscosity

IUCLID Data Set does not reference this issue. The value of 0.0262 mPa·s was given by Sax (1985), while the Safety Data Sheet of Bayer indicate a measured value of 2.37 mPa·s, the same gathered in Kirk-Othmer, 1996 (Encyclopedia of Chemical Technology).

Henry's constant

Several measured values, 5.673 Pa·m³/mole and 5.343 Pa·m³/mol, are mentioned in the SIDS Assessment Report of the OCDE, 1994. The experimental dimensionless value given by Altschuh, J. *et al.* (1999), 5.1·10⁻⁴ will be used after conversion to the units needed by means of the equation 22 of the new Technical Guidance Document (TGD), giving a value of 1.2 Pa·m³/mol.

Surface tension

Only a reference from Kirk-Othmer, 1996 (Encyclopedia of Chemical Technology), has been found. It gives a value of 44.1 mM/m at 20 °C.

Table 1.1: Summary of physico-chemical properties

Property	Value	Comments
Physical state	Liquid	
Melting point	- 9.55 °C	Kirk-Othmer, 1996
Boiling point	221.7 °C	Kirk-Othmer, 1996
Relative density	1.16 g/cm ³	at 20 °C. Verschueren, K., 1996
Vapour pressure	0.028 kPa	at 25 °C. Hine and Mookerjee, 1975
Water solubility	437 mg/l	at 20 °C. Bayer AG 2001
Partition coefficient n-octanol/water (log value)	2.3	Hansch & Leo, 1979
Granulometry	Not applicable	n.a.
Conversion factors	n.a.	n.a.
Flash point	95 °C (DIN 51755)	Bayer AG, 2001
Autoflammability	Ignition temperature: ca. 420 °C (DIN 51794)	Bayer AG, 2001
Flammability	n.a.	n.a.
Explosive properties	Lower limit : 1.47% by vol Upper limit : 8.8% by vol	Bayer AG, 2001
Oxidizing properties	n.a.	n.a.
Viscosity	2.37 mPa·s	Bayer AG, 2001
Henry's constant	1.2 (Pa·m ³ /mol)	Experimental dimensionless Henry's law constant value proposed by Altschuh, J. <i>et al.</i> 1999, transformed into the required units
Surface tension	44.1 mM/m	At 20 °C; Kirk-Othmer, 1996

1.4 CLASSIFICATION

1.4.1 Current classification

The classification of 2-nitrotoluene in Annex I to Directive 67/548/EEC was revised in the 29th ATP:

Carc. Cat. 2; R45: May cause cancer

Muta. Cat. 2; R46: May cause heritable genetic damage

Repr. Cat. 3; R62: Possible risk of impaired fertility

Xn; R22: Harmful if swallowed

N; R51/53: Toxic to aquatic organisms/May cause long-term adverse effects in the aquatic environment.

1.4.2 Proposed classification

It is proposed to keep the same classification as currently:

Carc. Cat. 2; R45: May cause cancer

Muta. Cat. 2; R46: May cause heritable genetic damage

Repr. Cat. 3; R62: Possible risk of impaired fertility

Xn; R22: Harmful if swallowed

N; R51/53: Toxic to aquatic organisms/May cause long-term adverse effects in the aquatic environment.

2 GENERAL INFORMATION ON EXPOSURE

2.1 PRODUCTION

2.1.1 Production processes

2-nitrotoluene is a light yellow liquid with characteristic odour, non-hygroscopic, non-corrosive and with a good storage life.

2-nitrotoluene can be produced by either a batch or continuous process in closed systems, by the nitration of toluene. The mononitration of toluene results in the formation of a mixture of the three isomers of nitrotoluene: ortho (2-nitrotoluene), meta (3-nitrotoluene) and para (4-nitrotoluene).

In a typical process, the toluene is fed into the nitrator and cooled to about 25 °C. The nitrating acid (52-56 wt% H₂SO₄, 28-32 wt% HNO₃ and 12-20 wt% H₂O) is added slowly below the surface of the toluene and the temperature of the reaction mixture is maintained at 25 °C by adjusting the feeding rate of the nitrating acid and the amount of cooling. After all the acid is added, the temperature is raised slowly to 35-40 °C. After completion of the reaction, the mixture is put into a separator where the spent acid is withdrawn from the bottom and reconcentrated. The crude product is washed in several steps with dilute caustic and then water. The product is steam distilled to remove excess toluene and then dried by distilling the remaining traces of water. The resulting product contains 55-60 wt% 2-nitrotoluene, 3-4 wt% 3-nitrotoluene and 35-40 wt% 4-nitrotoluene. The separation of the three isomers is carried out by a combination of fractional distillation and crystallization. In a fractional vacuum distillation step, the distillate, obtained at a head temperature of 96-97 °C at 1.6 kPa, is fairly pure 2-nitrotoluene and can be purified further by crystallization. The washing water is first cleaned by extraction with toluene, then the remaining toluene is stripped from the washing water and finally the washing water is led into the industrial wastewater treatment plant (WWTP).

If the pure isomer is required, it can be prepared by indirect method, treating 2,4-dinitrotoluene with ammonium sulphide followed by diazotisation and boiling with ethanol (Kirk-Othmer, 1994).

2.1.2 Production capacity

Since 1996, the principal producers and/or processors of 2-nitrotoluene in Europe are located in Germany, the United Kingdom and Italy, as can be seen in Table 2.1.

According to recent information from industry, Italy is the country with the major production. There were two producers located in Italy, but one industry ceased its 2-nitrotoluene production in September 2003 and the other is at present the largest European producer. According to the information supplied by this second industry, the production in 2002 was 49,200 tonnes and they were used as an intermediate for 2,4-dinitrotoluene production.

The principal part of the German company production was mainly directed in the past to obtain specialty isocyanates, but then it reduced its output of this raw material for polyurethane foams and coatings. Finally, a large part of 2-nitrotoluene output is preferably

converted into o-toluidine. In the IUCLID Data Set, a production between 10,000 and 50,000 tons/year is indicated for 1993, 1996 and 1999. But more recent information has been reported by industry for year 2000, when the total production of 2-nitrotoluene of the plant placed in Germany was 34,400 tons.

The United Kingdom was initially considered the second producer in Europe; almost all 2-nitrotoluene was hydrogenated into o-toluidine, with minor amounts going to the merchant market to several producers of TNT or non integrated producers of o-toluidine (Srouf, R.,1997). However, industry has indicated that 2-nitrotoluene is no longer manufactured in the United Kingdom since 2001, although it is still imported from inside Europe and used as a raw material.

Estimated production and/or processing amount of 2-nitrotoluene by country is given hereafter in metric tons.

Table 2.1: Production and/or processing volume in metric tons

Country	Volume
Germany ^a	34,400
United Kingdom ^b	3,744.56
Italy ^c	49,200
Total	87,344.56

^a data from industry, 2000

^b imported volume. Data from industry, 2003

^c data from industry, 2002

2.2 USES

2.2.1 Introduction

2-nitrotoluene can be used in the synthesis of intermediates for the manufacture of agricultural and rubber chemicals, explosives, heat sensitive colorants, azo and sulphur dyes, and in the organic synthesis of a wide variety of compounds including petrochemicals, pesticides and pharmaceuticals (OECD, 1994).

Oxidation with potassium permanganate or potassium dichromate causes the formation of o-nitrobenzoic acid. When boiled with a sodium hydroxide solution, 2-nitrotoluene exhibits the phenomena of autooxidation and reduction and yields anthranilic acid. When oxidation is carried out with manganese dioxide and sulphuric acid, o-nitrobenzoic acid or o-nitrobenzaldehyde is formed, depending on the reaction conditions. Alkaline reduction with iron or zinc leads in a stepwise fashion to azoxy, azo and hydrazo compounds, depending on the reaction conditions. Nitration of 2-nitrotoluene gives 2,5-dinitrotoluene and 2,6-dinitrotoluene, used in explosives and dyestuffs. Chlorination of 2-nitrotoluene in the absence of iron yields o-nitrobenzyl chloride, o-chlorotoluene or o-chlorobenzyl chloride, depending on the reaction conditions. In the presence of iron, chlorination results in the formation of 2-nitro-6-chlorotoluene and 2-nitro-4-chlorotoluene, for use in pharmaceuticals and pigments, respectively (Kirk-Othmer, 1994). Finally, one method of reducing 2-nitrotoluene to o-toluidine is by iron powder and hydrochloric acid, and this is used in the agricultural market segment for herbicides and in the dyestuffs and rubber industry.

However, information obtained from literature shows that even though 2-nitrotoluene can be used for the manufacture of a broad range of products, o-toluidine seems to be the largest outlet for this substance. The manufacture of o-toluidine accounted for nearly 90% of total 2-nitrotoluene consumption in 1997 in West Europe. In addition, information from an industry settled in Italy indicates that 2-nitrotoluene is also used there as an intermediate in the synthesis of 2,4-dinitrotoluene. Minor amounts went into some specialty intermediates and some TNT; nevertheless, the production of TNT from 2-nitrotoluene has decreased considerably in recent years and it is now very infrequent and may cease in the fairly near future, as an English company has lately pointed out. Therefore this minor use has not been considered in the present risk assessment report.

To summarize, data reported by the German industry about the breakdown of 2-nitrotoluene uses in 2000 indicate that 31,200 tons (90.70%) were used for processing to o-toluidine, 500 tons (1.45%) for processing to dinitrotoluene 65/35, 100 tons (0.30%) for processing to 2-nitrotoluene-4-sulfonic acid and 2,600 tons (7.55%) for merchandise.

An industry from the United Kingdom has indicated that 3,698.7 tons of 2-nitrotoluene were converted to o-toluidine, via catalytic hydrogenation, in 2003, while 45.86 tons were resold.

Finally, recent information has been reported by the Italian industry on the breakdown uses of the 2-nitrotoluene which indicates that 49,200 tons of 2-nitrotoluene were used as an intermediate for 2,4-dinitrotoluene production in 2002.

Therefore, the percentage of total use has been proposed regarding the information from industry.

Table 2.2: Quantity of 2-nitrotoluene used in 2000, per uses

Industry category	Use category	Quantity used (metric tons)	Percentage of total use
Chemical industry: used in synthesis of o-toluidine	Intermediate	34,898.7	40%
Chemical industry: used in synthesis 2,4-dinitrotoluene	Intermediate	49,200	56.3%
Chemical industry: used in synthesis of other chemicals	Intermediate	3,245.86	3.7%
Total		87,344.56	100%

Data from industry

2.2.2 Scenarios

The substance is produced and used in closed systems as an intermediate for further synthesis. 2-nitrotoluene is mainly synthesised by the nitration of toluene and once manufactured is mainly used on-site for production of o-toluidine or 2,4-dinitrotoluene. Therefore, only one scenario will be considered, the one in which production and use of 2-nitrotoluene as intermediate takes place in the same place. However, as there are several production sites in Europe, three cases will be assessed, in the scope of the environment exposure, inside this scenario:

Table 2.3: Site specific information of the volume used (metric tons/year)

Scenario	Country	Volume
Site A	Germany	34,400
Site B	United Kingdom	3,744.56
Site C	Italy	49,200

2.3 TRENDS

In West Europe, the number of producers of 2-nitrotoluene has decreased in the last years and the use pattern of this compound has changed because 2-nitrotoluene demand has been subject to many fluctuations. In 1991 2-nitrotoluene was produced by five companies, two in Germany, one in the United Kingdom, one in Sweden and another two in Italy. The Swedish producer was the one that used 2-nitrotoluene for TNT production, but it stopped this production in 1992.

The German producers used 2-nitrotoluene mainly for o-toluidine synthesis, though there were some other uses. Nowadays, only one of the German companies continues as 2-nitrotoluene producer and it has increased the capacity for producing o-toluidine to cope with higher demand from metolachlor, a herbicide. A mayor part of its production was mainly directed in the past to the production of specialty isocyanates, TDI 65:35 and TDI 100. Increasing competition in the early eighties from other major of these isocyanates, using either the distillation of crude TDI or the 2-nitrotoluene route, forced the industry to reduce its output of these raw materials for polyurethane foams and coatings. The collapse of the demand for TDI 65:35 in Russia since the early nineties had also a severe impact on the demand and pricing of these isocyanates, which no longer provided a good value for 2-nitrotoluene. Therefore, the German producer was pushed to shutdown its isocyanate facility and a large part of 2-nitrotoluene output is preferably converted into o-toluidine since 1985.

Another producer of 2-nitrotoluene in Western Europe, placed in the United Kingdom, had a nitration capacity which was formerly used for the manufacture of nitrochlorobenzenes, but since 1986, 2-nitrotoluene is almost entirely hydrogenated into o-toluidine in this site. However, recent information from the industry settled in the United Kingdom shows that its production of 2-nitrotoluene ceased during 2001, although it is still used as a raw material. In 2003, 3,698.7 tons were imported for processing and 45,86 tons were resold.

Table 2.4: Evolution of the quantity of 2-nitrotoluene produced and used in Western Europe in the last 25 years

Year	1978	1983	1985	1987	1991	1992	1996	
Production	48,000	50,800	53,500	50,000	52,500	56,000	54,500	
Demand	o-Toluidine	6,400	17,600	22,200	20,300	31,300	35,500	46,000
	65:35 TDI	24,500	19,500	16,600	17,600	18,400	16,500	-
	TNT	7,000	8,000	10,000	7,000	1,500	2,000	-
	o-Tolidine	1,500	1,500	1,500	1,000	800	-	-
	6-Chloro-2-nitrotoluene	700	800	900	900	600	500	800
	Others	1,000	1,000	1,000	1,000	1,300	1,500	2,000
	Total	41,000	48,400	52,400	47,800	53,900	56,000	48,800

Data from Srour, 1997

In relation to the Italian companies, one had the lowest production in Europe, based on information published in Srour, R., 1997, which was used in the dinitration stream in its TDI facility. But its production stopped in 2003. The other one is nowadays the major producer of 2-nitrotoluene in Europe.

Finally, one conclusion can be put forward: the introduction of metolachlor, in the late seventies increased significantly the demand for o-toluidine to a level much higher than any other outlet for this isomer, except for 2,4-dinitrotoluene production, while hardly any 2-nitrotoluene has been offered to TNT producers over the last years (Srour, R.,1997; Industry, 2001).

2.4 LEGISLATIVE CONTROLS

In Germany there are several legal regulations related to 2-nitrotoluene:

- Regulations on safeguarding health at the workplace: the maximum workplace concentration is 5 ppm or about 30 mg/m³ at 20 °C and 1013 hPa.
- “GesfStoffV” Regulations on Hazardous Substances: 2-nitrotoluene may only be dispensed by a company employee who has proven his or her knowledge and it must be kept under lock and stored so that access is restricted to competent personnel.
- Regulations in German Technical Guidelines for Air Pollution Control: 2-nitrotoluene is assigned to class I, where the mass flow is 0.1 kg/h or more, the mass concentration shall not exceed 20 mg/m³.
- Regulations for facilities which store, fill or handle water-hazardous substances: 2-nitrotoluene is classified as a water-hazard substance.
- Regulations on combustible fluids: on the basis of its physical properties, 2-nitrotoluene is assigned to class A III.
- Regulations on the transport of hazardous goods: according to the provisions covering the transport of hazardous goods, 2-nitrotoluene is classified as follows:
 - Hazardous Goods Regulations, Railroad: Cl. 6.1, No. 12 b
 - Hazardous Goods Regulations, Road: Cl. 6.1, No. 12 b
 - Hazardous Goods Regulations, Sea: Cl. 6.1, Un No. 1664
 - International regulations on the transportation of dangerous goods by rail / European agreement on the international transportation of dangerous goods by road: Cl. 6.1, No. 12 b
 - International Civil Aviation Organization / International Air Transport Association – Dangerous Goods Regulation (ICAO/IATA-DGR): Cl. 6.1 1664 II
 - Regulations on the transportation of dangerous goods on the Rhine: Cl. 6.1, No. 21 L Cat.

3 ENVIRONMENT

3.1 ENVIRONMENTAL EXPOSURE

3.1.1 General discussion

The level of exposure to a chemical in the environment depends on the quantity released and its subsequent degradation, distribution and accumulation. Following sections discuss the releases of 2-nitrotoluene to the environment and its behaviour once in each compartment. The methods and calculations described in the TGD for the risk assessment of new and existing substances have been used.

3.1.2 Environmental releases

2-nitrotoluene may enter the environment via the air and waste water during its production and/or use in the manufacture of other products. The manufacturer and/or processors of 2-nitrotoluene in West Europe, settled in Germany, the United Kingdom and Italy, have reported data from emission to air and water.

In addition, information provided by industry from site C indicates that this site is located in a coastal zone. Therefore, the risk for the marine environment will be also assessed for that site in this report.

3.1.2.1 Release from production and processing

Data on releases from production and processing of 2-nitrotoluene have been reported by industry for all production and/or processing sites.

The company of site A has an air purification unit and an industrial WWTP, and the reported releases are 25 kg/year to the air and 207 kg/year to water, data which will be taken as representative of local releases from site A, where 365 days of production and processing have been assumed.

Regarding site B, where waste waters are discharged to the on-site effluent treatment plant, industry has indicated an annual emission to water lower than 5.5 kg while releases of 2-nitrotoluene to air are considered to be trivial, due to the processes used and the abatement in use (chilled condensers, scrubbers and activated carbon beds). Nevertheless, no information on the value of the emission to air was supplied and therefore, the default factor of 10^{-5} indicated in the A-table will be considered in this site.

Finally, industry of site C indicates an emission to surface water as WWTP effluent of 357 kg/year, over a production period of 11 months. On the other hand, all air streams that could contain 2-nitrotoluene are sent to an incinerator and values of total organic content measured in the air emissions are always below 0.5 mg/m^3 . Nevertheless, no data on the volume emitted have been supplied and therefore the default factor of 10^{-5} indicated in the A-table will be considered in this site to estimate the releases to air.

Table 3.1: Local releases from production and processing (kg·day⁻¹)

Compartment	Site A	Site B	Site C
Air	0.07	0.125*	1.64*
Waste water	0.57	0.015	1.08

*No data received. Releases based on TGD emission factors

For regional level, according to TGD, it can be assumed that 10% of the amount that is produced and used in the EU is produced/used within a region, unless specific information on use or emission is available. However, in this case the industry of each site has provided information on the production volume and consequently, it is more appropriate to use specific values from the largest source as input for the regional model. Site C will be considered as the worst case for regional calculations. Finally, the continental emissions will be calculated using the total amount that is produced and used in the EU minus the regional amount.

Continental and regional emissions will be used to calculate predicted environmental concentrations (PECs) for these levels. PEC_{regional} is then used as background concentration and added to the local environmental concentrations to give PEC_{local} .

Table 3.2: Regional and continental releases from production and processing (kg·day⁻¹)

Scale	Compartment	
	Air	Waste water
Regional	1.35	0.978
Continental	1.05	0.758

Considering the terrestrial compartment, the information provided by industry of sites A and C shows that no spreading of sewage sludge from the industrial sewage treatment plants is done. Therefore, the emissions have been calculated with EUSES, only considering atmospheric deposition. However, industry from site B has indicated that the sludge from the sewage treatment plant is sent off-site for composting prior to spreading on land as an agricultural fertilizer, therefore the spreading of the sludge has been considered as worst case for this site.

No data have been reported by industry related to the emissions to industrial soil, therefore the emission of 1.35 kg/day to industrial soil for regional scale and 1.05 kg/day for continental scale, obtained with EUSES for site C, will be considered as worst case in calculations.

3.1.2.2 Release from formulation

Formulation has not been considered in the present risk assessment report because it is not a predictable step in the life cycle of 2-nitrotoluene.

3.1.2.3 Release from private use

2-nitrotoluene is not known to be employed in any private use.

3.1.2.4 Release from disposal

Practically all 2-nitrotoluene is used as an intermediate in the production of o-toluidine or 2,4-dinitrotoluene and release from disposal is not expected.

3.1.2.5 Summary of releases

The local site emissions to each compartment, in kg/day, are summarised in Table 3.3:

Table 3.3: Summary of release estimates (kg·day⁻¹)

Compartment	Local			Regional ^b	Continental
	Site A	Site B	Site C		
Air	0.07 ^a	0.125	1.64	1.35	1.05
Waste water	0.57 ^a	0.015 ^a	1.08 ^a	0.978 ^a	0.758

^a Calculated with emission data provided by industry

^b Calculated from emission data and production volume of site C as worst case

3.1.3 Environmental fate

2-nitrotoluene may be released into the environment during its production and processing. Emissions to water and air are expected to be the most important entry routes of 2-nitrotoluene to the environment. General characteristics of 2-nitrotoluene which are relevant for the exposure assessment are discussed in the following sections.

3.1.3.1 Degradation in the environment

3.1.3.1.1 Atmospheric degradation

When released to the atmosphere, 2-nitrotoluene is expected to exist entirely in the vapour phase. The dominant removal mechanisms would be reaction with photochemically generated hydroxyl radicals (estimated half-life 8h) and direct photolysis. The rate at which 2-nitrotoluene reacts with OH radicals has been measured experimentally; the velocity constant K_{OH} was $0.7 \cdot 10^{-12} \text{ cm}^3 \cdot \text{molecule}^{-1} \cdot \text{s}^{-1}$ (Meylan and Howard, 1993), equivalent to a half-life period ($t_{1/2}$) of 23 days, assuming a mean tropospheric OH radical concentration of $5 \cdot 10^5$ molecules per cm^3 . In view of the UV absorption of 2-nitrotoluene in the sunlight range ($\lambda > 295 \text{ nm}$) there is a possibility of direct photolysis under tropospheric conditions (BUA, 1989).

The photochemical reaction of 2-nitrotoluene was studied by Nojima and Kanno (1977). One millilitre of a solution of 2-nitrotoluene in hexane ($4.46 \cdot 10^{-5} \text{ moles/ml}$) was taken in a litre reaction vessel, followed by removal of n-hexane and substitution with air or nitrogen free from nitrogen oxides. The residual nitrotoluene in air was irradiated at $\lambda > 300 \text{ nm}$ for 5 hours and this yielded nitrophenol derivatives, as shown in Scheme 3.1. The reaction does not give the hydroxylated 2-nitrotoluene but 2-methyl-6-nitrophenol (6.1% yield) and 2-methyl-4-nitrophenol (7.5% yield). From this results it might be considered that the compound was converted at first to the corresponding phenol and cresol by the liberation of nitrogen monoxide through the nitronitrite rearrangement, followed by nitration with nitrogen dioxide,

3.1.3.1.2 Aquatic degradation (incl. sediment)

Abiotic

Chemical hydrolysis and oxidation of 2-nitrotoluene are not expected to be important removal processes. However, 2-nitrotoluene may be susceptible to photolysis. Because nitroaromatic compounds absorb sunlight strongly in the ultraviolet and blue spectral region, they are generally susceptible to photochemical transformation in aquatic systems. In a study by Simmons and Zeep (1985), carried out through the year under full exposure to sunlight and surface conditions at 40 °N latitude, the photodegradation rates of several nitroaromatic compounds were determined. Saturated solutions of 2-nitrotoluene were made up in distilled water (organic-free water) and were centrifuged at 15,000 rpm for 30 min. The supernatant was removed carefully, this stock solution was diluted to concentration levels of 10^{-6} – 10^{-5} M and then exposed to midday sunlight. The kinetic results for 2-nitrotoluene indicated a half life of 0.79 days.

The effect of humic substances in natural waters on photolysis was investigated in the same study and it was shown that they enhanced the sunlight-induced photodegradation rates compared to distilled water results. This was especially pronounced for 2-nitrotoluene, for which the degradation rate in natural waters was increased by a factor of 6 - 7.5.

Photoreaction in seawater may differ from those occurring in other aqueous media. The photolysis rate for 2-nitrotoluene in filter-sterilised seawater was $5.3 \cdot 10^{-3} \text{ min}^{-1}$ ($t_{1/2} = 130$ min). In unsterilised seawater, the rate was not significantly different, $5.5 \cdot 10^{-3} \text{ min}^{-1}$ ($t_{1/2} = 126$ min), and in neither case any loss of nitrotoluene was observed in the dark. The rate in unsterilised seawater collected from a more polluted site, which was presumed to have a larger microbial population, was also determined. Again, no degradation was observed in the dark, and a half-life of $5.7 \cdot 10^{-3} \text{ min}^{-1}$ ($t_{1/2} = 122$ min) was determined in the light, which is within 3% of that calculated in unsterilised seawater (Toole, 1988).

The studies referred above are based on direct photolysis under laboratory conditions. As half life under environmental conditions is not available, it was assumed that only 3.3% of the solved substance is exposed to photodegradation, since the Handbook of Estimation Methods for Chemicals (eds Boethling and Mackay, Lewis Publishers) suggests a diffuse attenuation coefficient of 0.1 cm^{-1} as an average. For a depth of 3 metres as in the TGD regional model, the average rate over this depth would be $1/30^{\text{th}}$ of the rate at the surface (3.3%). Considering the lowest photodegradation rate (0.79 days) as a worst case, an effective half-life of 24 days is estimated. The corresponding $K_{\text{photo}_{\text{water}}}$ of $1.2 \cdot 10^{-3} \text{ h}^{-1}$ has been used in the risk assessment.

Biodegradation

Information provided in this chapter includes a set of data on biodegradability. Unfortunately, it has not been possible to validate all information provided going back to the original publications (particularly for the non-biodegradable conclusion). This non-validated information has been accepted and included as useful information due to the quality of the institution which performed the study and the no-contradiction with other validated reports. In Table 3.5 it is compiled the information on the different biodegradation assays considered.

The MITI List (1986) classifies 2-nitrotoluene as **non readily biodegradable**. Same conclusions can be extracted from other MITI assay (1992) after a period of 14-d in which a biodegradation of 0.5% of BOD was measured after mixing 100 mg of substance plus 30 mg/l of sludge.

Nevertheless, some biodegradation test results show that 2-nitrotoluene undergoes biodegradation after a period of acclimation. BUA (1989), reports a die-away test after the adaptation of the inoculum, which shows 2-nitrotoluene as biodegradable. Investigations of the biological oxygen demand (BOD) of 2-nitrotoluene were carried out with an adapted bacterial mixed culture, which was taken from the sludge of an experimental purification plant for industrial and communal sewage (14 days aeration in the presence of the substance). They showed a BOD₁₀ of 42.8% and BOD₂₀ of 82.7%, based on a theoretical chemical oxygen demand (COD) of 1,635.04 mg O₂/g nitrotoluene (BUA, 1989).

Pitter (1976) reported a study of the biodegradability of 2-nitrotoluene with activated sludge from a sewage plant whose microorganisms had been adapted for 20 days. The substance was the only source of carbon for the microbes of the inoculum. Pitter's test was performed in a batch system and the concentration of 2-nitrotoluene was gradually increased so that, after 20 days of adaptation, it reached the equivalent value of 200 mg O₂/l. The baker was placed in a dark room with a roughly constant temperature of 20 ± 3 °C. Samples were taken at prescribed intervals till there was no decrease of COD. After 120 h of incubation, the 98% of the initial COD was removed and the degradation rate was 32.5 mg COD/g nitrotoluene·h. Pitter's test finishes when the chemical oxygen demand (COD) ceases to decrease, since its reduction is the basis for determining the biodegradation. If no constant COD has been attained after 20 days, another experiment is started with a lower initial concentration of the test substance. The study was performed in an open system and, loss of test substance by volatilisation may be likely to occur, due to the Henry's constant of the substance (1.2 Pa·m³/mol). So, in this case, the evaluation of degradability in dependence on the decrease of organic carbon from the biological medium can give a very good image of the total amount of removable substrate.

In another study, Canton *et al.* (1985) carried out a biodegradability study of 2-nitrotoluene in the dynamic system "Pitter's test", developed by Pitter (1976), with both, no-adapted and adapted inoculums, resulting in a half-life >> 4 weeks and between 1-2 weeks, respectively. The results presented by these authors indicate that the **test compound cannot be biodegraded in this kind of test without the adaptation of the inoculum**. Information from non acclimatized inoculum was obtained from a semi-static system (modified OECD test, 1971; Repetitive Die Away test (RDA)).

Another study, carried out by Struijs and Stoltenkamp (1986), utilized a modification of the adaptation procedure employed by the test method described by Pitter (1976). Adaptation period consisted of a 1:1 (v/v) mixture from a domestic sewage treatment plant and a solution containing organic material extracted from river mud. Addition of synthetic feed during a period of 3 weeks, in which the sludge is exposed to an increasing amount of the test compound from 2 mg of carbon per litre at the beginning of the adaptation period to 25 mgC/l after 10 days and maintained until day 21. The cultivated sludge was introduced in such amount that the concentration of the inoculum in the die-away test was adjusted to either 10 and 100 mg/l of 2-nitrotoluene, and afterwards incubated at 25 °C in the dark under rotary shaking. After 2 weeks biodegradation was almost complete. Even inoculation with a 10-fold higher concentration of adapted sludge resulted in 100% oxidation within 1 week. This procedure could fulfil the inherent biodegradability test conditions.

Robertson *et al.*, (1992) studied the capability of other aerobic toluene-grown microorganisms to metabolise 2-nitrotoluene. *Pseudomonas putida* F1 and *Pseudomonas* sp. strain JS150 initiated toluene degradation by incorporating molecular oxygen into the aromatic nucleus to form *cis*-1,2-dihydroxy-3-methylcyclohexan-3,5-diene. When toluene-grown cells were

incubated with 2-nitrotoluene, the major product identified was 2-nitrobenzyl alcohol. Toluene-grown cells of *Pseudomonas* sp. strain JS150 and *P. putida* F1 catalysed the almost stoichiometric oxidation of 2-nitrotoluene to 2-nitrobenzyl alcohol within 7 hours. Experiments with $^{18}\text{O}_2$ showed the incorporation of one atom of molecular oxygen, indicating that 2-nitrobenzyl alcohol is formed by a monooxygenation of 2-nitrotoluene. JS150 also produced small amounts of a transient metabolite. The preferential oxidation of the methyl group in 2-nitrotoluene by *P. putida* F1 and JS150 was unexpected. The same nitrobenzylalcohols were formed by *E. coli* JM109 (pDTG601).

Bayer (1977) also gives information, regarding the degradability of the 2-nitrotoluene, using an adapted domestic sewage. The test used to assess the biodegradation was the Geschlossener Flaschentest. After measuring the O_2 consumption it was determined a biodegradation of 70% after 20 days. This information will be considered as additional information since the only information provided was given through the IUCLID database, but there was no possibility of validating the original report.

As in the previous case, the IUCLID database provides information regarding the capability of inherent biodegradation of 2-nitrotoluene, measuring a degradation of >90% after 15 days under the inherent Zahn-Wellens test, using an industrial activated sludge (Hoech, 1993). There was no possibility of validating this information, so it will be considered as additional information.

The biodegradability of 2-nitrotoluene by soil organisms was also examined by Bringman and Kühn (1971) in a 2-stage sewage plant model. The first stage involved an aeration process. The cylinder serving as aeration vessel was ventilated by a water-jet jump. Once an hour, 10 ml of synthetic effluent, containing the corresponding nitrotoluene, were pumped into the aerating vessel. The first stage used an enriched culture of the soil bacterium *Azotobacter agilis*. The effluent from the aeration vessel flowed into a collecting tank and the contents were pumped hourly onto a trickling filter filled with coke (second stage). The second stage was inoculated with *Azotobacter agilis* and activated sludge from a model biological purification plant from communal sewage. 2-nitrotoluene was applied as the only source of carbon. At an initial concentration of 132 mg 2-nitrotoluene/l synthetic effluent (inlet), less than 1 mg/l, was still chemically detectable in the effluent of the first stage. Amounts below 1 mg/l were not quantitatively determined. Thus, the substances had already undergone degradation of more than 99% during the first stage. Duration of the exposure is not indicated. General test conditions are closer to aquatic biodegradation processes than soil degradation processes. Therefore, the test is described in the aquatic biodegradation section. The data are included as additional information.

No specific information has been provided on anaerobic biodegradation, so OECD comments on this issue have been included which indicate that, in general, anaerobic biodegradation of nitroaromatic compounds results in the reduction of the nitro group to an amino group (OECD, 1994).

On the view of the provided information, and in terms of the environmental modelling for the risk assessment, it will be assumed that 2-nitrotoluene is **inherent biodegradable**, fulfilling the specific criteria, for the aerobic industrial sewage treatment plant, so a rate constant of 0.1 hours^{-1} , as given in the Technical Document, will be used. On the contrary, it will be considered as **non-biodegradable** in surface water, as in running waters, the water column microbial population has a very high spatial and temporal variability, and the condition of

continuous exposure of the same population allowing its acclimatization cannot be guaranteed even for continuous point emissions.

Table 3.5: Biodegradation test results for 2-nitrotoluene

Type	Source of microorganisms	Method	Detection parameter	Result	Comments	Reference
Aerobic	Activated sludge	MITI (1)	O ₂ consumption	0.5% after 14 days. No biodegradation observed	V	MITI, 1992
Aerobic	Adapted bacterial mixed culture	Die-away test	O ₂ consumption	80% after 14 days	V	BUA, 1989
Aerobic	Activated sludge (adapted)	Other, ca. Inherent	COD	98% after 5 days.	V	Pitter, 1976
Aerobic	Activated sludge (adapted)	OECD (RDA), Pitter's test	O ₂ consumption	t _{1/2} 1-2 weeks	V	Canton <i>et al.</i> , 1985
Aerobic	Activated sludge (non adapted)	OECD (RDA), Pitter's test	O ₂ consumption	t _{1/2} >>4 weeks	V	Canton <i>et al.</i> , 1985
Aerobic	Aerobic microorganisms (<i>Pseudomonas</i> sp.)	Other	Appearance of nitrobenzyl alcohol	100% after 7 hours	VWR Additional information	Robertson <i>et al.</i> , 1992
Aerobic	Activated sludge (adapted)	Other Die-away test; ca. inherent	O ₂ consumption	90% after 14 days	V	Struijs and Stoltenkamp, 1986
Aerobic	<i>Azotobacter agilis</i> , adapted to 2,4,6-TNT	Bringmann & Kühn, 1971	Colorimetric measurement	100% (no time indication)	NA (Additional information)	Bringmann and Kühn, 1971
Aerobic	Domestic sewage (adapted)	Other, Geschlossener Flaschentest	O ₂ consumption	70% after 20 days	Additional information	Bayer AG, 1977 (in IUCLID database)
Aerobic	Industrial activated sludge	Inherent Zahn-Wellens	O ₂ consumption	> 90% after 15 days	Additional information	Hoech AG, 1993 (in IUCLID database)
Aerobic	Soil microorganisms	Other ca. inherent	Photometrical measurement of ring cleavage	0% within 64 days	NA (Additional information)	Alexander and Lustigman, 1966

V: Valid; VWR: Valid With Restrictions; NA: Not Assignable;

3.1.3.1.3 Degradation in soil

Biodegradation

If released to soil, 2-nitrotoluene may be resistant to oxidation and chemical hydrolysis. An earlier study (Alexander and Lustigman, 1966) worked with a procedure based on the loss of ultraviolet absorbance when the benzene ring is cleaved by mixed population of microorganisms derived from a soil inoculum under aerobic conditions in aqueous mineral salts media. 2-nitrotoluene was inoculated at an initial concentration of 10 µg/l of test compound as the sole carbon source. Another series identical to this was set up to determine if the chemical concentration was toxic to the microflora. The authors determined the rate of degradation of 2-nitrotoluene by a spectrophotometric technique. It relied upon the loss of ultraviolet absorbance when the benzene ring was cleaved by microorganisms derived from a soil inoculum. The solution contained the test compound as the sole carbon source to support microbial proliferation. The wavelength chosen was 263 nm, at which the light absorption was at, or near, the absorption maximum for the test substance, and so, considered to be sufficiently high for convenient use. Results indicate that 2-nitrotoluene was quite difficult to degrade, being the ultraviolet light retained for periods in excess of 2 months. The designation >64 indicates that significant ring cleavage was not detected even on the 64th day, which would correspond to the most persistent compounds. This study is considered not assignable for this assessment but included, as additional information, regarding the possible incapability of soil microorganisms to degrade the 2-nitrotoluene, even after a period enough for the acclimation of soil microorganisms.

According to equilibrium partitioning equations, degradation half-life for (bulk) soil, partly based on $K_{p_{soil}}$ is followed. Measured $K_{p_{soil}}$ values are preferred, but as not available, a calculated $K_{p_{soil}} = 4.19$ l/kg for 2-nitrotoluene (see section 3.1.3.2.1) will be used for estimations. And so, according to the Technical Guidance Document, the half-life for the soil compartment will be assumed to be 300 days.

Regarding the degradability on sediment compartment, and following the equilibrium partitioning method according to the Technical Guidance Document equations:

$$K_{bio_{sed}} = \ln 2 \cdot Fa_{er_{sed}} / DT50_{bio_{soil}}$$

DT50_{bio_{soil}} half-life for biodegradation in bulk soil (days).
 K_{bio_{sed}} first order rate constant for degradation in bulk sediment (days⁻¹).
 Fa_{er_{sed}} fraction of the sediment compartment that is aerobic (0.10)

$$K_{bio_{sed}} = \ln 2 \cdot 0.1 / 300 = 2.3 \cdot 10^{-4} \text{ days}^{-1}$$

This implies that the total half-life for the sediment compartment will be a factor of ten higher than the half-life in soil. That means $DT_{50_{bio_{sed}}} = 3,014$ days (8.3 years).

Regarding the information, and in terms of the environmental modelling of the risk assessment, it is assumed the biodegradation rate in sediment and soil compartment given in the Technical Guidance Document, as can be seen in Table 3.6.

Table 3.6: Summary of calculated ultimate biodegradation rate constants in soil and sediment used in EUSES

Compartment	Reaction rate constant	Half-life	Reference
Soil ($K_{p_{soil}} = 4.19$)	$2.3 \cdot 10^{-3} \text{ d}^{-1}$	300 d	TGD
Sediment ($K_{p_{soil}} = 4.19$)	$2.3 \cdot 10^{-4} \text{ d}^{-1}$	3,014 d	TGD

3.1.3.1.4 Summary of environmental degradation

The available biodegradation data show that 2-nitrotoluene can undergo primary biodegradation to form several products. Ultimate mineralisation to form carbon dioxide or methane appears to be low over the timeframe of the available studies (\gg than 4 weeks). Furthermore on the light of the available information, 2-nitrotoluene should be degraded by biological sewage treatment when suitable acclimation is provided to the cultures, so it can be classified as inherent biodegradable (not readily biodegradable).

The ultimate biodegradation rate constants and half-lives that will be used in the environmental modelling are summarized in Table 3.7.

Table 3.7: Environmental degradation

Compartment		Half life
Atmospheric		23 days
Aquatic	Abiotic degradation	24 days
	Biodegradation	∞ days
Sediment		3,014 days
Soil		300 days

Values utilised in EUSES calculations

3.1.3.2 Distribution

According to the fugacity model of Mackay (level 1) the theoretical distribution at equilibrium can be estimated. Nearly 67.6% of the total amount of 2-nitrotoluene is expected to be in the atmosphere and about 31.4% to remain in the water. Only 0.5% is expected to be allocated in the soil compartment and the same percentage is expected to end up in the sediments. Finally, less than 0.01% is expected to be in the suspended sediment and biota (IUCLID Data Set). Therefore, 2-nitrotoluene has a strong tendency to migrate to air and water, whereas potential for migration to soil and sediment is very low.

Table 3.8: Level I environmental partitioning of 2-nitrotoluene

Compartment	Percentage
Air	67.6
Water	31.4
Soil	0.5
Sediment	0.5
Suspended sediment	< 0.01
Biota	< 0.01
Aerosol	< 0.01

Data from IUCLID Data Set

The IUCLID Data Set indicates that the input parameters and the physico-chemical properties used in the Mackay Fugacity Model Level 1 are the following:

- molar mass: 137.13 g/mol
- log K_{ow} : 2.30
- vapour pressure: 19.5 Pa
- water solubility: 437 g/m³
- melting point: -10.6 °C
- volumes used (m³):
 - air: 6·10⁹
 - water: 7·10⁶
 - soil: 4.5·10⁴
 - sediment: 2.1·10⁴
 - susp. sediment: 35.0
 - biota (fish): 7.00
 - aerosol: 0.120

3.1.3.2.1 Adsorption

According to the TGD equation ($\log K_{oc} = 0.77 \cdot \log K_{ow} + 0.55$) and considering a log K_{ow} value of 2.3, the result obtained for K_{oc} is 209.41 l/kg. This K_{oc} value is within the range of the values calculated with the empirical regression equations by Kenega and Goring (1980) published in Lyman (1990) which are 155 (obtained from $\log K_{oc} = -0.55 \cdot \log S + 3.64$, where S = solubility in mg/l, in this case 437 mg/l) and 425 (obtained from $\log K_{oc} = 0.544 \cdot \log P_{ow} + 1.377$, where $P_{ow} = 2.3$). Therefore, a K_{oc} of 209.41 l/kg will be considered in all model calculations of this report. This K_{oc} value suggests that 2-nitrotoluene would be highly mobile in soil and would adsorb slightly to suspended solids and sediments in water.

The K_{oc} value can be used to derive the solid-water partition coefficient (K_p) of 2-nitrotoluene for each compartment, soil, sediment and suspended matter (Equation 23, Chapter 3 of the new TGD). These may also be expressed as dimensionless partition coefficients (Equation 24, Chapter 3 of the new TGD):

$K_{oc} = 209.41$ l/kg	Partition coefficient organic carbon-water
$K_{p_{susp}} = 20.9$ l/kg	Partition coefficient solids-water in suspended matter
$K_{p_{sed}} = 10.5$ l/kg	Partition coefficient solids-water in sediment
$K_{p_{soil}} = 4.19$ l/kg	Partition coefficient solids-water in soil
$K_{soil-water} = 6.48$ m ³ /m ³	Soil-water partitioning coefficient
$K_{susp-water} = 6.14$ m ³ /m ³	Suspended matter-water partitioning coefficient
$K_{sed-water} = 6.04$ m ³ /m ³	Sediment-water partitioning coefficient

The sorption of 2-nitrotoluene from aqueous solution to various natural clay minerals were investigated by Haderlein *et al.* (1996) in batch experiments in the presence of K^+ , Na^+ or Ca^{2+} electrolytes. Adsorption coefficient to clay minerals was determined as $K_d = 4.6$ l/kg classifying as a weakly adsorbing compound. Adsorption to suspended solids and sediments and bioaccumulation in aquatic organisms are not expected to be significant fate processes. In the light of these results, nitro-reduction, an important transformation pathway of NACs in

the subsurface, may enhance the mobility of contaminants due to their lower K_d values. According to Haderlein *et al.* (1996), no correlation between $\log K_d$ and $\log K_{ow}$ values exists, confirming that the hydrophobicity of a given nitroaromatic compound is not a significant factor in determining the specific adsorption of these compounds to clay minerals.

3.1.3.2.2 Precipitation

A 2-nitrotoluene lifetime of 20 days, based upon the reaction with hydroxyl radicals, may lead to a certain removal of 2-nitrotoluene from the atmosphere by precipitation. Even so, it is unlikely to be transported long distances from its point of emission and possible concentrations due to precipitation of 2-nitrotoluene from the atmosphere are therefore expected to be larger next to the point of emission.

3.1.3.2.3 Volatilisation

The volatilisation half-life of 2-nitrotoluene from 1 m deep water, flowing 1 m/s with a wind speed of 3 m/s, has been calculated to be 21 hours based on measured Henry's Law constant of 5.6×10^{-5} atm-m³/mole at 25 °C. A vapour pressure of 0.1 mmHg at 20 °C for 2-nitrotoluene suggests that this compound will volatilise slowly from dry soil surfaces (OCDE, 1994).

3.1.3.2.4 Distribution in wastewater treatment plants

The distribution of 2-nitrotoluene in wastewater treatment plants has been obtained using EUSES. The calculation has been done assuming inherently biodegradable conditions for 2-nitrotoluene in the plant and in the environment, as it was said in section 3.1.3.1.2, and it is only valid for industrial wastewater treatment plants, as the biodegradation test on which the rate constant of 0.1 h^{-1} is based was performed with adapted activated sludge.

Table 3.9: Estimated distribution in a sewage treatment plant

Fraction of emission directed to air (%)	1.42
Fraction of emission directed to water (%)	56.8
Fraction of emission directed to sludge (%)	2.25
Fraction degraded (%)	39.5

3.1.3.3 Accumulation and metabolism

Fish (experimentally)

Estimated calculations and values cited in good quality reports have been included in this section, even though it was not possible to validate the original publications. This assumption regards particularly the MITI (1992) and BUA (1989) reports..

Cyprinus carpio was exposed during 42 days to 0.1 mg/l of 2-nitrotoluene resulting in a BCF ranging from 12.5 to 29.9. A similar range (BCF from 6.6 to 29.7) was measured after an exposure of 0.01 mg/l for 42 days (MITI, 1992). A similar value, a BCF *ca.* 20 was cited, for 2-nitrotoluene in guppies (*Poecilia reticulata*), by BUA (1989). Data provided by Canton *et*

al. (1985) give a BCF of 20 for 2-nitrotoluene in the guppy, based on fresh weight (this datum is considered as additional information since no further experimental details are given).

Deneer *et al.*, (1987b) carried out another study on the accumulation of 2-nitrotoluene in the guppy (*P. reticulata*) giving a log BCF of 2.28 (BCF: 190.5), based on fat content, in a 3-day test, using concentration of 6.6 mg 2-nitrotoluene/l. For this bioconcentration test, female guppies (5 to 8 months old) were used. The wet weight varied from 60 to 450 mg at the end of the experiment; mean fat content was $8 \pm 2\%$. This is also in agreement with the information from Hendriks *et al.*, (2001), considering the average percentage of lipid content in *P. reticulata* around 10%. Under these considerations, a BCF of 19 could be estimated for the whole fish. It was previously established, in preliminary experiments, that the type of short-term assay used to determine BCF would not lead to erroneous results, since the concentrations found in fish and water showed that the steady state value for fish $C(\text{fish})/C(\text{water})$ was reached after approximately 6 h. This fact was confirmed by the authors, who indicated no apparent differences between BCFs determined on subsequent days.

In another experiment (Wang *et al.*, 1999), 2-nitrotoluene was selected as a model compound for a laboratory continuous-flow-experiment where the uptake of the compound by the goldfish (*Carasius auratus*) and a triolein SemiPermeable Membrane Device (SPMD) was followed. 40 goldfish and the SPMDs were placed in an exposure chamber. A concentrated mixture of 9 nitroaromatics was added at a constant rate (1 ml/min) into the main water flow to the chamber by a peristaltic pump. The experiment was conducted for 20 days with a concentration of 1.63 mg/l of 2-nitrotoluene in the exposure chamber. Measured, BCF was determined as 4.4 and 312.6 for fish and SPMDs, respectively. Information extracted from these figures is very relevant since it gives an indication of the importance of the rapid elimination metabolism in fish. The higher BCF value estimated from SPMDs would indicate higher accumulation rates in those organisms with a lower elimination metabolism. The dialysis recovery for the nitroaromatic compound from goldfish in recovery studies were from 57% to 71%, and the deviation of the averaged recovery did not exceed 16%. It can be assumed that the final values can be slightly underestimated. Based on the K_{ow} and the uptake rate constant, Wang *et al.*, (1999) estimated the time to reach 90% steady-state concentration, in SPMD and in goldfish, in 4.08 and 6.64 days, respectively, and the elimination rate in 0.57 (hours^{-1}) and 0.35 (hours^{-1}) for SPMDs and goldfish, respectively.

Canton *et al.* (1985) determined experimentally a BCF of 20 for 2-nitrotoluene on fish (*P. reticulata*).

Fish (calculated):

The log K_{ow} (2.3) for 2-nitrotoluene indicates a weak bioaccumulation potential. According to the TGD, BCF data for fathead minnows (*P. promelas*) for substances with $\log K_{ow} < 6$ can be estimated following the equation:

$$\text{Log BCF} = 0.85 * \log K_{ow} - 0.70 = 0.85 * 2.3 - 0.70 \quad \mathbf{BCF = 17.99}$$

Additionally, Canton *et al.*, (1985) calculated a theoretically value of $\text{BCF} = 39$, according to the view of octanol/water partition coefficient, in aquatic species using the formula $\log \text{BCF} = 0.76 * \log P_{ow} - 0.23$. This value was not considered for the assessment, since experimental data and a calculated value according to TGD are available.

Sabljić (1987) predicted the bioconcentration factor of 2-nitrotoluene from the molecular connectivity model $\log \text{BCF} = 1.58$ ($\text{BCF} = 38$), calculated by the computer program GRAPH

III. Molecular connectivity is a method of describing molecular structure based solely on bonding and branching patterns rather than physical or chemical characteristics.

In summary, the biconcentration factor (BCF) for 2-nitrotoluene has been experimentally determined to be between 4.4 (based on fat content) and 29.9. BCF values of 17.99 and 39 have been calculated based on different models using the log octanol water partition coefficient ($\log K_{ow}$) of 2.30. These BCF values suggest that 2-nitrotoluene can be considered as non accumulative ($BCF < 100$) and will not bioaccumulate significantly in fish. Table 3.10 compiles information on the different bioaccumulation studies considered within this section.

Table 3.10: Different bioaccumulation studies results for 2-nitrotoluene

Specie	Exp. Period (days)	Conc. (mg/l)	BCF	Elimination rate (hours ⁻¹)	Comments	Reference
<i>Cyprinus carpio</i>	42	0.1	12.5-29.9	-	V	MITTI, 1992
<i>Cyprinus carpio</i>	42	0.01	6.6-29.7	-	V	MITTI, 1992
<i>Carassius auratus</i>	20	1.63	4.4	0.35	V	Wang <i>et al.</i> , 1999
<i>Poecilia reticulata</i>	3	6.6	190.5 (based on fat content)	-	V	Deneer <i>et al.</i> , 1987
<i>Poecilia reticulata</i>	-	-	20	-	V	Canton <i>et al.</i> , 1985
<i>Poecilia reticulata</i>	-	-	ca. 20	-	V	BUA, 1989
Aquatic species	-	-	39	-	NA Calculated value	Canton <i>et al.</i> , 1985
Aquatic species	-	-	38	-	NA Calculated value	Sabljić (1987)
Aquatic species	-	-	17.99	-	VWR Calculated value accor. to TGD	This report

V: Valid; VWR: Valid With Restrictions; NA: Not Assignable.

Mammals (experimentally)

Following oral administration to male F344 rats, 2-nitrotoluene is rapidly and extensively absorbed, from 60% (Elwell, 1996) to 95% (Chism *et al.*, 1984), from the gastrointestinal tract by male F344 rats, as indicated by 73-86% excretion radioactivity in the urine after 24 h after a single gavage administration of 2-nitrotoluene (200 [¹⁴C]mg/kg) (Chism *et al.*, 1984). According to these authors, after 72 h most of the compound (80-90%) has been excreted, being the urine the major route of excretion with 70-85% of the dose being excreted by that route (faeces: 4.6% and expired air: 0.1%). So, it can be assumed a half-life less than 24 h in rats. The maximum excretion rates of the metabolites of 2-nitrotoluene that had not undergone nitro group reduction occurred within the first 4 h following the administration.

According to de Bethizy and Rickert (1984), the metabolism of each nitrotoluene appears to be initially oxidized at the methyl group, by a cytochrome P-450-dependent process, in the hepatic microsomes to the respective nitrobenzyl alcohols, which are biotransformed by three

different routes, depending on the nitrotoluene isomer: (i) o-nitrobenzyl alcohol, the principal metabolite of 2-nitrotoluene, was primarily conjugated with glucuronic acid (28% of the metabolized nitrotoluene), (ii) a smaller percentage (3%) is oxidized to nitrobenzoic acid in the first pathway in the hepatocytes and, (iii) the third pathway is conjugation with glutathione.

2-nitrotoluene, but not 3-nitrotoluene nor 4-nitrotoluene, is genotoxic due to hepatic metabolism. 2-nitrobenzyl alcohol is primarily converted to 2-nitrobenzyl alcohol glucuronide. This is also the metabolic pathway followed by the benzyl alcohols arising from hepatic metabolism of 2,4-dinitrotoluene (Rickert *et al.*, 1981; Long and Rickert, 1982). The glucuronide is excreted by the females mainly in the urine and by the males mainly in the bile (Chism and Rickert, 1985; Rickert, 1987). Metabolites secreted in the bile are reduced by intestinal bacteria at the nitro group and reabsorbed from the intestine and further metabolised by hepatic enzymes generating reactive products in the liver (Rickert *et al.*, 1981; Bond *et al.*, 1981; Long and Rickert, 1982). These reactive metabolites form covalent bonds with macromolecules in the rat liver DNA. It is indicative the fact that, in male rats three times as much 2-nitrotoluene was covalently bound to hepatic macromolecules as in female rats (BUA, 1989). These can be the reason due to which female rats are less susceptible than males to the hepatic genotoxic effects of 2-nitrotoluene. It is possible that a similar mechanism of bioactivation is operative with 2-nitrotoluene.

In this sense, alteration of the gastrointestinal flora by daily gavage administration of antibiotics did not affect the pattern or severity of toxicity at any site or the development of mesothelioma in rats exposed to 2-nitrotoluene, although cholangiocarcinomas that occurred in rats with the normal flora did not occur in groups with the altered intestinal flora. Antibiotic regime had little effect on obligate anaerobes, which are thought to play a major role in nitro group reduction (Elwell, 1996).

According to the BUA (1989), differences in metabolism could not be attributed to microsomal mechanisms, but rather to differing reaction rates with glucuronyl transferase.

Recent studies on the metabolism of cetaceans indicate that these marine mammals' detoxifying capacity is limited. In fact, these animals have a low capacity for degradation of certain compounds due to a specific mode of their cytochrome P450 enzyme system (Tanabe and Tatsukawa, 1992). Significant differences in Mixed Function Oxidase Activity (BPMO) levels have been found in some marine mammals (Fossi *et al.*, 2000; Fossi *et al.*, 2001).

These metabolic disadvantages could make marine mammals, and other organisms in the same circumstances, more susceptible to the effects of certain contaminants.

Summary of accumulation data

The BCF for 2-nitrotoluene has been experimentally determined to be between 4.4 and 29.9. BCF values of 17.99 and 39 have been calculated based on different models using the log octanol water partition coefficient ($\log K_{ow}$) of 2.30.

Bioconcentration data are only available for freshwater fish. A BCF of 20 has been chosen as an average value for further calculations. This value suggests that 2-nitrotoluene can be considered as a substance with low accumulative potential ($BCF < 100$), and will not bioaccumulate significantly in fish, due to the rapid elimination metabolism in these organisms (elimination rate of 0.35 hours^{-1} ; half life of *ca.* 2 hours).

Regarding mammals, 2-nitrotoluene is rapidly and extensively absorbed up to 95% from the gastrointestinal tract by male F344 rats, being metabolized initially by a cytochrome P-450-dependent process. Most of compound (80-90%) is excreted after 72 h, being the urine the major route of excretion. So, according to this information, it seems that, in general, 2-nitrotoluene has also a low accumulative potential in mammals (rats F344). Although it should be accounted the metabolic differences of some species, especially regarding marine mammals, since there are indications about the fact that some cetaceans have a low detoxifying capacity for some chemicals due to a specific mode of their cytochrome P-450 enzyme system.

3.1.4 Aquatic compartment (incl. sediment)

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

3.1.4.1.1 Calculation of PEC_{local} for production and processing

Releases from production are considered altogether with releases from processing, because production and processing happen one just after the other in the same place in sites A and C. Therefore, only a PEC_{local} for production and processing is calculated with EUSES for these sites, using the equations given in the TGD. In site B, there is not a production step and consequently only a PEC_{local} for processing has been estimated.

In site C, the flow of 37,152 m³/d of waste water indicated by industry has been used. Waste waters are discharged into a lagoon, and as no dilution factor has been provided, the default factor of 10 given in the TGD has been used. Due to the fact that the place is located at a coastal zone, the marine exposure will be assessed.

Finally, the dilution factor for sites A (698) and B (494) was calculated with data provided by industry. The flow of the WWTP was provided as well, and it is 130,000 m³/d and 3,850 m³/d, respectively.

$$PEC_{local(water)} = C_{local(water)} + PEC_{regional(water)}$$

$$C_{local(water)} = C_{eff} / (1 + K_{p_{susp}} \cdot C_{susp} \cdot 10^{-6}) \cdot D$$

$$C_{eff} = W \cdot (100 - P) / 100 \cdot Q$$

where:

$C_{local(water)}$ = local concentration in surface water during emission episode

$PEC_{regional(water)}$ = regional concentration in surface water

C_{eff} = concentration of the chemical in the WWTP effluent

$K_{p_{susp}}$ = solids-water partitioning coefficient of suspended matter

C_{susp} = concentration of suspended matter in the river

D = dilution factor

W = emission rate (kg/day)

P = percentage removal in the WWTP (%)

Q = volume of waste water

Considering that 2-nitrotoluene is inherently biodegradable and taking into account release data provided by industry for site A, the PEC_{local} in surface water calculated for 2-nitrotoluene production and processing is $6.58 \cdot 10^{-3}$ µg/l. In groundwater, the PEC_{local} under agricultural

soil is $6.88 \cdot 10^{-3}$ $\mu\text{g/l}$, and the $\text{PEC}_{\text{local}}$ in sediment during emission episode is $3.51 \cdot 10^{-2}$ $\mu\text{g/kg}$ wet wt.

In site B, where only processing takes place, the $\text{PEC}_{\text{local}}$ in surface water calculated from emissions indicated by industry is $1.12 \cdot 10^{-2}$ $\mu\text{g/l}$. In groundwater, the $\text{PEC}_{\text{local}}$ under agricultural soil is $0.195 \mu\text{g/l}$, considering spreading of sludge, and the $\text{PEC}_{\text{local}}$ in sediment during emission episode is $5.99 \cdot 10^{-2}$ $\mu\text{g/kg}$ wet wt.

Finally, the $\text{PEC}_{\text{S}_{\text{local}}}$ obtained for site C, which is the one with the highest amount of 2-nitrotoluene produced and processed, are $1.66 \mu\text{g/l}$ in surface water, $0.145 \mu\text{g/l}$ in groundwater under agricultural soil and $8.83 \mu\text{g/kg}$ wet wt in sediment during emission episode.

Table 3.11: Predicted levels in aquatic compartment

Compartment	Site	Concentration
Surface water	A	$6.58 \cdot 10^{-3} \mu\text{g/l}$
	B	$1.12 \cdot 10^{-2} \mu\text{g/l}$
	C	$1.66 \mu\text{g/l}$
Ground water	A	$6.88 \cdot 10^{-3} \mu\text{g/l}$
	B	$0.195 \mu\text{g/l}$
	C	$0.145 \mu\text{g/l}$
Sewage Treatment Plant	A	$2.49 \cdot 10^{-3} \text{mg/l}$
	B	$3.98 \cdot 10^{-3} \text{mg/l}$
	C	$1.65 \cdot 10^{-2} \text{mg/l}$
Sediment	A	$3.51 \cdot 10^{-2} \mu\text{g/kg wet wt}$
	B	$5.99 \cdot 10^{-2} \mu\text{g/kg wet wt}$
	C	$8.83 \mu\text{g/kg wet wt}$

Regarding the marine environment, the default emission factors proposed in the TGD have been applied for site C, which is located at a coastal zone, and the resulting PECs in sea water and marine sediment during emission episode are $0.29 \mu\text{g/l}$ and $1.55 \mu\text{g/l}$, respectively.

3.1.4.2 Measured levels

Several measured exposure data have been gathered in relation with production and use of 2-nitrotoluene within the EU, all of them taken in Rhine and Elbe rivers. In Rhine river, values of $< 0.02 - 0.35 \mu\text{g/l}$ were measured in Wiesbaden, $0.05 - 0.37 \mu\text{g/l}$ in Köln, $0.08 - 0.46 \mu\text{g/l}$ in Düsseldorf (BUA, 1989) and $\leq 2.45 \mu\text{g/l}$ in Lippe, all in West Germany (LWA, 1990). In Elbe river the monitoring data come from Schmilka, $0.26 \mu\text{g/l}$ in the left bank and $0.20 \mu\text{g/l}$ in the right; Zehren, $0.11 \mu\text{g/l}$ in the left bank and $0.12 \mu\text{g/l}$ in the right; Domnitzsch, $0.11 \mu\text{g/l}$ and Schnackenburg, $0.4 \mu\text{g/l}$. These monitoring data are relatively old and as no information on the conditions of the measurements and their proximity to the production and processing sites has been found, they cannot be assigned to the local emission sites.

More recent monitoring data are available from 1999 for three German rivers, Elbe, Rhin and Danube, in Umweltpolitik Wasserwirtschaft in Deutschland, Teil II Gewässergüte oberirdischer Binnengewässer, and they are the followings: $< 0.02 \mu\text{g/l}$ in river Danube, $< 0.5 \mu\text{g/l}$ in river Rhine and $0.06 \mu\text{g/l}$ in river Elbe. According to industry information, no 2-

nitrotoluene has been detected in the river Rhine, with a detection limit of 0.5 µg/l over the last 10 years.

Regarding ground water, some measured levels from the Netherlands, 4.5, 18.1 and 0.3 µg/l (Meijers *et al.*, 1976) and one from Germany, 10 µg/l (Howard, 1976), have been reported. These values are old, but they are the only ones available for comparison. Related to the sediment, only values for Japan have been found. The concentration measured was 3.4 - 140 µg/l (BUA, 1989).

Table 3.12: Measured levels in aquatic compartment

Compartment	Site	Concentration	Year
Surface water	Brakel; The Netherlands (Waal) ^a	3.1 – 16 µg/l	1972
	Maasluis; The Netherlands (Rhine) ^a	1 – 10 µg/l	1978
	Lobith; The Netherlands (Rhine) ^a	3 – 10 µg/l	1978
		1 – 3 µg/l	1979
		3.0 µg/l (max.)	1981
		1.6 µg/l (max.)	1982
		2.0 µg/l (max.)	1983
		0.8 µg/l (max.)	1984
	Gorinchem; The Netherlands (Rhine) ^a	3 µg/l (max.)	1981
	Wiesbaden; Germany (Rhine) ^a	< 0.02 - 0.35 µg/l	1987
	Köln; Germany (Rhine) ^a	0.05 - 0.37 µg/l	1987
	Düsseldorf; Germany (Rhine) ^a	0.08 - 0.46 µg/l	1987
	Lippe; Germany (Rhine) ^b	≤ 2.45 µg/l	1989
	Schmilka; Germany (Elbe) ^c	0.15 µg/l	1997
		0.26 µg/l	
		0.20 µg/l	
	Zehren; Germany (Elbe) ^c	0.11 µg/lc	1997
Domnitzsch; Germany (Elbe) ^c	0.11 µg/lc	1997	
Schnackenburg; Germany (Elbe) ^c	0.4 µg/lc	1997	
Danube ^d	< 0.02 µg/l (90%)	1999	
Rhine ^d	< 0.5 µg/l (90%)	1999	
Elbe ^d	0.06 µg/l (max.)	1999	
Ground water	The Netherlands (Waal) ^e	4.5 µg/l (avg.)	1974
	The Netherlands (Waal) ^e	18.1 µg/l (max.)	1974
	The Netherlands (Maas) ^e	0.3 µg/l (max.)	1974
	Germany (Rhine) ^f	10 µg/l	-
Sediment	Japan ^a	3.4 - 140 µg/l	1976

^a BUA, 1989

^c Wassegütedaten der Elbe, Zahlentafeln 1997

^e Meijers *et al.*, 1976

^b LWA, 1990

^d Umweltpolitik Wasserwirtschaft in Deutschland, 2001

^f Howard, 1976

3.1.4.3 Comparison between predicted and measured levels

A clear trend could be seen in the measured levels reported in table 3.12, which is the result of the technical improvements in production and processing and the better WWTP technology.

However, most of the monitoring data are relatively old and as no information on the conditions of the measurements and their proximity to the production and processing sites has been found, they cannot be assigned to the local emission sites. Regarding the more current values, that information has not been supplied either. Even so, these data can provide, at least, a basis for comparing the calculated exposure data with the orders of magnitude which are to be expected.

Therefore, compared with the monitoring levels recently obtained for the three German rivers, predicted concentrations of 2-nitrotoluene in surface water of site A are lower than the values measured. Concerning site B, with a smaller amount processed than the other ones, the PEC_{local} in surface water calculated is similar to the measured levels. On the other hand, PEC_{local} in surface water of site C is higher than the monitoring ones.

Regarding PEC_{local} in ground water, the values of sites A, B and C are below the monitoring data from the Netherlands and Germany, especially the value obtained for site A.

Finally, only some old data of sediment levels are available from Japan showing a wide range of measured values. PECs local calculated with EUSES for sites A and B are lower than those reported, whereas values for site C are in the range.

3.1.5 Terrestrial compartment

3.1.5.1 Calculation of PEC_{local}

According to the information provided by industry of sites A and C, no spreading of sewage sludge from an industrial sewage treatment is done. Therefore, PECs have been calculated with EUSES for natural soil, agricultural soil and grassland, only considering atmospheric deposition. The calculated Koc indicates that if 2-nitrotoluene is released to soil, it is not expected to geoaccumulate significantly.

However, industry from site B has indicated that the sludge from the sewage treatment plant is sent off-site for composting prior to spreading on land as an agricultural fertilizer.

3.1.5.1.1 Calculation of PEC_{local} for production and processing

Table 3.13: Predicted levels in terrestrial compartment (in $\mu\text{g}/\text{kg}$ wet wt)

	Site A	Site B	Site C
Soil (total) averaged over 30 days	0.0262	0.903	0.551
Agricultural soil (total) averaged over 180 days	0.0262	0.742	0.551
Grassland (total) averaged over 180 days	0.0434	0.311	0.916

3.1.5.2 Measured levels

No monitoring data of concentration of 2-nitrotoluene in soil have been found.

3.1.6 Atmosphere

3.1.6.1 Calculation of PEC_{local}

The PEC_{local} is calculated with the method described in the TGD Chapter 3, section 2.3.8.2. For PEC_{local} calculation, emissions from point sources and sewage treatment plants need to be considered. The sewage treatment plant is assumed to be a point source and the concentration of the chemical is calculated 100 m off. The indirect emission of a chemical from a sewage treatment plant to air is calculated from the fraction of emission directed to air. For 2-nitrotoluene this is 1.42% (see Table 3.9). Direct releases to air have been reported by industry as being 0.07 kg/day.

The direct emission to air is multiplied by the concentration in air at a source strength of 1 kg/d ($C_{std_{air}} 2.78 \cdot 10^{-4} \text{ mg/m}^3$) to give the local air concentration. The local concentration can be converted to an annual concentration if the number of emission days per year is known.

$$C_{local_{air}} = \max(E_{local_{air}}, Estp_{air}) \cdot C_{std_{air}}$$

$$C_{local_{air,ann}} = C_{local_{air}} \cdot T_{emission} / 365$$

where:

$E_{local_{air}}$: Local direct emission rate to air during emission episode

$Estp_{air}$: Local indirect emission to air during emission episode

$T_{emission}$: Number of days per year that the emission takes place

The PEC_{air} for the regional and continental scenarios is calculated using EUSES. The regional PEC is taken as a background concentration and added to the local concentrations to give the PEC_{local} .

3.1.6.1.1 Calculation of PEC_{local} for production and processing

The annual average PEC_{local} in air (total) obtained with EUSES from data of emission reported by industry of site A is $0.0167 \mu\text{g/m}^3$. As no information on the emission to air of site B has been reported, the estimation of PEC_{local} has been done with default parameters of EUSES, and a value of $0.0285 \mu\text{g/m}^3$ has been obtained.

Regarding site C, all air steams are sent to an incinerator and industry has indicated that values of total organic carbon measured in the air emissions are always below 0.5 mg/m^3 , as said in section 3.1.2.1. Nevertheless, the default factors indicated in the A-tables have been considered too, because no data on the volume emitted have been supplied and therefore, the PEC_{local} obtained is $0.375 \mu\text{g/m}^3$.

3.1.6.2 Measured levels

Measured levels of concentration of 2-nitrotoluene in air have not been found in Europe. Two monitoring data have been provided, one from Japan, $44 \mu\text{g/m}^3$ (Kosuge, 1993) and another

from USA, $0.047 \mu\text{g}/\text{m}^3$. These data are very different and no further information on the monitoring details has been obtained.

3.1.6.3 Comparison between predicted and measured levels

Considering the releases to air indicated by the industry, the value of $\text{PEC}_{\text{local}}$ obtained for site A is of the same order of magnitude but lower than the lowest monitoring data, measured in the USA, and this may be explained by the existence of an air purification unit (information given by industry). Considering the $\text{PEC}_{\text{local}}$ of site B, it is somewhat smaller than the data from the USA. All these values are, nevertheless, far away from the one from Japan. Regarding site C, predicted value with default EUSES values is higher than the level measured in the USA, but it is also distant from the Japanese monitoring value.

3.1.7 Secondary poisoning

According to the Technical Guidance Document, the risk characterisation for secondary poisoning is required if three specific criteria/situations are fulfilled.

These criteria can be summarized as:

- Indirect exposure likely,
- Indication of bioaccumulation potential and,
- Potential to cause toxic effects if accumulated in higher organisms.

2-nitrotoluene is toxic for mammalian species. Some critical effects of concern in higher animals are *in vivo* genotoxicity and mutagenicity, “presumptive” carcinogenicity and reproductive toxicity. But the assessment of the potential impact of substances on top predators is based on the accumulation through the food chain and, regarding this point, 2-nitrotoluene has a low potential for bioaccumulation which indicates that secondary poisoning is of low concern.

According to the TGD strategy for secondary assessment, for substances with a $\log K_{\text{ow}} < 4.5$ the primary uptake route is directly from the water phase. In the absence of data on other uptake routes, it is assumed that the direct uptake accounts for 100% of the intake. This strategy takes into account the $\text{PEC}_{\text{aquatic}}$, the direct uptake, and the resulting concentration in food-prey of aquatic organisms and its toxicity for higher mammalian and avian oral-predators.

$$\text{PEC}_{\text{oral,predator}} = \text{PEC}_{\text{water}} \cdot \text{BCF}_{\text{fish}} \cdot \text{BMF}$$

According to the TGD it has been applied the more appropriate scenario where 50% of the diet comes from a local area and 50% of the diet comes from a regional area. So, the average $\text{PEC}_{\text{water}}$ value has been considered within the assessment. $1.66 \cdot 10^{-3} \text{ mg/l}$ has been selected as worst case scenario for the local $\text{PEC}_{\text{water}}$, while calculated $\text{PEC}_{\text{regional}}$ is $3.39 \cdot 10^{-6} \text{ mg/l}$. This value was calculated for site C, according to the TGD default values:

$$\text{PEC}_{\text{oral,predator}} = [(1.66 \cdot 10^{-3}/2) + (3.39 \cdot 10^{-6}/2)] \cdot 20 \cdot 1 = 1.66 \cdot 10^{-2} \text{ mg/kg}$$

According to the low bioaccumulation potential and the rapid elimination of this compound in fish and mammals, no secondary poisoning potential is expected from this substance.

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

The calculations of PECs at a regional and continental scale were done using the EUSES model and the values obtained for the worst-case scenario of a regional production of 2-nitrotoluene. The results are shown below:

Table 3.14: Regional and continental PECs

Compartment	Regional	Continental
Surface water (total) ($\mu\text{g/l}$)	$3.39 \cdot 10^{-3}$	$4.42 \cdot 10^{-5}$
Surface water (dissolved) ($\mu\text{g/l}$)	$3.39 \cdot 10^{-3}$	$4.42 \cdot 10^{-5}$
Air ($\mu\text{g/m}^3$)	$3.45 \cdot 10^{-5}$	$4.73 \cdot 10^{-6}$
Agricultural soil (total) ($\mu\text{g/kg wet wt}$)	$1.97 \cdot 10^{-3}$	$3.47 \cdot 10^{-5}$
Pore water of agricultural soils ($\mu\text{g/l}$)	$5.17 \cdot 10^{-4}$	$9.09 \cdot 10^{-6}$
Natural soil (total) ($\mu\text{g/kg wet wt}$)	$2.87 \cdot 10^{-4}$	$3.93 \cdot 10^{-5}$
Industrial soil (total) ($\mu\text{g/kg wet wt}$)	0.643	$5.7 \cdot 10^{-3}$
Sediment (total) ($\mu\text{g/kg wet wt}$)	0.0173	$2.38 \cdot 10^{-4}$

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

3.2.1 Aquatic compartment (incl. sediment)

The provided information includes a set of data on the toxicity of 2-nitrotoluene only for fresh water organisms. No information has been provided regarding toxicity on marine organisms.

Provided data have been summarised in tables 3.15, 3.16 and 3.17 for aquatic vertebrates, aquatic invertebrates and algae, respectively. It has not been possible to validate all data going back to the original publications (particularly in relation to fish test), and some values have been included in the assessment when cited in good quality reports. Other non-validated data have been used as additional information to support the assessment produced from the available reports. Values used to establish the proposed PNEC have been checked and validated against the original publications.

3.2.1.1 Toxicity test results

3.2.1.1.1 Fish

In this section, the toxicity test results on fish are presented. Comments on tests have been focused especially on those which have been considered for the assessment. Provided information has been summarized in Table 3.15.

Acute toxicity

Ramos *et al.*, (1998) determined an EC₅₀ for guppies (*Poecilia reticulata*) of 30.1 mg/l. Acute toxicity tests on fish were performed according to the OECD guideline 203, applying 12 h of photoperiod during the experiments. Animals were starved from 24 h before the tests. 5 concentration levels were tested with a dilution factor of 2.

Deneer *et al.*, (1987) gave an LC₅₀ = 32.9 mg/l for the chronic effects of 2-nitrotoluene to guppies in a 14-day test. Fish used in the toxicity experiments were male and female guppies, varying in age from two to three months, and in weight from 60 to 450 mg (mean fat content 8 ± 2%). Procedure for obtaining the 14d-LC₅₀ values is according to Könemann (1981). Water samples were taken regularly on, at least, four days during the experimental period, both before and after renewal of the solutions and analysed after hexane extraction. Upon analysis after 24 h of exposure, up to 40% of the compound added was converted into the corresponding aniline. This effect only occurred when fish were present in the solution. Nitroaryl compounds belong to the class of bioactivated chemicals, since they are reduced *in vivo* to highly active intermediates like arylnitroso-compounds and arylhydroxylamines. Therefore, it seems very likely that an adequate description of their toxicity involves their tendency to be reduced.

Bailey and Spanggord (1983) conducted ecotoxicity tests, with several individual substances, on fathead minnows (*Pimephales promelas*). The test fish were juveniles averaging 2.4 cm in

total length and 0.28 g in weight. Fish were not fed for 24 h before or during any of the tests. As a general rule, test solutions were aerated only when DO dropped below 60% of saturation in the first 48 h of exposure or below 40% of saturation in the last 48 h of exposure. It is not indicated whether this is the case for 2-nitrotoluene, since aeration which could introduce a disturbing procedure that could lead to the disappearance of the test substance from the water. The 96h-LC₅₀ was determined in 37.1 mg/l (ranging from 34.6 to 39.9 mg/l of 2-nitrotoluene). Toxicity appears to be related to the ease of reduction of the nitro group to form nitroso free radicals, which would then block life-supporting processes. Although reduction is believed to be an important pathway for detoxifying foreign nitro-bodies, the ease of formation of intermediate free radicals may overwhelm protective mechanisms available to the organisms.

In other experiment, carried out by Liu *et al.* (1983), it is provided a 96h-LC₅₀ of 37.1 mg/l (ranging from 34.6 to 39.9 mg/l of 2-nitrotoluene). Acute toxicity tests were performed on *P. promelas* under static conditions. Fish were immature and no food was provided to the organisms during the test. These figures are in agreement with Bailey and Spanggard (1983) results.

Canton *et al.* (1985) performed a 24-h short-term toxicity tests on *Poecilia reticulata*, in analogy with the OECD (1979) proposal, resulting in an LC₅₀ of 29 mg/l and in a EC₅₀ (behaviour) of 18 mg/l. To obtain an indication of the biological availability of the test compound, the decline of a non-aerated standardized medium was studied at room temperature. A recovery of 92% after 8 days was measured. Other short-term tests, performed with *Oryzias latipes* by the same authors, resulted in an LC₅₀ of 37 mg/l and an EC₅₀ (behaviour) of 7 mg/l. Test duration is not given in Canton *et al.* (1985) itself, but reference concerning the tests methods is made to other publications of Canton and his co-workers. In these publications exposure of fish in short-term tests was either 48 or 96 h. Test concentrations were adjusted 3 times a week.

Bayer (1986) also provides information within the IUCLID database regarding an exposure period of 96 hours in a static test, giving an EC₅₀ of 65 mg/l on *Brachidario rerio*. This information has not been considered for the assessment, since there was no possibility to check the original report.

Yoshioka *et al.* (1986) indicated an EC₅₀ of 86 mg/l for *Oryzia latipes*, after an exposure period of 48 hours. A two days exposure time test was considered not assignable for the assessment.

Canton *et al.* (1985) referred some information on semi-chronic toxicity tests after an exposure period of 28 days resulted in both, an LC₅₀ and EC_{50S} (behaviour) of 9.4 mg/l. The NOEC (mortality and behaviour) was determined as 1.9 mg/l.

Juhnke and Lüdemann (1978) also presented some data on *Leuciscus idus*, which have been considered as not assignable, since not enough information has been provided for its validation.

Long-term toxicity

There is no information provided on long-term toxicity on aquatic vertebrates.

Table 3.15: Toxicity of 2-nitrotoluene to aquatic vertebrates

Species	Test type	Measured/ Nominal	Test	Duration	Toxicity Endpoint (mg/l)	Comments	Reference
Short-term results							
<i>Poecilia reticulata</i>	Semistatic	Measured	OECD 203	96 h	LC ₅₀ = 30.1	V	Ramos, EU <i>et al.</i> , 1998
<i>Poecilia reticulata</i>	Semistatic	Measured	Other	14 d	LC ₅₀ = 32.9	V	Deener, <i>et al.</i> , 1987
<i>Poecilia reticulata</i>	Static	Nominal	Other	24 h	LC ₅₀ = 29 EC ₅₀ (behaviour) = 18	NA	Canton <i>et al.</i> , 1985
<i>Brachydario rerio</i>	Static	-	-	96 h	LC ₅₀ = 64.9	NA	Bayer AG, 1986
<i>Leuciscus idus</i>	Static	-	-	48 h	LC ₀ = 11 LC ₅₀ = 29 LC ₁₀₀ = 57	NA	Juhnke and Lüdeman, 1978
<i>Pimephales promelas</i>	Static Aerated	Nominal	-	96 h	LC ₅₀ = 37.1 (34.6-39.9)	V	Bailey and Spanggard, 1983
<i>Pimephales promelas</i>	Static	Measured	-	96 h	LC ₅₀ = 37.1 (34.6-39.9)	V	Liu <i>et al.</i> , 1983
<i>Oryzias latipes</i>	Static	Nominal	Other	48 h-96 h	LC ₅₀ = 7.0 EC ₅₀ (mort + behaviour) = 37	NA	Canton <i>et al.</i> , 1985
<i>Oryzias latipes</i>	-	-	Other	48 h	LC ₅₀ = 86	NA	Yoshioka, <i>et al.</i> , 1986
<i>Oryzias latipes</i>	Static	Nominal	Other	28 d	LC ₅₀ = 9.4 EC ₅₀ (behaviour) = 9.4	Additional information	Canton <i>et al.</i> , 1985
<i>Oryzias latipes</i>	Static	Nominal	Other	28 d	NOEC (mortality and behaviour) = 1.9	Additional information	Canton <i>et al.</i> , 1985

V: Valid; VWR: Valid With Restrictions; NA: Not Assignable.

3.2.1.1.2 Aquatic invertebrates

In this section, toxicity test results on aquatic invertebrates are presented. Comments on tests have been focused especially on those considered for the assessment. Provided information has been summarized in Table 3.16.

Acute toxicity

Ramos *et al.*, (1998) published several toxicity data of 2-nitrotoluene on the water flea (*Daphnia magna*) and the pond snail (*Lymnaea stagnalis*). Toxicity tests were performed according to the OECD guidelines for testing chemicals. The authors provided a 24h-EC₅₀ of 13.2 mg/l (12.0-14.5 mg/l) and a 48h-EC₅₀ of 12.3 mg/l (10.9-13.9 mg/l) on *Daphnia magna*. The result corresponding to the standard exposure period of 24 hours will not be considered for the assessment. The test for the snail *L. stagnalis* was adapted from the one described for fish (OECD 203 guideline): a 96 hours lethality test performed at 21-24 °C in semi-static conditions. The authors provided different results at different exposure times.

Deneer *et al.*, (1989) performed a 48-h immobilisation test for *D. magna* in static test conditions, according to the protocol of the Dutch Standards Organization, NEN 6501 (1980). During the tests, daphnids were fed with *Chlorella pyrenidosa*, which at the start of the experiments was present at a concentration of $1 \cdot 10^8$ cells/l. All daphnids used were <24 hours old at the beginning of the experiments. A 48h-EC₅₀ of 10.9 mg/l was determined. Another value of 21d-IC₅₀ of 7.4 mg/l for *Daphnia magna* was also established.

Liu *et al.*, (1983) performed a 48-h exposure static test on *Daphnia magna*. It is indicated that no food was provided to the test organisms during the test, but no full description of the test procedure is shown. An LC₅₀ >77mg/l is provided.

In other paper, Canton *et al.* (1985) carried out short-term toxicity tests on crustaceans (*D. magna*) in analogy with the OECD 202 proposal (1979). The authors provided LC₅₀ (immobilisation) values ranging between 5.4 and 8.8 mg/l after a 2 days exposure period. Additional information cited in the paper provides (semi)chronic toxicity test information on immobilisation ranging from 2.6 and 7.5 mg/l on *Daphnia magna*. No information regarding the exposure period is provided.

Zhao and Wang (1995) carried out an acute short test on *Daphnia magna*. *Daphnia magna* was cultured partenogenetically in an environmental chamber at 22 ± 1 °C, with a photoperiod of 14 daylight/10 hours darkness. Daphnids were fed a diet of green algae and 6-24 hours old Daphnids were used for toxicity tests. Acute toxicity tests were conducted with a static method for 24 hours, with 10 daphnids in 25 ml of test water. It produced a 24h-EC₅₀ of 3.91 mg/l. This result has been included as additional information, but has been considered as not assignable for the assessment.

Bringmann and Kühn (1981), carried out an acute short test on *Daphnia magna*. The effects of 2-nitrotoluene were measured on 24-h individuals after 24-h exposure period. Following results are provided: an EC₀ of 6.8 mg/l, an EC₅₀ of 16 mg/l (14-18 mg/l) and an EC₁₀₀ of 26 mg/l. This result has been included as additional information, but considered as not assignable for the assessment.

Long-term toxicity

Deneer *et al.* (1989) carried out a study on *D. magna*, in semi-static test conditions, following the protocol of the Dutch Standards Organization NEN 6502 (1980). Test organisms were fed with $3 \cdot 10^8$ cells/l·day of *Chlorella pyrenoidosa*. All daphnids used were < 24 h old at the start of the experiments. A 21d-LOEC of 1.86 $\mu\text{mol/l}$ (21d-LOEC of 9.9 mg/l) was determined for 21-d test as the lowest concentration tested that significantly decreased the length and the intrinsic growth rate of *Daphnia magna* population.

Canton *et al.* (1985) determined the following values for *Daphnia magna* after a semi-chronic exposure period: 2.5 mg/l as the concentration that reduced the reproduction in a 50% and, 0.5 mg/l as the no observed inhibition of reproduction.

Table 3.16: Toxicity of 2-nitrotoluene to aquatic invertebrates

Species	Test type	Test	Duration	Toxicity Endpoint (mg/l)	Comments	Reference
Short-term results						
<i>Daphnia magna</i>	Static nominal concentrations	OECD 202	48 h	EC ₅₀ = 12.3 (10.9-13.9)	V	Ramos, EU <i>et al.</i> , 1998
<i>Daphnia magna</i>	Static	OECD 202	24 h	EC ₅₀ = 13.2 (12.0-14.5)	NA Additional information	Ramos, EU <i>et al.</i> , 1998
<i>Daphnia magna</i>	Static	Dutch Standards Organisation NEN 6501(1980)	48 h	EC ₅₀ = 10.9	V	Deener <i>et al.</i> , 1989
<i>Daphnia magna</i>	Static	Other exposure of first instars	48 h	LC ₅₀ >77	NA Additional information	Liu <i>et al.</i> 1983
<i>Daphnia magna</i>	Static	OECD 202	48 h	LC ₅₀ = 5.4 LC ₅₀ = 8.8	V	Canton <i>et al.</i> , 1985
<i>Daphnia magna</i>	-	Other	24 h	EC ₀ = 6.8 EC ₅₀ = 16 (14-18) EC ₁₀₀ = 26	NA NA NA	Bringmann and Kühn., 1981
<i>Daphnia magna</i>	Static	OECD 202	Semichronic	EC ₅₀ (mort + behaviour) = 2.6 LC ₅₀ = 7.5	V	Canton <i>et al.</i> , 1985
<i>Daphnia magna</i>	Static	Other	24 h	EC ₅₀ = 10.01	NA	Zhao and Wang, 1995
<i>Culex pipiens</i>	Static	Adaptation OECD 202	48 h	LC ₅₀ = 22	NA	Canton <i>et al.</i> , 1985
<i>Lymnaea stagnalis</i>	-	Other	48 h	LC ₅₀ = 28	NA	Canton <i>et al.</i> , 1985
<i>Lymnaea stagnalis</i>	Semi-static	Adaptation of the OECD 203	24 h 48 h 72 h 96 h	EC ₅₀ = >65.5 EC ₅₀ = 46.5 EC ₅₀ = 40.3-49.6* EC ₅₀ = 35.9-49.3*	Additional information Additional information Additional information Additional information	Ramos, EU <i>et al.</i> , 1998
<i>Daphnia magna</i>	Semi-static, measured	Immobilisation NEN 6502	21 d	IC ₅₀ = 7.4	V	Deener <i>et al.</i> , 1989
Long-term results						
<i>Daphnia magna</i>	Semi-static	Population growth, NEN 6502	21 d	LOEC = 9.9	V	Deneer <i>et al.</i> , 1989
<i>Daphnia magna</i>	-	OECD 202	Semichronic	NOEC = 0.5	V	Canton <i>et al.</i> , 1985
<i>Daphnia magna</i>	-	OECD 202	Semichronic	EC ₅₀ (reproduction) = 2.5	V	Canton <i>et al.</i> , 1985

V: Valid; VWR: Valid With Restriction; NA: Not Assignable; *95% confidence limits

3.2.1.1.3 Algae

In this section, the results of the toxicity test on algae are presented. Comments on tests have been focused especially on those considered for the assessment. Provided information has been summarized in Table 3.17. When clearly stated, indication of the growth phase of the algae culture has also been included.

Acute and long-term toxicity

Ramos *et al.* (1999) tested the effects on the population growth of the algae *Chlorella pyrenoidosa* according to the OECD 201 guideline (1984). The NOEC was 4.4. Tests were carried out for 72h. Algae were exposed, in triplicate, to five concentration levels and one control. The pH and temperature values in the tests were approximately 7.4 and 22 °C, respectively, and the average growth rate of the controls was 1.0 day⁻¹. The study, considered valid for the assessment, provided an EC₅₀ of 22 mg/l (ranging between 16 and 30 mg/l).

Derneer *et al.* (1989) determined the effects of 2-nitrotoluene on the population growth of *Chlorella pyrenoidosa*, as described in the OECD guideline 201 (1984), including some slight modifications indicated elsewhere (van Leeuwen *et al.*, 1985). The 96h-log EC₅₀ was 2.54 µmol/l (EC₅₀ = 47.5 mg/l). According to these authors, it appears therefore, that the aromatic mononitro compounds are only, slightly, if at all, more toxic than narcosis type of chemicals to daphnids and algae. The study has been considered valid with restrictions, and included as additional information.

Canton *et al.*, (1985) reported a minimum inhibitory concentration (MIC) for the algae *Scenedesmus pannonicus* of 2.8 mg/l and a NOEC of 1.3 mg/l (dividing the MIC by 2 and correcting the values from stability tests results conducted with the chemical, according to the information published by Bringhman and Kühn (1978). This original paper, by Bringhman and Kühn, has not been available, and therefore not validated without a clear indication of the used test methodology, so the data have been considered as additional information. Canton *et al.*, (1985) also studied the disappearance of the test compound from the test solution. The result of the stability experiment indicated a decay of 8% of 2-nitrotoluene in the test medium during 8 days.

Bringmann (1975) determined the Lowest Observed Effect Concentration for the cell growth inhibition test on cyanobacterium/bluealgae, *Mycrocystis aeruginosa*, after an exposure period of 8 days of 3.1 mg/l. Cultures were shaken daily and final turbidity was measured and compared to controls, using a Hg-lamp wavelength of 578 nm. Measure chamber 10 mm thickness. Based on the results of the previous stability experiment, this toxicity datum is expected to be reliable, although no information on the exponential growth of the culture is indicated. So, it will be considered as not assignable for the assessment, but included as additional information.

Bringham and Kühn (1977), conducted a study on *Scenedesmus quadricauda*, after an exposure time of 8 days, the growth rate was determined by measuring the turbidity using an Hg-lamp wavelength of 578 nm. A toxicity threshold of 28 mg/l was determined. Based on the results of the previous stability experiment, this toxicity datum is expected to be reliable, although no information on the exponential growth of the culture is indicated. So, it will be considered as not assignable for the assessment, but included as additional information.

Table 3.17: Toxicity of 2-nitrotoluene to algae

Species	Test type	Tests	Duration	Toxicity End point (mg/l)	Comments	Reference
Short-term results						
<i>Chlorella pyrenoidosa</i>	OECD 201	Growth rate	72 h	EC50 = 22 (16-30)	V	Ramos <i>et al.</i> , 1999
<i>Chlorella pyrenoidosa</i>	Modified OECD 201	Growth rate	96 h	EC50 = 47.5	VWR	Deener <i>et al.</i> , 1989
Long-term results						
<i>Microcystis aeruginosa</i>	Other: Bringmann	Growth rate	8 d	LOEC = 3.1	NA Additional information	Bringmann, 1975
<i>Scenedesmus pannonicus</i>	Other: Bringmann and Kühn	Growth rate	Semichronic	LOEC = 2.8 NOEC = 1.3	NA Additional information	Bringmann & Kühn 1978 reported by Canton <i>et al.</i> , 1985
<i>Scenedesmus quadricauda</i>	Other: Bringmann and Kühn	Growth rate	8d	TT = 28	NA Additional information	Bringmann & Kühn 1977
<i>Chlorella pyrenoidosa</i>	OECD 201	Growth rate	72 h	NOEC = 4.4	V	Ramos <i>et al.</i> , 1999

V: Valid; VWR: Valid With Restrictions; NA: not assignable.

3.2.1.1.4 Microorganisms

In this section, toxicity test results on microorganisms are presented. Comments on tests have been focused especially on those ones considered for the assessment. Provided information has been summarized in Table 3.18.

Yoshioka *et al.*, (1986) carried out the “activated sludge, respiration inhibition test” proposed by the OECD providing an EC₅₀ of 665 mg/l on inhibiting the respiration of activated sludge, after a three-hour exposure period. The activated sludge was obtained from a treatment plant, treating predominantly domestic sewage.

Derneer *et al.* (1989) indicated a log EC₅₀ of 1.13 µmol/l (EC₅₀ of 1.8 mg/l) for the bioluminescence of *Photobacterium phosphoreum*. Tests with *P. phosphoreum*, and calculations causing 50% reduction of bioluminescence after 15 min of exposure were carried out as described in the Beckman Instruments Manual (1982). This test was considered not assignable for the assessment evaluation.

In the same way, Zhao and Wang (1995) provided information on *P. phosphoreum*. Ecotoxicological descriptors were the EC₅₀ inhibition of bioluminescence after 15 minutes exposure at 20 °C, according to the procedures described in the Instrument Manual. It results in an EC₅₀ of 16.9 mg/l. This assay is considered not assignable for the assessment purpose.

Bringman and Kühn (1977) tested 2-nitrotoluene on *Pseudomonas putida*. Exposure time was 16h at 25 °C. Growth rate was determined measuring the final turbidity that was measured and compared to controls, using a Hg-lamp wavelength of 436 nm. Measure chamber 10 mm thickness. The toxicity threshold concentration (EC₃) on *P. putida* was determined as 18 mg/l.

There are two data included in the IUCLID database by Hoechst (1984). The authors carried out a growth inhibition test on industrial activated sludge, using the ETAD fermentation tube method. For an exposure period of 24 hours it is indicated a NOEC of 60 mg/l and an EC₅₀ of 80 mg/l. These values are included as additional information but it will not be accounted for the assessment estimations since no validation of the original report was possible.

There is also some information, which are presented below, regarding the effects on unicellular protozoa.

In another study, Yoshioka *et al.* (1985) measured an EC₅₀ of 100 mg/l for the growth inhibiting effect of 2-nitrotoluene on the protozoa *Tetrahymena pyriformis*, after an exposure period of 24 hours.

Bringmann and Kühn, (1981) gave the following Toxic Threshold Concentrations (EC₅) measuring the cell growth inhibition rate of various protozoa. Quantitative estimation of initial cell number and cell growth is achieved through a culture counter. The following EC₅s are presented: 46 mg/l on *Entosiphon sulcatum* (bacteriovorous flagellate protozoa, consuming flagellate) after 72h exposure; 24 mg/l on *Uronema parduczi holozoic* (bacteriovorous ciliate protozoa, consuming ciliate) after 20h exposure and 27 mg/l on *Chilomonas paramecium* (saprozoic flagellate protozoa) after 48h exposure.

Table 3.18: Toxicity of 2-nitrotoluene to aquatic microorganisms

Species	Test type	Test	Duration	Endpoint (mg/l)	Comments	Reference
Activated sludge (mainly domestic)	OECD 209		3h	EC ₅₀ = 665	V	Yoshioka, Y, <i>et al.</i> , 1986
Activated sludge (industrial)	ETAD fermentation tube method		24 h	EC ₅₀ = 80	Additional information	Hoechst 1984 (in IUCLID)
Activated sludge (industrial)	ETAD fermentation tube method		24 h	NOEC = 60	Additional information	Hoechst 1984 (in IUCLID)
<i>Pseudomonas putida</i> (Bacteria)	-	Inhibition of cell multiplication	16 h	TT5 (EC ₃)=18	V	Bringmann and Kühn, 1977
<i>Chilomonas paramecium</i> (Protozoa)	-	Inhibition of cell multiplication	48 h	TT(EC ₅) = 27	V	Bringmann and Kühn, 1981
<i>Tetrahymena pyriformis</i> (Protozoa)	-	Inhibition of cell multiplication	24 h	EC ₅₀ = 100	V	Yoshioka, <i>et al.</i> , 1985
<i>Entosiphon sulcatum</i> (Protozoa)	-	Inhibition of cell multiplication	72 h	TT(EC ₅) = 46	V	Bringmann and Kühn, 1981
<i>Uronema parduzci</i> (Protozoa)	-	Inhibition of cell multiplication	20 h	TT(EC ₅)= 24	V	Bringmann and Kühn, 1981
<i>Photobacterium phosphoreum</i> (Bacteria)	Microtox-test	Unsuitable test system	15 min	EC ₅₀ = 16.9	NA	Zhao and Wang, 1995
<i>Photobacterium phosphoreum</i> (Bacteria)	Microtox-test	Unsuitable test system	15 min	EC ₅₀ = 1.8	NA	Deneer <i>et al.</i> , 1989

V: Valid; VWR: Valid With Restrictions; I: Invalid; NA: Not Assignable.

⁵ TT = Toxicity Threshold

3.2.1.1.5 Amphibians

There are only two data regarding amphibians. This information was provided by Canton *et al.* (1985), without providing the description of the test procedure. There was only information indicating an exposure period ranging from 1 to 4 days. So, this information is considered as assignable and is included in the assessment report as additional information. (Table 3.19).

Table 3.19: Toxicity of 2-nitrotoluene to amphibians.

Species	Phase	Measured / Nominal	Test	Duration	Endpoint (mg/l)	Comments	Reference
<i>X. laevis</i>	-	Nominal	Other	1-4 d	LC ₅₀₋₁₀	NA	Canton <i>et al.</i> , 1985
<i>X. laevis</i>	-	Nominal	other	1-4 d	EC ₅₀ = 3.4	NA	Canton <i>et al.</i> , 1985

3.2.1.2 Calculation of Predicted No Effect Concentration (PNEC) for aquatic organisms

There are no data on chronic toxicity to vertebrate aquatic organisms. Anyway, some NOECs (behaviour and mortality) are assessed, after an exposure period of 28 days, by Canton *et al.* (1985). Nevertheless, these endpoints cannot be applied when considering long-term effects.

Invertebrates seem to be the most sensitive taxonomic group, however, the large variation in the acute toxicity effects, even for those results conducted on the same species, i.e. *D. magna*, creates difficulties in comparisons.

A long-term NOEC of 0.5 mg/l on aquatic invertebrates (*Daphnia magna*) has been selected as the lowest value, and used for the PNEC_{aquatic organisms} calculation. There are long-term information on aquatic invertebrate and algae, but only short-term information on aquatic vertebrates. Therefore, according to the TGD, an assessment factor of 50 is applied:

$$\text{PNEC}_{\text{aquatic organisms}} = \text{lowest end chronic toxicity range} / 50 = 0.5 / 50 = \mathbf{10 \mu\text{g/l}}$$

In rapporteur's opinion, the sound PNEC for the sea water organisms can not be derived appropriately with the available information on fresh water effects. However, following the agreement adopted by the TC NES II 04, a PNEC for marine environment has been derived, according to the Technical Guidance Document, by applying an assessment factor of 500 to the lowest chronic toxicity data on freshwater organisms:

$$\text{PNEC}_{\text{seawater}} = \text{lowest chronic freshwater toxicity} / 500 = 0.5 / 500 = \mathbf{1 \mu\text{g/l}}$$

Sewage Treatment Plants

A validated EC₅₀ value of 665 mg/l, obtained according to the OECD guideline 209, has been used for calculations, to which it would be applied a factor of 100.

$$\text{PNEC}_{\text{microorganisms}} = \text{respiration} / 100 = 665 / 100 = \mathbf{6.65 \text{ mg/l}}$$

It has not been possible to check and validate the information regarding the lowest fermentation data (NOEC = 60 mg/l) provided by Hoechst (1984), which was included in the IUCLID database. Assuming that this value may be correct, and applying an assessment

factor of 10 as recommend for a NOEC, a PNEC of 6 mg/l should be proposed. Taking into account that the former PNEC, derived from the effects on respiration (6.65 mg/l), is very similar to the PNEC obtained from the NOEC on fermentation, there is no need for requesting formally the fermentation study as no differences in the PNEC are expected.

The calculated PNEC is lower than the toxicity threshold values provided for the different protozoa, and therefore, the proposed PNEC is considered to be protective for the role of these organisms in WWTP.

3.2.1.3 Toxicity test results for sediment organisms

The only available Data Set information on sediment organisms includes a single datum on the effect of 2-nitrotoluene on *Tubifex* sp. The EC₅₀ obtained for this organism is much more higher than the other for the aquatic invertebrates. For the oligochaeta *Tubifex* sp., 2-nitrotoluene has shown a 24-h LC₅₀ of 410 mg/l and 48-h LC₅₀ of 370 mg/l (these results must be considered under the light of the fact that the mortality in the control was above 10%) (Yoshioka *et al.*, 1986b). (It was no possible to validate this information, since only the abstract was provided in English, and so, even the exposure route could not be determined appropriately).

No data have been provided regarding toxicity on marine sediment organisms.

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

No relevant sediment toxicity information has been provided. So, taking into account the physical-chemical properties of the substance, the equilibrium partitioning method is considered to be appropriate for the PNEC_{sediment} derivation. The PNEC for sediment is calculated according to the Technical Guidance Document.

$$\text{PNEC}_{\text{sed}} = \frac{K_{\text{susp-water}}}{\text{RHO}_{\text{susp}}} \times \text{PNEC}_{\text{water}} \times 1000$$

where:

$K_{\text{susp-water}}$ = suspended sediment-water coefficient = 6.14 m³/m³ for 2-nitrotoluene (see section 3.1.3.2.1)

RHO_{susp} = bulk density of suspended sediment = 1150 kg/m³

$$\text{PNEC}_{\text{sed}} = 6.14 \text{ m}^3/\text{m}^3 \times 10 \text{ } \mu\text{g}/\text{l} \times 1000 / 1150 \text{ kg}/\text{m}^3 = \mathbf{53.4 \text{ } \mu\text{g}/\text{kg ww}}$$

Following the same approach, and using the equilibrium partitioning method, the PNEC for the marine sediment will be:

$$\text{PNEC}_{\text{marine-sediment}} = \frac{K_{\text{susp-water}}}{\text{RHO}_{\text{susp}}} \times \text{PNEC}_{\text{saltwater}} \times 1000$$

$$\text{PNEC}_{\text{marine-sediment}} = 6.14 \text{ m}^3/\text{m}^3 \times 1 \text{ } \mu\text{g}/\text{l} \times 1000 / 1150 \text{ kg}/\text{m}^3 = \mathbf{5.34 \text{ } \mu\text{g}/\text{kg ww}}$$

3.2.2 Terrestrial compartment

Regarding the terrestrial compartment, no toxicological information has been provided.

3.2.2.1 Toxicity test results

There are no ecotoxicity data available for this compartment.

3.2.2.1.1 Plants

There is not available information regarding the toxicity on plants.

3.2.2.1.2 Earthworm

There is not available information regarding the toxicity on soil dwelling invertebrates.

3.2.2.1.3 Microorganisms

There is not available information on nitrification or carbon mineralization regarding soil microorganisms.

3.2.2.1.4 Other terrestrial organisms

There is no information on toxicity on terrestrial organisms.

3.2.2.2 Calculation of Predicted No Effect Concentration (PNEC)

Taken into account the lack of data, and according to the Technical Guidance Document, the equilibrium partitioning method can be applied as a conservative calculation method to identify a potential risk to the soil compartment. Thus, the PNEC has been calculated using the equilibrium partitioning method with the PNEC for aquatic organisms.

$$\text{PNEC}_{\text{soil}} = \frac{K_{\text{soil-water}}}{\text{RHO}_{\text{soil}}} \times \text{PNEC}_{\text{water}} \times 1000$$

where:

$K_{\text{soil-water}}$ = soil-water partition coefficient = 6.48 m³/m³ for 2-nitrotoluene (see section 3.1.3.2.1)

RHO_{soil} = bulk density of wet soil = 1700 kg/m³

$$\text{PNEC}_{\text{soil}} = 6.48 \text{ m}^3/\text{m}^3 \times 10 \text{ } \mu\text{g}/\text{l} \times 1000 / 1700 \text{ kg}/\text{m}^3 = \mathbf{38.11 \text{ } \mu\text{g}/\text{kg}}$$

Considering the PNEC for microorganisms, it can be assumed that the equilibrium partitioning method would protect also the soil microbial community from undesirable effects. But there is no information in relation to the possibility of covering also the effects on vascular plants.

3.2.3 Atmosphere

No information is available on the effects of 2-nitrotoluene to plants and other organisms exposed via air, although volatilisation to the atmosphere may be likely to be limited due to the vapour pressure of the substance.

3.2.4 Secondary poisoning

According to the low bioaccumulation potential and the rapid elimination of this compound in fish and mammals, no secondary poisoning potential is expected from this substance.

3.3 RISK CHARACTERISATION ⁶

3.3.1 Aquatic compartment (incl. sediment)

Local, regional and continental PECs for aquatic and sediment compartments have been compared to a $PNEC_{aquatic}$ of 10 $\mu\text{g/l}$ and a $PNEC_{seawater}$ of 1 $\mu\text{g/l}$, derived from available information. And a $PNEC_{sediment}$ of 53.4 $\mu\text{g/kg ww}$ and a $PNEC_{marine_sediment}$ of 5.34 $\mu\text{g/kg ww}$, calculated using the equilibrium partitioning method according to the TGD. For the assessment of the Sewage Treatment Plants, a $PNEC_{microorganisms}$ of 6.65 mg/l , will be used.

Table 3.20: Risk characterisation for surface water

Local Compartment	Site	PEC	PEC/PNEC
Surface water ($\mu\text{g/l}$)	A	$6.58 \cdot 10^{-3}$	$6.58 \cdot 10^{-4}$
	B	$1.12 \cdot 10^{-2}$	$1.12 \cdot 10^{-3}$
	C	1.66	0.166
Sea water ($\mu\text{g/l}$)	C	0.29	0.29
Sewage Treatment Plants (mg/l)	A	$2.49 \cdot 10^{-3}$	$3.74 \cdot 10^{-4}$
	B	$3.98 \cdot 10^{-3}$	$5.98 \cdot 10^{-4}$
	C	$1.65 \cdot 10^{-2}$	$2.48 \cdot 10^{-3}$
Sediment ($\mu\text{g/kg wet wt}$)	A	$3.51 \cdot 10^{-2}$	$6.57 \cdot 10^{-4}$
	B	$5.99 \cdot 10^{-2}$	$1.12 \cdot 10^{-3}$
	C	8.83	0.16
Marine sediment ($\mu\text{g/kg wet wt}$)	C	1.55	0.29

Regional Compartment	PEC	PEC/PNEC
Surface water (total) ($\mu\text{g/l}$)	$3.39 \cdot 10^{-3}$	$3.39 \cdot 10^{-4}$
Surface water (dissolved) ($\mu\text{g/l}$)	$3.39 \cdot 10^{-3}$	$3.39 \cdot 10^{-4}$
Sediment (total) ($\mu\text{g/kg wet wt}$)	$1.73 \cdot 10^{-2}$	$3.24 \cdot 10^{-4}$

Continental Compartment	PEC	PEC/PNEC
Surface water (total) ($\mu\text{g/l}$)	$4.42 \cdot 10^{-5}$	$4.42 \cdot 10^{-6}$
Surface water (dissolved) ($\mu\text{g/l}$)	$4.42 \cdot 10^{-5}$	$4.42 \cdot 10^{-6}$
Sediment (total) ($\mu\text{g/kg wet wt}$)	$2.38 \cdot 10^{-4}$	$4.45 \cdot 10^{-6}$

⁶ Conclusion (i) There is a need for further information and/or testing.
 Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.
 Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Conclusions to the risk assessment for the aquatic compartment:

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

Conclusion (ii) applies to the aquatic compartment.

3.3.2. Terrestrial compartment

Local, regional and continental PECs for the terrestrial compartment have been compared to the $PNEC_{soil}$ of 38.11 $\mu\text{g}/\text{kg}$, calculated using the equilibrium partitioning method according to the TGD.

Table 3.21: Predicted levels in terrestrial compartment

Local Compartment	Site	PEC	PEC/PNEC
Soil (total) averaged over 30 days ($\mu\text{g}/\text{kg}$ wet wt)	A	0.0262	$6.87 \cdot 10^{-4}$
	B	0.903	0.023
	C	0.551	0.014
Agricultural soil (total) averaged over 180 days ($\mu\text{g}/\text{kg}$ wet wt)	A	0.0262	$6.87 \cdot 10^{-4}$
	B	0.742	0.0194
	C	0.551	0.014
Grassland (total) averaged over 180 days ($\mu\text{g}/\text{kg}$ wet wt)	A	0.0434	$1.13 \cdot 10^{-3}$
	B	0.311	$8.16 \cdot 10^{-3}$
	C	0.916	0.024

Regional Compartment	PEC	PEC/PNEC
Agricultural soil (total) ($\mu\text{g}/\text{kg}$ wet wt)	$1.97 \cdot 10^{-3}$	$5.16 \cdot 10^{-5}$
Natural soil (total) ($\mu\text{g}/\text{kg}$ wet wt)	$2.87 \cdot 10^{-4}$	$7.53 \cdot 10^{-6}$
Industrial soil (total) ($\mu\text{g}/\text{kg}$ wet wt)	0.643	0.016

Continental Compartment	PEC	PEC/PNEC
Agricultural soil (total) ($\mu\text{g}/\text{kg}$ wet wt)	$3.47 \cdot 10^{-5}$	$9.10 \cdot 10^{-7}$
Natural soil (total) ($\mu\text{g}/\text{kg}$ wet wt)	$3.93 \cdot 10^{-5}$	$1.03 \cdot 10^{-6}$
Industrial soil (total) ($\mu\text{g}/\text{kg}$ wet wt)	$5.7 \cdot 10^{-3}$	$1.5 \cdot 10^{-4}$

Conclusions to the risk assessment for the terrestrial compartment:

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

Conclusion (ii) applies to the terrestrial compartment.

3.3.3 Atmosphere

No effects on the atmosphere are likely in the regional and continental scenarios, because of the low predicted environmental concentrations of 2-nitrotoluene.

Table 3.22: Predicted levels in air compartment

Scale		PEC ($\mu\text{g}/\text{m}^3$)
Local	Site A	0.0167
	Site B	0.0285
	Site C	0.375
Regional		$3.39 \cdot 10^{-3}$
Continental		$4.42 \cdot 10^{-5}$

Nevertheless, a preliminary assessment can be done using data from the Human Health Assessment. Several values regarding inhalation toxicity could be used: the NOAEC value of $1086 \text{ mg}/\text{m}^3$ derived from an acute 8-hour inhalatory toxicity study in rats as a starting point, the corresponding human NAEC of $727.62 \text{ mg}/\text{m}^3$, and the LAEC of $175 \text{ mg}/\text{m}^3$ derived from an oral LOAEL of $25 \text{ mg}/\text{kg}$ bw in rats for repeated dose toxicity. Among them, the lowest concentration, $175 \text{ mg}/\text{m}^3$, has been compared with the highest $\text{PEC}_{\text{local}}$, $0.375 \mu\text{g}/\text{m}^3$, and the ratio obtained is $466 \cdot 10^3$, which indicates a low concern for inhalation exposure.

Conclusions to the risk assessment for the atmosphere:

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

Conclusion (ii) is applied to atmospheric compartment.

3.3.4 Secondary poisoning

According to the low bioaccumulation potential and the rapid elimination of this compound in fish and mammals, no secondary poisoning potential is expected from this substance.

Conclusions to the risk assessment for secondary poisoning:

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

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 General discussion

2-nitrotoluene is a yellow liquid at room temperature with a vapour pressure of 0.16 hPa at 20°C and a weak aromatic odour like bitter almond. The odour threshold is 0.05 ppm (Amoore and Hautala, 1983). The saturated vapour concentration at 20°C is calculated to be 0.90 g/m³.

It is soluble in most organic solvents and slightly soluble in water. It may be absorbed through the intestine, skin and through the respiratory tract.

Human exposure to 2-nitrotoluene occurs primarily through occupational sources. It has not been identified in consumer products.

4.1.1.2 Occupational exposure

The most probable route of human exposure to 2-nitrotoluene is inhalation and dermal contact of workers involved in the production and use of this substance.

2-nitrotoluene can be used in the synthesis of intermediates for azo dyes, sulfur dyes, rubber chemicals, and agriculture chemicals (Kirk-Othmer, 1996). The substance can be used in several ways:

1. Hydrogenated to 2-toluidine and sold for use in herbicides, dyestuffs and the rubber industry.
2. Nitrated to di-nitrotoluene and used in the production of polyurethane foams or sold for use in explosives and dyestuffs.
3. Chlorinated and sold for use in drugs and pigments.
4. Chlorinated and hydrogenated to either 6-chloro-2-toluidine or 4-chloro-2-toluidine and sold for use in pharmaceuticals and pigments respectively.
5. Sold as 2-nitrotoluene (for use in manufacture of explosives).
6. Sulfonated to 2-nitrotoluene-4-sulfonic acid.

Even though 2-nitrotoluene can be used for the manufacture of a broad range of products, o-toluidine seems to be the largest outlet for this substance. The manufacture of o-toluidine now account for nearly 85% of total 2-nitrotoluene consumption with minor amounts going into some specialty intermediates and some TNT (Srour, 1997; Industry, 2001).

In addition, there is information about this substance as a non isolated intermediate in the synthesis of dinitrotoluene (Industry, 2004b).

The substance is synthesised and used in closed systems. It is mainly used within the manufacturers as an intermediate for further synthesis. Exposure is therefore expected in those activities where the system is breached. Given the high captive consumption of this substance, one scenario is only considered. External exposure by inhalation and dermal routes during 2-nitrotoluene manufacture and further processing is assessed. Oral exposure is assumed to be prevented by good hygiene practices.

According to the Srour report, in 1997 three manufacturers produced 2-nitrotoluene in EU. It was estimated that one manufacturer is responsible for around 75-80 % of total European production of this substance. Therefore, information from this manufacturer will be specially considered in the assessment. A more recent information from Industry (2004a), indicates that the production of 2-nitrotoluene by the considered second producer ceased during 2001.

There is also information from Industry (2004b) showing that a company produces an important quantity of 2-nitrotoluene as a non isolated intermediate in the synthesis of dinitrotoluene.

Occupational exposure information, including monitoring data, has been obtained from the main European manufacturer of this substance and from a company that produces 2-nitrotoluene as an intermediate in the synthesis of dinitrotoluenes. Relevant information from SIDS Initial Assessment on 2-nitrotoluene (KemI, 1994) has also been used in the assessment. Additional monitoring data from the literature have not been found.

Quality and recent measured data will be preferred to derive 90th percentile values representing 8hTWA RWC or short term RWC. When the data set is small, values near the highest end of the concentration range will be used. Typical values are also derived.

EASE model (EASE for Windows Version 2.0) has been used to estimate both inhalation and dermal exposure.

The assessment has been carried out without taking into account the exposure reducing effect of PPE. Information reported by industry about the use of PPE is not enough to know if PPE is correctly selected, worn and maintained. When PPE is used in accordance with Directive 89/656/EEC (this is, in fact, obligatory around the EU) exposure will be reduced considerably.

The occupational exposure limits in different countries are summarised in the table 4.1.1.2.

Table 4.1.1.2: Occupational Exposure Limits for 2-nitrotoluene

Country	8h-TWA exposure limit		STEL-short term exposure limit		Skin notation	References
	Ppm	mg/m ³	ppm	mg/m ³		
USA (ACGIH)	2				YES	ACGIH (2001)
USA (NIOSH)	2*	11*			YES	NIOSH (2003)
Austria	2	11			YES	NIOSH (2003)
Belgium	2	11			YES	NIOSH (2003)
Denmark	2	12			YES	NIOSH (2003)
Germany		Carcinogen cat: 2 TRK**: 0.5			YES	DFG (2002)
Spain	5	29			YES	INSHT (2004)
Sweden	1	6	2	11	YES	NIOSH (2003)
United Kingdom (2001)	5	29	10	57	YES	HSE (2001)

*TWA limit for up to a 10-hour work shift in a 40-hour workweek.

** TRK: Technical exposure limit

Conversion factor: 1 ppm = 5.61 mg/m³

1 mg/m³ = 0.178 ppm

4.1.1.2.1 Occupational exposure from production

Scenario 1: Production and further processing of 2-nitrotoluene

Occupational exposure to 2-nitrotoluene could occur when it is manufactured or used as raw material or chemical intermediate. It is generally further processed within the manufacturing firms themselves. Therefore, one scenario is only considered.

2-Nitrotoluene is synthesised, by either batch or continuous process, by the nitration of toluene with an aqueous acidic mixture of sulphuric acid and nitric acid at a temperature that starts at 25°C and is slowly raised to 35-40°C. After being washing and dried, the resulting product contains 55-60 wt % *o*-nitrotoluene, 3-4 wt % *m*-nitrotoluene, and 35-40 wt % *p*-nitrotoluene. The separation of the isomers is carried out by a combination of fractional distillation and crystallization. This process is generally used to manufacture 2-nitrotoluene. However, 2-nitrotoluene can also be prepared by indirect methods if the pure isomer is required. It can be obtained by treating 2,4-dinitrotoluene with ammonium sulphide followed by diazotization and boiling with ethanol (Kirk-Othmer, 1996).

Once manufactured the substance is mainly used on site. It is largely further processed to *o*-toluidine by catalytic hydrogenation of 2-nitrotoluene in closed system followed by distillation (Industry, 2001).

Information regarding this substance as a non isolated intermediate in the synthesis of dinitrotoluene has also been provided (Industry, 2004b).

No information about the number of exposed workers has been received. A survey carried out by NIOSH in the USA (1973-1974) estimates that 285 workers were exposed to 2-nitrotoluene at that time (NIOSH, 1974). A company has reported a total of 37 workers in its plant, where 2-nitrotoluene is generated as a non isolated intermediate in the synthesis of dinitrotoluene. Following SIDS Initial Assessment on 2-nitrotoluene (KemI, 1994), the number of workers involved at two EU plants was 50 (manufacture, handling, maintenance and site use) and 23. It can be estimated that the number of workers potentially exposed within EU is about 150 workers.

The production and further processing of 2-nitrotoluene takes places in closed plant. Potential exposure may occur during activities such as, collection of samples for analysis, filling and maintenance. The process is described as continuous at least in two companies. Filling operations involve filling tank wagons or drums. Some technical measures at the UK plant are mentioned in the SIDS Initial Assessment on 2-nitrotoluene (KemI, 1994): automated enclosed pipe systems, storage areas vented through filters, local exhaust ventilation during drumming, ventilation to air during filling of road tankers. A company has reported low emission sampling station either within an encapsulated housing equipped with air filtration or a vent system, or equipped with a local air exhaust unit and operated from a remote position.

PPE are used in special tasks. The use of respiratory masks with ABEK filter, suitable gloves and other protective equipment has been reported from a company. The SDS of one company recommends the use of respiratory protection (combination filter, e.g. DIN 3181 ABEK), hand protection (gloves of Baypren, Viton or of PVC), eye protection (goggles) and other protective equipment. The SIDS Initial Assessment on 2-nitrotoluene (KemI, 1994) mentions that PPE are used at the UK and Italy plants.

Measured data

Sampling and analysis may be performed by collection of vapours using a silica gel adsorption tube with subsequent desorption using methanol and gas chromatographic analysis (NIOSH, 1998).

Long-term occupational exposure to 2-nitrotoluene during production and further processing has been provided by the main manufacturer and are showed in the table 4.1.1.2.1-1

Table 4.1.1.2.1-1: Long-term occupational exposure to 2-nitrotoluene during production and processing

Year	Process/Activity	Number of measures	Range mg/m ³	Mean mg/m ³	Remarks
1996-1999	Manufacturing: Nitration	12	0.017-0.280	0.075	Personal air sampler Full shift
1991-2000	Manufacturing: Distillation	10	0.004-0.120	0.057	Personal air sampler Full shift
1997-2000	Manufacturing: Regeneration of nitrating acid	5	0.005-0.012	0.009	Personal air sampler Full shift
1995-1998	Processing: Sulfonation (2-nitrotoluene-4-sulfonic acid)	10	0.004-0.140	0.062	Personal air sampler Full shift
1997-2000	Processing: Nitration (dinitrotoluene)	5	0.050-0.050	0.050	Personal air sampler Full shift
1997-1999	Tank station: Drum filling	3	0.007-0.097	0.052	Personal air sampler Full shift
1997-1999	Tank station. Filling tank wagons	4	0.020	0.030	Personal air sampler Full shift

Short-term exposure to 2-nitrotoluene during further processing obtained from the same company are showed in the table 4.1.1.2.1-2

Table 4.1.1.2.1-2: Short-term occupational exposure to 2-nitrotoluene during processing

Year	Process	Task	Number of measures	Range mg/m ³	Mean mg/m ³	Remarks
1997-1999	Processing: Sulfonation (2-nitrotoluene-4-sulfonic acid)	Product filling and sampling	5	0.046-0.200	0.109	Use of RPE
1997	Processing: Hydrogenation (2-toluidine)	Discharge of tank wagons	3	0.200-0.290	0.250	Use of RPE

The meaning of these measurements can only be assessed to a limited extent because information about the sampling and analytical method has not been provided and although mentioned, no details about the activities during measurement are available. Most data are reported as full shift personal air samples. Some of them have been reported as short-term measurements without specifying the measured period.

Data have been reported by a company that produces the substance as an intermediate in the synthesis of dinitrotoluene. The range of 19 measurements taken in 2002 is 0.01-0.0545 mg/m³. The NIOSH 2005 measurement method is deemed appropriate. Whether these data refer to short or long term exposures is not known. The monitored task is assumed to be sampling since, besides maintenance, this is the only activity where exposure has been reported to be possible.

Based on these measurements, despite their shortcomings, the highest value for the whole shift (0.280 mg/m³) can be regarded as RWC. Typical values can be in the order of 0.1 mg/m³. Short-term measurement data are in the same order of magnitude as long-term measurement data.

Very limited information from other companies has been found both in IUCLID and SIDS Initial Assessment on 2-nitrotoluene (KemI, 1994). Both sources mention that atmospheric monitoring of workplace air at a former manufacturing plant demonstrates that exposure was generally well below OEL of 30 mg/m³ (UK). Exposure levels lower than 0.01 ppm (0.06 mg/m³) are reported for the Italian plant.

Some old data are mentioned in the SIDS Initial Assessment on 2-nitrotoluene (KemI, 1994) but they are not considered here because they are 20 or 30 years old and they come from plants where the production and use of 2-nitrotoluene has ceased some years ago.

Modelled data

As it is said above, production and further processing of 2-nitrotoluene takes place in closed systems. Therefore, exposure may occur during activities that involve breaching the closed system such as sampling, loading and unloading of tanks and drums, and maintenance.

This substance is mainly used on site principally through enclosed pipe systems, so that transfer or filling are not very frequent activities. Information on these tasks has been found in the SIDS Initial Assessment on 2-nitrotoluene (KemI, 1994) for one former manufacturer: handling was mainly conducted using enclosed pipe systems, automated systems for drum and tanker filling. Drumming was carried out once every 2 or 3 months for short durations up to 100 tonnes (200 l steel drums). Road tankers (18 tonne capacity) were filled once every two weeks.

Information on sampling and maintenance activities has been sent by a company that produces the substance as an intermediate for DNT production:

Process samples are taken 3 times per day. Containers used to collect the samples are tightly coupled to the closed reaction system. The design of the sampling stations guarantees minimized emissions. The sampling arrangement is placed in an encapsulated housing equipped with exhaust air.

Maintenance activities do not occur on a daily basis, the equipment or the lines are breached only occasionally if maintenance is necessary. All maintenance activities require the prior approval by supervising plant personnel and an approved working instruction checklist. The relevant plant parts are emptied, flushed and rinsed with hot water. If it is not feasible to rinse the relevant parts with water, the system will be flushed with pressurized nitrogen in order to minimize residual contaminations in the plant, and cooled down before being breached for maintenance.

EASE model (EASE for Windows Version 2.0) has been used to estimate both inhalation and dermal exposure.

Accordingly, the estimated inhalation exposure (non dispersive use with LEV, $V_p = 0.016$ kPa) will be 0.5 – 1 ppm (2.85 – 5.61 mg/m³). However, these kinds of activities are not carried out along the whole shift. Assuming that they are performed for about one hour per day, an eight-hour TWA exposure range of (0.35 – 0.7 mg/m³) can be calculated.

Dermal exposure for these activities is also estimated by EASE considering non dispersive use and direct contact:

- Sampling: Incidental contact is assumed. The estimated exposure range is 0-21 mg/day, considering an exposed surface of 210 cm².
- Filling and emptying of tanks and drums: Incidental contact can also be assumed when road tankers are filled. When filling operations involves run off 2-nitrotoluene into drums, intermittent contact is assumed. The same level of contact is assumed when tanks or drums are emptied. Then, a worker could be dermal exposed to 420 mg/day of 2-nitrotoluene considering an exposed surface of 420 cm².
- Maintenance: It can be assumed an intermittent level of contact. An exposed surface of 840 cm² is considered for regular cleaning and maintenance tasks. Then, the exposure level will be in the range of 0 – 840 mg/day. The uncertainty around this estimation is very high. The estimation provided by EASE refers to the pure substance. Decontamination procedures before maintenance are established to prevent exposures. Exposure depends largely on the efficacy of decontamination procedures and on the proper use of PPE. On the other hand these are not daily activities, although information on their frequency is not available.

Therefore, a global RWC of 420 mg/day is assumed for workers dermal exposed to 2 - nitrotoluene in this scenario.

It could be assumed that the use of gloves is highly accepted by the involved companies. Information about the used glove materials is only available from one company. However, information about the suitability of the recommended materials for the substance under consideration is lacking. Therefore, the use of gloves has not been taken into account in the assessment. When PPE is used in accordance with Directive 89/656/EEC (this is, in fact,

obligatory around the EU) dermal exposure will be reduced considerably and therefore, it will be lower than the quoted range.

Conclusions

From measured exposure data, the highest value for the whole shift of 0.280 mg/m³ is regarded as RWC. This value is in agreement with the low part of the range estimated by EASE (0.35 mg/m³). Therefore, an estimated value of 0.3 mg/m³ based both in measured and modelled data will be used for purposes of risk characterisation.

Typical values can be considered in the order of 0.1 mg/m³ according to the measured data.

Short term measured data are similar to the highest part of the range of full shift measured data. Because the limited reliability of this dataset, a RWC short term value of twice as high as the full shift RWC value is assumed: up to 0.6 mg/m³. This is in agreement with the highest part of the range estimated by EASE for the full shift.

Dermal exposure is estimated by EASE as 420 mg/day for the whole scenario.

The assessment has been carried out without taking into account the exposure reducing effect of PPE. Information reported by industry about the use of PPE is not enough to know if PPE is correctly selected, worn and maintained. When PPE is used in accordance with Directive 89/656/EEC (this is, in fact, obligatory around the EU) exposure will be reduced considerably.

Conclusions of the occupational exposure assessment are showed in Table 4.1.1.2.1-3.

Table 4.1.1.2.1-3: Conclusions of the occupational exposure assessment

Inhalation exposure								
Scenario	Activity	Duration and frequency	RWC		Typical exposure		Short-term exposure	
			mg/m ³	method	mg/m ³	Method	mg/m ³	method
Production and further processing	Sampling Filling Maintenance	Full shift/daily (assumed)	0.3	Measured EASE	0.1	Measured	0.6	Calculated EASE
Dermal exposure								
Scenario	Activity	Frequency/contact level	Exposed area (cm ²)	Exposure range (mg/cm ² /day)	RWC (mg/day)	Method		
Production and further processing	Sampling Filling Maintenance	Daily/ Intermittent	420	0.1 - 1	420	EASE		

4.1.1.3 Consumer exposure

2-nitrotoluene can be used in the chemical industry in the synthesis of intermediates for azo dyes, sulfur dyes, rubber chemicals, and agriculture chemicals. There is not information about 2-nitrotoluene in consumer products. However, given the intended use, the exposure of consumers to the substance is expected to be negligible.

4.1.1.4 Humans exposed via the environment

Indirect exposure via the environment is calculated using data for oral intake via food, drinking water and air. The same three local scenarios A, B and C were considered, as well as the data for the regional scenario. The resultant daily doses for the uptake of 2-nitrotoluene are in the table below:

Table 4.1.1.4: Daily Human Doses (mg·kg⁻¹·d⁻¹)

Intake route	Site A (Germany)	Site B (United Kingdom)	Site C (Italy)	Regional
Drinking water	1.96·10 ⁻⁷	5.56·10 ⁻⁶	3.89·10 ⁻⁵	9.67·10 ⁻⁸
Fish	1.76·10 ⁻⁷	2.89·10 ⁻⁷	4.02·10 ⁻⁵	1·10 ⁻⁷
Leaf crops	1.69·10 ⁻⁶	3.04·10 ⁻⁶	3.95·10 ⁻⁵	3.72·10 ⁻⁹
Root crops	1.18·10 ⁻⁷	3.33·10 ⁻⁶	2.47·10 ⁻⁶	8.82·10 ⁻⁹
Meat	1.94·10 ⁻¹⁰	5.66·10 ⁻¹⁰	5.97·10 ⁻⁹	4.44·10 ⁻¹²
Milk	5.74·10 ⁻⁹	1.67·10 ⁻⁹	1.76·10 ⁻⁸	1.31·10 ⁻¹¹
Air	3.43·10 ⁻⁶	6.12·10 ⁻⁶	8.03·10 ⁻⁵	7.39·10 ⁻⁹
Total	5.63·10 ⁻⁶	1.84·10 ⁻⁵	2.01·10 ⁻⁴	2.17·10 ⁻⁷

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

4.1.2.1 Toxicokinetics, metabolism and distribution

4.1.2.1.1 Studies in animals

In vivo studies

Inhalation

No data available.

Dermal

No data available.

Oral

Studies on the toxicokinetics following oral administration of 2-nitrotoluene have been performed in experimental animals, especially in rats. Most of them are mainly research orientated and published in the open literature. Consequently, the protocols were not specifically designed according to OECD guideline 417 and addressed only some of the parameters described.

Rats

Albino rats (6 animals/group, weighing about 150 g) were administered orally 0 and 48 mg/kg b.w. of 2-nitrotoluene (purity not given) daily for 7 days to study its metabolism by estimating the amount of hippuric acid excretion in urine samples collected daily for 24 h from the first day of the experiment (Maitrya and Vyas, 1970). Moderate amounts of hippuric acid were excreted in urine (the mean of six independent experiments \pm SE of the mean was 8.64 ± 0.06 mg/kg b.w.). Conjugation normally takes place with glycine although other compounds like glucuronic acid also participate. Therefore, ultimate products excreted in urine will be hippuric acid in the case of glycine and glucuronides in the case of glucuronic acid. Taking into account that this study is confined to the excretion of hippuric acid only, it is considered premature to postulate the actual mechanism of conjugation of 2-nitrotoluene. In addition, the validity of this study is questionable because it is a short communication with no details on methodology for the estimation of hippuric acid (Quik's method), a metabolite that has not been detected in the remaining toxicokinetics studies.

The metabolism and excretion of 2-nitrotoluene were studied in male Fischer-344 rats (Chism, Turner and Rickert, 1984). Rats (3 animals/group, 80-90 days old) were given orally 200 mg/kg of [ring-U- 14 C] 2-nitrotoluene (>99% purity) dissolved in corn oil by gavage. This dose was selected because it induced UDS in hepatocytes of treated rats. Urine from 2, 4, 8, 12, 24, 36, 48, 60 and 72 hours after dosing and feces from 12, 24, 36, 48, 60 and 72 hours after dosing were collected. Most of the 2-nitrotoluene excretion occurred during the first 24 hours (86% of the dose). By 72 hours after treatment, 91% of the dose was excreted in the urine (86%), feces (5%) and expired air (0.1%). The urinary metabolites were 2-nitrobenzoic acid (29%), an unidentified metabolite (16%), 2-nitrobenzyl glucuronide (14%), S-(2-nitrobenzyl)-N-acetylcysteine (12%), an unidentified metabolite (6%), S-(2-nitrobenzyl)-glutathione (4%), 2-aminobenzoic acid (2%), 2-nitrobenzyl sulfate (0.5%) and 2-nitrobenzyl alcohol (0.4%). The peak excretion rates of metabolites which had not undergone nitro group reduction occurred within the first 4 h following the dose. 2-aminobenzoic acid and peaks 2 and 3 (unidentified metabolites) had maximal excretion rates between 4 and 12 h after dose. In summary, based on the nature of the metabolites found and taking into account *in vitro* data from deBethizy and Rickert (1984), the metabolism of 2-nitrotoluene appears to be the result of further biotransformation of nitrobenzyl alcohol. There also appear to be three pathways of 2-nitrobenzyl alcohol metabolism *in vivo*: 1) oxidation to 2-nitrobenzoic acid; 2) conjugation with glucuronic acid; and 3) conjugation with glutathione.

The role of enterohepatic circulation on bioactivation of 2-nitrotoluene was studied in male and female Fischer-344 rats (Chism and Rickert, 1985). Rats (3/sex, 80-90 days old) were anesthetized with methoxyflurane and the common bile duct was cannulated. The cannula was connected to a glass receptacle with a side arm. The receptacle was implanted in the abdomen and the side arm was exteriorized to allow periodic removal of bile. Sham-operated rats (3/sex) were taken through the surgery and the glass receptacle was implanted but the bile duct was not cannulated. Control rats (3/sex) were not anesthetized or taken through the surgery. Rats were given orally 200 mg/kg b.w. of [ring-U- 14 C] 2-nitrotoluene (>99% purity) dissolved in corn oil, one hour after the rats regained the righting reflex. Bile from 1, 2, 3, 4, 6, 9 and 12 hours after dosing, urine from 6 and 12 hours after dosing and feces from 12 h after dosing were collected. Rats were killed 12 hours after dosing and their livers were removed for analysis of total and covalently bound 14 C. The bile excretion in cannulated rats was 29% (♂) and 10% (♀) of the dose. Urine was the predominant route of excretion in males and females, regardless of surgical manipulation. In males, urinary excretion was 75% of the dose (controls), 52% of the dose (sham operation) or 36% (bile duct cannulation). In females,

urinary excretion was 80% of the dose (controls), 63% of the dose (sham operation) or 33% (bile duct cannulation). Faecal excretion accounted for < 2%. Biliary metabolites (expressed as % of the dose) were: 2-nitrobenzyl glucuronide (22% in ♂, 8% in ♀), S-(2-nitrobenzyl) glutathione (5% in ♂, 0.4% in ♀), S-(2-nitrobenzyl)-N-acetylcysteine (1% in ♂, 0.4% in ♀), and 2-nitrobenzyl sulfate (1% in ♂, 0.1% in ♀). Urinary metabolites in controls (expressed as % of the dose) were: 2-nitrobenzoic acid (25% in ♂, 31% in ♀), unknown metabolite 1 (14% in ♂, 6% in ♀), 2-nitrobenzyl glucuronide (13% in ♂, 25% in ♀), S-(2-nitrobenzyl)-N-acetylcysteine (11% in ♂, 7% in ♀), unknown metabolite 2 (5% in ♂, 4% in ♀), S-(2-nitrobenzyl)-glutathione (3% in both sexes), 2-aminobenzoic acid (2% in ♂), 2-nitrobenzyl sulfate (0.5% in ♂, 1% in ♀), and 2-nitrobenzyl alcohol (<0.5% in both sexes). The urinary excretion of the most abundant metabolite in both sexes, 2-nitrobenzoic acid, was decreased by ≥60% in both sexes (bile duct cannulation) and by 42% in ♂ and 12% in ♀ (sham operation). The urinary excretion of 2-nitrobenzyl glucuronide was not markedly affected by either bile duct cannulation or sham operation. The urinary excretion of S-(2-nitrobenzyl)-glutathione and S-(2-nitrobenzyl)-N-acetylcysteine was decreased by bile duct cannulation while sham operation had no effect. The two unknown urinary metabolites (1, 2) were the most affected by bile duct cannulation, decreasing especially in males by 81% (1) and 95% (2). Sham operation also decreased the urinary excretion of unknown metabolites, especially in males by 60% (1) and 84% (2). Concentrations of total hepatic radioactivity in males were higher than in females. Bile duct cannulation decreased the hepatic concentration in males but not in females and sham operation had no effect. There was covalent binding to hepatic macromolecules in both sexes (3 times more in males). Sham operation and bile duct cannulation decreased covalent binding in both sexes. Bile duct reduced covalent binding in males by 98% when compared to controls and by 93% when compared to sham operated animals. The decreased covalent binding in sham-operated rats may reflect a decreased in biliary excretion of 2-nitrobenzyl glucuronide due to the anesthetic (methoxyflurane) that temporarily reduced hepatic concentrations of UDP-glucuronic acid. In summary, the results of this study indicate that the enterohepatic circulation is obligatory to the hepatic macromolecular covalent binding of 2-nitrotoluene in rats and suggest that 2-nitrobenzyl glucuronide is a precursor to the active metabolite. The proposed pathway for 2-nitrotoluene bioactivation is the following: 2-nitrobenzylglucuronide is excreted into the intestine via the bile where intestinal microflora hydrolyzes the glucuronic acid and reduces the nitro group to form 2-aminobenzyl alcohol, which is reabsorbed and further metabolized by hepatic enzymes to a species capable of covalent binding to hepatic DNA. Sex differences seen in covalent binding to hepatic macromolecules, genotoxicity and carcinogenicity could be explained by the greater biliary excretion in males than in females.

Good quality and GLP compliant studies were conducted by NTP (2002) in F344/N rats of approximately 9 to 14 (males) or 9 to 12 (females) weeks old at dosing to determine: a) the metabolism and excretion of 2-nitrotoluene following the administration of single and repeated doses by gavage; b) the effect of buthionine sulfoximine or pentachlorophenol on the 2-nitrotoluene metabolism after a single dose administration; c) the plasma concentration of 2-nitrotoluene after a single dose administration; and d) the binding of 2-nitrotoluene equivalents to hemoglobin after a single dose administration.

- a) In the initial study groups of 4 males or females were given 200 mg/kg b.w. of [¹⁴C]-2-nitrotoluene (≥ 98% purity) in Emulphor by gavage. Radioactivity was measured in urine and feces collected 4, 8, 24, 48 and 72 hours after dosing. 2-nitrotoluene was excreted mainly in urine with 86% (males) and 92% (females) of the dose in the first 24 hours or with 99% (males) and 101% (females) of the dose in the first 48 hours. Total recoveries of radiolabel for males and females were approximately 106% (103% in urine and 3% in

feces). The profiles of urinary metabolites were determined by HPLC. At least 8 metabolites were present in the urine of rats (expressed as % of the dose in urine from 0 to 48 hours of males): 2-nitrobenzoic acid (A, 21%), 2-nitrobenzyl glucuronide (B, 17%), an unidentified metabolite (C, 0.2%), 2-aminobenzyl alcohol (D, 18%), an unidentified metabolite (E, 4%), S-(2-nitrobenzyl)-N-acetylcysteine (F, 10%), 2-nitrobenzyl alcohol (G, 2%) and o-toluidine (H, 1%). A similar profile of major metabolites was observed in females, except the production of 2-aminobenzyl alcohol and S-(2-nitrobenzyl)-N-acetylcysteine was significantly less than that of males. After urine incubation with glucuronidase/sulfatase, the most pronounced change in the profile of metabolites was the loss of the peak B giving rise to peak G; and upon incubation with purified sulfatase, peak B remained, confirming it is a glucuronide rather than a sulfate conjugate. The identity of peak G was confirmed by coelution with the authentic standard, 2-nitrobenzyl alcohol. Thermospray mass spectral analysis of isolate metabolite E showed that while its retention time was the same as that of the synthetic standard, S-(2-nitrobenzyl) glutathione its mass spectrum did not match that of the synthesized compound.

In the second study, groups of 3 males or females were given 2 mg/kg b.w. of [¹⁴C]-2-nitrotoluene (≥ 98% purity) in Emulphor:ethanol:water (1:1:8) by gavage. Radioactivity was measured in urine and feces collected 24, 48 and 72 hours after dosing. Excretion of radioactivity was similar to that found after a 200 mg/kg dose. 2-nitrotoluene was excreted mainly in urine with 98% (males) and 97% (females) of the dose in the first 24 hours or with 104% (males and females) of the dose in the first 48 hours. Total recoveries of radiolabel were 112% (106% in urine and 5% in feces) for males and 113% (108% in urine and 4% in feces) for females. The metabolite profiles were similar to those seen after the 200 mg/kg dose; the most pronounced difference was a greater proportion of the dose excreted as 2-nitrobenzoic acid and 2-nitrobenzyl glucuronide after the lower dose. The sex-dependent variance in metabolite profile remained at the 2 mg/kg dose, with females excreting significantly less 2-aminobenzyl alcohol and S-(2-nitrobenzyl)-N-acetylcysteine than males, but more 2-nitrobenzoic acid.

In the third study, groups of 3 male rats were administered 200 mg/kg b.w. of [¹⁴C]-2-nitrotoluene (≥ 98% purity) in Emulphor:ethanol:water (1:1:8) daily by gavage for 14 days. The dose was nonradiolabeled for the first 11 days, radiolabeled on day 12, and nonradiolabeled on days 13 and 14. Radioactivity was measured in urine and feces collected 24, 48 and 72 hours after radiolabeled dosing. Excretion of radioactivity was similar to that found after a single dose. 2-nitrotoluene was excreted mainly in urine with 78% of the dose in the first 24 hours or with 85% of the dose in the first 48 hours. Total recovery of radiolabel was 98% (87% in urine and 11% in feces). The urinary profile after repeated doses showed decreased excretion of S-(2-nitrobenzyl)-N-acetylcysteine (50% less than that found after a single dose), but no other obvious changes.

- b) The effect of buthionine sulfoximine or pentachlorophenol on the 2-nitrotoluene metabolism was investigated in separate studies carried out in males administered single doses of [¹⁴C]-2-nitrotoluene (200 mg/kg.b.w) in Emulphor:ethanol:water (1:1:8) by gavage. Three rats were provided drinking water containing buthionine sulfoximine (glutathione synthesis inhibitor) for 6 days before and until 72 hours after the 2-nitrotoluene dose, and three rats were injected i.p. with pentachlorophenol (O-sulfation inhibitor) before 2-nitrotoluene administration. Radioactivity was measured in urine collected 24, 48 and 72 hours (buthionine sulfoximine group) or 4, 8 and 24 hours (pentachlorophenol group) after 2-nitrotoluene dose. Rats pretreated with buthionine sulfoximine excreted significantly less of the dose in urine (57% in 24 hours) than non-

pretreated rats; the amount of S-(2-nitrobenzyl)-N-acetylcysteine excreted was about halved and the amount of 2-nitrobenzyl alcohol excreted tripled relative to rats that were not pretreated. Rats pretreated with pentachlorophenol, also excreted significantly less of the dose (52% in 24 hours) in the urine than nonpretreated rats, and the amount of S-(2-nitrobenzyl)-N-acetylcysteine excreted was decreased from 10% to 1.5% of the dose. These data suggest 2-nitrobenzyl alcohol is converted to an alkylating species by O-sulfation, and that glutathione may serve in a protective role by conjugation with that reactive intermediate. Urinary excretion of 2-nitrobenzyl glucuronide and 2-aminobenzyl alcohol was also decreased by pentachlorophenol pretreatment from 15% to 8% of the dose and from 17% to 4% of the dose, respectively. 2-nitrobenzyl glucuronide urinary excretion was decreased, probably due to competition of pentachlorophenol for glucuronidation, and consequently there were decreases in its biliary excretion, nitro group reduction and deconjugation by gut microflora, which may have led to the observed decrease in the urinary excretion of 2-aminobenzyl alcohol.

- c) To determine plasma concentration of 2-nitrotoluene, 4 males from the initial study were anesthetized with an i.p. injection of ketamine:xylazine (7:1), implanted with indwelling jugular cannulae to allow serial blood sampling and returned to metabolism cages to recover for one day prior to dosing with 200 mg/kg b.w. of [¹⁴C]-2-nitrotoluene (≥ 98% purity) in Emulphor by gavage. Samples were collected 15 and 30 minutes and 1, 2, 4, 8 and 24 hours after dosing. 2-nitrotoluene concentrations in plasma peaked at nearly 10000 ng/g plasma 15 to 60 minutes after dosing, and rapidly decreased through 24 hours after dosing; they were below the limit of detection at 24 hours after dosing. The half-life of 2-nitrotoluene in plasma was about 1.5 hours.
- d) The binding of 2-nitrotoluene equivalents to hemoglobin was also determined in a separate experiment. Blood was collected via cardiac puncture from male and female rats (4 rats/sex) anesthetized with an i.p. injection of ketamine:xylazine that had been administered a single gavage dose (200 mg/kg b.w) of [¹⁴C]-2-nitrotoluene (≥ 98% purity) in Emulphor. Radioactivity was determined in plasma, erythrocytes and isolated protein. Of the total radioactivity in blood 72 hours after dosing, 89% was associated with red blood cells. Of the radioactivity in the red blood cells, approximately 40% was associated with the isolated, washed protein pellet. This pellet was subjected to continuous (Soxhlet) extraction to ensure that only covalently bound 2-nitrotoluene equivalents remained. There were 26 pmol-equivalents/mg globin for males and 29.9 pmol-equivalents/mg globin for females. These data do not indicate a marked sex-related difference in globin binding of 2-nitrotoluene equivalents and, contrary to the metabolite profiles, do not suggest a differential production of alkylating species by male and female rats.

Based on the metabolism studies of Chism, Turner and Rickert (1984) three urinary metabolites (2-nitrobenzoic acid, 2-nitrobenzylmercapturic acid and 2-aminobenzoic acid) were chosen as biomarkers of exposure to establish the correlation between exposure concentration and internal dose and to determine how metabolism of 2-nitrotoluene may change with chronic exposure and age. A carcinogenicity study was conducted by NTP (2002) in F344/N rats (6-7 weeks old) administered 0, 625, 1250 and 2000 ppm of 2-nitrotoluene (>99% pure) in the diet for 105 weeks days. From each treatment group, five males and five females were randomly selected for urinary metabolite analyses at 2 weeks and at 3, 12 and 18 months. Comparisons among the metabolite data were made using the metabolite/creatinine ratio. The ratios of 2-aminobenzoic acid to creatinine for control and exposed males and females were generally similar. The 2-nitrobenzoic acid/creatinine ratios were linearly related to exposure concentrations and generally larger for exposed females than

for males. The 2-nitrobenzylmercapturic acid/creatinine ratios were also linearly related to exposure concentrations and significantly smaller for exposed females than for males. There appear to be differences in metabolism between male and female rats, with males excreting more 2-nitrobenzylmercapturic acid and females excreting more 2-nitrobenzoic acid. Because the first step in the formation of either 2-nitrobenzoic acid or 2-nitrobenzylmercapturic acid is oxidation of the methyl group to a benzyl alcohol, metabolic differences must be either in further oxidation to the carboxylic acid or in formation of conjugates of the alcohol and further reaction with reduced glutathione.

Mice

Good quality and GLP compliant studies were conducted by NTP (2002) in B6C3F1 mice, of 9 to 11 weeks old at dosing, to determine the metabolism and excretion of 2-nitrotoluene following the administration of single doses.

In the initial study groups of 4 males were given 200 mg/kg b.w. of [¹⁴C]-2-nitrotoluene (≥ 98% purity) in Emulphor by gavage. Radioactivity was measured in urine and feces collected 4, 8, 24, 48 and 72 hours after dosing. 2-nitrotoluene was excreted mainly in urine with 66% of the dose in the first 24 hours or with 74% of the dose in the first 48 hours. Total recovery of radiolabel was 87% (78% in urine and 9% in feces). The profile of urinary metabolites was determined by HPLC. There were only two major urinary metabolites, 2-nitrobenzoic acid (A, 38%), and 2-nitrobenzyl glucuronide (B, 24%). After urine incubation with glucuronidase/sulfatase the most pronounced change, in the profile of metabolites, was the loss of the peak B giving rise to peak G; and upon incubation with purified sulfatase, peak B remained, confirming it is a glucuronide rather than a sulfate conjugate. The identity of peak G was confirmed by coelution with the authentic standard, 2-nitrobenzyl alcohol.

In the second study, groups of 3 males were given 2 mg/kg b.w. of [¹⁴C]-2-nitrotoluene (≥ 98% purity) in Emulphor:ethanol:water (1:1:8) by gavage. Radioactivity was measured in urine and feces collected 24, 48 and 72 hours after dosing. Excretion of radioactivity was similar to that found after a 200 mg/kg dose, with about 60% of the dose excreted in the urine in the first 24 hours after dosing or with 69% of the dose in the first 48 hours. Total recovery of radiolabel was 108% (85% in urine and 23% in feces). Mice again excreted primarily 2-nitrobenzoic acid and 2-nitrobenzyl glucuronide in urine, but a small amount of 2-aminobenzyl alcohol (4%) was also detected.

Three urinary metabolites (2-nitrobenzoic acid, 2-nitrobenzylmercapturic acid and 2-aminobenzoic acid) were chosen as biomarkers of exposure to establish the correlation between exposure concentration and internal dose and to determine how metabolism of 2-nitrotoluene may change with chronic exposure and age. A carcinogenicity study was conducted by NTP (2002) in B6C3F1 mice (6 weeks old) administered 0, 1250, 2500 and 5000 ppm of 2-nitrotoluene (>99% pure) in the diet for 105 weeks. From each treatment group, five males and five females were randomly selected for urinary metabolite analyses at 2 weeks and at 3, 12 and 18 months. Comparisons among the metabolite data were made using the metabolite/creatinine ratio. At time points with sufficient data to permit determinations, the ratios of 2-nitrobenzoic acid to creatinine excreted in urine appeared to be linearly related to exposure concentration in males and females. The concentrations of 2-nitrobenzylmercapturic acid and 2-aminobenzoic acid were generally below the limit of quantification. Sex differences were not observed.

In vitro studies

The metabolism of 2-nitrotoluene by both isolated hepatocytes and hepatic microsomes of male Fischer 344 rats was investigated (deBethizy and Rickert, 1984). Hepatocytes were incubated with [ring-U-¹⁴C] 2-nitrotoluene (>99% purity) at concentrations from 10 to 1000 µM for varying lengths of time. Recovery of total radioactivity added to the incubation was >90%. Cell viability was unaffected by incubation for up to 90 min. 2-nitrotoluene (200 µM) after 45-min incubation was converted to 2-nitrobenzyl alcohol (52%), 2-nitrobenzyl alcohol glucuronide (28%), an unidentified metabolite (20%), and 2-nitrobenzoic acid (3%). Varying the concentration of 2-nitrotoluene in the hepatocyte incubation there were no significant changes in the percentage of total metabolism accounted for by any metabolite. The half-life for disappearance of 2-nitrotoluene from the incubation mixture was approximately 27 min. 2-nitrobenzyl alcohol increased linearly with time over the first 20 min. The unidentified metabolite and 2-nitrobenzyl alcohol glucuronide increased linearly with time over the 45-min incubation period after a lag phase of 10 min. When hepatic microsomes were incubated with [ring-U-¹⁴C] 2-nitrotoluene for up to 90 min, the only metabolite formed was the nitrobenzyl alcohol and its rate of formation appeared to be linear for the first 20 min. In summary, experiments with hepatic microsomes indicate that 2-nitrotoluene is initially oxidized at the methyl group, by a cytochrome P-450-dependent process, and isolated rat hepatocytes metabolize the resulting 2-nitrobenzyl alcohol by conjugation with glucuronic acid (major pathway) or by oxidation to 2-nitrobenzoic acid (minor pathway).

The role of 2-aminobenzyl alcohol on metabolism of 2-nitrotoluene, as a substrate for microsomal N-hydroxylation, was investigated (Rickert, Chism and Kedderis, 1986). 2-aminobenzyl alcohol (0.5 mM final concentration) was incubated with rat liver microsomes in the presence of NADPH for 30 min. 2-aminobenzyl alcohol yielded metabolites capable of reducing Fe³⁺. The rate of formation was 0.29 ± 0.01 nmol reducing equivalents/min/mg microsomal protein. HPLC analysis of ethyl acetate extracts of reaction mixtures revealed the formation of 2 metabolites which were capable of reducing ferric iron. Comparison of the retention times of the products of microsomal metabolism with the synthesized 2-hydroxylaminobenzyl alcohol standard showed that one metabolite co-eluted with the hydroxylamine. The second metabolite was not identified, but it was suggested as a ring hydroxylated aminobenzyl alcohol. These data are consistent with the proposed mechanism for activation: 2-nitrotoluene is converted to 2-nitrobenzyl glucuronide which is excreted in the bile. The action of intestinal microflora can convert the glucuronide into 2-aminobenzyl alcohol which is the substrate for hepatic cytochrome P-450-dependent N-hydroxylation. Since, the *in vivo* administration of sulfotransferase inhibitors decreases covalent binding to hepatic DNA by >96% (to see Rickert *et al.*, 1984) it seems possible that the ultimate reactive metabolite of 2-nitrotoluene is an unstable N,O-sulfate which decomposes to electrophilic nitrenium ions.

In order to determine the metabolic pathway involved in the activation of 2-aminobenzyl alcohol, [ring-U-¹⁴C] 2-aminobenzyl alcohol was incubated in the presence of calf thymus DNA with either male Fischer-344 rat or male Rhesus monkey hepatic cytosol and 3'-phosphoadenosine 5'-phosphosulfate (PAPS), microsomes and NADPH or microsomes and cytosol with PAPS and NADPH; 2,6-dichloro-4-nitrophenol (DCNP) was added to some incubations. DNA was isolated and analysed for covalently bound ¹⁴C. For both species, 2-aminobenzyl alcohol was activated to a metabolite capable of covalently binding to DNA only when PAPS and cytosol were present. The PAPS-dependent binding (5 times higher in rat cytosol than in monkey cytosol) was inhibited by DCNP. HPLC analysis of the incubations revealed one major PAPS-dependent, acid labile metabolite (11% and 5% of added substrate

for rat and monkey, respectively) which is lowered by the addition of DNCP (2% of added substrate). Results of the study (reported as an abstract) suggest that the precursor to the metabolite of 2-nitrotoluene responsible for covalently binding to DNA is a sulfate conjugate of 2-aminobenzyl alcohol, most likely 2-aminobenzyl sulfate (Chism and Rickert, 1987).

The possible pathways for activation of 2-aminobenzyl alcohol were studied *in vitro* by using various enzyme sources and cofactors in appropriate combinations (Chism and Rickert, 1989). Calf thymus DNA and [ring-U-¹⁴C] 2-aminobenzyl alcohol (98% purity) were incubated with male Fischer-344 rat hepatic cytosol and PAPS, microsomes and NADPH, or microsomes and cytosol with PAPS, NADPH, and acetyl coenzyme A. In addition, DCNP or NADP was added to the cytosolic incubations following generation of PAPS but prior to the addition of substrate. ¹⁴C] 2-aminobenzyl alcohol was also incubated with cytosol, PAPS and hepatic nuclei from male Fischer-344 rats for determining radiolabel bound to DNA and protein. Acid hydrolysis by adjusting the sample pH to 3 with HCl, and enzymatic hydrolysis by incubating the sample with sulfatase were performed. Analysis of cytosolic incubations revealed radiolabel bound covalently to DNA, as well as one metabolite labile in both sulfatase and acid, identified tentatively as 2-aminobenzyl sulfate (M1). The appearance of each required the presence of PAPS and was inhibited by DCNP. NADP did not alter the formation of the metabolite M1 but reduced radiolabel bound to DNA. In incubations with microsomes, there was very little radiolabel bound to DNA, regardless of the presence and absence of NADPH but the radiolabel bound to microsomal protein increased in the presence of NADPH and was about twice as great as that bound cytosolic proteins. HPLC analysis revealed the NADPH-dependent formation of a metabolite identified tentatively as 2-(N-hydroxylamino)-benzyl alcohol (M2). When microsomes and cytosol were incubated together, generation of radiolabel bound to DNA or the appearance of metabolite M1 was detected only when PAPS was included in the incubations. Inclusion of NADPH resulted in the detection of metabolite M2. The addition of acetyl coenzyme A resulted in the formation of a metabolite identified tentatively as 2-(N-acetylamino) benzyl alcohol (M3). In the absence of PAPS, inclusion of NADPH and acetyl coenzyme A resulted in no radiolabel bound to DNA. Compared to incubations that contained cytosol and microsomes and no added cofactors, radiolabel bound to protein was increased by NADPH, lowered by acetyl coenzyme A, and either not affected or slightly increased by PAPS. Incubation of hepatic nuclei instead of thymus DNA with cytosol, PAPS and [ring-U-¹⁴C] 2-aminobenzyl alcohol resulted in radiolabel bound to DNA and protein; removal of cytosol and PAPS reduced the radiolabel bound to DNA by 70% and increased the radiolabel bound to protein. These data suggest two enzyme-mediated pathways of 2-aminobenzyl alcohol metabolism that can result in binding to cellular macromolecules. One pathway required PAPS and hepatic cytosolic enzymes and generated a compound that bound covalently to DNA. DCNP, an inhibitor of sulfotransferase, decreased the covalent binding to DNA and inhibited the PAPS-dependent formation of a sulfatase-labile metabolite. Therefore, it seems likely that the active metabolite is 2-aminobenzyl sulfate. The other pathway required hepatic microsomal enzymes and NADPH and resulted in an intermediate that bound covalently to protein. PAPS appeared to play no role in the formation of a protein binding species and acetyl coenzyme A decreased the covalent bound to protein. Therefore, these data suggest that 2-(N-hydroxylamino) benzyl alcohol or a phenolic metabolite of 2-aminobenzyl alcohol is the precursor to the reactive compound that bound covalently to protein whereas the formation of 2-(N-acethylamino) benzyl alcohol, mediated by acetyl coenzyme A, may be a route of detoxification.

In order to determine the role of sulfation in the conjugation of 2-nitrobenzyl alcohol with glutathione, 2-nitrobenzyl alcohol was incubated with rat liver cytosol in the presence or absence of a PAPS-generating system. No evidence for the formation of glutathione

conjugates with 2-nitrobenzyl alcohol was found by HPLC analysis of the incubation mixtures (NTP, 2002).

4.1.2.1.2 Studies in humans

In vivo studies

Inhalation

No data available.

Dermal

No data available

Oral

No data available.

In vitro studies

No data available.

4.1.2.1.3 Other information

The following studies are not specific toxicokinetic studies but provide relevant information on the relationship of metabolism to toxicity and by this reason they have been included in this section.

The disposition and hepatic macromolecular covalent binding of ^{14}C -2-nitrotoluene was investigated in F-344 rats following oral administration (200 mg/kg b.w) (Long and Rickert, 1983). Livers, small intestines, cecal contents and portal vein blood were collected at 3, 6, 12, 24, 48 and 96 hours. Tissue homogenates and blood were assayed for radioactivity and for individual metabolites by HPLC, and covalent binding to hepatic macromolecules was estimated by exhaustive extraction of TCA-precipitable material. Covalent binding of ^{14}C -2-nitrotoluene to hepatic macromolecules reached its peak after 12 hours and decreased slowly. 96 hours after 2-nitrotoluene administration it was still markedly above the first measurement (at 3 hours). Appearance of 2-nitrotoluene metabolites in the intestines and portal blood preceded peak concentrations (12 hr) of covalently bound ^{14}C , suggesting that enterohepatic circulation was involved in the generation of the reactive metabolites. In the intestines, the nitrobenzyl alcohol glucuronide (NBA1cG) was present in greater quantity following 2-nitrotoluene administration. Two unknown metabolites and the acetamido-benzoic and nitrobenzoic acids were also present. These data suggest that 2-nitrotoluene is probably activated following metabolism to NBA1cG.

The effects of the sulfotransferase inhibitors on covalent binding of 2-nitrotoluene to hepatic macromolecules, including DNA, were determined in male Fischer-344 rats (Rickert *et al.*, 1984). Rats (18 animals, 70-80 days old) were given orally 200 mg/kg of [ring- ^{14}C] 2-nitrotoluene (>99% purity) dissolved in corn oil. 3 rats from each group were anesthetized with methoxyflurane at 3, 6, 12, 24, 48 and 96 h after the dose. Livers were removed and

analyzed for total and covalently bound radiolabel. While concentrations of total radioactivity peaked at 6-12 h after the dose and declined rapidly between 12 and 24 h, concentrations of covalently bound material peaked at 12 h after the dose and declined slowly thereafter. When DNA was isolated from livers of rats, covalent binding was observed at levels above the detection limits of the assay. The effects of the sulfotransferase inhibitors, PCP and DCNP, were tested in a group of 27 animals. 9 rats received an i.p. injection of DCNP (40 µmol/kg in propane-1,2-diol), 9 rats received an i.p. injection of PCP (40 µmol/kg in propane-1,2-diol) and 9 rats received only propane-1,2-diol. 45 min later 3 animals from each pretreatment group were given orally 150 mg/kg of [ring-U-¹⁴C] 2-nitrotoluene (>99% purity) dissolved in corn oil. Animals were killed by cervical dislocation 12 h later. Livers were removed and analyzed for covalently bound radiolabel. Prior administration of DCNP and PCP decreased the hepatic covalent binding to macromolecules by 63% and 57%, respectively, and to DNA by >96%. These results suggest that 2-nitrotoluene requires the action of sulfotransferase for its conversion to a species capable of covalently binding to hepatic DNA

In a study performed by Marques *et al.*, 1997 (cited in NTP, 2002), focused on reaction at the nitrogen of the arylamine, it was observed that the conformation of DNA adducts is a determinant factor in the genotoxic response induced by *ortho*-, *meta*- or *para*-substituted arylamines. Adducts or *ortho*-analogues tend to adopt a *syn* conformation while *meta*- and *para*-analogues tend to adopt an *anti* conformation. This fact may be also applied for reaction at the benzylic carbon of an *o*-nitrotoluene metabolite since *o*-nitrotoluene, but not *m*- or *p*-nitrotoluene, induced DNA repair in the *in vivo* UDS assay in male rats.

4.1.2.1.4 Summary of toxicokinetics, metabolism and distribution

There are not available data on toxicokinetics of 2-nitrotoluene for humans but several studies following oral administration have been performed in experimental animals, especially in rats. *In vitro* studies provide additional information on the metabolism.

Absorption

2-nitrotoluene is rapidly absorbed, extensively metabolised and rapidly excreted in rats and mice.

2-nitrotoluene concentrations in plasma of male rats peaked 15 to 60 minutes after a dose of 200 mg/kg b.w., rapidly decreased through 24 hours after dosing and were below the limit of detection at 24 hours after dosing; the half-life of 2-nitrotoluene in plasma was about 1.5 hours (NTP, 2002).

The excretion of radioactivity was measured in cannulated rats 12 hours after a single dose of 200 mg/kg b.w. The bile excretion of radioactivity was 29% (males) and 10% (females); the urinary excretion of radioactivity was 36% (males) and 33% (females), and fecal elimination accounted for <2%. Since fecal elimination accounted for <2% it is presumed that the label is reabsorbed from the gut.

Oral absorption in rats was determined to be 100% within 24 hours based on excretion of radioactivity (more than 95% in urine from both sexes) obtained after a single oral dose of 2 mg/kg b.w. There were no differences between sexes. In addition, similar results were obtained in rats administered a single or a repeated oral dose of 200 mg/kg b.w.

Oral absorption in male mice was determined to be 100% within 72 hours based on excretion of radioactivity (85% in the urine) obtained after a single oral dose of 2 mg/kg b.w. Similar results were obtained in mice administered a single oral dose of 200 mg/kg b.w.

No data are available for inhalation exposure route. Then, the worst case inhalation absorption should be assumed (i.e. 100%).

No data are available for dermal exposure route. Then, a default value for dermal absorption of 100% should be applicable based on both the physico-chemical properties of the substance (MW= 137.14, log P_{ow} = 2.3) and the oral excretion data.

Distribution

Pertinent data were not located. However, 2-nitrotoluene appears to be well distributed as indicated by toxicity in various organs of rats or mice orally exposed (to see sections corresponding to repeated dose toxicity studies). In rats, toxicity was observed mainly in liver, kidney, spleen, testes and haematopoietic system. In addition, based on excretion data (a total recovery of radioactivity in urine and feces of rats and mice 24 or 72 hours after dosing) it is appropriate to state “no evidence of accumulation”.

Metabolism

No parent compound was detected in urinary samples of rodents. The urinary metabolites that have been identified in rats and mice are shown in Figure 1.

In rats, at least nine urinary metabolites were identified: 2-nitrobenzoic acid, 2-nitrobenzyl glucuronide, S-(2-nitrobenzyl)-N-acetylcysteine, S-(2-nitrobenzyl)-glutathione, 2-nitrobenzyl sulfate, 2-nitrobenzyl alcohol, 2-aminobenzoic acid, 2-aminobenzyl alcohol and *o*-toluidine; (Chism, Turner and Rickert, 1984; NTP, 2002) The metabolite profiles after 2 mg/kg dose were similar to those seen after the 200 mg/kg dose; the most pronounced difference was a greater proportion of the dose excreted as 2-nitrobenzoic acid and 2-nitrobenzyl glucuronide after the lower dose; the urinary profile after repeated doses showed decreased excretion of S-(2-nitrobenzyl)-N-acetylcysteine but no other obvious changes; there was a sex-dependent variance in metabolite profile with females excreting less 2-aminobenzyl alcohol and S-(2-nitrobenzyl)-N-acetylcysteine than males, but more 2-nitrobenzoic acid (NTP, 2002).

Male mice excreted primarily 2-nitrobenzoic acid and 2-nitrobenzyl glucuronide in urine after 200 mg/kg b.w. dose, but a small amount of 2-aminobenzyl alcohol was also detected after 2 mg/kg b.w. dose (NTP, 2002).

The major metabolite excreted in bile of rats following 2-nitrotoluene administration was 2-nitrobenzyl glucuronide. Males excreted about 3 times as much of this metabolite as did females. The next most abundant biliary metabolite was S-(2-nitrobenzyl)-glutathione, and males excreted 10 times as much of this metabolite in the bile as did females (Chism and Rickert, 1985). Sex differences seen in covalent binding to DNA and genotoxicity could be explained by the greater biliary excretion in male than in female rats.

On the basis of all data from *in vivo* and *in vitro* studies, it can be said that the metabolism of 2-nitrotoluene proceeds firstly by cytochrome-P450 mediated oxidation to nitrobenzyl alcohol which then undergoes metabolism by four pathways: a) oxidation to 2-nitrobenzoic acid; b) conjugation with glutathione to 2-nitrobenzylmercapturic acid; c) nitro-group reduction to 2-aminobenzoic acid; and d) conjugation with glucuronic acid to 2-nitrobenzyl glucuronide (Figure 1). This latter pathway appears as the responsible of 2-nitrotoluene bioactivation

(Figure 2). The glucuronide metabolite secreted in the bile is believed to be converted to 2-aminobenzyl alcohol by hydrolytic and reductive activities of intestinal microflora, and then systemically reabsorbed. The final activation step is dependent upon sulfotransferase, since sulfation of 2-aminobenzyl alcohol lead to covalent binding to macromolecules. Two enzyme-mediated pathways are involved. One of them requires *in vitro* PAPS and cytosolic enzymes and generates a compound (likely, 2-aminobenzyl sulfate) that binds covalently to DNA. The reactivity of 2-aminobenzyl sulfate with DNA could be related to the ease formation of a reactive benzyl cation due to electron-donating ability of the amino group. The other pathway requires *in vitro* hepatic microsomal enzymes and NADPH, and results in an intermediate that binds covalently to protein. There is evidence that oxidation of 2-aminobenzyl to 2-(N-hydroxylamino) benzyl alcohol followed by sulfation yields an unstable N-sulphate which decomposes to an electrophilic nitrenium and/or carbonium ions. Since *o*-nitrotoluene, but not *m*- or *p*-nitrotoluene, induced DNA repair in the *in vivo* UDS assay, the *syn* conformation of DNA adducts (focused on reaction at the benzylic carbon of an *o*-nitrotoluene metabolite) appears to be a determinant factor in the genotoxic response, as demonstrating previously for *ortho*-arylamines (focused on reaction at the nitrogen-group).

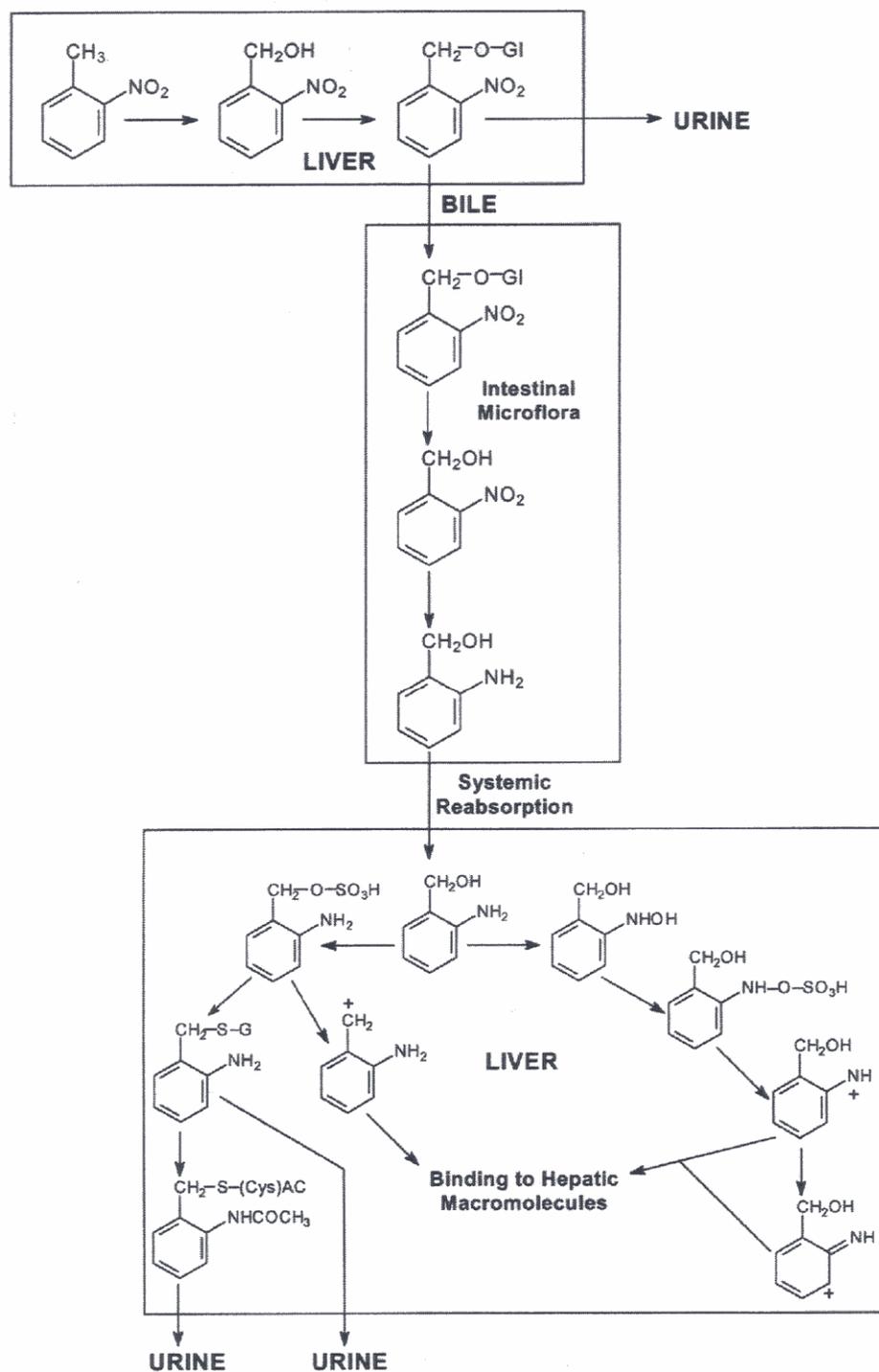


Figure 2: Proposed pathway for bioactivation of *o*-nitrotoluene (Chism and Rickert, 1985)

Excretion

Following oral administration of 2-nitrotoluene, the substance and its metabolites are excreted rapidly and extensively in urine, feces and in expired air. There is no evidence of accumulation in organs and tissues.

The routes of excretion were similar in rats and mice, with the predominant route being via urine. By 72 hours after a single oral dose of 2 mg/kg b.w., the percentages of the radioactivity recovered in the urine were 100% (rats) and 85% (mice), and fecal excretion accounted for 4-5% (rats), and 23% (male mice). The higher amount of radioactivity in feces of mice can be attributed to contamination of the feces by urine (NTP, 2002). Minimal amounts of radiolabel (0.1%) were captured in expired air (Chism, Turner and Rickert, 1984).

The rate of excretion was more rapid in rats, with about 100% of the radioactivity excreted in urine in the first 24 hours. Less than 70% of the administered radioactivity was excreted in urine by mice in the same time period.

For male and female rats similarly treated, biliary excretion measured after 12 h was greater for males (29%) than for females (10%). Since proportionately less label is excreted via the feces it is presumed that the label is reabsorbed from the gut. In addition, cannulation of the bile duct inhibits covalent binding in the liver indicating the involvement of the enterohepatic circulation (Chism and Rickert, 1985).

4.1.2.2 Acute toxicity

4.1.2.2.1 Studies in animals

In vivo studies

Several studies have been carried out using different species and administration routes. They are summarized in table 4.1.2.2.1.

Inhalation

The acute inhalation toxicity of 2-nitrotoluene has been investigated in three studies in rats and in one mouse study. The studies were reported over the period 1972-77, pre-guideline performed and GLP non compliant. None of these studies were conducted to modern guidelines. In addition, the purity of the test substance was not reported.

Rats

ChR-CD male rats were exposed to 2-nitrotoluene vapours at an average analytical concentration of 209 ppm (1.17 mg/L) for 1 h. 2-nitrotoluene was metered, using a syringe drive, into a heated stainless steel tube. Houseline air carried the resulting vapours into a 20-liter battery jar containing 10 animals. Neither gross nor histopathological examination was performed on any animals tested. The animals displayed only mild clinical signs (face-pawing, grooming, labored respiration, red-tinged discharge from eyes) during exposure and had a normal rate of weight gain following exposure (Brown & Reinhardt, 1972).

SPF Wistar male rats (6 animals) were exposed to saturated vapour of 2-nitrotoluene, i.e. at a concentration of 190.8 ppm (1.086 mg/L) for 8 h. There were not mortalities, toxicity and gross lesions within 14-day observation period (Hollander and Weigand, 1975a)

Sprague-Dawley CFE male rats were exposed to saturated vapours of 2-nitrotoluene, i.e. at a concentration of 320 ppm (1.795 mg/L) for 4 h. The percentage of saturation for this concentration was calculated to be 77%. Production of saturated vapours was accomplished by bubbling dry air through a fritted disc immersed in the sample. The resultant vapours were then passed through a 9-liter glass chamber containing 10 animals. There were not mortalities, toxicity and gross lesions within 14-day observation period. All animals gained weight normally during the 14-day observation period (Kinkead *et al.*, 1977).

Mice

Sprague-Dawley CF-1 male mice were exposed to saturated vapours of 2-nitrotoluene, i.e. at a concentration of 354 ppm (1.986 mg/L) for 4 h. The percentage of saturation for this concentration was calculated to be 85%. Production of saturated vapours was accomplished by bubbling dry air through a fritted disc immersed in the sample. The resultant vapors were then passed through a 9-liter glass chamber containing 10 animals. There were not mortalities, toxicity and gross lesions within 14-day observation period. All animals gained weight normally during the 14-day observation period (Kinkead *et al.*, 1977).

Dermal

The acute dermal toxicity of 2-nitrotoluene has been investigated in two studies in rabbits and in one rat study. The studies were reported over the period 1972-77, pre-guideline performed and GLP non compliant. However, they appear to be broadly similar to modern test guidelines although the purity of the test substance was not reported.

Rats

A limit test was carried out at one dose level, 5000 mg/kg b.w. in a group of 6 female SPF-Wistar rats. The test substance was applied undiluted on the clipped back for 24 hours, covered by a plaster and then washed, not observing either mortality or toxicity within 14-day observation period. (Hollander and Weigand, 1975b).

Rabbits

A limit test was carried out at one dose level, 20000 mg/kg b.w. in a group of 3 female New Zealand albino rabbits. The test substance was applied undiluted to the clipped back and was kept in place by gauze patches, latex rubber, dental dam and elastic adhesive tape for 24 hours, and then removed. All rabbits were symptom free and gained weight normally during the subsequent 14-day observation period.

2-nitrotoluene was applied to the intact clipped on the dorsal skin of 6 male albino rabbits at a dose level of 200 mg/kg b.w. The rabbits' trunks were then wrapped with a layer of Saran® Wrap, stretch gauze bandage and elastic adhesive tape. After 24 hours, the wrappings were removed and the skin washed and dried. The animals were fitted with plastic collars to prevent ingestion of any residue and were observed for a further 48 hours. There were not mortality and clinical signs (McDonnell and Reinhardt, 1972).

Oral

The acute oral toxicity of 2-nitrotoluene has been investigated in rats, mice and rabbits. The studies were reported over the period 1972-85, pre-guideline performed and GLP non compliant. Most of the available studies have limited quality, especially with respect to the identity of the test substance and the description of test methods.

The studies of best quality were performed by Ciss in rats (Ciss, 1978; Ciss *et al.*, 1980a). 2-nitrotoluene (>99% purity) was administered by gavage to Wistar rats, using neutralized olive oil as vehicle. Initially, four groups of three males were treated with doses of 1700, 2900, 5800 and 11600 mg/kg b.w. There were deaths in all treated groups: 1/3 (1700 mg/kg b.w.group, 24 h after dosing), 2/3 (2900 mg/kg b.w. group, 24 and 48 h after dosing), 3/3 (5800 mg/kg b.w.group, 24 h after dosing) and 3/3 (11600 mg/kg b.w. group, 24 h after dosing). Accordingly, six groups consisting of 10 males and 10 females were treated with doses of 1000, 1500, 2000, 2500, 3000 and 4000 mg/kg b.w. Mortalities were observed at 1500 mg/kg b.w and above as follows: At 1500 mg/kg b.w there were 3/20 deaths (1/10 ♂ and 2/10 ♀ 18-24 hours post-dosing); at 2000 mg/kg b.w. there were 9/20 deaths (2/10 ♂ and 4/10 ♀ 18-24 hours post-dosing, and 3/10 ♂ 48 hours post-dosing); at 2500 mg/kg b.w. there were 15/20 deaths (5/10 ♂ and 6/10 ♀ 18-24 hours post-dosing, and 2/10 ♂ and 2/10 ♀ 48 hours post-dosing); at 3000 mg/kg b.w. there were 16/20 deaths (6/10 ♂ and 8/10 ♀ 18-24 hours post-dosing, and 2/10 ♂ 48 hours after dosing); and at 4000 mg/kg b.w. all 20 animals (10 ♂ and 10 ♀) died 18-24 hours after dosing. The LD₅₀ was determined to be 2100 ± 145 mg/kg b.w for both males and females, using the Miller and Tainter's method (1944) following the directions of Barlett (1937). Gross pathological results were not documented. Clinical signs of toxicity were characterized by excitation which occurred five or ten minutes after the beginning of the treatment. There was an acceleration of the respiratory rhythm and convulsions. It was followed by a phase of slumber which could be last 24 hours. Mortalities occurred up to 2–days post-dosing, otherwise the recovery was gradually made to be complete at the end of the week. In addition, Wistar rats (10 animals) were orally administered 2-nitrotoluene at a single dose of 3000 mg/kg b.w. for determining in blood extracted from eye sinus biochemical parameters such as -SH group and methaemoglobin rates (Ciss, 1978). Mortalities (8/10) occurred 24 hours post-dosing (3/10), 48 hours post-dosing (4/10) and 1 week post-dosing (1/10). SH group rates were: 10.76 ± 0.11 mmoles/L (pre-dosing), 10.76 ± 0.10 mmoles/L (4 hours post-dosing), 10.5 ± 0.10 mmoles/L (24 hours post-dosing), 10.3 ± 0.14 mmoles/L (48 hours post-dosing), 10.1 ± 0.18 mmoles/L (1 week post-dosing), and 10.1 ± 0.19 mmoles/L (2 weeks post-dosing). Methaemoglobin rates (expressed as percentages of total haemoglobin) were 0.73% (pre-dosing) and 1.07% (48 hours post-dosing). Most mortality occurred again during the first 2 days post-dosing. The slight decreases in SH group rate observed can be the consequence of an indirect toxicity mechanism that results in immobilization or elimination of essential amino acids for growth such as cysteine.

Table 4.1.2.2.1: Summary of acute toxicity of 2-nitrotoluene in experimental animals

Route	Species	Dosage	LC ₅₀ (mg/L) or LD ₅₀ (mg/kg b.w.)	Comments	References
Inhalatory	Rat, ChR-CD (♂) 10 rats/group	209 ppm = 1.17 mg/L (1 h)	>1.17 mg/L	Purity not given	Brown and Reinhardt (1972)
	Rat, SPF Wistar (♂) 6 rats/group	190.8 ppm = 1.086 mg/L (8 h)	>1.086 mg/L	Purity not given	Hollander and Weigand (1975a)
	Rat, Sprague-Dawley CFE (♂) 10 rats/group	320 ppm = 1.795 mg/L (4 h)	>1.795 mg/L	Purity not given	Kinkead <i>et al.</i> (1977)
	Mouse, Sprague-Dawley CF-1 (♂) 10 mice/group	354 ppm = 1.986 mg/L (4 h)	>1.986 mg/L	Purity not given	Kinkead <i>et al.</i> (1977)
Dermal	Rat, SPF Wistar (♀) 6 rats/group	5000 mg/kg b.w. (24 h)	>5000 mg/kg b.w.	Purity not given Limit test	Hollander and Weigand (1975b)
	Rabbit, albino (♂) 6 rabbits/group	200 mg/kg b.w. (24 h)	>200 mg/kg b.w.	Purity not given	McDonnell and Reinhardt (1972)
	Rabbit, albino New Zealand (♀) 3 rabbits/group	20000 mg/kg b.w. (24 h)	>20000 mg/kg b.w.	Purity not given Limit test	Kinkead <i>et al.</i> (1977)
Oral	Rat		891 (530-1584) mg/kg b.w.	Purity not given. Method not reported.	Back, Thomas and MacEwen (1972)
	Rat, SPF Wistar (♀) 10 rats/group	1600, 2000, 2500, 3200, 4000 mg/kg b.w. (by gavage in sesame oil).	2546 (2343-2766) mg/kg b.w.	Purity not given.	Hollander and Weigand (1975c)
	Rat		1610 mg/kg b.w.	Purity not given.	Vasilenko and Kovalenko (1976)
	Rat, Sprague-Dawley (♂)		890 (500-1580) mg/kg b.w.	Purity not given. Experimental details not documented	Vernot <i>et al.</i> (1977)
	Rat, Wistar (♂, ♀) 10 rats/sex/group	1000, 1500, 2000 2500, 3000, 4000 mg/kg bw. (by gavage in olive oil).	2100 ± 145 mg/kg b.w.	>99% purity. ↓-SH group and ↑methaemoglobin (3000 mg/kg bw). Clinical signs related with the formation of methaemoglobin.	Ciss (1978)* Ciss <i>et al.</i> (1980a)*
	Rat		891 mg/kg b.w.	Purity not given. Method not reported.	NIOSH (1985)
	Mouse		2462 (1789-3390) mg/kg b.w.	Purity not given. Method not reported.	Back, Thomas and MacEwen (1972)
	Mouse		970 mg/kg b.w.	Purity not given. Method not reported.	Vasilenko and Kovalenko. (1976)
	Mouse, CF-1 (♂)		2460 (1790-3390) mg/kg b.w.	Purity not given. Experimental details not documented.	Vernot <i>et al.</i> (1977)
	Mouse		970 mg/kg b.w.	Purity not given. Method not reported.	NIOSH (1985)
	Rabbit		1750 mg/kg b.w.	Purity not given. Method not reported	Vasilenko and Kovalenko. (1976)

*) Studies of good quality for risk assessment

On the other hand, the effect of a single oral dose on hematopoietic system has been studied in cats (Hollander and Weigand, 1975d). Two female cats were given orally 100 mg/kg (dose <LD₅₀) of 2-nitrotoluene (purity not given), dissolved in sesame oil, by gavage. 3 hours later, the animals' total leucocyte count was increased and their differential blood picture showed a rise in neutrophilic granulocytes and a decrease in lymphocytes. These changes were largely reversible within 24 hours. An increase in Heinz bodies was also observed (with a maximum of 11.5% in one cat and 15% in the other). No methaemoglobin formation was detected after 1 to 48 hours. The methaemoglobin formation may not have been detectable at the measuring time selected (after 1, 3, 7, 24 and 48 hours) especially in view of the fact that the number of Heinz bodies was already increased after 1 hour. The appearance and behaviour of the animals were unchanged.

In vitro studies

No data available.

4.1.2.2.2 Studies in humans

In vivo studies

Specific data on acute toxicity are limited to inhalation exposure.

However, according to information of different references cited in the HSDB (2004) it is stated that: a) 2-nitrotoluene is toxic by all routes (inhalation, ingestion and dermal absorption); b) it produces methaemoglobin causing hypoxia, but a low potency; c) target organs were blood, central nervous system, gastrointestinal, cardiovascular system and skin; d) clinical signs and symptoms were headache, flushing of face, dizziness, dyspnea, cyanosis, nausea, vomiting, muscular weakness, increased pulse and respiratory rate, irritability and convulsions. Nevertheless, it is impossible to determine the exposure-response relationship because original data are not available.

Inhalation

In order to assure that men and women may work in the chemical industry without harm, the toxic concentrations of various gases, dusts, fumes and metals in the atmosphere were established as well as the upper limit to satisfactory conditions (Goldblatt, 1955). With respect to 2-nitrotoluene vapour, the information is limited to a table of concentrations where 200 ppm (1140 mg/m³) is considered a concentration causing severe toxic effects in persons after exposure for 60 min; 40 ppm (228 mg/m³) is considered a no tolerated concentration because if exposure continues for more than a short time, may lead to symptoms of illness; and 1 ppm (5.7 mg/m³) is considered the upper limit to satisfactory conditions. The table of concentrations was elaborated taking into account data on human effects derived from a searching examination of clinical and experimental records.

In addition, an IDLH (Immediately Dangerous to Life or Health) of 200 ppm has been established by NIOSH (NTIS publication n° PB-94-195047, May 1994)

Dermal

No data available.

Oral

No data available.

In vitro studies

No data available.

4.1.2.2.3 Summary of acute toxicity

The acute toxicity of 2-nitrotoluene has been investigated in rats and mice (inhalatory), rats and rabbits (dermal), and rats, mice and rabbits (oral).

The available studies on inhalatory toxicity have limited quality especially with respect to the identity of the test substance (purity not reported). However, because of results were similar they can be useful for risk assessment. At saturated vapour concentrations, 190.8 ppm (1.086 mg/L) for 8 h or 320 ppm (1.795 mg/L) for 4 h in rats and 354 ppm (1.986 mg/L) for 4 h in mice, 2-nitrotoluene, did not produce mortalities, toxicity and gross lesions within 14-day observation period. Therefore, according to EU criteria, no classification is necessary.

The available studies on dermal toxicity have limited quality especially with respect to the identity of the test substance (purity not reported). However, because of results were similar they can be useful for risk assessment. In a limit test, 2-nitrotoluene (5000 mg/kg b.w. in rats and 20000 mg/kg b.w. in rabbits) did not produce either mortality or toxicity within 14-day observation period. Therefore, according to EU criteria, no classification is necessary.

The available studies on acute oral toxicity have limited quality (only in one study purity was reported). However, because of results were similar they can be useful for risk assessment. The oral LD₅₀ value ranged from 890 to 2546 mg/kg b.w. in rats, from 970 to 2462 mg/kg b.w. in mice and was determined to be 1750 mg/kg b.w. in rabbit. Clinical signs of toxicity were related with methaemoglobin formation. Based on the lower oral LD₅₀ value (890 mg/kg b.w. in rats), according to EU criteria, 2-nitrotoluene is classified as Xn R22. The only numerical value of any reliability for risk characterization obtained from these studies is the LD₅₀.

The reported effects in humans exposed by different routes are most likely due to methaemoglobin formation but data on acute toxicity (Goldblatt, 1955) are limited to inhalation exposure. However, we have disregarded them because a NOAEC/LOAEC could not be determined as starting point for risk characterization for acute toxicity because the exposure duration was not reported.

Therefore, the risk assessment for acute toxicity has been derived from animal data.

4.1.2.3 Irritation

Acute dermal and ocular irritation studies performed are summarized in table 4.1.2.3.

Table 4.1.2.3: Summary of acute toxicity (irritation) of 2-nitrotoluene in experimental animals

Exposure	Species	Protocol	Effect	References
Dermal	Rabbit, albino 6 rabbits	The test substance (99.2% purity) is applied undiluted (0.5 ml/rabbit) to intact areas of skin. 4 h exposure, observations at 24 and 48 h.	Not irritating	Edwards and Reinhardt (1973)*
	Rabbit, SPF albino Himalayan 6 rabbits	The test substance (purity not given) is applied undiluted (0.5 ml/rabbit) and diluted in sesame oil (0.5 ml 10% dilution/ rabbit) to clipped intact and scarified areas of skin. 24 h exposure, observations at 24, 48 and 72 h.	Not irritating	Hollander and Weigand (1975e)
	Rabbit, albino New Zealand (♀) 6 rabbits	The test substance (purity not given) is applied undiluted (0.5 ml/rabbit) to intact and abraded areas of skin. 24 h exposure, observations at 24 and 72 h.	Not irritating	Kinkead <i>et al.</i> (1977)
	Rabbit, albino (♂) 6 rabbits	The test substance (>99% purity) is applied undiluted (0.5 ml/rabbit) to intact and abraded areas of skin. 24 h exposure, observations at 24 and 72 h.	Not irritating	Ciss (1978)* Ciss <i>et al.</i> (1981)*
Ocular	Rabbit, SPF albino Himalayan 6 rabbits	The test substance (purity not given) is applied undiluted (0.1 ml/rabbit). 24 h exposure, observations at 1, 7, 24, 48 and 72 h.	Not irritating	Hollander and Weigand (1975e)
	Rabbit, albino (♂) 6 rabbits	The test substance (>99% purity) is applied undiluted (0.1 ml/rabbit). ≥ 24 h exposure, observations at 24, 48 and 72 h.	Not irritating	Ciss (1978)* Ciss <i>et al.</i> (1981)*

*) Studies considered of good quality for risk assessment

4.1.2.3.1 Skin

Studies in animals

The skin irritation of 2-nitrotoluene has been investigated in rabbits. Four studies were reported over the period 1973-81, pre-guideline performed and GLP non compliant. Only two of them (Edwards and Reinhardt, 1973; Ciss, 1978 and Ciss *et al.*, 1981) were considered for

risk assessment because of the limited quality of the remaining, especially with respect to identity of the test substance (purity not reported). In both studies, 6 rabbits were used.

In the first study (Edwards and Reinhardt, 1973), 2-nitrotoluene was applied undiluted (0.5 ml/rabbit) to an intact area and covered with a gauze patch loosely wrapped with rubber sheeting. After 4 h, the wrapping and gauze patch were removed and any skin reactions were evaluated. The test sites were then washed. Readings were again made at 24 and 48 h after the initial application. Neither the irritation evaluation method nor scores were reported.

In the second study (Ciss, 1978 and Ciss *et al.*, 1981), the test substance was also applied undiluted (0.5 ml/rabbit) to the gauze patch and then applied that to the intact and abraded areas. The gauze patch was loosely wrapped with rubber sheeting. After 24 h, the wrapping and gauze patch were removed and any skin reactions were evaluated. The test sites were then washed. Two readings were made at 24 and 72 h after the initial application, and animals were kept in observation for one week. The irritation evaluation was made by means the method described by Draize *et al.*, 1944. The results were the following:

Erythema and eschar formation	Reading times	Rabbit 1	Rabbit 2	Rabbit 3	Rabbit 4	Rabbit 5	Rabbit 6	Mean value
Intact skin	24 h	0	0	0	0	0	0	0
Intact skin	72 h	0	0	0	0	0	0	0
Abraded skin	24 h	2	1	2	2	1	1	1.5
Abraded skin	72 h	0	0	0	0	0	0	0

Oedema formation	Reading times	Rabbit 1	Rabbit 2	Rabbit 3	Rabbit 4	Rabbit 5	Rabbit 6	Mean value
Intact skin	24 h	0	0	0	0	0	0	0
Intact skin	72 h	0	0	0	0	0	0	0
Abraded skin	24 h	2	1	0	1	0	0	0.66
Abraded skin	72 h	0	0	0	0	0	0	0

According to these results, 2-nitrotoluene was considered non irritant to intact skin. With respect to abraded skin, although erythema and eschar formation was observed in 3 animals at 24 hours, it did not persist at the end of the observation time, i.e. at 72 hours.

Studies in humans

No data available. However, in the HSDB (2004) it is stated that 2-nitrotoluene is irritating to skin.

4.1.2.3.2 Eye

Studies in animals

The ocular irritation of 2-nitrotoluene has been investigated in rabbits. Two studies were reported over the period 1975-81, pre-guideline performed and GLP non compliant. In one study, the purity of the test substance was not reported; therefore, only that performed by Ciss (Ciss, 1978; Ciss *et al.*, 1981) was considered reliable for risk assessment. 6 male albino rabbits were used and 2-nitrotoluene was applied undiluted (0.1 ml/rabbit). The exposure time was ≥ 24 h. The eyes of each animal were examined at 24, 48 and 72 h. Irritation evaluation was made by means the method described by Draize *et al.*, 1944. The values for corneal opacity, iris lesion, redness of the conjunctivae and chemosis were always 0 for each animal at the specified reading times. Therefore, 2-nitrotoluene was considered non irritant to eye.

Studies in humans

No data available. However, in the HSDB (2004) it is stated that 2-nitrotoluene is irritating to eye. It is also stated that this substance causes eye-redness and ocular pain (IPCS INCHEM, 2000).

4.1.2.3.3 Respiratory tract

Studies in animals

Acute inhalation toxicity studies in rodents did not reveal any sign of irritation on respiratory tract. However, in two mouse dietary studies on subchronic toxicity (NTP, 1992) and carcinogenicity (NTP, 2002), olfactory epithelial degeneration was observed in both sexes at 1250 ppm and above. It is possible the olfactory changes reflected an irritant effect related to inhalation of the chemical. Nevertheless, this effect was not seen with the other isomers (*m*- and *p*-nitrotoluene) which have the same volatility, and did not occur in rats. Severities of this lesion increased with increasing exposure concentrations. Olfactory degeneration was a complex lesion and consisted of necrosis, atrophy, regeneration, hyperplasia, hypertrophy, and metaplasia. The most prominent changes were atrophy of the olfactory epithelium, Bowman's glands and olfactory nerve bundles accompanied by replacement of the olfactory epithelium with a respiratory-type epithelium composed of ciliated columnar cells that covered the dorsal meatus surface and extended into the mucosa to form dilated pseudoglands. The mechanism for the development of the olfactory epithelium degeneration and metaplasia in these studies is unknown.

Studies in humans

No data available. However, in the HSDB (2004) it is stated that 2-nitrotoluene is irritating to respiratory tract.

4.1.2.3.4 Summary of irritation

In the HSDB (2004) it is stated that 2-nitrotoluene is irritating to skin, eye and respiratory tract. However, without further information, and taking into account that there are no human available data, the assessment of irritation has been derived from animal studies.

The acute toxicity irritation of 2-nitrotoluene (dermal and ocular) has been investigated in rabbits. Based on results, according to EU criteria, no classification is necessary.

The acute inhalation studies have not revealed any signs of respiratory irritation. The olfactory degeneration was observed in both subchronic toxicity and carcinogenicity dietary studies in mice and did not occur in rats. This effect was not seen with the other isomers, which have the same volatility. Accordingly, the olfactory degeneration is a systemic effect observed only in mice and considered not appropriate to classify 2-nitrotoluene for respiratory irritation.

In conclusion, it can be said that 2-nitrotoluene is not irritating for the skin, eye and the respiratory tract.

4.1.2.4 Corrosivity

The studies in animals (see 4.1.2.3) indicate that 2-nitrotoluene is not corrosive for the skin, eye or the respiratory tract.

4.1.2.5 Sensitisation

No data available concerning skin and respiratory sensitization. However, it is considered significant the absence of positive reports on such effects in humans.

4.1.2.6 Repeated dose toxicity

4.1.2.6.1 Studies in animals

In vivo studies

Sub-acute toxicity studies

Inhalation

No data available.

Dermal

No data available.

Oral

The sub-acute oral toxicity of 2-nitrotoluene has been investigated in rats and mice. The studies were reported over the period 1973-1993 and summarized in table 4.1.2.6.1-1.

Rats

F344/N rats (5 animals/sex/group, 42 days of age) were administered 0, 625, 1250, 2500, 5000 and 10000 ppm (0, 56, 98, 178, 383 and 696 mg/kg b.w. in males; 0, 55, 102, 190, 382 and 779 mg/kg b.w. in females) of 2-nitrotoluene (>96% pure) in the diet for 14 days after

acclimation periods of 13-15 days to determine the high dose selected for the 13-week toxicity study (NTP, 1992). There was no evidence of mortality or clinical signs of toxicity other than decreased main body weight gain and feed consumption in the two highest male dose groups. There were not clinical biochemistry and haematology determinations. The only tissue examined microscopically was liver. There was minimal oval cell hyperplasia in the high dose male group. No lesions were observed in the liver of females. This study is non OECD 407 guideline but GLP compliant and useful to select the high dose (10000 ppm) for the 13-week toxicity study.

Wistar rats (10 animals/sex/group weighing about 200 g) were administered 0, 500 and 1000 mg/kg b.w of 2-nitrotoluene (99% pure) in olive oil by gavage for 28 days, 5 days/week (Ciss, 1978; Ciss *et al.* 1980a). Mortality was observed within 1 week at 1000 mg/kg b.w. A slight fatigue during the 3rd or 4th intoxication day was observed at 500 mg/kg b.w. There were not either clinical biochemistry and haematology determinations or histopathology examinations. This study is non OECD 407 guideline, non GLP compliant and considered inadequate to determine a NOAEL.

Wistar rats (6 animals/sex/group) received a daily intragastric administration of 2-nitrotoluene (purity not given) in corn oil (0, 3.6, 18, 90 and 450 mg/kg) for 28 days. Additional two groups of animals exposed to 0 and 450 mg/kg were used for investigation of subsequent recovery for 2 weeks (Kaneko *et al.*, 1993). Hematological and biochemical examination revealed decreases in the number of erythrocytes, volume of haemoglobin, values of hematocrit and concentration of total serum protein in both 90 and 450 mg/kg groups. On histopathological examination, hemosiderin deposition, congestion and extramedullary hematopoiesis were observed in spleen in the 90 and 450 mg/kg groups, and hepatocytes swelling and bile duct proliferation were observed in liver in the 450 mg/kg group. After the recovery period, these symptoms were diminished or weaker, suggesting that all the effects were reversible. This study was published as an abstract and therefore cannot be used for risk assessment.

Rats were administered 322 mg/kg b.w of 2-nitrotoluene (purity not given) by gavage for 30 days to determine effects on haematopoietic system (Kovalenko, 1973). Effects on haematological parameters were suggestive of anaemia. Slight sulphahemoglobinemia and prolonged blood clotting time were observed. This study is non OECD 407 guideline, non GLP compliant and considered inadequate to determine a NOAEL.

Mice

B6C3F₁ female mice were administered 600 mg/kg of 2-nitrotoluene (purity not given) by gavage for 14 days to determine effects on humoral immunity by estimating the IgM antibody forming cell response to sheep erythrocytes (Lysy *et al.*, 1988). 2-nitrotoluene did not alter the IgM response.

B6C3F₁ mice (5 animals/sex/group, 42-49 days of age) were administered 0, 388, 675, 1250, 2500 and 5000 ppm (0, 63, 106, 204, 405 and 854 mg/kg b.w. in males; 0, 134, 217, 397, 631 and 1224 mg/kg b.w. in females) of 2-nitrotoluene (>96% pure) in the diet for 14 days after acclimation periods of 13-14 days to determine the high dose selected for the 13-week toxicity study (NTP, 1992). There was no evidence of mortality. Body weight gains were variable but appeared less than controls in mice given the diet with the highest concentration. Other than effects on body weight, there were no clear chemically related clinical signs of toxicity. There were not clinical biochemistry and haematology determinations. At necropsy, liver weights were increased somewhat in the highest 3 dose groups of males. The only tissue

examined microscopically was liver. No lesions were observed in the liver of both males and females. This study is non OECD 407 guideline but GLP compliant and useful to select the high dose (10000 ppm) for the 13-week toxicity study.

Table 4.1.2.6.1-1: Summary of oral sub-acute toxicity studies in experimental animals administered 2-nitrotoluene

Species, sex and number	Protocol	Results and Comments	References
Rat, F344/N (♂, ♀) 5/sex/group	14 days, daily, diet 0, 625, 1250, 2500, 5000, 10000 ppm (0, 56, 98, 178, 383, 696 mg/kg b.w. in males; 0, 55, 102, 190, 382, 779 mg/kg b.w. in females). >96% purity. GLP	↓ b.w. gain and ↓ feed consumption from 5000 ppm and minimal oval cell hyperplasia in liver at 10000 ppm (♂). Therefore, a high dose of 10000 ppm was selected for the 13-week toxicity study. No determinations on clinical biochemistry and haematology.	NTP (1992)*
Rat, Wistar (♂, ♀) 10/sex/group	28 days (5 days/week), gavage, olive oil 0, 500, 1000 mg/kg b.w. 99% purity.	Mortality within 1 week at 1000 mg/kg b.w. A slight fatigue during the 3 rd or 4 th intoxication day at 500 mg/kg b.w. Not information on clinical biochemistry, haematology or histopathology	Ciss (1978) Ciss <i>et al.</i> (1980a)
Rat, Wistar (♂, ♀) 6/sex/group	28 days, daily, intra-gastric, corn oil 0, 3.6, 18, 90, 450 mg/kg Purity not given An additional group of rats exposed to 450 mg/kg was used for investigate subsequent recovery for 2 weeks	<u>Haematopoietic system</u> : ↓erythrocytes, ↓volume of haemoglobin, ↓ hematocrit and ↓ concentration of total serum protein from 90 mg/kg. <u>Spleen</u> : hemosiderin deposition, congestion and extramedullary haematopoiesis from 90 mg/kg. <u>Liver</u> : hepatocytes swelling and bile duct proliferation at 450 mg/kg group. After the recovery period, these symptoms were diminished or weaker, suggesting that all the effects were reversible.	(Kaneko <i>et al.</i> , 1993).
Rat	30 days, daily, gavage 322 mg/kg b.w Purity not given.	Sulphahaemoglobinemia and prolonged blood clotting time. Study designed to observe effects on haematopoietic system	Kovalenko (1973)
Mouse B6C3F1 (♀)	14 days, gavage 600 mg/kg Purity not given.	Not altered IgM response. Study designed to determine effects on humoral immunity by estimating the IgM antibody forming cell response to sheep erythrocytes	Lysy <i>et al</i> (1988)
Mouse, B6C3F1 (♂, ♀) 5/sex/group	14 days, daily, diet 0, 388, 675, 1250, 2500, 5000 ppm (0, 63, 106, 204, 405, 854 mg/kg b.w. in males; 0, 134, 217, 397, 631, 1224 mg/kg b.w. in females) >96% purity. GLP.	↓ b.w. gain at 5000 ppm. Therefore, a high dose of 10000 ppm was selected for the 13-week toxicity study. No determinations on clinical biochemistry and haematology	NTP (1992) *

*) Studies considered of good quality for risk assessment.

Sub-chronic toxicity studies

Inhalation

No data available.

Dermal

No data available.

Oral

The sub-chronic oral toxicity of 2-nitrotoluene has been investigated in rats and mice. The studies were reported over the period 1973-1996.

Rats

Rats were administered 322 mg/kg b.w. of 2-nitrotoluene (purity not given) by gavage for 3 months (3 days/week) to determine effects on haematopoietic system (Kovalenko, 1973). Effects on haematological parameters suggested anaemia. Slight sulphahemoglobinemia and prolonged blood clotting time were observed. This study is non OECD 408 guideline, non GLP compliant and considered inadequate to determine a NOAEL.

In a study performed in essence according to OECD guideline 408 and GLP compliant, F344/N rats (10 animals/sex/group, 6 weeks of age) were administered 0, 625, 1250, 2500, 5000 and 10000 ppm (0, 45, 89, 179, 353 and 694 mg/kg b.w. in males; 0, 44, 87, 178, 340 and 675 mg/kg b.w. in females) of 2-nitrotoluene (>96% pure) in the diet for 13 weeks after acclimation periods of 10-15 days (NTP, 1992; Dunnick, Elwell and Bucher, 1994). There were no effects on survival and clinical signs of toxicity were limited to decreases in mean body weight gain and feed consumption in the three highest dose groups, most evident in males. According to the results from haematological and clinical biochemistry determinations, necropsy and histopathology examinations, the main treatment-related effects are those described below (per organ or system) and summarized qualitatively in Table 4.1.2.6.1-2.1. The incidence and severity of lesions are summarized in Table 4.1.2.6.1-2.2.

Liver: At necropsy, gross lesions were observed in the liver of all males administered 10000 ppm, which appeared larger than in controls, pale and with mottled focus. Relative liver weights were increased with increasing doses in both sexes from 625 ppm. By the end of 13 weeks, the predominant biochemical effect in the serum of males and females was mild to moderate increases in bile acid concentration in both sexes in the highest 1 or 2 dose groups. These changes were associated with minimal increases in ALT and SDH activities only in males; these findings are consistent with cholestasis and/or decreased hepatocellular function. Microscopic nonneoplastic lesions were seen only in males receiving 2500 ppm and above and consisted of cytoplasmic vacuolization, oval cell hyperplasia and inflammation. The relationship of the inflammation to treatment was less clear than for the other hepatic lesions. Although the incidence of inflammation was slightly increased at the higher exposures, the severity was similar among all dosed and control groups.

Kidney: Relative kidney weights increased from 1250 ppm in females and from 2500 ppm in males. Treatment-related lesions occurred in the kidney of males and females; in both sexes there was an accumulation of brown pigment globules in the cytoplasm of the tubular epithelium of the renal cortex, observed from 2500 ppm in females and from 5000 ppm in males. In addition to the presence of pigment, in the kidney of males there was a hyaline droplet nephropathy in groups fed diets containing 1250 ppm or more 2-nitrotoluene, attributed to an increase in the level of α -2 μ globulin.

Hematopoietic system/Spleen: At necropsy, gross lesions were observed in the spleen of 5 males administered 10000 ppm, which appeared darker and/or slightly thicker than in controls. Biochemical changes in blood were consistent with a mild regenerative anaemia.

The mechanism of the anaemia is assumed to involve oxidative damage to haemoglobin leading to Heinz body formation and decreased erythrocyte survival. Microscopic lesions were characterised by an increase in haematopoiesis (from 2500 ppm in males, from 5000 ppm in females) and hemosiderin accumulation (from 2500 ppm in both sexes). Both haematopoiesis and hemosiderin pigment increased in incidence and/or severity with increasing dose. In addition, there was capsular fibrosis (from 1250 ppm in males, from 5000 ppm in females) with an incidence of 9/10 males and 2/10 females in the 10000 ppm group.

Reproductive system: In females, the only treatment-related effect was an increase in oestrous cycle length in the 10000 ppm dose group. In males, at necropsy, testes from the highest dose group appeared smaller than in controls, pale and with mottled focus. There was a decrease in relative testis weight in the 10000 ppm dose group, and absolute epididymal weights were markedly lower than controls in the 2500, 5000 and 10000 ppm groups. Impaired testicular function occurred in the 5000 and 10000 ppm dose groups, as shown microscopically (degeneration of the seminiferous tubules) and by sperm measurement (decreases in density, motility and number). In addition, 2 males of the 10000 ppm group had mesothelial cell hyperplasia of the tunica vaginalis on the surface of the epididymis, and mesotheliomas occurred at the same anatomic lesion in 3 males of the 5000 ppm dose group.

Table 4.1.2.6.1-2.1: Summary of main treatment-related effects observed in the rat NTP (1992) study

RATS		Male	Female
Final Body Weight (90% or less than control)		↓(3) ^a	↓(3)
Liver			
	Relative weight	↑(1)	↑(1)
	ALT	↑(4)	–
	SDH	↑(3)	–
	Bile Acids	↑(4)	↑(5)
	Nonneoplastic lesions	+(3)	–
Kidney			
	Relative weight	↑(3)	↑(2)
	Nonneoplastic lesions	+(2)	+(3)
Spleen			
	Hematology	(3)	(3)
	Nonneoplastic lesions	+(2)	+(3)
Testis			
	Sperm count	↓(4)	
	Nonneoplastic lesions	+(4)	
Epididymis			
	Neoplastic and preneoplastic lesions	+(4)	
Estrous cycle length			↑(5)

^a Lowest dose group in which an effect was seen, 1 = 625 ppm (45 mg/kg b.w in ♂, 44 mg/kg b.w in ♀); 2 = 1250 ppm (89 mg/kg b.w in ♂, 87 mg/kg b.w in ♀); 3 = 2500 ppm (179 mg/kg b.w in ♂, 178 mg/kg b.w in ♀); 4 = 5000 ppm (353 mg/kg b.w in ♂, 340 mg/kg b.w in ♀); 5 = 10000 ppm (694 mg/kg b.w in ♂, 675 mg/kg b.w in ♀).

⁺ Presence of treatment-related histopathology.

Table 4.1.2.6.1-2.2: Incidence and severity of the lesions observed in the rat NTP (1992) study^a

Dose (ppm)	0	625	1250	2500	5000	10000
MALE						
<i>Liver</i>						
oval cell hyperplasia	0	0	0	2 (1.0)	10 (1.2)	10 (2.2)
vacuolization	0	0	0	6 (1.3)	9 (1.8)	10 (3.0)
inflammation	5 (1.8)	5 (1.0)	5 (1.6)	10 (1.5)	10 (1.8)	8 (1.8)
<i>Kidney</i>						
nephropathy, hyaline droplet	0	0	6 (1.0)	10 (1.6)	10 (2.8)	9 (2.6)
regeneration	2 (1.0)	6 (1.0)	2 (1.0)	2 (1.0)	5 (1.0)	6 (1.1)
pigment	0	0	0	0	1 (1.0)	10 (1.0)
<i>Spleen</i>						
hematopoiesis	0	0	0	6 (1.3)	10 (2.0)	10 (2.0)
hemosiderin pigment	0	0	0	7 (1.3)	10 (2.0)	10 (2.0)
capsular fibrosis	0	0	1 (1.0)	1 (2.0)	1 (1.0)	9 (1.9)
<i>Testis</i>						
degeneration	0	0	0	0	10 (2.3)	10 (4.0)
<i>Epididymis</i>						
mesothelial hyperplasia	0	0	0	0	0	2
mesothelioma	0	0	0	0	3	0
FEMALE						
<i>Kidney</i>						
pigment	0	0	0	3 (1.0)	10 (1.1)	10 (1.8)
<i>Spleen</i>						
hematopoiesis	0	0	0	0	1 (1.0)	10 (1.0)
hemosiderin pigment	0	0	0	5 (1.0)	9 (2.0)	10 (2.0)
capsular fibrosis	0	0	0	0	1 (1.0)	2 (1.0)

^a Incidence and severity score () based on a scale of 1 to 4; 1 = minimal, 2 = mild; 3 = moderate; 4 = marked. Severity scores are averages based on the number of animals with lesions from groups of 10

Wistar rats (10 animals/sex/group weighing about 200 g) were administered 0 and 200 mg/kg b.w. of 2-nitrotoluene (99% pure) in olive oil by gavage for 6 months, 5 days/week, after an acclimation period of 1 week (Ciss, 1978; Ciss *et al.* 1980b). There were no effects on survival. Clinical signs of toxicity were not observed. From haematological and clinical biochemistry determinations, necropsy and histopathology examinations, the main treatment-related effects are those described below (per organ or system). **Haematopoietic system:** 10% haemoglobin level decrease in both sexes. **Kidney:** Hyaline droplet accumulation in the tubule, in females (7/9) and in males (3/8). **Spleen:** Slight modifications in males.

F344 male rats (10 animals/group; aged 6-7 weeks) were administered 0 and 5000 ppm (375 mg/kg b.w.) of 2-nitrotoluene (99 ± 1% pure) in the diet for 13 and 26 weeks, and for 13 weeks followed by control diet until necropsy at 26 weeks (Ton *et al.*, 1995). The livers were weighed and examined for gross and histopathological lesions. Livers were stained immunohistochemically for determine placental glutathione-S-transferase (PGST), a marker of hepatic preneoplasia, and quantified stereologically using computer-assisted image analysis. All treated groups showed decreased mean final body weight, average daily feed consumption and mean body weigh gains, and increased absolute and relative liver weight compared to those of the respective control groups. Cytoplasmic vacuolization and oval cell hyperplasia were observed in the liver of all treated rats after 13 weeks of exposure. The cytoplasmic vacuolization ranged from minimal to mild severity at 13 weeks and increased to a moderate severity at 26 weeks in the continuous and stop-exposure groups. The severity of oval cell hyperplasia was similar in all treated groups. PGST positive (PSGT+) liver foci were

observed in all treatment groups. The 26-week continuous-exposure group produced more PSGT+ liver foci and greater mean focus volume than the 13-week continuous-exposure group. In the 26-week stop-exposure group, there were fewer PSGT+ liver foci than observed with continuous exposure at 13 or 26 weeks; however, the mean focal volume in the stop-exposure group at 26 weeks was greater than that at 13 or 26 weeks of continuous exposure. In summary, the persistence and increase in size of these foci, even in the absence of chemical exposure, suggest the potential for a hepatocarcinogenic effect in long-term studies for 2-nitrotoluene.

F344 male rats (10 and 20 animals per control and exposed group respectively, aged 45 days) were administered 0 and 5000 ppm (0 and 292-296 mg/kg b.w.) of 2-nitrotoluene (99.8 ± 0.3% pure) in the diet, after an acclimation period of 9 days, for 13 and 26 weeks, and for 13 weeks followed by control diet until necropsy at 26 weeks (NTP, 1996). The dose of 2-nitrotoluene selected was based on the previous 13-week study where a concentration of 5000 ppm in the diet caused epididymis mesotheliomas. Additional groups were included in an attempt to determine the effect of altered gastrointestinal flora on the toxicity or carcinogenicity of 2-nitrotoluene. Rats in the altered gastrointestinal flora groups received a single gavage dose of an antibiotic mixture in deionized water daily for 6 days before the start of the study and daily for 13 weeks thereafter (this treatment produced a reduction of aerobic flora greater than 97% compared to controls in a pilot study). Following the 13-week administration, 10 rats from each control group (normal and altered gastrointestinal flora) and 20 rats from each exposure group (normal and altered gastrointestinal flora) were killed for the 13-week interim evaluation. Following the 13-week administration and the 13-week maintenance period on the control diet, 20 rats from each exposure group (normal and altered gastrointestinal flora) were killed for the stop-exposure evaluation. Following the 26-week administration, 20 rats from each normal gastrointestinal flora exposure group were killed for the 26-week evaluation. 10 rats from each control group (normal and altered gastrointestinal flora) were killed at 26 weeks for the stop-exposure and 26-week evaluations. There were no effects on survival and clinical signs of toxicity were limited to decreases in mean body weight gain and feed consumption in all exposure groups. Taking into account results from necropsy and histopathology examinations (summarized in Table 4.1.2.6.1-3 for normal gastrointestinal flora groups) as well as supplemental evaluations such as microbial colony counts in cecal specimens and placental glutathione-S-transferase (PGST) determinations in liver, the main treatment-related effects are described below.

Liver: Progressive, irreversible increases in liver weight and irreversible increases in the incidence of both cytoplasmic vacuolization and oval cell hyperplasia. PGST positive liver foci occurred after 13-week exposure and the number and size of foci were increased after 26 weeks of continuous exposure; during the recovery period the number of PGST positive liver foci decreased slightly but the size of the foci continued to increase. After 26 weeks, cholangiocarcinoma occurred in normal gastrointestinal flora groups, two rats in the stop-exposure group and one rat in the continuous-exposure group.

Kidney: Slight increases in relative weights. An accumulation of hyaline droplets in the renal tubule epithelium was observed after 13 week-exposure, which did not increase in severity after 26 weeks of continuous exposure and regressed during the recovery period. After a continuous exposure for 26-weeks, the incidence and severity of other renal lesions typically associated with nephropathy (regeneration and protein casts) were greater than those in the controls, but did not differ from those seen in the stop-exposure groups.

Spleen: Slight increases in relative weights. Microscopic lesions were characterised by increased incidences of haematopoiesis, hemosiderin accumulation and capsular fibrosis,

slightly most severe after 26 weeks of continuous exposure. During the recovery period, incidences of haematopoiesis and hemosiderin accumulation were decreased but the capsular fibrosis did not resolve.

Testis and epididymis: Significantly lower testis and epididymis weights and greater incidences of degeneration of the seminiferous tubules than those in the controls occurred in all rats exposed. Those effects increased in severity with continuous exposure and showed no evidence of recovery in the stop-exposure groups. Mesothelial cell hyperplasia and/or mesotheliomas occurred on the mesothelial surface of the tunica vaginalis on the testis and epididymis in the stop- and continuous-exposure groups.

Table 4.1.2.6.1-3: Summary of main treatment-related lesions in the rat NTP (1996) study

	Control (0 ppm)	o-Nitrotoluene (5,000 ppm)
Body weights		Less than controls at all time points
Lesions 13 weeks	<u>Kidney</u> : regeneration (3/10)	<u>Kidney</u> : hyaline droplet accumulation (20/20); regeneration (16/20) <u>Liver</u> : cytoplasmic vacuolization (20/20); oval cell hyperplasia (20/20) <u>Spleen</u> : hematopoietic cell proliferation (20/20); hemosiderin pigmentation (20/20); capsule, fibrosis (1/20)
	<u>Spleen</u> : hematopoietic cell proliferation (2/10)	<u>Testis/epididymis</u> : degeneration (20/20)
Stop-exposure	<u>Kidney</u> : protein casts (3/10); regeneration (7/10)	<u>Kidney</u> : protein casts (20/20); regeneration (19/20) <u>Liver</u> : cytoplasmic vacuolization (20/20); oval cell hyperplasia (20/20); cholangiocarcinoma (2/20) <u>Spleen</u> : hematopoietic cell proliferation (18/20); hemosiderin pigmentation (17/20); capsule, fibrosis (7/20)
	<u>Spleen</u> : hematopoietic cell proliferation (3/10); hemosiderin pigmentation (3/10)	<u>Testis/epididymis</u> : degeneration (20/20); mesothelial hyperplasia (2/20); mesothelioma (5/20)

Table 4.1.2.6.1-3 (cont): Summary of main treatment-related lesions in the rat NTP (1996) study

	Control (0 ppm)	o-Nitrotoluene (5,000 ppm)
Lesions		
26 weeks	<p><u>Kidney</u>: protein casts (3/10); regeneration (7/10)</p> <p><u>Spleen</u>: hematopoietic cell proliferation (3/10); hemosiderin pigmentation (3/10)</p>	<p><u>Kidney</u>: hyaline droplet accumulation (20/20); protein casts (20/20); regeneration (19/20)</p> <p><u>Liver</u>: cytoplasmic vacuolization (20/20); oval cell hyperplasia (20/20); cholangiocarcinoma (1/20)</p> <p><u>Spleen</u>: hematopoietic cell proliferation (20/20); hemosiderin pigmentation (20/20); capsule, fibrosis (3/20)</p> <p><u>Testis/epididymis</u>: degeneration (20/20); mesothelial hyperplasia (2/20); mesothelioma (7/20)</p>

The only effect that may be related to the altered gastrointestinal flora diet was the absence of cholangiocarcinomas, which were seen in rats administered 2-nitrotoluene in the normal diet. However, the altered gastrointestinal flora did not prevent increased liver weight or hepatic toxicity (cytoplasmic vacuolization, PGST-positive foci and oval cell hyperplasia). Oval cell hyperplasia is considered to be a morphological change preceding and associated with the development of cholangial neoplasms. Based on these findings, the short duration of the study and the small number of these liver neoplasms seen in the normal gastrointestinal flora group (3/40) it considered unlikely that the altered gastrointestinal flora prevented the development of cholangiocarcinoma. In addition, it was determined that the antibiotic regimen used was effective only in reducing the gut population of aerobic microorganisms and had little effect on obligate anaerobes, which are thought to play a major role in nitro group reduction. In summary, the results of this study have confirmed the carcinogenicity of 2-nitrotoluene (high incidence of mesotheliomas and small number of cholangiocarcinomas) in male rats after only 13 or 26 weeks of exposure. However, no conclusion could be done about the role played by the intestinal microflora on carcinogenicity.

Mice

In a GLP compliant study, B6C3F₁ mice (5 animals/sex/group, 6 weeks of age) were administered 625, 1250, 2500, 5000 and 10000 ppm (104, 223, 415, 773 and 1536 mg/kg b.w. in males; 132, 268, 542, 1007 and 1712 mg/kg b.w. in females) of 2-nitrotoluene (>96% pure) in the diet for 13 weeks after acclimation periods of 12-14 days (NTP, 1992; Dunnick, Elwell and Bucher, 1994). According to the results from necropsy and histopathology examinations, the main treatment-related effects are those described below (per organ or system) and summarized qualitatively in Table 4.1.2.6.1-4.1. The incidence and severity of lesions are summarized in Table 4.1.2.6.1-4.2.

There were no effects on survival and clinical signs of toxicity were limited to decreases in main body weight gain and feed consumption in the two highest dose groups in both sexes. At necropsy, no gross lesions related to treatment were found. With respect to relative organ weight, there were increases in liver and testis and decreases in kidney in the highest 2 or 3 dose groups (males) and increases in liver and kidney from 1250 ppm and in heart and lungs from 5000 ppm (females). The only histopathological lesion observed was degeneration and metaplasia of the olfactory epithelium in both sexes from 1250 ppm. In addition, sperm motility was significantly decreased in males from the 10000 ppm group compared to controls.

Table 4.1.2.6.1-4.1: Summary of main treatment-related effects observed in the mouse NTP (1992) study^a

MICE	Male	Female
Final Body Weight (90% or less than control)	↓(3)	↓(3)
Nose		
Nonneoplastic lesions	+(2)	+(2)
Liver		
Relative weight	↑(3)	↑(2)

^a Lowest dose group in which an effect was seen; 1 = 625 ppm (104 mg/kg b.w in ♂, 132 mg/kg b.w in ♀); 2 = 1250 ppm (223 mg/kg b.w in ♂, 268 mg/kg b.w in ♀); 3 = 2500 ppm (415 mg/kg b.w in ♂, 542 mg/kg b.w in ♀); 4 = 5000 ppm (773 mg/kg b.w in ♂, 1007 mg/kg b.w in ♀); 5 = 10000 ppm (1536 mg/kg b.w in ♂, 1712 mg/kg b.w in ♀).

+ Presence of treatment-related histopathology.

Table 4.1.2.6.1-4.2: Incidence and severity of the lesions observed in the mouse NTP (1992) study^a

Dose (ppm)	0	625	1250	2500	5000	10000
MALE						
Nose						
olfactory epithelium, degeneration/metaplasia	0	0	1 (1.0)	2 (1.0)	10 (2.0)	10 (3.0)
FEMALE						
Nose						
olfactory epithelium, degeneration/metaplasia	0	0	2 (1.5)	9 (1.0)	10 (1.9)	10 (2.9)

^a Incidence and severity score () based on a scale of 1 to 4; 1= minimal, 2= mild; 3= moderate; 4= marked. Severity scores are averages based on the number of animals with lesions from groups of 10

Chronic toxicity studiesInhalation

No data available.

Dermal

No data available.

Oral

No data are available from a specific chronic toxicity study but many non-neoplastic lesions were reported in a two year carcinogenicity study in rats and mice (NTP, 2002). The study has been included at the section 4.1.2.8. However, it is mentioned here (only methodology description and summary of the main non-neoplastic lesions) in order to consider its relevance for the derivation of a chronic-toxicity NOAEL/LOAEL.

Rats

In the rat core study, performed in essence according to OECD guideline 451 and GLP compliant, F344/N rats (60 animals/sex/group, 6-7 weeks old) were administered 0 (females only), 625, 1250 and 2000 ppm (25, 50 and 90 mg/kg b.w. in males; 0, 30, 60 and 100 mg/kg b.w. in females) of 2-nitrotoluene (>99% pure) in the diet for 105 weeks after acclimation periods of 12-14 days; in a 3-month stop-exposure study, groups of 60 males were fed diets containing 0, 2000 and 5000 ppm (0, 125 and 315 mg/kg b.w.) for 13 weeks followed by undosed feed for the remainder of the study (NTP, 2002). Increased incidences of nonneoplastic lesions from 625 ppm were observed in liver, bone marrow, spleen and lung for both sexes and in mammary gland and mandibular lymph node only for females (Table 4.1.2.6.1-5)

Table 4.1.2.6.1-5: Summary of the main non-neoplastic lesions in the rat carcinogenicity study (2-year evaluation)^a

2-nitrotoluene	0 ppm	625 ppm	1,250 ppm	2,000 ppm	2,000 ppm (Stop-exposure)	5,000 ppm (Stop-exposure)
Male Rats						
<i>Average Daily Dose (mg/kg)</i>	0	25	50	90	125	315
<i>Body weights</i>			Less than controls	Less than controls	Less than controls	Less than controls
<i>Survival</i>	39/60	18/60	3/60	0/60	11/60	0/60
<i>Liver</i>						
Eosinophilic focus	7/60	18/60	29/60	24/60	15/60	13/60
Mixed cell focus	5/60	7/60	12/60	6/60	12/60	8/60
Clear cell focus	29/60	29/60	34/60	31/60	30/60	34/60
Mixed cell cellular infiltration	1/60	5/60	11/60	20/60	15/60	33/60
<i>Bone marrow</i>						
Hyperplasia	2/60	25/60	43/60	45/60	37/60	33/60
<i>Spleen</i>						
Hematopoietic cell proliferation	7/60	33/60	38/60	47/60	36/60	35/60
<i>Lung</i>						
Alveolar epithelial hyperplasia	2/60	8/60	3/60	7/60	15/60	29/60

	2-nitrotoluene	0 ppm	625 ppm	1,250 ppm	2,000 ppm
Female Rats					
Average Daily Dose (mg/kg)		0	30	60	100
Body weights					Less than controls
Survival		47/60	47/60	39/60	33/60
Mammary gland Hyperplasia		14/60	36/60	30/60	19/60
<i>Liver</i>					
Eosinophilic focus		5/60	12/59	25/60	32/60
Mixed cell focus		6/60	9/59	11/60	28/60
Clear cell focus		16/60	30/59	28/60	33/60
Basophilic focus		51/60	56/59	60/60	54/60
<i>Bone marrow</i>					
Hyperplasia		2/60	7/60	15/60	24/60
<i>Spleen</i>					
Hematopoietic cell proliferation		22/60	38/59	48/60	48/59
<i>Lung</i>					
Alveolar epithelial hyperplasia		6/60	14/60	16/60	9/60
<i>Lymph node (mandibular)</i>					
Lymphoid hyperplasia		3/60	5/60	6/59	15/59

^a NTP, 2002

^b Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver and lung; for other tissues, denominator is number of animals necropsied.

Mice

In the mouse study, performed in essence according to OECD guideline 451 and GLP compliant, B6C3F₁ mice (60 animals/sex/group, 6 weeks old) were administered 0, 1250, 2500 and 5000 ppm (0, 165, 360 and 700 mg/kg b.w. in males; 0, 150, 320 and 710 mg/kg b.w. in females) of 2-nitrotoluene (>99% pure) in the diet for 105 weeks after acclimation periods of 12 days (NTP, 2002). Increased incidences of nonneoplastic lesions from 1250 ppm were observed in liver, kidney and nose for both sexes (Table 4.1.2.6.1-6)

Table 4.1.2.6.1-6: Summary of the main non-neoplastic lesions in the mouse carcinogenicity study (2-year evaluation)^a

	2-nitrotoluene	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Male Mice					
Average Daily Dose (mg/kg)		0	165	360	700
Body weights			Less than controls	Less than controls	Less than controls
Survival		52/60	34/60	0/60	0/60
<i>Liver</i>					
Eosinophilic focus		3/60	14/59	1/57	1/60
Basophilic focus		0/60	6/59	4/57	0/60
Necrosis		1/60	15/59	27/57	30/60
Focal hepatocyte syncytial alteration		16/60	26/59	43/57	39/60
<i>Kidney</i>					
Renal tubule pigmentation		1/58	6/59	32/58	35/60
<i>Nose</i>					
Olfactory epithelial degeneration		0/60	36/60	60/60	60/60

	2-nitrotoluene	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Female Mice					
Average Daily Dose (mg/kg)		0	150	320	710
Body weights				Less than controls	Less than controls
Survival		52/60	46/60	47/60	5/60
<i>Liver</i>					
Eosinophilic focus		2/60	3/59	6/59	28/60
Basophilic focus		1/60	6/59	2/59	6/60
Necrosis		3/60	0/59	2/59	13/60
Focal hepatocyte necrosis		0/60	0/59	0/59	6/60
Focal hepatocyte cytoplasmic vacuolization		1/60	2/59	2/59	9/60
<i>Kidney</i>					
Renal tubule pigmentation		0/59	1/56	3/58	35/59
<i>Nose</i>					
Olfactory epithelial degeneration		0/60	28/60	59/59	57/57

^a NTP, 2002

^b Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver and lung; for other tissues, denominator is number of animals necropsied.

4.1.2.6.2 Studies in humans

In vivo studies

According to PIHT, 1981-82 (cited in HSDB, 2004), 2-nitrotoluene is suspected of causing anaemia in chronic exposures. Nevertheless, it is impossible to determine the exposure-response relationship because original data are not available, and in addition, the exposure route is not reported.

Inhalation

No data available

Dermal

No data available.

Oral

No data available.

4.1.2.6.3 Summary of repeated dose toxicity

There were not available studies in experimental animals following inhalatory or dermal exposure but several studies have been investigated the toxicity of 2-nitrotoluene following repeated oral administration to rats and mice. Most of feed studies were performed in essence according to OECD guidelines and in conformity with GLP and therefore selected for risk assessment.

Rat was the most susceptible species of the ones tested for repeated dose toxicity of 2-nitrotoluene.

In the 14-day studies (NTP, 1992), 2-nitrotoluene, administered up to 5000 ppm (mice) or 10000 ppm (rats), did not cause either effects on survival or clinical signs of toxicity, although 5000 ppm animals showed decreases in body weight gains relative to controls. In addition, a minimal oval cell hyperplasia in liver was observed only in 10000 ppm male rats. Therefore, 10000 ppm was selected the high concentration for 13-week studies.

At the 13-week toxicity studies, relative liver weights were increased from 625 ppm in both sexes of rats. However, at this dose level there was not treatment-related histopathology. Non-neoplastic lesions occurred at dose levels of 1250 ppm and above. Therefore, the NOAEL for subchronic-toxicity was considered to be 625 ppm (45 mg/kg b.w.) based on capsular fibrosis observed in spleen of male rats at 1250 ppm (89 mg/kg b.w.). In mice, the only histopathological lesion observed was degeneration and metaplasia of the olfactory epithelium in both sexes from 1250 ppm (223 and 268 mg/kg b.w. for males and females, respectively).

At the two-year carcinogenicity study, non-neoplastic lesions occurred at the lower dose level tested of 625 ppm in rats and 1250 ppm in mice. Therefore, the LOAEL for chronic toxicity was considered to be 625 ppm in rats (25 and 30 mg/kg b.w. in males and females, respectively) based on lesions observed in liver, bone marrow, spleen and lung for both sexes and in mammary gland and mandibular lymph node only for females.

4.1.2.7 Mutagenicity

Several test methods for investigating the mutagenicity and genotoxicity of 2-nitrotoluene are available *in vitro* and *in vivo*.

4.1.2.7.1 Studies in vitro

Bacterial studies

2-nitrotoluene has been tested for genotoxicity in several studies in bacteria; these are listed in **Table 4.1.2.7.1-1**.

Chiu *et al.* (1978) tested 2-nitrotoluene for mutagenicity in *Salmonella typhimirium* TA98 and TA100 without metabolic activation, at 10, 1 and 0.1 $\mu\text{mol}/\text{plate}$. The results were negative.

Tokiwa *et al.* (1981) investigated mutagenicity of 2-nitrotoluene in *strains* TA98 and TA100 with and without metabolic activation, using liver S9 fraction prepared from rats treated with Aroclor 1254. The protein concentration of S9 fraction was 39 mg/ml. The S9 fraction was used at 0.15 ml/plate. The doses of 2-nitrotoluene were 1000 and 100 $\mu\text{g}/\text{plate}$. The results were negative.

Miyata *et al.* (1981), using *Salmonella typhimirium*, strains TA92 TA94, TA98, TA100, TA1535 and TA1537, tested 2-nitrotoluene in the absence and in the presence of a hepatic 9000xg supernatant (S9) containing NADPH-generating system. The doses were 30, 100, 300, 1000 and 3000 $\mu\text{g}/\text{plate}$. The results were negative, 1000 and 3000 $\mu\text{g}/\text{plate}$ doses were toxic in the absence of S9 mix, 3000 $\mu\text{g}/\text{plate}$ was toxic in the presence of S9 mix.

The mutagenicity of 2-nitrotoluene was investigated by Spanggord *et al.* (1982) with five strains of *Salmonella typhimirium*, TA98, TA100, TA1535, TA1537 and TA1538. The results were negative with and without metabolic activation. The range tested was 10-5,000 μg .

Haworth *et al.* (1983), tested 2-nitrotoluene in the Ames test, using preincubation, strains TA98, TA100, TA1535 and TA1537 of *Salmonella typhimurium*. Liver S9 fractions were prepared from male Sprague-Dawley rats and male Syrian hamsters that were injected, i.p., with Aroclor 1254. The concentrations of 2-nitrotoluene used were 3.3, 10.0, 33.0, 100.0 and 333.0 µg/plate. The results were negative, 333.0 µg/plate resulted toxic for the strains TA 100, TA 1537 and TA 98 without S9 mix.

Sundvall *et al.* (1984) analyzed wastewater from a chemical process industry producing primarily nitrobenzoic acids and nitrotoluenes. *Salmonella typhimurium*, TA1535, TA1538, TA98 and TA100 were used with and without metabolic activation. 2-NT was only tested in TA 100, without metabolic activation, the mutagenic response at 13 µg per ml wastewater was negative.

Suzuki *et al.* (1983), studied the mutagenicity of mono-nitrobenzene derivatives, among them, 2-nitrotoluene, in order to evaluate the effect of norharman, a substance found in tobacco tar. The mutagenicity assay used was *Salmonella typhimurium*, strains TA 98 and TA100. The concentrations used were 100, 200 and 300 µg/plate, with and without S9 mix and 200 µg/plate of norharman. The result was positive for the ortoisomers of every nitro-compound, among them 2-nitrotoluene, in the presence of norharman when *Salmonella typhimurium* TA 98 with S9 mix was used. This study is important from the viewpoint of environmental carcinogenesis, since norharman is found in tobacco tar and nitro aromatics are found in atmosphere particulate matter and in the exhaust gas from diesel engines.

Nohmi *et al.* (1984) tested the mutagenicity of 2-nitrotoluene with the *Salmonella*/microsome test according to the method of Ames with preincubation during 20 min. at 37°C. *S. typhimurium* TA98 and TA100 were used, the concentration of 2-nitrotoluene was 10 mM. The result was negative, both with and without metabolic activation.

Shimizu and Yano (1986) studied 2-nitrotoluene in the Ames test. TA98, TA100, TA1535, TA1537, TA1538 were used. The doses used by plate, with and without S9, were 0.01, 0.05, 0.1, 0.5, 1 and 5 µl. The result was negative.

Mutagenicity of 2-nitrotoluene was tested by pre-incubation method using *Salmonella typhimurium* TA100 and TA98 strains with and without S9 mix. The result was negative (Kawai *et al.*, 1987).

Negative results were found when 2-nitrotoluene was tested in *S. typhimurium* TA100 and TA98 strains with and without S9 at dose levels of 0.5-5.0 µM per plate (Gupta *et al.*, 1987).

A mutagenicity assay was carried out with 2-nitrotoluene according to the guidelines of Ministry of Labour Japan (JETOC, 1996). *Salmonella typhimurium* strains TA98, TA100, TA102, TA104, TA1535, TA1537 and TA1538 were obtained from Dr. Ames and *Escherichia coli* WP2uvrA and WP2uvrA/pKM101 were from Dr. Ishizawa. DMSO was added to give a final concentration of 2.8% (v/v). Metabolic activation (S9 mix) was obtained using sodium phenobarbital and 5,6-benzoflavone as an inducer of the rat metabolic activation system. A preincubation procedure was performed. The concentrations used were 0.0763, 0.305, 1.22, 4.88, 19.5, 78.1, 313, 1250 and 5000 µg/plate. The mutagenicity results for 2-nitrotoluene in bacterial test was negative, both *Salmonella typhimurium* and *Escherichia coli*.

In the recombination assay on *Bacillus subtilis*, strains H17 and M45, without addition of S9 mix, 2-nitrotoluene showed a weakly effect at 5 µl/plate (Shimizu and Yano, 1986).

2-nitrotoluene was tested for its ability to inactivate *Bacillus subtilis* transforming DNA. Incubation with 5 and 10 mM of 2-nitrotoluene dissolved in DMSO, during 60 min at 37°C, did not inactivate transforming DNA (Nohmi *et al.*, 1984).

Table 4.1.2.7.1-1: Genotoxicity tests in bacteria by 2-nitrotoluene

TEST SYSTEM	SOURCE AND PURITY OF CHEMICAL	RESULT ^{a)}		DOSE ^{b)} (LED/HID)	REFERENCE
		Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium: TA98, TA100	Eastman Organic chemicals, Rochester NY. Purity not given	-	NT	10 µmol/plate	Chiu <i>et al.</i> (1978)
Salmonella typhimurium: TA98, TA100	Aldrich chemical Co. Purity not given	-	-	1000 µg/plate	Tokiwa <i>et al.</i> (1981)
Salmonella typhimurium: TA92, TA94, TA98, TA100, TA1535, TA1537	Not given	-	-	1000 µg/plate	Miyata <i>et al.</i> (1981)
Salmonella typhimurium: TA98, TA100, TA1535, TA1537, TA 1538	Not given	-	-	5000 µg/plate	Spanggord <i>et al.</i> (1982)
Salmonella typhimurium: TA98, TA100, TA1535, TA1537(with preincubation)	Eastman. Purity > 99%	-	-	333 µg/plate	Haworth <i>et al.</i> (1983)
Salmonella typhimurium: TA100	2NT extracted from wastewater	-	NT	13 µg/ml wastewater	Sundvall <i>et al.</i> (1984)
Salmonella typhimurium: TA98, TA100	Tokyo Kasei Kogyo Co. Ltd. Purity not given	-	+ (TA98) with Norharman (200µg/plate)	300 µg/plate	Suzuki <i>et al.</i> (1983)
Salmonella typhimurium: TA98, TA100 (with preincubation 20 min. at 37 °C)	Not given	-	-	10 mM	Nohmi <i>et al.</i> (1984)
Salmonella typhimurium: TA98, TA 100, TA1535, TA1537, TA 1538	Tokyo Kasei Kogyo Co. Ltd. Purity 99% min	-	-	5 µl/plate	Shimizu and Yano (1986)
Salmonella typhimurium: TA98, TA 100	Merck Schuhardt Purity not given	-	-	5 µM/plate	Gupta <i>et al.</i> (1987)
Salmonella typhimurium: TA98, TA 100	Wako Purity not given	-	-	Not given	Kawai <i>et al.</i> (1987)
Salmonella typhimurium: TA98, TA100, TA 102, TA104, TA1535, TA 1537, TA 1538 (with preincubation) E. coli WP2uvra and WP2uvra/PKM101 (with preincubation)	Tokyo Kasei Kogyo Co. Ltd. Purity 99% min	-	-	5000 µg/plate	JETOC (1996)
Bacillus subtilis recombination assay, H17 and M45	Tokyo Kasei Kogyo Co. Ltd. Purity 99%	(+)	NT	5 µl/plate	Shimizu and Yano (1986)
Bacillus subtilis assay for loss of transforming DNA activity	Not given	-	NT	10 mM	Nohmi <i>et al.</i> (1984)

Notes:

- a) +, positive; (+) weak positive; ?, inconclusive; -, negative; NT, not tested
b) LED, lowest effective dose; HID, highest ineffective dose

Mammalian cell studies

In the **Table 4.1.2.7.1-2**, are listed the *in vitro* studies for genetic and related effect in cultured mammalian cells by 2-nitrotoluene.

A clonal sub-line of fibroblasts derived from lung tissue of Chinese hamster lung (CHL) cells was used to evaluate the mutagenicity of 2-nitrotoluene in a cytogenetic assay. The concentrations used were up to 250 µg/ml. The assay was carried out without metabolic activation. The duration of exposure was 48 h., without a recovery period. No structural aberrations were obtained despite a significant increase in the proportion of polyploid cells (Ishidate, Harnois and Sofuni, 1988).

A cytogenetic assay to detect chromosome aberrations, in cultured Chinese hamster ovary (CHO) cells, was carried out by Galloway *et al* (1987). Concentrations of 2-nitrotoluene up to 425 µg/ml was tested in the absence and presence of S9 mix. The chromosome aberration rate did not differ from that of controls. In a sister chromatid exchange assay on CHO cells, 2-nitrotoluene concentrations in the 177-218 µg/ml range, without addition of S9 mix, caused a very slight increase in the sister chromatid exchange rate, while the increase was pronounced at concentrations of 355-423 µg/ml with S9 mix (Galloway *et al.*, 1987).

The genotoxicity of 2-nitrotoluene was evaluated by the chromosome aberrations test in cultured human peripheral lymphocytes (Huang, Wang and Han., 1995). Lymphocytes were obtained from a healthy male donor. Concentrations at 0.005, 0.050, 0.40 and 1.00 mmol/L of 2-nitrotoluene dissolved in DMSO were added to lymphocytes cultures at 48h after culture initiation. Five duplicate cultures were made for each subject. The cultures were incubated for additional 24h after treatment, and 5µg/ml colchicine was added 2h before the end of incubation. Chromosome preparations were made and stained with Giemsa solution. The number of cells with chromosome aberrations among 100 well-spread metaphase cells in one culture was recorded (gaps were not regarded as aberration). 2-nitrotoluene exhibited genotoxic activity, the percentage of aberrant cells (PAC ± S.E) was: 7.6 ± 1.1 , 14.8 ± 1.0 , 29.8 ± 1.4 and 46.2 ± 2.2 , the $-\text{LogEC}_{50}$ value was -0.60. The control (10µl DMSO), exhibited a PAC of 1.8 ± 0.3 .

The genotoxicity of 2-nitrotoluene has been evaluated using *in vitro* DNA repair as unscheduled DNA synthesis (UDS) in rat spermatocytes and spermatides (Working and Butterworth, 1984). Freshly isolated cells were added to William's Medium E containing 10 µCi/ml ³H-thymidine and 10, 100 and 1000 µM (1.37 to 137 µg/ml) of 2-nitrotoluene in DMSO. The cell cultures were incubated for 18 hr., in the absence of metabolic activation. Grain counts were made on at least five replicates (50 cells/replicate). 2-nitrotoluene did not induce DNA repair synthesis (UDS) either in rat spermatocytes or spermatids, 1000 µM dose resulted toxic.

Doolittle, Sherrill and Butterworth (1983) evaluated the genotoxicity of 2-nitrotoluene in the *in vitro* UDS assay. Hepatocytes were isolated from untreated male F344 rats by an EGTA-collagenase perfusion procedure. 2-nitrotoluene at 10, 100 and 1000 µM and ³H-thymidine were incubated with the hepatocytes for 18 h. 2-nitrotoluene do not induce DNA repair in F344 rat hepatocytes, 1000 µM dose resulted toxic. The positive control, 10 µM acetylaminofluorene, markedly increased both the net nuclear grains and the percentage of hepatocytes with ≥ 5 net nuclear grains, demonstrating that the hepatocytes were metabolically competent and capable of DNA repair.

Negative results were obtained by Furihata and Matsushima (1987) in an Unscheduled DNA synthesis assay using rat hepatocytes and concentrations up to 1mM in DMSO of 2-nitrotoluene.

Brambilla and Martelli (1990) compared the 2-nitrotoluene potency of inducing unscheduled DNA synthesis (UDS) in human and rat hepatocytes. The range of concentrations tested was 0.01-1 mM. 2-nitrotoluene did not induce DNA repair synthesis in hepatocytes of both species.

Butterworth *et al.* (1989), investigated the genotoxic potential of 2-nitrotoluene in a human hepatocyte UDS assay. For purposes of comparison 2-nitrotoluene was evaluated in the *in vitro* rat hepatocyte DNA repair assay. Concentrations at 0.01, 0.1 and 1 mM of 2-nitrotoluene prepared in DMSO were used. Procedures were optimized to prepare primary cultures of human hepatocytes from discarded surgical material: a 6-year-old female who underwent surgery to remove a liver sarcoma, cells were 86% viable; a 73-year-old male who underwent a left lobectomy for the removal of a metastatic tumor, cells were 82% viable; a 25-year-old female who underwent surgery for a colon cancer metastasis, cells were 84% viable; a 16-year-old female suicide victim, cells were 75% viable; a 56-year-old male who underwent surgery for the removal of liver metastases, cells were 87% viable; the last case was a liver resection, the viability of the primary hepatocyte culture prepared from the tissue was 89%. The cells were incubated with ³H-thymidine and test chemical for periods of 18 to 24 h. The qualitative DNA repair response was negative and the same from all six human cases and rat hepatocytes.

2-nitrotoluene was evaluated in the *in vitro* UDS assay employing a serum-free procedure (Parton, Yount and Garriot, 1995). Primary rat hepatocytes were prepared by the collagenase method and incubated with 2 ml of the concentrations 100, 50, 10, 5, 1, 0.5 and 0.1 µg/ml of 2-nitrotoluene. Two chambers were used for each concentration. Serum-free WEM, containing 10 µCi/ml of ³H-thymidine, was used to prepare the appropriate concentrations. Concentration of 1% of DMSO was used as control. The results showed cytotoxicity at 50 and 100 µg/ml; at 10 µg/ml the increase in the net nuclear grain count was significantly greater than the vehicle control ($p \leq 0.01$).

Table 4.1.2.7.1-2: In vitro test for genetic and related effect in cultured mammalian cells by 2-nitrotoluene

TEST SYSTEM	SOURCE AND PURITY OF CHEMICAL	RESULT ^{a)}		DOSE ^{b)} (LED/HID)	REFERENCE
		Without exogenous metabolic system	With exogenous metabolic system		
Cytogenetic assay, Chinese hamster lung (CHL) (structural aberration)	Not given	- P	NT	250 µg/ml	Ishidate <i>et al.</i> (1988)
Chromosomal aberrations, Chinese hamster ovary CHO cells in vitro	Radian Corp. Austin, TX Purity not given	-	NT	425 µg/ml	Galloway <i>et al.</i> (1987)
Sister chromatid exchange assay, Chinese hamster ovary CHO cells in vitro	Radian Corp. Austin, TX Purity not given	(+)	-	177-218 µg/ml	Galloway <i>et al.</i> (1987)
			+	355-423 µg/ml	
Chromosome aberrations test in cultured human peripheral lymphocytes	Not given	+	NT	0.005 mmol/L	Huang, Wang and Han. (1995)
Unscheduled DNA synthesis (UDS), rat spermatocytes and spermatides	Aldrich Chemical Co. Purity 99%	-	NT	100 µM	Working and Butterworth (1984)
UDS assay rat hepatocytes	Aldrich Chemical Co. Purity 99%	-	NT	100 µM	Doolittle <i>et al.</i> (1983)
UDS assay rat hepatocytes	Not given	-	NT	up 1mM	Furihata and Matsushima (1987)
UDS assay human and rat hepatocytes	Not given	-	NT	1 mM	Brambilla and Martelli (1990)
UDS assay human hepatocytes	Aldrich Chemical Co. Purity 99%	-	NT	1 mM	Butterworth <i>et al.</i> (1989)
UDS assay (serum – free) rat hepatocytes	Aldrich Chemical Co. Purity > 98%	+	NT	10 µg/ml	Parton <i>et al.</i> (1995)

Notes:

- a) +, positive; (+) weak positive; ? inconclusive; -, negative; NT, not tested
b) LED, lowest effective dose; HID, highest ineffective dose
P Polyploid cells increased significantly

4.1.2.7.2 Studies in vivo

The *in vivo* test for the genotoxicity of 2-nitrotoluene are listed in **Table 4.1.2.7.2-1**.

Insect studies

A cytogenetic assay has been carried out to test the genotoxic potential of 2-nitrotoluene in a mosquito *Culex fatigans* (Sharma, Chaudhry and Ahluwalia, 1989). Larvae of mosquito *Culex fatigans* were treated for 24 h., with a concentration of 0.01 µg/ml of 2-nitrotoluene dissolved in DMSO. The chromosomal preparations were made from the ovaries of 12-15 hours old adult females. 2-nitrotoluene is found to be clastogenic as the frequencies of the various chromosomal aberrations are statistically different from their respective control. The various

aberrations encountered were breaks, translocations, fragments and aneuploids. However, no polyploid cells were observed.

A Dominant Lethal assay has been carried out to test the genotoxic potential of 2-nitrotoluene in a mosquito *Culex fatigans* (Sharma, Chaudhry and Ahluwalia, 1989). Treated males were crossed with normal females. Each egg raft was analysed for hatched and unhatched eggs. The dominant lethality was determined in terms of the percentage frequency of the unhatched eggs. 2-nitrotoluene failed to induce dominant lethals.

DNA Adducts

The information from the two studies described below is only qualitative because in both cases it derives from the abstract and not from the full study.

Male WELS-Fohm rats were dosed chronically with 2-nitrotoluene, 5 days a week for 12 weeks in order to determine the formation of hemoglobin and DNA adducts (Jones *et al.*, 2003). After mild base treatment of hemoglobin, 2-methylamine (2MA) was released and quantified by GC/MS. 2'-deoxyguanosine (dG) and 2'-deoxyadenosine (dA) adducts of 2MA were identified in hepatic DNA by electrospray-MS. The dG adduct found *in vivo* did not co-elute with N-(2'-deoxyguanosine-8-yl)-2MA which is the expected adduct for arylamines. The dG adduct detected in the dosed rats was not present in calf thymus-DNA (ct-DNA) modified *in vitro* with N-acetoxy-2-methylaniline. The dA adduct detected in rats was very minor product in ct-DNA modified *in vitro*. The dG and dA adducts found in the 2-nitrotoluene dosed rats increased with the dose and the same increase was seen for the Hb adduct measured in the same animals. The increased DNA and hemoglobin adduct levels were supralinear. There was a very strong linear relationship between the level of dG-2MA adducts and dA-2MA adducts in hepatic DNA from rats administered 2-nitrotoluene over the whole dose range studied. A strong linear relationship also existed between the levels of dG-2MA or dA-2MA adducts, in hepatic DNA, and hemoglobin adducts, over the whole dose range. Thus, according to these results there was strong evidence to support the notion that hemoglobin adducts were an effective surrogate marker for the hepatic DNA damage of rats chronically administered 2-nitrotoluene.

In vitro and *in vivo* experiments were carried out with arylamines and nitroarenes in order to identify DNA adducts (Jones and Sabbioni, 2003). Calf thymus DNA was modified *in vitro* by reaction with an activated N-hydroxylarylamine (2-methylaniline). Two female Wistar rats were given a single dose of 2-methylaniline and its analogous nitro derivative (2-nitrotoluene) by oral gavage and sacrificed after 24 hours. Hepatic DNA and *in vitro* modified DNA were hydrolyzed enzymatically to individual 2'-deoxyribonucleosides. Adducts were determined using HPLC/MS/MS by comparison to synthesized standards. The hydrolysis efficiency was monitored by HPLC with UV detection. After *in vitro* reaction with DNA, 2-methylaniline formed adducts to 2'-deoxyguanosine (dG) and 2'-deoxyadenosine (dA). DNA adducts were not detected in rats dosed with either 2-methylaniline or 2-nitrotoluene. Nevertheless, both compounds formed hydrolyzable hemoglobin adducts. Therefore, biologically available N-hydroxylarylamine yielded hemoglobin adducts but not hepatic DNA adducts.

Mammalian studies

An acute micronucleus test, using male F344/N rats, was performed using two protocols. The initial test used a single intraperitoneal injection of 625, 1250 and 2500 mg/Kg of 2-nitrotoluene dissolved in corn oil, followed by bone marrow analysis 24 h later. In the second test bone marrow was harvested for analysis 48 h after a single intraperitoneal injection of 625

and 2500 mg/Kg of 2-nitrotoluene. The positive control animals received injection of cyclophosphamide. Two thousand polychromatic erythrocytes (PCEs) were scored in up to five animals per dose group. The frequency of micronucleated cells among PCEs was analysed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the control group. In neither test was a positive response observed. The micronucleated PCEs/1000 PCEs (24-hour sample) was 1.50 ± 0.70 ; 0.33 ± 0.33 and 0.80 ± 0.25 , at doses 625, 1250 and 2500 mg/Kg. The micronucleated PCEs/1000 PCEs in the positive control, cyclophosphamide, was 9.80 ± 2.18 , and in the vehicle control, corn oil, 0.60 ± 0.19 . The micronucleated PCEs/1000 PCEs (48-hour sample) was 1.30 ± 0.41 and 0.75 ± 0.25 at doses 625 and 2500 mg/Kg respectively, the micronucleated PCEs/1000 PCEs in the positive control, cyclophosphamide, was 13.40 ± 1.76 and in the vehicle control, corn oil, 1.10 ± 0.19 . In order to select the doses for testing, a preliminary range-finding study was performed. Factors affecting dose selection included chemical solubility and toxicity and the extent of cell cycle delay induced by 2-nitrotoluene exposure. However, results of the preliminary range-finding study were not reported. In addition, there was no information on PCE/NCE (NTP, 2002).

Male B6C3F₁ mice were injected intraperitoneally (three times at 24 h. intervals) with 100, 200, 300 and 400 mg/kg of 2-nitrotoluene dissolved in corn oil. The mice were killed 24h after the third injection. Blood smears were prepared from bone marrow cells obtained from the femurs; 2000 polychromatic erythrocytes (PCEs) were scored in up to five animals per dose group. The result was negative, although a small increase in the frequency of micronucleated PCEs was observed at all doses tested (NTP, 2002). The micronucleated PCEs/1000 PCEs was 1.50 ± 0.16 ; 1.30 ± 0.60 ; 1.60 ± 0.37 and 1.80 ± 0.30 at doses 100, 200, 300 and 400 mg/Kg, respectively. The micronucleated PCEs/1000 PCEs in the positive control, cyclophosphamide, was 6.20 ± 1.15 , in the vehicle control, corn oil, 0.90 ± 0.10 . In order to select the doses for testing, a preliminary range-finding study was performed. Factors affecting dose selection included chemical solubility and toxicity and the extent of cell cycle delay induced by 2-nitrotoluene exposure. However, results of the preliminary range-finding study were not reported. In addition, there was no information on PCE/NCE (NTP, 2002).

Concentrations of 625, 1250, 2500, 5000 and 10000 ppm of 2-nitrotoluene was administered in feed of mice during 13 week. At the end of this toxicity study, peripheral blood samples were obtained from male and female mice. Slides were scanned to determine the frequency of micronuclei in 2000 normochromatic (mature) erythrocytes (NCEs) in each of 10 animals per dose group. 2-nitrotoluene did not increase the frequency of micronucleated NCEs in peripheral blood of female mice. However, a small increase in the frequency of micronucleated NCEs was noted in male mice at the highest dose tested, 10000 ppm. The increase in male mice was sufficient to generate a significant trend test ($P = 0.003$), but because none of the frequencies in individual dose groups were significantly increased over the corresponding control group and because the increase in the frequency of micronuclei in the 10000 ppm group was small, the test in male mice was judged to be equivocal. The percentage of PCEs among the total erythrocyte population in the peripheral blood was scored for each group as a measure of toxicity. The percentage of PCEs for untreated controls was 1.6 and 1.5 in males and females, respectively. When compared with untreated controls, a slight decrease in the percentage of PCEs was only observed in the highest concentration treated group, 1.1% and 1% for males and females, respectively (NTP, 2002).

An UDS assay of 2-nitrotoluene in F344/N rats and B6C3F₁ mice was carried out according to the methods of Mirsalis *et al.*, 1985 (NTP, 1992). The animals with approximately 11 to 12

weeks of age were given a single oral gavage dose of 0, 100, 200 or 500 mg/kg for male rats, and 0, 200, 500 or 750 mg/kg for female rats and male and female mice, 2,6-dinitrotoluene was used as positive control. Both, material test and positive control were formulated in corn oil for dosing. At specified time points, 3 rats or 6 mice were selected from each group for the collection of hepatocytes for UDS determination. For each dose, 3 slides were scored for each of 3 animals (6000 total cells). A positive response for UDS was seen at 200 and 500 mg/kg in male rats, and at 750 mg/kg in female rats. There was a negative response for UDS in mice. 2-nitrotoluene was also found to increase the number of hepatocytes in S-phase in both male and female rats but not in mice.

The genotoxicity of 2-nitrotoluene was evaluated using an *in vivo-in vitro* hepatocyte DNA repair assay (Butterworth *et al.*, 1982, Doolittle, Sherrill and Butterworth, 1983;). Doses at 200, 500 mg/kg b.w. in corn oil were administered by gavage to male F344 rats. Twelve hours after administration the animals were sacrificed, the hepatocytes isolated and incubated with ³H-thymidine, and UDS assay assessed by quantitative autoradiography. 2-nitrotoluene induced pronounced DNA repair in both dose groups. Twenty-four hours following treatment with 2-nitrotoluene, a 50-fold increase in the number of hepatocytes in S-phase was observed and indicated that 2-nitrotoluene induces cell division in addition to DNA repair. The results obtained by Butterworth *et al.*, (1982), shown > 20 Net grains for both doses 200 and 500 mg/Kg. The result in the positive control NDMA was > 20 Net grains. Doolittle *et al.*, (1983), obtained 15.4 ± 4.6 net nuclear grains at 200 mg/Kg dose and 38.5 ± 1.0 net nuclear grains at 500 mg/Kg dose. The result in the positive control, Dimethylnitrosamine 10 mg/Kg, was 34.9 ± 7.6 .

Butterworth *et al.*, (1982), carried out a preliminary study about the role of gut flora in metabolic activation of 2-nitrotoluene. Germ-free Fisher-344 male rats were obtained from Charles River Laboratories. Two weeks before shipment one group was separated and inoculated with Charles River Associated Flora (CRAF), a mixture of bacteria similar to the normal flora. As with the conventional animals, 2-NT produced a positive response in the hepatocytes from CRAF associated animals (>5 net grains/nucleus), whereas no DNA repair was seen in the hepatocytes isolated from 2-NT treated germ-free animals, NDMA at 0.2 ml/100 gr b.w. was used as positive control. Cecal contents were taken at the time of liver perfusion for quantitative bacteriology to confirm bacterial status. These data showed that 2-NT induces DNA repair in the hepatocytes of treated animals and that metabolism by gut flora is obligatory for this activity.

Doolittle, Sherrill and Butterworth, (1983), confirmed the role of the intestinal flora in the metabolic activation of 2-nitrotoluene, male F344 rats (germ-free, and germ-free male rats inoculated with Charles River Altered Schaedler Flora) were gavaged with 2-nitrotoluene. The cecal bacterial status was confirmed at sacrifice. 2-nitrotoluene did not induce DNA repair in germ-free animals (200 mg/kg:-3.8 NG), whereas DNA repair was induced in Charles River Altered Schaedler Flora-associated animals (200 mg/kg: 5.4 NG). When F344 female rats with conventional intestinal microflora were gavaged with 2-nitrotoluene and primary hepatocyte cultures were prepared, no unscheduled DNA synthesis was observed (200 mg/kg: -2.6 NG). Male and female F344 rats were shown to have similar populations of intestinal bacteria, however, at the doses used, females are resistant to the genotoxic action of 2-NT. These results indicate the obligatory role of intestinal bacteria in the metabolic activation of 2-nitrotoluene, showing that the genotoxic potential of 2-nitrotoluene is dependent upon the sex of the animal under study.

Molecular studies

The focus of this study was to evaluate spontaneous and 2-nitrotoluene-induced hemangiosarcomas for mutations in cancer genes important in the pathogenesis of human cancer (NTP, 2002). 15 subcutaneous, mesentery, and skeletal muscle hemangiosarcomas from mice exposed to 2-nitrotoluene at 1250, 2500 or 500 ppm during the carcinogenicity study, and 13 subcutaneous hemangiosarcomas from control mice from previous NTP studies were first screened for *p53* and β -catenin protein expression by immunohistochemical analysis using the avidin-biotin-peroxidase detection system. Wild-type *p53* protein has a short half-life but, when mutated, can be detected in the nucleus of neoplastic cells. Wild type β -catenin protein expression is quickly degraded in normal cells, but when a genetic alteration occurs in the β -catenin gene, increased expression can be detected in the cell membrane, cytoplasm, or nucleus of neoplastic cells. Tissue specimens from a *p53* transgenic mouse (mutation in codon 135 of *p53*) and a mouse hepatoblastoma where β -catenin point mutation was confirmed by direct sequencing were used as positive controls.

No indication of *p53* and β -catenin protein accumulation was observed in the hemangiosarcomas from the control mice and consequently, no further analysis of DNA from these hemangiosarcomas was performed. However, *p53* protein had accumulated in all 15 hemangiosarcomas from 2-nitrotoluene-exposed mice. β -catenin had accumulated in seven of the 2-nitrotoluene-induced hemangiosarcomas. Therefore, these 2-nitrotoluene-induced hemangiosarcomas were also examined for genetic alterations in the *K-ras*, *H-ras*, *p53* and β -catenin genes.

Mutations in at least one of these genes were identified in 13 of 15 (87%) of the 2-nitrotoluene induced hemangiosarcomas, and missense mutations in *p53* exons 5 through 8 were detected in 11 of 15 (73%) of these lesions.

Mutations in *p53* were identified at codons 190, 195, 200, 205, 210, 220, 235, 241, 243, 250, 263 and 264. Of the 15 *p53* mutations identified, six were G-to-A-transitions, three were A-to-G-transitions, three were C-to-T transitions, and the other three were various base pair alterations. Four hemangiosarcomas from the 5000 ppm group exhibited double mutations in *p53*; one of these hemangiosarcomas also had a β -catenin mutation, while another had a CTA mutation at codon 61 of *K-ras*. The lack of *p53* mutations in some of the hemangiosarcomas that were positive by immunohistochemical methods for the *p53* protein may be due to mutations outside the exons 5 to 8 evaluated, or to changes in the expression of other genes that influence the expression of *p53*.

Mutations in *ras* genes or the β -catenin gene were not detected in five of the 2-nitrotoluene-induced hemangiosarcomas that had *p53* mutations. Seven of 15 (47%) hemangiosarcomas from mice exposed to 2-nitrotoluene had splice site mutations at the beginning of exon 2 or deletions in the β -catenin gene. Two 2-nitrotoluene-induced hemangiosarcomas had β -catenin genetic alterations but not *p53* or *ras* mutations. There was a generally good correlation (six of seven; 86%) between hemangiosarcomas that had membrane staining for the β -catenin protein and genetic alterations in the β -catenin gene.

The occurrence of *p53* and β -catenin mutations in the 2-nitrotoluene-induced hemangiosarcomas, but not in the spontaneous hemangiosarcomas, suggest that the pathways leading to 2-nitrotoluene-induced cancer differ from the pathways in spontaneous hemangiosarcomas.

On the other hand, mutations in *ras*, *p53* and β -catenin genes observed in mouse hemangiosarcomas were also present in different human neoplasms (Marion, Froment and Trepo, 1991; Hollstein *et al.*, 1994; Gamallo *et al.*, 1999).

The finding of chemical-associated mouse hemangiosarcomas with *p53* and β -catenin mutations suggests these neoplasms are good models for the human equivalent. Moreover, the human and mouse neoplasms may have similar carcinogenic pathways with implications for human risk from exposure to this chemical.

Table 4.1.2.7.2-1: *In vivo* test for the genotoxicity of 2-nitrotoluene

TEST SYSTEM	SOURCE AND PURITY OF CHEMICAL	RESULT ^{a)}	DOSE ^{b)} (LED/HID)	REFERENCE
Chromosomal aberrations (ovaries) Culex Faligans	Department of Pharmacy Panjab University Chandigarh	+	0,01 µg/ml	Sharma, Chaudhaj and Ahluwalya (1989)
Dominant letal Culex Fatigans	Department of Pharmacy Panjab University Chandigarh	-	0,01 µg/ml	Sharma, Chaudhaj and Ahluwalya (1989)
DNA adducts and hemoglobin adducts male Wels –Fohm rats	Not given	+	5 days /wk 12 wks	Jones <i>et al.</i> (2003)
DNA adducts and hemoglobin adducts	Not given	- (DNA)	1, p.o.	Jones and Sabbioni (2003)
		+ (haemoglobin)		
Micronucleus test male F344/N rats bone-marrow cells	Not given	- (24 hr)	2500 mg/kg 1,i.p.	NTP (2002)
		- (48 hr)		
Micronucleus test male B6C3F1 mice bone-marrow cells	Not given	-	400 mg/kg 3,i.p.	NTP (2002)
Micronucleus test male and female B6C3F1 mice peripheral blood cells	Not given	? (male)	10000 ppm 13 weeks, feed	NTP (2002)
		- (female)		
UDS Assay male and female F344/N rats hepatocytes	Aldrich Chemical Co. Purity > 96%	+ (male)	200 mg/kg 1,p.o.	NTP (1992)
		+ (female)	750 mg/kg 1,p.o.	
UDS Assay, male and female F6C3F1 mice hepatocytes	Aldrich Chemical Co. Purity > 96%	-	750 mg/kg 1,p.o.	NTP (1992)
UDS Assay, male F344 rats hepatocytes	Aldrich Chemical Co. Purity > 99%	+	200 mg/kg, 1,p.o.	Doolittle <i>et al.</i> (1983)
UDS Assay, male F344 rats hepatocytes	Not given	+	200 mg/kg 1,p.o.	Butterworth <i>et al.</i> (1982)
UDS male F344 rats (germ free and CRAF)	Not given	- (germ free)	200 mg/kg 1,p.o.	Butterworth <i>et al.</i> (1982)
		+ (CRAF)		
UDS male F344 rats (germ free and CRAF), female F344 rats (conventional intestinal flora)	Aldrich Chemical Co. Purity > 99%	- (male germ free)	500 mg/kg 1,p.o.	Doolittle <i>et al.</i> (1983)
		+ (male CRAF)	200 mg/kg 1,p.o.	
		- (female conventional intestinal flora)	200 mg/kg 1,p.o.	

Notes:

c) +, positive; (+) weak positive; ? inconclusive; -, negative; NT, not tested

d) LED, lowest effective dose; HID, highest ineffective dose

CRAF: Charles River Altered Schaedler Flora (mixture of 8 bacteria, 2 Lactobacillus sp., bacteroides distasonis, 4 fusiform-shaped bacteria and spirochete) intended to stimulate the autochthonous gastrointestinal flora

4.1.2.7.3 Summary of mutagenicity

There are several data available on the genotoxicity of 2-nitrotoluene *in vitro* and *in vivo* studies.

The information in bacteria indicates that 2-nitrotoluene is not mutagenic in any of several strains of *Salmonella typhimurium* with or without metabolic activation enzymes (S9). However, the addition of norharman produced a positive result in the presence of S9 mix. On *Bacillus subtilis*, 2-nitrotoluene had a genotoxic effect.

In cytogenetic tests on *Chinese Hamster Ovary* cells, 2-nitrotoluene increased the Sister Chromatid Exchange rate, being this more pronounced in the presence of S9mix. In the Chromosomal Aberrations test the result was negative with or without S9 mix. There was an increase in polyploidy cells when 2-nitrotoluene was tested in cultures of Chinese hamster lung (CHL) cells in the absence of S9 mix. 2-nitrotoluene induced chromosomal aberrations in human lymphocytes.

No induction of unscheduled DNA synthesis was observed in isolated rat spermatids, spermatocytes or hepatocytes.

2-nitrotoluene was found to be clastogenic when tested in larvae of mosquito *Culex fatigans* *in vivo*. However, for a Dominant Lethal test in the same species was negative.

2-Nitrotoluene induced adducts in haemoglobin and hepatic DNA in male Wels-Fohm rats dosed chronically 5 days a week for 12 weeks. However, Wistar rats dosed with a single dose of 2-nitrotoluene by oral gavage not induced DNA adducts, nevertheless formed haemoglobin adducts.

2-nitrotoluene did not induce a significant increase in the frequency of micronuclei in bone marrow polychromatic erythrocytes of male rats or male mice when administered by intraperitoneal injection. Results of a peripheral blood micronucleus test were equivocal for male mice and negative for female mice administered o-nitrotoluene in feed for 13 weeks.

Positive results were found in the UDS test for both male and female rats administered 2-nitrotoluene. Males were more sensitive to genotoxicity of 2-nitrotoluene. A sex difference in biliary excretion may explain the sex difference in the genotoxicity of 2-nitrotoluene. In addition, 2NT did not induce DNA repair in germ-free animals, whereas DNA repair was induced in Charles River Altered Schaedler Flora-associated animals. Male and female F344 rats were shown to have similar populations of intestinal bacteria; however at the doses used, females were resistant to the genotoxic action of 2NT. These results indicate the obligatory role of intestinal bacteria in the metabolic activation of 2NT, showing that the genotoxic potential of 2-NT is dependent upon the sex of the animal under study.

Gene mutations in *ras*, *p53* and *β-catenin* genes were observed in hemangiosarcomas from B6C3F₁ mice exposed to 2-nitrotoluene, for 2 years, in feed. These *in vivo* data suggest 2-nitrotoluene is metabolized to mutagenic intermediates and that could be the reason why most of the *in vitro* genotoxicity tests were negative. 2-nitrotoluene has a number of potentially active metabolites that could account for the mutation profile observed in these tumours, as described in the proposal pathway for bioactivation (See 4.1.2.1.4).

In conclusion, 2-nitrotoluene is mutagenic in somatic cells. In addition, it reaches the germ cells since toxicity was observed in testis and epididymis (to see section 4.1.2.6). According to the TGD (2005), *it is reasonable to assume that a somatic cell mutagen also has the potential to cause mutations in germ cells. The likelihood of mutagenicity occurring in the*

germ cells in vivo is determined by the toxicokinetics of the substance and its ability to reach the target tissues in sufficient amounts to elicit the effect. Therefore, although there is not direct evidence relating to mutagenicity in the germ cells, in view of the above exposed, we support the current (29th ATP) classification of 2-NT as a mutagenic category 2 (T, R46).

4.1.2.8 Carcinogenicity

4.1.2.8.1 Studies in animals

In vivo studies

Inhalation

No data available.

Dermal

No data available

Oral

Rats

In the core study, performed in essence according to OECD guideline 451 and GLP compliant, F344/N rats (60 animals/sex/group, 6-7 weeks old) were administered 0 (females only), 625, 1250 and 2000 ppm (25, 50 and 90 mg/kg b.w. in males; 0, 30, 60 and 100 mg/kg b.w. in females) of 2-nitrotoluene (>99% pure) in the diet for 105 weeks after acclimation periods of 12-14 days; in a 3-month stop-exposure study, groups of 70 males were fed diets containing 0, 2000 and 5000 ppm (0, 125 and 315 mg/kg b.w.) for 13 weeks followed by undosed feed for the remainder of the study, 10 males from each stop exposure and control group were sacrificed at 3 months (NTP, 2002). All 2000 ppm core study, all 5000 ppm stop-exposure and all but three core study 1250 ppm male rats died before the end of the studies. Survival of 625 ppm core study and 2000 ppm stop-exposure males and of 2000 ppm females was significantly less than that of the controls. Mean body weights of all exposed groups of males except the 625 ppm group were generally less than those of the controls throughout the study. Mean body weights of 2000 ppm females were less than those of the controls during year 2 of the study. Feed consumption by exposed groups was similar to that by the controls throughout the study. Clinical findings included large subcutaneous masses in the torso, head and appendages of exposed males and females, which increased in number with exposure; males in both 2000 ppm groups and the 5000 ppm stop-exposure had small ears and thin tails. The main toxicological findings are described below and summarised in Tables 4.1.2.8.1-1 and 4-1.2.8.1-2.

Mesothelium: At the 3-month interim sacrifice, no mesotheliomas or mesothelial hyperplasia were observed in exposed males. However, as time progressed, chemical-induced mesotheliomas occurred in all exposed male groups, including the stop-exposure groups. The incidences of malignant mesothelioma were significantly greater in exposed groups than in controls and exceeded the 2-year historical control ranges. Mesotheliomas were mainly associated with the tunica vaginalis of the testis or epididymis.

Skin: Significant increases in the incidences of subcutaneous fibroma, fibrosarcoma, fibroma or fibrosarcoma (combined) and lipoma in all exposed groups of males and of fibroma and fibroma or fibrosarcoma (combined) in 1250 and 2000 ppm females. These increases occurred with positive trends and exceeded the 2-year historical control ranges.

Mammary gland: In all exposed groups of males and females except 2000 ppm core study males, the incidences of fibroadenoma were significantly greater than those in the controls and exceeded the 2-year historical control ranges. The incidences of hyperplasia, a precursor of fibroadenoma, were significantly increased in 625 and 1250 ppm females.

Liver: Liver weights of 5000 ppm stop-exposure males were significantly greater than those of the controls at 3 months. The incidences of hepatocellular adenoma in 2000 ppm core study males and females and of hepatocellular adenoma or carcinoma (combined) in 2000 ppm core study and 5000 ppm stop-exposure males were significantly greater than those in the controls and generally exceeded the 2-year historical control ranges. Cholangiocarcinoma occurred in three 5000 ppm stop-exposure males, and a single hepatocholangiocarcinoma occurred in a 625 ppm male and in a 2000 ppm core study male. In exposed groups there was an increase of several nonneoplastic lesions such as eosinophilic, mixed cell and clear cell foci in both sexes, mixed cell infiltrate in males, and basophilic focus in females.

Lung: Incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined) were significantly increased in 5000 ppm stop-exposure males and exceeded the 2-year historical control ranges, as were alveolar epithelial hyperplasia in most exposed groups of both sexes.

Haematopoietic system: The incidences of hyperplasia in the **bone marrow** (all exposed males, 1250 and 2000 ppm exposed females) and of haematopoietic cell proliferation in the **spleen** (all exposed groups) and of hyperplasia of the **mandibular lymph node** (2000 ppm females) were significantly increased.

Blood: The incidences of mononuclear cell leukemia were significantly decreased in all groups of males exposed to 1250 ppm or greater and in all exposed groups of females and were less than the 2-year historical control ranges. Decreased incidences of leukemia was most likely related to the splenic toxicity.

Testis: The incidence of testicular interstitial cell adenoma was significantly decreased in 5000 ppm stop-exposure males, likely related to testicular toxicity.

Table 4.1.2.8.1-1: Summary of the main neoplastic lesions in the rat carcinogenicity study (2-year evaluation)^{a,b,c}

2-nitrotoluene	0 ppm	625 ppm	1,250 ppm	2,000 ppm	2,000 ppm (Stop-exposure)	5,000 ppm (Stop-exposure)
Male Rats						
<i>Average Daily Dose (mg/kg)</i>	0	25	50	90	125	315
<i>Body weights</i>			Less than controls	Less than controls	Less than controls	Less than controls
<i>Survival</i>	39/60	18/60	3/60	0/60	11/60	0/60
<i>Mesothelium Malignant Mesothelioma</i>	2/60	20/60	29/60	44/60	44/60	54/60
<i>Skin (Subcutaneous)</i>						
Lipoma	0/60	4/60	13/60	13/60	10/60	12/60
Fibroma	5/60	46/60	52/60	59/60	45/60	52/60
Fibrosarcoma	0/60	7/60	17/60	20/60	8/60	12/60
Fibroma or Fibrosarcoma	5/60	47/60	55/60	59/60	47/60	53/60
<i>Mammary Gland</i>						
Fibroadenoma	0/60	7/60	10/60	2/60	13/60	20/60
<i>Liver</i>						
Hepatocellular Adenoma	2/60	3/60	3/60	7/60	3/60	4/60
Hepatocellular Adenoma or Carcinoma	3/60	3/60	3/60	8/60	3/60	6/60
Cholangiocarcinoma	0/60	0/60	0/60	0/60	0/60	3/60
Hepatocholangiocarcinoma	0/60	1/60	0/60	1/60	0/60	0/60
<i>Lung</i>						
Alveolar/bronchiolar Adenoma	1/60	5/60	1/60	2/60	3/60	8/60
Alveolar/bronchiolar adenoma or Carcinoma	2/60	5/60	1/60	2/60	3/60	11/60
2-nitrotoluene	0 ppm	625 ppm	1,250 ppm	2,000 ppm		
Female Rats						
<i>Average Daily Dose (mg/kg)</i>	0	30	60	100		
<i>Body weights</i>				Less than controls		
<i>Survival</i>	47/60	47/60	39/60	33/60		
<i>Skin (Subcutaneous)^c</i>						
Fibroma	3/59	3/60	18/60	19/60		
Fibroma or Fibrosarcoma	3/60	3/60	21/60	22/60		
<i>Mammary Gland</i>						
Fibroadenoma	23/60	47/60	52/60	56/60		
<i>Liver</i>						
Hepatocellular Adenoma	1/60	0/59	1/60	6/60		

^a NTP, 2002^b Most treated animals (all exposed groups of males and the high exposure group of females) died before getting 2 years old (in contrast to the controls). Thus, the necropsy results for the treated animals refer to the time-points when they actually died. Early deaths among treated animals were due to the development of neoplasms. For males, malignant mesotheliomas were the reason for early deaths.^c Incidences of neoplasms are given as number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver and lung; for other tissues, denominator is number of animals necropsied.

Table 4.1.2.8.1-2: Summary of the main non-neoplastic lesions in the rat carcinogenicity study (2-year evaluation)^{a,b,c}

2-nitrotoluene	0 ppm	625 ppm	1,250 ppm	2,000 ppm	2,000 ppm (Stop-exposure)	5,000 ppm (Stop-exposure)
Male Rats						
Average Daily Dose (mg/kg)	0	25	50	90	125	315
Body weights			Less than controls	Less than controls	Less than controls	Less than controls
Survival	39/60	18/60	3/60	0/60	11/60	0/60
<i>Liver</i>						
Eosinophilic focus	7/60	18/60	29/60	24/60	15/60	13/60
Mixed cell focus	5/60	7/60	12/60	6/60	12/60	8/60
Clear cell focus	29/60	29/60	34/60	31/60	30/60	34/60
Mixed cell cellular infiltration	1/60	5/60	11/60	20/60	15/60	33/60
<i>Bone marrow</i>						
Hyperplasia	2/60	25/60	43/60	45/60	37/60	33/60
<i>Spleen</i>						
Hematopoietic cell proliferation	7/60	33/60	38/60	47/60	36/60	35/60
<i>Lung</i>						
Alveolar epithelial hyperplasia	2/60	8/60	3/60	7/60	15/60	29/60
2-nitrotoluene	0 ppm	625 ppm	1,250 ppm	2,000 ppm		
Female Rats						
Average Daily Dose (mg/kg)	0	30	60	100		
Body weights				Less than controls		
Survival	47/60	47/60	39/60	33/60		
<i>Mammary gland</i>						
Hyperplasia	14/60	36/60	30/60	19/60		
<i>Liver</i>						
Eosinophilic focus	5/60	12/59	25/60	32/60		
Mixed cell focus	6/60	9/59	11/60	28/60		
Clear cell focus	16/60	30/59	28/60	33/60		
Basophilic focus	51/60	56/59	60/60	54/60		
<i>Bone marrow</i>						
Hyperplasia	2/60	7/60	15/60	24/60		
<i>Spleen</i>						
Hematopoietic cell proliferation	22/60	38/59	48/60	48/59		
<i>Lung</i>						
Alveolar epithelial hyperplasia	6/60	14/60	16/60	9/60		
<i>Lymph node (mandibular)</i>						
Lymphoid hyperplasia	3/60	5/60	6/59	15/59		

^a NTP, 2002^b Most treated animals died before getting 2 years old (in contrast to the controls). Thus, the necropsy results for the treated animals refer to the time-points when they actually died.^c Incidences of non-neoplastic lesions are given as number of non-neoplastic lesions-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver and lung; for other tissues, denominator is number of animals necropsied.

Mice

In a study, performed in essence according to OECD guideline 451 and GLP compliant, B6C3F₁ mice (60 animals/sex/group, 6 weeks old) were administered 0, 1250, 2500 and 5000 ppm (0, 165, 360 and 700 mg/kg b.w. in males; 0, 150, 320 and 710 mg/kg b.w. in females) of 2-nitrotoluene (>99% pure) in the diet for 105 weeks after acclimation periods of 12 days (NTP, 2002). All 2500 and 5000 ppm males died before the end of the study. Survival of 1250

ppm males and 5000 ppm females was significantly less than that of the controls. Mean body weights of exposed males and 5000 ppm females were generally less than those of the controls throughout the study, and those of 2500 ppm females were less during the second year of the study. Feed consumption by 5000 ppm males was less than that by the controls. Clinical findings included large subcutaneous masses in the torso, head and appendages of exposed males and females. The main toxicological findings are described below and summarised in Table 4.1.2.8.2-1 and 4.1.2.8.2-2.

Circulatory system: The incidences of hemangiosarcoma in all exposed groups of males and in 5000 ppm females were significantly greater than those in the controls and exceeded the 2-year historical control ranges. Hemangiosarcomas occurred primarily in the mesentery, skeletal muscle and subcutaneous skin.

Large intestine: The incidences of carcinoma of the cecum in 1250 and 2500 ppm males were significantly increased and exceeded the 2-year historical control ranges. Although the increased incidences of carcinomas in exposed females were not significant, they were considered to be exposure related because this neoplasm is very rare and has not been observed in historical female controls.

Liver: The incidences of adenoma in 2500 and 5000 ppm females, carcinoma in 5000 ppm females, and adenoma or carcinoma (combined) in 2500 and 5000 ppm females were significantly greater than those in the controls and exceeded the 2-year historical control ranges. Exposed males died early because of the development of hemangiosarcomas, which may explain why the later-developing hepatocellular neoplasms were not seen in males. The incidences of nonneoplastic lesions, eosinophilic foci in 1250 ppm males and 5000 ppm females, basophilic foci in 1250 and 2500 ppm males and 1250 and 5000 ppm females, necrosis in all exposed males and in 5000 ppm females were significantly increased; also present were focal hepatocyte syncytial alteration in exposed males and focal hepatocyte cytoplasmic vacuolization in 5000 ppm females.

Kidney: The incidences of renal tubule pigmentation in all exposed males and 5000 ppm females were significantly greater than those in the controls.

Nose: Olfactory epithelial degeneration occurred in males and females exposed from 1250 ppm, and the severity of this lesion increased with increasing exposure concentration.

Table 4.1.2.8.2-1: Summary of the main neoplastic lesions in the mouse carcinogenicity study (2-year evaluation)^{a,b,c}

	2-nitrotoluene	0 ppm	1250 ppm	2,500 ppm	5,000 ppm
Male Mice					
<i>Average Daily Dose (mg/kg)</i>		0	165	360	700
<i>Body weights</i>			Less than controls	Less than controls	Less than controls
<i>Survival</i>		52/60	34/60	0/60	0/60
<i>Circulatory System</i> Hemangiosarcoma		4/60	17/60	55/60	60/60
<i>Large Intestine (Cecum)</i> Carcinoma		0/56	12/49	9/36	0/44
<i>Lung</i> Alveolar/bronchiolar Adenoma or Carcinoma		14/60	7/60	6/60	0/60
	2-nitrotoluene	0 ppm	625 ppm	1,250 ppm	2,000 ppm
Female Mice					
<i>Average Daily Dose (mg/kg)</i>		0	150	320	710
<i>Body weights</i>				Less than controls	Less than controls
<i>Survival</i>		52/60	46/60	47/60	5/60
<i>Circulatory System</i> Hemangiosarcoma		0/60	2/60	3/60	50/60
<i>Large Intestine (Cecum)</i> Carcinoma		0/60	1/60	4/60	3/60
<i>Liver</i> Hepatocellular Adenoma		7/60	5/59	19/59	29/60
Hepatocellular Carcinoma		2/60	4/59	6/59	16/60
Hepatocellular Adenoma or Carcinoma		9/60	9/59	24/59	39/60

^a NTP, 2002^b Most treated animals (all exposed groups of males and the high exposure group of females) died before getting 2 years old (in contrast to the controls). Thus, the necropsy results for the treated animals refer to the time-points when they actually died. Early deaths among treated animals were due to the development of neoplasms. Hemangiosarcomas were the reason for early deaths in both males and females.^c Incidences of neoplasms are given as number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver and lung; for other tissues, denominator is number of animals necropsied.

Table 4.1.2.8.2-2: Summary of the main non-neoplastic lesions in the mouse carcinogenicity study (2-year evaluation)^{a,b,c}

	2-nitrotoluene	0 ppm	1250 ppm	2,500 ppm	5,000 ppm
Male Mice					
Average Daily Dose (mg/kg)		0	165	360	700
Body weights			Less than controls	Less than controls	Less than controls
Survival		52/60	34/60	0/60	0/60
<i>Liver</i>					
Eosinophilic focus		3/60	14/59	1/57	1/60
Basophilic focus		0/60	6/59	4/57	0/60
Necrosis		1/60	15/59	27/57	30/60
Focal hepatocyte syncytial alteration		16/60	26/59	43/57	39/60
<i>Kidney</i>					
Renal tubule pigmentation		1/58	6/59	32/58	35/60
<i>Nose</i>					
Olfactory epithelial degeneration		0/60	36/60	60/60	60/60
Female Mice					
Average Daily Dose (mg/kg)		0	150	320	710
Body weights				Less than controls	Less than controls
Survival		52/60	46/60	47/60	5/60
<i>Liver</i>					
Eosinophilic focus		2/60	3/59	6/59	28/60
Basophilic focus		1/60	6/59	2/59	6/60
Necrosis		3/60	0/59	2/59	13/60
Focal hepatocyte necrosis		0/60	0/59	0/59	6/60
Focal hepatocyte cytoplasmic vacuolization		1/60	2/59	2/59	9/60
<i>Kidney</i>					
Renal tubule pigmentation		0/59	1/56	3/58	35/59
<i>Nose</i>					
Olfactory epithelial degeneration		0/60	28/60	59/59	57/57

^a NTP, 2002

^b Most treated animals died before getting 2 years old (in contrast to the controls). Thus, the necropsy results for the treated animals refer to the time-points when they actually died.

^c Incidences of non-neoplastic lesions are given as number of non-neoplastic lesions-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver and lung; for other tissues, denominator is number of animals necropsied.

Other route

Mice

In a lung-tumour induction test, young A/Jax male mice (30 animals/group) were injected i.p. with 2-nitrotoluene (98% pure) in corn oil at doses of 1200, 3000 and 6000 mg/kg 3 times a week for 8 consecutive weeks. Mice were killed 16 weeks after the last injection, and lung tumours were counted (Slaga *et al.*, 1985). No treatment related effect on body weight. There was an apparently dose-related but statistically non-significant increased incidence of lung tumours. This study is non OCDE (451 or 453) guideline, non GLP compliant and considered inadequate for risk assessment.

In vitro studies

No data available.

4.1.2.8.2 Studies in humans

No data available.

4.1.2.8.3 Other information

In a tumour initiation-promotion test using TPA as promoter, 2-nitrotoluene (98% pure) in acetone at doses of 24, 120 and 240 mg was applied once onto the skin of SENCAR mice, followed by once weekly promotion with 4 µg of TPA for 30 weeks (Slaga *et al.*, 1985). Mice survival was 90% or more. No treatment related effect on body weight. There was a statistically non-significant increased incidence of papillomas and carcinomas at 240 mg when compared with the TPA only treated mice. It was concluded that 2-nitrotoluene might have weak skin tumour initiating activity.

4.1.2.8.3 Summary of carcinogenicity

Only a long-term feed carcinogenicity study in rodents was available (NTP, 2002), performed in essence according to OECD guideline 451 and GLP compliant, and therefore considered adequate for risk assessment.

There was clear evidence of carcinogenic activity of 2-nitrotoluene in rats, based on increased incidences of malignant mesothelioma, subcutaneous skin neoplasms, mammary gland fibroadenoma and liver neoplasms in males and increased incidences of subcutaneous skin neoplasms and mammary gland fibroadenoma in females. The increased incidences of lung neoplasms in males and of hepatocellular adenoma in females were also considered to be exposure related. Malignant mesotheliomas occurred with incidences of 33%, 48% and 73% in the 625, 1250 and 2000 ppm core study male rat groups, respectively. The incidences of malignant mesotheliomas were 73% and 90% in the 2000 and 5000 ppm stop-exposure male rat groups. The incidence of mesothelioma was higher in the 2000 stop-exposure group than in the 625 ppm even though the latter group received approximately 50% more total exposure to 2-nitrotoluene. The incidences of mesotheliomas were similar in the 2000 ppm core study and stop-exposure groups of male rats. Thus, critical events leading to mesothelioma occurred early in the study, and this damage was irreversible. The molecular pathogenesis of mesotheliomas is not well understood. Decreased incidences of mononuclear cell leukaemia and of testicular interstitial cell adenoma in exposed groups were related to splenic and testicular toxicity, respectively.

There was clear evidence of carcinogenic activity of 2-nitrotoluene in male and female mice based on increased incidences of hemangiosarcoma, carcinoma of the large intestine (cecum), and hepatocellular neoplasms (females only because males died early due to the development of hemangiosarcomas). The occurrence of *p53* or β -catenin mutations in 2-nitrotoluene-induced hemangiosarcomas, but not in spontaneous hemangiosarcomas, suggest that the pathways leading to 2-nitrotoluene-induced cancer differ from the pathways in spontaneous hemangiosarcomas.

No 2-nitrotoluene epidemiology studies on carcinogenesis have been reported in the literature. However, according to Ward *et al.*, 1991 (cited in NTP, 2002) excess cancers have been found in workers exposed to a related chemical, *o*-toluidine.

In summary, there is a good evidence of an increase in tumour incidence at multiple sites in both rats and mice. There is also evidence that time to onset is very short. These observations

are consistent with genotoxic aetiology, which is consistent with the findings from the genotoxicity studies.

Therefore, according to EU criteria, 2-nitrotoluene is considered carcinogenic category 2 and then classified as T R45.

4.1.2.9 Toxicity for reproduction

4.1.2.9.1 Effects on fertility

Studies in animals

Oral

Rats

Within the framework of a study on subchronic and reproductive toxicity, Wistar rats (10 animals/sex/group weighing about 200 g) were administered 0 and 200 mg/kg b.w. of 2-nitrotoluene (99% pure) in olive oil by gavage 5 days/week for 3 months, after an acclimation period of 1 week. Then, 5 treated males were in each case mated with 5 untreated or treated females and 5 untreated males were in each case mated with 5 untreated or treated females. Treated females were dosed throughout pregnancy but undosed during the lactation period of the offspring; in addition, 2 untreated females, which have been mated with untreated males, were dosed during the lactation period of the offspring. Pups, non-treated, were sacrificed 3 month post-partum (Ciss, 1978; Ciss *et al.* 1980b). There was not treatment related histopathology on either testes or ovaries of parents. In addition, the number of pups from treated and untreated animals was the same. Therefore, it can be said that at the dose used in this study, 2-nitrotoluene did not have any influence on the fertility (more details on subchronic toxicity in section 4.1.2.6.1).

Within the framework of a reproduction/developmental toxicity screening study performed by Huntingdon, 1994 (cited in KemI 1994), male and female CD rats received 2-nitrotoluene at daily doses of 0, 50, 150 or 450 mg/kg/d by gavage in corn oil over a total period of approximately 10 weeks (2 weeks prior to mating, 2 weeks' mating period, 20 days' pregnancy and 21 days post-partum). Pups were sacrificed on day 21 post-partum. For the 450 mg/kg/d group wet coats generally occurred for both sexes and brown stained coats were also observed in all males followed by most females. Lower body weight gain and food consumption occurred for males from 150 mg/kg/d. For females lower body weight gain occurred during pregnancy for the 450 mg/kg/d group but recovered Day 21 post partum. Food consumption was lower from 150 mg/kg/d Days 1 to 13 post partum. The parent animals showed increases in liver weight, from 50 mg/kg/d (♀) and 150 mg/kg/d (♂), and in both kidney and spleen weights (from 150 mg/kg/d in both sexes). In relation to the reproductive system, there was a dosage-related decrease of epididymis, seminal vesicles and prostate weights from 150 mg/kg/d and of testes at 450 mg/kg/d. In the absence of further details, these effects are not considered enough evidence of impaired fertility.

Within the framework of a study on subchronic toxicity, performed in essence according to OECD guideline 408 and GLP compliant, F344/N rats (10 animals/sex/group, 6 weeks of age) were administered 0, 625, 1250, 2500, 5000 and 10000 ppm (0, 45, 89, 179, 353 and 694 mg/kg b.w. in males; 0, 44, 87, 178, 340 and 675 mg/kg b.w. in females) of 2-nitrotoluene

(>96% pure) in the diet for 13 weeks after acclimation periods of 10-15 days (NTP, 1992; Dunnick, Elwell and Bucher, 1994). At 5000 ppm and above, degeneration of the testes with reduction in sperm count and motility occurred among the males and a prolongation of the menstrual cycle among the females. These dose levels are located in the toxic range (more details in section 4.1.2.6.1).

Within the framework of a study on subchronic toxicity, F344 male rats (10 and 20 animals per control and exposed group respectively, aged 45 days) were administered 0 and 5000 ppm (0 and 292-296 mg/kg b.w.) of 2-nitrotoluene (99.8 ± 0.3% pure) in the diet, after an acclimation period of 9 days, for 13 and 26 weeks, and for 13 weeks followed by control diet until necropsy at 26 weeks (NTP, 1996). Degeneration of the seminiferous tubules increased in severity with continuous exposure and showed no evidence of recovery in the stop-exposure groups. This dose level is considered toxic (more details in section 4.1.2.6.1).

Mice

Within the framework of a study on subchronic toxicity, GLP compliant, B6C3F₁ mice (5 animals/sex/group, 6 weeks of age) were administered 625, 1250, 2500, 5000 and 10000 ppm (104, 223, 415, 773 and 1536 mg/kg b.w. in males; 132, 268, 542, 1007 and 1712 mg/kg b.w. in females) of 2-nitrotoluene (>96% pure) in the diet for 13 weeks after acclimation periods of 12-14 days (NTP, 1992; Dunnick, Elwell and Bucher, 1994). Sperm motility was reduced among the males of the 10000 ppm group (more details in section 4.1.2.6.1).

Studies in humans

No data available.

4.1.2.9.2 Developmental toxicity

Studies in animals

Oral

Rats

Within the framework of a study on subchronic and reproductive toxicity, Wistar rats (10 animals/sex/group weighing about 200 g) were administered 0 and 200 mg/kg b.w. of 2-nitrotoluene (99% pure) in olive oil by gavage 5 days/week for 3 months, after an acclimation period of 1 week. Then, 5 treated males were in each case mated with 5 untreated or treated females and 5 untreated males were in each case mated with 5 untreated or treated females. Treated females were dosed throughout pregnancy but undosed during the lactation period of the offspring; in addition, 2 untreated females, which have been mated with untreated males, were dosed during the lactation period of the offspring. Pups, non-treated, were sacrificed 3 month post-partum (Ciss, 1978; Ciss *et al.* 1980b). Mortality, vitality and behaviour of pups from treated and untreated animals were the same. In addition, no histopathological changes in organs occurred among the young animals regardless on treatment. Therefore, it can be said that at the dose used in this study, 2-nitrotoluene did not induce developmental toxicity. Furthermore, there was no toxicity derived from transfer of the substance through the milk (more details on subchronic toxicity in section 4.1.2.6.1).

Within the framework of a reproduction/developmental toxicity screening study performed by Huntingdon, 1994 (cited in KemI 1994), male and female CD rats received 2-nitrotoluene at daily doses of 0, 50, 150 or 450 mg/kg/d by gavage in corn oil over a total period of approximately 10 weeks (2 weeks prior to mating, 2 weeks' mating period, 20 days' pregnancy and 21 days post-partum). Pups were sacrificed on day 21 post-partum. For the 450 mg/kg/d group wet coats generally occurred for both sexes and brown stained coats were also observed in all males followed by most females. Lower body weight gain and food consumption occurred for males from 150 mg/kg/d. For females lower body weight gain occurred during pregnancy for the 450 mg/kg/d group but recovered Day 21 post partum. Food consumption was lower from 150 mg/kg/d Days 1 to 13 post partum. The parent animals showed increases in liver weight, from 50 mg/kg/d (♀) and 150 mg/kg/d (♂), and in both kidney and spleen weights (from 150 mg/kg/d in both sexes). In relation to the reproductive system, there was a dosage-related decrease of epididymis, seminal vesicles and prostate weights from 150 mg/kg/d and of testes at 450 mg/kg/d. 3 out of 12 females from the 450 mg/kg/d group died in the immediate post natal period (Day 1 or 2 post-partum), and had dead implantations in the uteri. A dosage-related retardation in pup growth was apparent in all treated groups from the 4th or 8th day post-partum. The mortality of 3 among 12 dams treated with 450 mg/kg/d of 2-nitrotoluene is attributed to systemic toxicity of the test material; consequently, the foetus mortality is seen as a secondary effect of maternal toxicity. The retardation in pup growth could be indicative of developmental toxicity; however, in the absence of further details on its severity we are not sure if this effect could be used for classification or it is only a minor developmental change. In addition, if the retardation in pup growth was considered a toxic effect, it cannot be ruled out that some toxicity was due to the transfer of the substance through the milk.

Studies in humans

No data available.

4.1.2.9.3 Summary of toxicity for reproduction

The only information available on fertility is from non-standard studies in experimental animals. In rats, 2-nitrotoluene administered in feed at 5000 ppm for 13 weeks causes damage to the testes and the epididymis with a simultaneous reduction in the sperm count and the motility of the sperm in males, and a prolongation of the menstrual cycle among the females. Reduced sperm motility was also observed at 10000 ppm (the highest dose level tested) for the mouse. The testicular effects indicate a need for fertility classification but because they occur at toxic dose levels, while clear-cut effects on fertility seem absent, indeed only toxic for reproduction category 3 (Xn, R 62) is justified according to EU criteria. In addition, the NOAEL for reproduction toxicity is considered to be 2500 ppm (179 mg/kg bw).

The only information available on developmental toxicity is derived from two non-standard reproduction studies in experimental animals. In one of them carried out in CD rats, the only effect considered as indicative of developmental toxicity was the retardation in pup growth; however, because of the absence of further details on its severity we are not sure if this effect could be used for classification; in addition, if this effect was considered as toxic, it cannot be ruled out that some toxicity was due to the transfer of the substance through the milk. In the other study carried out in Wistar rats, mortality, vitality and behaviour of pups from both treated and untreated animals were the same and no histopathological changes in organs occurred among the young animals regardless on treatment; therefore, 2-nitrotoluene did not

induce developmental toxicity; furthermore, there was no toxicity derived from transfer of the substance through the milk. Differences on results between studies could be due to differences on sensitivity between strains. The classification for developmental toxicity based on available data and the EU criteria is not justified. However, assuming the worst case, it is necessary a risk characterization for developmental toxicity using the LOAEL of 50 mg/kg.

4.1.3 Risk characterisation ⁷

4.1.3.1 General aspects

There are not available data on toxicokinetics of 2-nitrotoluene for humans but several studies following oral administration have been performed in experimental animals, especially in rats. *In vitro* studies provide additional information on the metabolism.

In rats, 2-nitrotoluene is rapidly absorbed, extensively metabolised and rapidly excreted. Its half-life in plasma was about 1.5 hours. Oral absorption was determined to be 100% within 24 hours based on excretion of radioactivity (more than 95% in urine from both sexes) obtained after a single oral dose of 2 mg/kg b.w. There were no differences between sexes. In addition, similar results were obtained in rats administered a single or a repeated oral dose of 200 mg/kg b.w.

Oral absorption in male mice was determined to be 100% within 72 hours based on excretion of radioactivity (85% in the urine) obtained after a single oral dose of 2 mg/kg b.w. Similar results were obtained in mice administered a single oral dose of 200 mg/kg b.w.

No data are available for inhalation exposure route. Then, the worst case inhalation absorption should be assumed (i.e. 100%).

No data are available for dermal exposure route. Then, a default value for dermal absorption of 100% should be applicable based on both the physico-chemical properties of the substance (MW= 137.14, log P_{ow} = 2.3) and the oral excretion data.

Pertinent data were not located on distribution. However, 2-nitrotoluene appears to be well distributed as indicated by toxicity in various organs of rats or mice orally exposed. In rats, toxicity was observed mainly in liver, kidney, spleen, testes and haematopoietic system. In addition, based on excretion data (a total recovery of radioactivity in urine and feces of rats and mice 24 or 72 hours after dosing) it is appropriate to state “no evidence of accumulation”.

No parent compound was detected in urinary samples of rodents. In rats, at least nine urinary metabolites were identified, four major: 2-nitrobenzoic acid, 2-nitrobenzyl glucuronide, 2-aminobenzyl alcohol and S-(2-nitrobenzyl)-N-acetylcysteine, and five minor: S-(2-nitrobenzyl)-glutathione, 2-nitrobenzyl sulfate, 2-nitrobenzyl alcohol, 2-aminobenzoic acid and *o*-toluidine. The metabolite profiles after single (using two dose levels) and repeated doses were similar; there was a sex-dependent variance in metabolite profile with females excreting significantly less 2-aminobenzyl alcohol and S-(2-nitrobenzyl)-N-acetylcysteine than males, but more 2-nitrobenzoic acid. In mice, only two major metabolites, 2-nitrobenzoic acid and 2-nitrobenzyl glucuronide, were identified in urine.

⁷ Conclusion (i) There is a need for further information and/or testing.
 Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.
 Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

The major metabolite excreted in bile of rats following 2-nitrotoluene administration was 2-nitrobenzyl glucuronide. Males excreted about 3 times as much of this metabolite as did females.

The metabolism of 2-nitrotoluene proceeds firstly by cytochrome-P450 mediated oxidation to nitrobenzyl alcohol which then undergoes metabolism by four pathways: a) oxidation to 2-nitrobenzoic acid; b) conjugation with glutathione to 2-nitrobenzylmercapturic acid; c) nitro-group reduction to 2-aminobenzoic acid; and d) conjugation with glucuronic acid to 2-nitrobenzyl glucuronide. This latter pathway appears as the responsible of 2-nitrotoluene bioactivation. The glucuronide metabolite secreted in the bile is believed to be converted to 2-aminobenzyl alcohol by hydrolytic and reductive activities of intestinal microflora, and then systemically reabsorbed. The final activation step is dependent upon sulfotransferase. Two enzyme-mediated pathways are involved. One of them requires *in vitro* PAPS and cytosolic enzymes and generates a compound (likely, 2-aminobenzyl sulfate) that binds covalently to DNA. The reactivity of 2-aminobenzyl sulfate with DNA could be related to the ease formation of a reactive benzyl cation due to electron-donating ability of the amino group. The other pathway requires *in vitro* hepatic microsomal enzymes and NADPH and results in an intermediate that binds covalently to protein. There is evidence that oxidation of 2-aminobenzyl to 2-(N-hydroxylamino) benzyl alcohol followed by sulfation yields an unstable N-sulphate which decomposes to an electrophilic nitrenium and/or carbonium ions. Since *o*-nitrotoluene, but not *m*- or *p*-nitrotoluene, induced DNA repair in the *in vivo* UDS assay in male rats, the *syn* conformation of DNA adducts appears to be a determinant factor in the genotoxic response.

The routes of excretion were similar in rats and mice, with the predominant route being via urine. By 72 hours after a single oral dose of 2 mg/kg b.w., the percentages of the radioactivity recovered in the urine were 100% (rats) and 85% (mice), and fecal excretion accounted for 4-5% (rats), and 23% (male mice). Minimal amounts of radiolabel (0.1%) were captured in expired air. The rate of excretion was more rapid in rats, with about 100% of the radioactivity excreted in urine in the first 24 hours. Less than 70% of the administered radioactivity was excreted in urine by mice in the same time period.

For male and female rats similarly treated, biliary excretion measured after 12 h was greater for males (29%) than for females (10%). Since proportionately less label is excreted via the feces it is presumed that the label is reabsorbed from the gut. In addition, cannulation of the bile duct inhibits covalent binding in the liver indicating the involvement of the enterohepatic circulation.

The only numerical value of any reliability for risk characterization obtained from acute toxicity studies in animals is the LD₅₀. Accordingly, 2-nitrotoluene is of low toxicity, being classified as Xn R22. The reported effects in humans exposed by different routes are most likely due to methaemoglobin formation but data on acute toxicity are limited to inhalation exposure; however, we have disregarded them because a NOAEC/LOAEC could not be determined as starting point for risk characterization for acute toxicity because the exposure duration was not reported. Therefore, the risk assessment for acute toxicity has been derived from animal data. Starting points for the risk assessment are the 8-hour inhalation value of 1.086 mg/L (1086 mg/m³) in rats and the dermal value of 2000 mg/kg in both rabbits and rats, considered as the NOAEC and NOAEL for inhalation and dermal exposure, respectively.

Based on results derived from good quality animal studies it can be said that 2-nitrotoluene is not either irritant or corrosive for the skin and eyes. In relation to the respiratory tract, the acute inhalation toxicity studies in rodents have not revealed any signs of irritation. However,

an olfactory degeneration was observed in both subchronic toxicity and carcinogenicity dietary studies in mice, and did not occur in rats. This effect was not seen with the other isomers, which have the same volatility. Accordingly, the olfactory degeneration is considered a mouse specific systemic effect. Therefore, the classification for respiratory tract irritation is not justified. In addition, data of dietary studies are not considered appropriate to assess quantitatively the respiratory irritation/corrosion risk.

There are no data on skin or respiratory sensitisation to 2-nitrotoluene in animals or in humans. However, it is considered significant the absence of positive reports on such effects in humans.

There were not available data in humans and neither in experimental animals following inhalatory or dermal exposure but several studies have been investigated the toxicity of 2-nitrotoluene following repeated oral administration to rats and mice. The LOAEL for chronic toxicity was considered to be 625 ppm in rats (25 and 30 mg/kg b.w. in males and females, respectively) based on lesions observed in liver, bone marrow, spleen and lung for both sexes and in mammary gland and mandibular lymph node only for females.

Most of the *in vitro* genotoxicity tests carried out with 2-nitrotoluene were negative. However, 2-nitrotoluene was an *in vivo* genotoxic agent for somatic cells. Positive results were found in the UDS test for both male and female rats. Males were more sensitive to genotoxicity of 2-nitrotoluene. A sex difference in biliary excretion may explain the sex difference in the genotoxicity of 2-nitrotoluene. In addition, 2NT did not induce DNA repair in germ-free animals, whereas DNA repair was induced in Charles River Altered Schaedler Flora-associated animals. Male and female F344 rats were shown to have similar populations of intestinal bacteria; however at the doses used, females were resistant to the genotoxic action of 2NT. These results indicate the obligatory role of intestinal bacteria in the metabolic activation of 2NT, showing that the genotoxic potential of 2-NT is dependent upon the sex of the animal under study. On the other hand, gene mutations in *ras*, *p53* and *β-catenin* genes were observed in hemangiosarcomas from B6C3F₁ mice exposed to 2-nitrotoluene in feed for 2 years, but not in spontaneous hemangiosarcomas. These *in vivo* data suggest 2-nitrotoluene is metabolized to mutagenic intermediates and that could be the reason why most of the *in vitro* genotoxicity tests were negative. 2-nitrotoluene has a number of potentially active metabolites that could account for the mutation profile observed in these tumours, as described in the proposal pathway for bioactivation (See 4.1.2.1.4). In conclusion, 2-nitrotoluene is mutagenic in somatic cells, and it reaches the germ cells since toxicity was observed in testis and epididymis of rats (to see section 4.1.2.6). According to the TGD (2005) criteria the classification of 2-NT as mutagenic category 2 (T, R46) is justified. Therefore, we support the current classification (29th ATP).

There is a clear evidence of an increase in tumour incidence at multiple sites in both rats and mice. There is also evidence that time to onset is very short. These observations are consistent with genotoxic aetiology, which is consistent with the findings from the genotoxicity studies. Therefore, according to EU criteria, 2-nitrotoluene is considered carcinogenic category 2 and then classified as T R45.

There are no data on toxicity for reproduction in humans. The only animal data are derived from non-standard reproduction studies.

Damage in testes and epididymis of rats indicates a need for fertility classification but because they occur at toxic dose levels, while clear-cut effects on fertility seem absent, indeed only

toxic for reproduction category 3 (Xn, R 62) is justified according to EU criteria. The NOAEL for impair fertility is considered to be 2500 ppm (179 mg/kg bw).

With respect to the development, only the retardation on pup growth observed in one CD rat study could be considered an indication of toxicity. However, because of the absence of further details on its severity, this effect cannot be used for classification. In addition, it cannot be ruled out that some toxicity is due to the transfer of the substance through the milk. This effect was not observed in another Wistar rat study: Differences on results between studies could be due to differences on sensitivity between strains. Therefore, based on available data and the EU criteria, the classification for developmental toxicity is not justified. However, assuming the worst case, a quantitative risk assessment for developmental toxicity is considered appropriate using the LOAEL of 50 mg/kg derived from the CD rat study.

4.1.3.2 Workers

The most probable route of human exposure to 2-nitrotoluene is inhalation and dermal contact of workers involved in the production and use of this substance.

The following assumptions have been made:

- The body weight of the average worker is 70 kg and the worker breathes 10 m³ of air during an 8-hours working day.
- In the absence of quantitative data on the bioavailability of 2-nitrotoluene, 100% absorption by inhalation and dermal routes has been assumed.

The exposure estimations used in the workers risk characterisation are summarised in section 4.1.1.2 (Table 4.1.1.2.1-3).

Comparison of exposure and effects

When considering the risks to human health arising from occupational exposure to 2-nitrotoluene, the key areas of concerns are for mutagenicity and carcinogenicity.

Acute Toxicity

Inhalation exposure

Using the NOAEC of 1086 mg/m³ derived from an acute inhalatory toxicity study in rats as a starting point, the standard respiratory volume of 6.7 m³/person and the worker respiratory volume of 10 m³/person, a corresponding human NAEC of 727.62 mg/m³ was derived. The calculated MOS, the ratio between the human NAEC (727.62 mg/m³) and the estimated short-term inhalation exposure (0.6 mg/m³), is 1212.7. The minimal MOS is considered to be 12.5 based on the following assessment factors: 2.5 for interspecies differences and 5 for intraspecies differences. Comparing minimal MOS with the calculated MOS indicates no concern for inhalation exposure and **conclusion (ii)** is reached.

Dermal exposure

Using the NOAEL of 2000 mg/kg b.w. derived from acute dermal toxicity studies in both rats and rabbits as a starting point and a human body weight of 70 kg, a corresponding human NAEL of 140000 mg/person/day was derived. The calculated MOS, the ratio between the

human NAEL (140000 mg/person/day) and the RWC dermal exposure (420 mg/person/day), is 333.33. The minimal MOS is considered to be 50 based on the following assessment factors: 4 x 2.5 for interspecies differences and 5 for intraspecies differences. Comparing minimal MOS with the calculated MOS indicates no concern for dermal exposure and **conclusion (ii)** is reached.

Irritation/Corrosivity

2-nitrotoluene is considered not irritant to skin, eye or the respiratory tract and **conclusion (ii)** is reached.

Sensitization

No data is available concerning skin and respiratory sensitization. The absence of positive reports on such effects in humans is considered to provide some reassurance that 2NT may at least not be a potent sensitiser. Still, there is a data gap for the end-point skin sensitization, and conclusion (i) should apply. However, considering that 2NT is a non-threshold, genotoxic carcinogen, and strict risk reduction measures are already required, it is proposed to put the conclusion (i) “on hold”. Thus, **conclusion (i) “on hold”** is proposed for skin sensitization, assuming that the knowledge that the substance be a skin sensitiser would not lead to stricter control measures than need to be applied for a genotoxic carcinogen.

Repeated dose toxicity

The LOAEL of 25 mg/kg b.w. derived from the chronic oral carcinogenicity study in rats is used as a starting point for the risk assessment for repeated dose toxicity.

The minimal MOS is considered to be 150 based on the following assessment factors: 4 x 2.5 for interspecies differences, 5 for intraspecies differences and 3 for dose-response curve (LOAEL instead of NOAEL).

Inhalation Exposure

Using the oral LOAEL of 25 mg/kg b.w. in rats as a starting point, a worker respiratory volume of 10 m³/8 hs, a human body weight of 70 kg, and oral and inhalation absorption values of both 100%, a corresponding human inhalatory LAEC of 175 mg/m³ was derived. This human LAEC is compared with the exposure information (table 4.1.3.2-1). The calculated MOS is 583. Comparing minimal MOS with the calculated MOS indicates no concern for inhalation exposure and **conclusion (ii)** is reached.

Table 4.1.3.2-1: Risk characterisation for repeated dose toxicity (inhalation)

Exposure scenario	Duration and frequency	Inhalation			
		Shift average value mg/m ³	LAEC mg/m ³	MOS	Conclusion
Production and further processing	Full shift/daily	0.3 ⁽¹⁾	175	583	(ii)

⁽¹⁾RWC

Dermal Exposure

Using the oral LOAEL of 25 mg/kg b.w. in rats as a starting point, a human body weight of 70 kg, and oral and dermal absorption values of both 100%, a corresponding human dermal LAEL of 1750 mg/person/day was derived. This human LAEL is compared with the exposure information (table 4.1.3.2-2). The calculated MOS is 4.2. Comparing minimal MOS with the calculated MOS indicates concern for dermal exposure and **conclusion (iii)** is reached.

Table 4.1.3.2-2: Risk characterisation for repeated dose toxicity (dermal exposure)

Exposure scenario	Duration and frequency	Dermal contact				
		Exposure range mg/cm ² /d	RWC mg/p/d	LAEL mg/p/d	MOS	Conclusion.
Production and further processing	Daily/Intermittent	0.1 – 1	420	1750	4.2	(iii)

Combined exposure

Systemic health effects due to combined exposure have to be assessed in addition to route specific risk assessment.

Combined exposure is calculated by the formula:

$$\text{MOS} = \frac{[\text{LOAEL}_{\text{oral-rat}} * \text{ABS}_{\text{oral-rat}}]}{[\text{Expo}_{\text{inh-human}} * (\text{RV}_{\text{human/bw human}}) * \text{ABS}_{\text{inh-human}}] + [\text{Expo}_{\text{derm-human}} * \text{ABS}_{\text{derm-human}}] + [\text{Expo}_{\text{oral-human}} * \text{ABS}_{\text{oral-human}}]}$$

The calculated MOS_{comb} is 4.2. Comparing minimal MOS (150) with the calculated MOS_{comb} (4.2) indicates concern for combined exposure because of dermal exposure and **conclusion (iii)** is reached.

Mutagenicity

2-nitrotoluene is mutagenic in somatic cells, and it reaches the germ cells since toxicity was observed in testis and epididymis of rats (to see section 4.1.2.6). According to the TGD (2005) criteria the current classification of 2-NT (29th ATP) as mutagenic category 2 (T, R46) is justified. Therefore, **conclusion (iii)** is reached.

Carcinogenicity

2NT is classified as carcinogenic. Carcinogenicity of 2NT was established in rodents, with a clear evidence of an increase in tumours incidence at multiple sites in both rats and mice. There was also evidence that time to onset was very short. These observations are consistent with a genotoxic mechanism of carcinogenicity supported by findings from the genotoxicity studies. Thus, a NOAEL cannot be established.

A quantitative risk characterization for carcinogenicity according to the T25 approach (EC, 2005) was performed for 2-NT in order to estimate the level of concern for the only exposure scenario of production and further processing. The dose-descriptor T25, the chronic dose rate that will give 25% of the animals' tumours at a specific tissue site after correction for spontaneous incidence, within the standard life time of that species, was calculated according to Expression 1. D_c was the exposure/observation-period-corrected dose of 2-NT (mg/kg bw/day) and i was cancer incidence after correction for spontaneous incidence.

$$T25 = D_c \cdot \frac{0.25}{i} \quad \text{Exp. 1}$$

D_c was calculated by using Expression 2 where w_1 and w_2 were the exposure and observation period (months), respectively.

$$D_c = D \cdot \frac{w_1}{24} \cdot \frac{w_2}{24} \quad \text{Exp. 2}$$

Incidence after correction for spontaneous incidence was computed with Expression 3.

$$i = \frac{\text{treatment incidence} - \text{control incidence}}{1 - \text{control incidence}} \quad \text{Exp. 3}$$

The animal dose descriptor (T25) was converted into a human dose descriptor (HT25) by using the Expression 4.

$$HT25 = \frac{T25}{\left(\frac{bw_{human}}{bw_{animal}}\right)^{0.25}} \quad \text{Exp. 4}$$

It was assumed a human body weight of 70 kg and the typical values for rodent lifetime studies of 500 and 350 g b.w. for male and female rats, respectively; and 30 and 25 g b.w. for male and female mice, respectively.

The data for calculating T25 were obtained from the life-time oral carcinogenicity studies performed in rodents. In rats, 2-NT increased the incidences of malignant mesothelioma, subcutaneous skin neoplasms, mammary gland fibroadenoma and liver neoplasms in males and those of subcutaneous skin neoplasms and mammary gland fibroadeoma in females; the increased incidences of lung neoplasms in males and of hepatocellular adenoma in females were also considered to be exposure related. In male and female mice, 2-NT increased the incidences of hemangiosarcoma, carcinoma of the large intestine (cecum) and hepatocellular neoplasms (only females because males died early due to the development of hemangiosarcomas). Consequently, a dose descriptor value was determined for each tumour type (Table 4.1.3.2-3). Nevertheless, regarding carcinogenicity as a starting point for the quantitative risk characterization, the HT25 value must be derived from malignant tumours or from benign tumours that are suspected of possibly progressing to malignant tumours.

Table 4.1.3.2-3: HT25 obtained from T25 calculated for each rodent tumour observed in each site

Species	Exposure/ observation (months)	Tumour	Incidence (control)	Incidence (2-NT)	Corrected incidence	Dose	T25	HT25
Male rats	24/24	<i>Mesothelium malignant</i> Mesothelioma	3.3%	33.3%	31%	25	20.1	5.9*
		<i>Skin (subcutaneous)</i> Lipoma	0%	21.7%	21.7%	50	57.7	17
		Fibroma	8.3%	76.7%	74.5%	25	8.4	2.5
		Fibrosarcoma	0%	28.3%	28.3%	50	44.1	13*
		Fibroma or fibrosarcoma	8.3%	78.3%	76.4%	25	8.2	2.4*
		<i>Mammary gland</i> Fibroadenoma	0%	16.7%	16.7%	50	75	22.1
		<i>Liver</i> Adenoma	3.3%	11.7%	8.6%	90	261	76.8
		Adenoma or carcinoma	5%	13.3%	8.8%	90	256.5	75.4
		Hepatocolangio- carcinoma	0%	1.7%	1.7%	25	375	110.3*
		<i>Lung</i> Alveolar/bronquial adenoma	1.7%	8.3%	6.8%	25	92.2	27.1
		Alveolar/bronquial adenoma or carcinoma	3.3%	8.3%	5.2%	25	120.8	35.5
Female Rats	24/24	<i>Skin (subcutaneous)</i> Fibroma	5.1%	30%	26.3%	60	57.1	15.04
		Fibroma or fibrosarcoma	5%	35%	31.6%	60	47.5	12.5
		<i>Mammary gland</i> Fibroadenoma	38.3%	78.3%	64.9%	30	11.6	3.04
		<i>Liver</i> Hepatocellular adenoma	1.7%	10%	8.5%	100	295	77.6
Male mice	24/24	<i>Circulatory system</i> Hemangiosarcoma	6.7%	28.3%	23.2%	165	177.7	25.4*
		<i>Large intestine</i> Carcinoma	0%	24.5%	24.5%	165	168.4	24.1*
Female mice	24/24	<i>Circulatory system</i> Hemangiosarcoma	0%	83%	83%	710	213	29.2*
		<i>Large intestine</i> Carcinoma	0%	6.7%	6.7%	320	120	164.4*
		<i>Liver</i> Adenoma	11.7%	32.2%	23.2%	320	344.1	47.2
		Carcinoma	3.3%	26.7%	24.1%	710	735.4	100.7*
		Adenoma or carcinoma	15%	40.7%	30.2%	320	264.8	36.3

(*) HT25 values relevant for carcinogenicity risk characterisation; HT25 of 2.4 mg/kg b.w./d, derived from skin fibromas plus fibrosarcomas in male rats, was used for carcinogenicity risk characterization

Therefore, the lowest HT25 value of 2.4 mg/kg b.w./d, derived from the T25 value of 8.2 mg/kg b.w./d calculated for the incidence of skin fibromas plus fibrosarcomas in male rats, was considered the critical value to be used for carcinogenicity risk characterization. The progression from benign to malignant tumours is clear because approximately one-third of the animals with fibroma (incidence 78%) also had fibrosarcoma (incidence 28%).

2-NT can be considered a carcinogen of medium potency (1 mg/kg b.w./day < T25 < 100 mg/kg b.w./day) based on both the T25 value (8.2 mg/kg b.w./day) and the starting assumptions, i.e. a linear dose-response relationship, genotoxic activity, mechanistic relevance to humans and toxicokinetic behaviour similar in animals and humans. However, according to site/species/strain/gender activity modifying element, the potency of 2-NT could be higher than the potency inferred from T25, since benign and malignant tumours occurred at different sites in both sexes of rats and mice.

The life-time cancer risk for workers (wLR) is obtained by Expression 5 where *wE* was worker exposure estimation (mg/kg b.w./day). For workers the exposure time is 8 hours per day, 5 days per week, 48 weeks per year for 40 years, i.e. a correction factor of 2.8 (Exp. 5).

$$wLR = \frac{\frac{wE}{2.8}}{\frac{HT25}{0.25}} \quad \text{Exp. 5}$$

The wLR calculated values are shown in Table 4.1.3.2-4.

Table 4.1.3.2-4: Life-time cancer risk for workers

Scenario	Route of exposure	Exposure ⁽¹⁾	Exposure ⁽²⁾	wLR ⁽³⁾
Production and further processing	Inhalation	0.3	0.042857143	0.0016
	Dermal	420	6	0.2232

⁽¹⁾ RWC; inhalation, mg/m³; dermal, mg/day

⁽²⁾ RWC; inhalation and dermal, mg/kg bw/day

⁽³⁾ wLR, life-time cancer risk for workers calculated by Exp. 5 ($wLR = (wE/2.8)/(HT25/0.25)$); HT25 value of 2.4 mg/kg bw/day.

By default, a life-time cancer risk for workers of less than 10⁻⁵ is considered a tolerable risk according to the Draft TGD (2005). However, since 2-NT is effective in multiple tissue sites and across species and genders, the risk may be higher than that based on the calculation for one specific tumour type. Anyway, for the exposure scenario life-time cancer risk for workers is clearly greater than the established default cancer risk value.

In summary, according to the quantitative risk characterization by concerns for carcinogenicity, **conclusion (iii)** is reached for the scenario of production and further processing.

Toxicity for reproduction

Fertility

Because of the lack of other relevant data, the NOAEL of 179 mg/kg b.w. derived from a 13-week rat oral toxicity study is used as a starting point for the risk assessment for impaired fertility.

The minimal MOS is considered to be 50 based on the following assessment factors: 4 x 2.5 for interspecies differences and 5 for intraspecies differences.

Inhalation Exposure

Using the oral NOAEL of 179 mg/kg b.w. in rats as a starting point, a worker respiratory volume of 10 m³/8 hs, a human body weight of 70 kg, and oral and inhalation absorption values of both 100%, a corresponding human inhalatory NAEC of 1253 mg/m³ was derived. This human NAEC is compared with the exposure information (Table 4.1.3.2-4). The calculated MOS is 4177. Comparing minimal MOS with the calculated MOS indicates no concern for inhalation exposure and **conclusion (ii)** is reached.

Table 4.1.3.2-5: Risk characterisation for impaired fertility (inhalation)

Exposure scenario	Duration and frequency	Inhalation			
		Shift average value mg/m ³	NAEC mg/m ³	MOS	Conclusion
Production and further processing	Full shift/daily	0.3 ⁽¹⁾	1253	4177	(ii)

⁽¹⁾RWC

Dermal Exposure

Using the oral NOAEL of 179 mg/kg b.w. in rats as a starting point, a human body weight of 70 kg, and oral and dermal absorption values of both 100%, a corresponding human dermal NAEL of 12530 mg/person/day was derived. This human NAEL is compared with the exposure information (table 4.1.3.2-5). The calculated MOS is 29.8. Comparing minimal MOS with the calculated MOS indicates concern for dermal exposure and **conclusion (iii)** is reached.

Table 4.1.3.2-6: Risk characterisation for impaired fertility (dermal exposure)

Exposure scenario	Duration and frequency	Dermal contact				
		Exposure range mg/cm ² /d	RWC mg/p/d	NAEL mg/p/d	MOS	Conclusion
Production and further processing	Daily/Intermittent	0.1 - 1	420	12530	29.8	(iii)

Combined exposure

Combined exposure is calculated by the formula:

$$\text{MOS} = \frac{[\text{NOAEL}_{\text{oral-rat}} * \text{ABS}_{\text{oral-rat}}]}{[\text{Expo}_{\text{inh-human}} * (\text{RV}_{\text{human}}/\text{bw}_{\text{human}}) * \text{ABS}_{\text{inh-human}}] + [\text{Expo}_{\text{derm-human}} * \text{ABS}_{\text{derm-human}}] + [\text{Expo}_{\text{oral-human}} * \text{ABS}_{\text{oral-human}}]}$$

The calculated MOS_{comb} is 29.8. Comparing minimal MOS (50) with the calculated MOS_{comb} (29.8) indicates concern for combined exposure because of dermal exposure and **conclusion (iii)** is reached.

Development

Because of the lack of other relevant data, the LOAEL of 50 mg/kg b.w. derived from a 10-week oral rat reproduction/developmental toxicity screening study is used as a starting point for the risk assessment for developmental toxicity.

The minimal MOS is considered to be 150 based on the following assessment factors: 4 x 2.5 for interspecies differences, 5 for intraspecies differences and 3 for dose-response curve (LOAEL instead of NOAEL).

Inhalation Exposure

Using the oral LOAEL of 50 mg/kg b.w. in rats as a starting point, a worker respiratory volume of 10 m³/8 hs, a human body weight of 70 kg, and oral and inhalation absorption values of both 100%, a corresponding human inhalatory LAEC of 350 mg/m³ was derived. This human LAEC is compared with the exposure information (Table 4.1.3.2-6). The calculated MOS is 1167. Comparing minimal MOS with the calculated MOS indicates no concern for inhalation exposure and **conclusion (ii)** is reached.

Table 4.1.3.2-7: Risk characterisation for developmental toxicity (inhalation)

Exposure scenario	Duration and frequency	Inhalation			
		Shift average value mg/m ³	LAEC mg/m ³	MOS	Conclusion
Production and further processing	Full shift/daily	0.3 ⁽¹⁾	350	1167	(ii)

⁽¹⁾RWC

Dermal Exposure

Using the oral LOAEL of 50 mg/kg b.w. in rats as a starting point, a human body weight of 70 kg, and oral and dermal absorption values of both 100%, a corresponding human dermal NAEL of 3500 mg/person/day was derived. This human LAEL is compared with the exposure information (table 4.1.3.2-7). The calculated MOS is 8.3. Comparing minimal MOS with the calculated MOS indicates concern for dermal exposure and **conclusion (iii)** is reached.

Table 4.1.3.2-8: Risk characterisation for developmental toxicity (dermal exposure)

Exposure scenario	Duration and frequency	Dermal contact				
		Exposure range mg/cm ² /d	RWC mg/p/d	LAEL mg/p/d	MOS	Conclusion
Production and further processing	Daily/Intermittent	0.1 - 1	420	3500	8.3	(iii)

Combined exposure

Combined exposure is calculated by the formula:

$$\text{MOS} = \frac{[\text{LOAEL}_{\text{oral-rat}} * \text{ABS}_{\text{oral-rat}}]}{[\text{Expo}_{\text{inh-human}} * (\text{RV}_{\text{human}}/\text{bw}_{\text{human}}) * \text{ABS}_{\text{inh-human}}] + [\text{Expo}_{\text{derm-human}} * \text{ABS}_{\text{derm-human}}] + [\text{Expo}_{\text{oral-human}} * \text{ABS}_{\text{oral-human}}]}$$

The calculated MOS_{comb} is 8.3. Comparing minimal MOS (150) with the calculated MOS_{comb} (8.3) indicates concern for combined exposure because of dermal exposure and **conclusion (iii)** is reached.

Summary of risk characterization for workers

When considering the risks to human health arising from occupational exposure to 2-nitrotoluene, the key areas of concern are for mutagenicity and carcinogenicity.

Overall, the available data do not allow the identification of a threshold level of exposure below which there would be no risk for the development of these effects in humans. In view of this, there are potential health concerns at all exposure levels. According to the quantitative risk characterization by concerns for carcinogenicity, the exposure scenario life-time cancer risk for workers by both inhalation and dermal routes is clearly greater than the established default cancer risk value. The same quantitative risk characterization was applied for somatic and germ cell mutagenicity. Consequently, **conclusion (iii)** is reached for both carcinogenicity and mutagenicity as a consequence of inhalation and dermal exposure.

In relation to sensitization, **conclusion (i) “on hold”** is proposed for skin sensitization, assuming that the knowledge that the substance be a skin sensitiser would not lead to stricter control measures than need to be applied for a genotoxic carcinogen

In addition, regarding repeated dose toxicity and toxicity for reproduction (fertility and development), the calculated MOS are judged not to be enough for workers exposed by dermal route. Although high standards of control are assumed for these industry sectors representing best practice, there is no evidence that these standards are applied across EU industry. Thus, there is no evidence that the appropriate equipment is in place in work places and that it is used and maintained in the correct manner. Therefore, **conclusion (iii)** is reached for both repeated dose toxicity and toxicity for reproduction (fertility and development) as a consequence of dermal exposure.

On the other hand, there is no concern for the remaining end-points: acute toxicity by inhalation and dermal route; irritation/corrosivity to skin, eye or the respiratory tract; repeated dose toxicity by inhalation, and toxicity for reproduction (fertility and development) by inhalation. Therefore, **conclusion (ii)** applies.

4.1.3.3 Consumers

Exposure of the consumers is not assumed to exist. Therefore, the **conclusion (ii)** is reached.

4.1.3.4 Humans exposed via the environment

The human exposure estimations (EUSES) via the environment for both local (three sites) and regional scales from the only scenario (production and further processing) are summarised in section 4.1.1.4 (Table 4.1.1.4). The daily human doses through total intake are 2.01×10^{-4} mg/kg b.w./day for the worst case of local scale (site C) and 2.17×10^{-7} mg/kg b.w./day for regional scale, and both routes, oral and inhalatory, are applicable.

Comparison of exposure and effects

When considering the risks to human health arising from indirect exposure to 2-nitrotoluene via the environment the key areas of concerns are for mutagenicity and carcinogenicity.

Repeated dose toxicity

The LOAEL of 25 mg/kg b.w. derived from the chronic oral carcinogenicity study with rats is used as starting point for the risk assessment for repeated dose toxicity. Comparing this LOAEL with the estimated internal total human daily intake levels, the calculated MOSs for both local (the worst case) and regional scale are 124000 and 115×10^6 , respectively. The

minimal MOS is considered to be 300 based on the following assessment factors: 4 x 2.5 for interspecies differences, 10 for intraspecies differences and 3 for dose-response curve (LOAEL instead of NOAEL). Comparing minimal MOS with the calculated MOSs indicates no concern for repeated dose toxicity and **conclusion (ii)** is reached.

Mutagenicity

2-nitrotoluene is mutagenic in somatic cells, and it reaches the germ cells since toxicity was observed in testis and epididymis of rats (to see section 4.1.2.6). According to the TGD (2005) criteria the current classification of 2-NT (29th ATP) as mutagenic category 2 (T, R46) is justified. Therefore, **conclusion (iii)** is reached.

Carcinogenicity

2NT is classified as carcinogenic. Carcinogenicity of 2NT was established in rodents, with a clear evidence of an increase in tumours incidence at multiple sites in both rats and mice. There was also evidence that time to onset was very short. These observations are consistent with a genotoxic mechanism of carcinogenicity supported by findings from the genotoxicity studies. Thus, a NOAEL cannot be established.

A quantitative risk characterization for carcinogenicity according to the T25 approach (Draft TGD 2005) was performed for 2-NT in order to estimate the level of concern for the different scenarios described in Section 4.1.1.4. The HT25 value of 2.4 mg/kg b.w./day derived from a T25 value of 8.2 mg/kg b.w./day was selected as the human descriptor dose for carcinogenicity risk characterization on the basis of skin fibromas plus fibrosarcomas observed at low-dose in male rats of the carcinogenicity study.

The lifetime cancer risks for humans exposed via the environment (eLR) are obtained directly from HT25 and scenario-specific exposures (eE) by the formula $eLR = eE/(HT25/0.25)$. The eLR calculated values are shown in Table 4.1.3.4.

Table 4.1.3.4: Life-time cancer risk for humans exposed via environment

Scenario	Exposure⁽¹⁾	eLR⁽²⁾
Local site A	5.63E-06	5.86E-07
Local site B	1.84E-05	1.92E-06
Local site C	2.01E-04	2.09E-05
Regional	2.17E-07	2.26E-08

⁽¹⁾ Exposure (mg/kg bw/day)

⁽²⁾ eLR, life-time cancer risk for humans exposed via environment calculated by $eLR = eE/(HT25/0.25)$; HT25 value of 2.4 mg/kg bw/day

By default, a life-time cancer risk for humans exposed via the environment of less than 10^{-5} is considered a tolerable risk according to the Draft TGD (2005). However, since 2-NT is effective in multiple tissue sites and across species and genders, the risk may be higher than that based on the calculation for one specific tumour type. Anyway, for the local site C life-time cancer risk for humans exposed via the environment is clearly greater than the established default cancer risk value.

Overall, by concerns for carcinogenicity, **conclusion (iii)** is reached for local site C scenario

Toxicity for reproduction

Fertility

Because of the lack of other relevant data, the NOAEL of 179 mg/kg b.w. derived from a 13-week rat oral toxicity study is used as a starting point for the risk assessment for impaired fertility. Comparing this NOAEL with the estimated internal total human daily intake levels, the calculated MOSs for both local (the worst case) and regional scale are 890547 and 825×10^6 , respectively. The minimal MOS is considered to be 100 based on the following assessment factors: 4×2.5 for interspecies differences and 10 for intraspecies differences. Comparing minimal MOS with the calculated MOSs indicates no concern for fertility and **conclusion (ii)** is reached.

Development

Because of the lack of other relevant data, the LOAEL of 50 mg/kg b.w. derived from a 10-week oral rat reproduction/developmental toxicity screening study is used as a starting point for the risk assessment for developmental toxicity. Comparing this LOAEL with the estimated internal total human daily intake levels, the calculated MOSs for both local (the worst case) and regional scale are 248756 and 230×10^6 , respectively. The minimal MOS is considered to be 300 based on the following assessment factors: 4×2.5 for interspecies differences, 10 for intraspecies differences and 3 for dose-response curve (LOAEL instead of NOAEL). Comparing minimal MOS with the calculated MOSs indicates no concern for developmental effects and **conclusion (ii)** is reached.

Summary of risk characterization for humans exposed via environment

When considering the risks to human health arising from indirect exposure to 2-nitrotoluene via environment the key areas of concern are for mutagenicity and carcinogenicity.

Overall, the available data do not allow the identification of a threshold level of exposure below which there would be no risk for the development of these effects in humans. In view of this, there are potential health concerns at all exposure levels. According to the quantitative risk characterization for carcinogenicity, the local site C is considered the only scenario of concern; the remaining sites are of very low concern. The same quantitative risk characterization was applied for somatic and germ cell mutagenicity. Therefore, **conclusion (iii)** is reached for both carcinogenicity and mutagenicity as a consequence of inhalation and oral exposure arising from the local site C.

The calculated MOS for total exposure (oral and inhalation routes) of man via the environment in both local and regional scales are judged to be enough regarding repeated dose toxicity and toxicity for reproduction (fertility and development) and **conclusion (ii)** is reached.

Combined exposure

Exposure to 2-nitrotoluene may reasonably be predicted to arise as a result of combined exposure from workplace and environmental sources. The risk to human health under conditions of combined exposure is dominated by occupational exposure.

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

4.2.1 Exposure assessment

4.2.1.1 Workers

2-nitrotoluene is not volatile, not flammable, and thermal and hazardous decompositions are not expected when handled correctly.

4.2.1.2 Consumers

2-nitrotoluene has not been detected in consumer products.

4.2.1.3 Humans exposed via the environment

Not applicable

4.2.2 Effects assessment: Hazard identification

4.2.2.1 Explosivity

The explosion limits are 1.47% in volume as lower limit and 8.8% in volume as upper limit.

4.2.2.2 Flammability

2-nitrotoluene is not a flammable liquid (flash point: 95°C). It does not ignite readily.

4.2.2.3 Oxidizing potential

2-nitrotoluene is not an oxidising agent.

4.2.3 Risk characterisation

There is not risk of concern in the industry setting, regarding its physico-chemical properties. Adequate safety measures are taken and information is provided on the label and safety data sheet. Therefore, since risk reduction measures already being applied are considered sufficient, **conclusion (ii)** is reached.

5 RESULTS ⁸

5.1 INTRODUCTION

5.2 ENVIRONMENT

Environment

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

Conclusion (ii) applies to the aquatic compartment (marine and freshwater), including sediments.

Conclusion (ii) applies to the terrestrial.

Conclusion (ii) applies to the atmospheric compartment.

And conclusion (ii) is also applied to secondary poisoning according to the low bioaccumulation potential and the rapid elimination of this compound in fish and mammals, no secondary poisoning potential is expected from this substance.

5.3 HUMAN HEALTH

5.3.1 Human health (toxicity)

5.3.1.1 Workers

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

This conclusion is reached because of:

- concerns for carcinogenicity and mutagenicity as a consequence of inhalation and dermal exposure.
- concerns for repeated dose toxicity and toxicity for reproduction (fertility and development) as a consequence of dermal exposure.

Conclusion (i) “on hold” There is need for further information and/or testing

⁸ Conclusion (i) There is a need for further information and/or testing.
Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.
Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

This conclusion is proposed for skin sensitization, assuming that the knowledge that the substance be a skin sensitizer would not lead to stricter control measures than need to be applied for a genotoxic carcinogen

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

This conclusion is reached for acute toxicity by inhalation and dermal routes; irritation/corrosivity to skin, eye or the respiratory tract; repeated dose toxicity by inhalation; and toxicity for reproduction (fertility and development) by inhalation, because these endpoints are of no concern.

5.3.1.2 Consumers

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

This conclusion is reached because exposure of consumers is not assumed to exist.

5.3.1.3 Humans exposed via the environment

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

This conclusion is reached because of:

- concerns for carcinogenicity and mutagenicity as a consequence of inhalation and oral exposure arising from the local site C.

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

This conclusion is reached for repeated dose toxicity and toxicity for reproduction (fertility and development) because the calculated MOS for total exposure (oral and inhalation routes) of man via the environment in both local and regional scales are judged to be enough for these endpoints.

5.3.1.4 Combined exposure

The risk to human health under conditions of combined exposure is dominated by occupational exposure.

5.3.2 Human health (risks from physico-chemical properties)

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

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

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ABBREVIATIONS

ADI	Acceptable Daily Intake
AF	Assessment Factor
ASTM	American Society for Testing and Materials
ATP	Adaptation to Technical Progress
AUC	Area Under The Curve
B	Bioaccumulation
BBA	Biologische Bundesanstalt für Land- und Forstwirtschaft
BCF	Bioconcentration Factor
BMC	Benchmark Concentration
BMD	Benchmark Dose
BMF	Biomagnification Factor
bw	body weight / <i>Bw</i> , <i>b.w.</i>
C	Corrosive (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
CA	Chromosome Aberration
CA	Competent Authority
CAS	Chemical Abstract Services
CEC	Commission of the European Communities
CEN	European Standards Organisation / European Committee for Normalisation
CMR	Carcinogenic, Mutagenic and toxic to Reproduction
CNS	Central Nervous System
COD	Chemical Oxygen Demand
CSTEE	Scientific Committee for Toxicity, Ecotoxicity and the Environment (DG SANCO)
CT ₅₀	Clearance Time, elimination or depuration expressed as half-life
d.wt	dry weight / <i>dw</i>
DCNP	2,6-dichloro-4-nitrophenol
dfi	daily food intake
DG	Directorate General
DIN	Deutsche Industrie Norm (German norm)
DNA	DeoxyriboNucleic Acid
DOC	Dissolved Organic Carbon
DT50	Degradation half-life or period required for 50 percent dissipation / degradation
DT90	Period required for 50 percent dissipation / degradation
E	Explosive (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
EASE	Estimation and Assessment of Substance Exposure Physico-chemical properties [Model]

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

Kow	octanol/water partition coefficient
Kp	solids-water partition coefficient
L(E)C50	median Lethal (Effect) Concentration
LAEL	Lowest Adverse Effect Level
LC50	median Lethal Concentration
LD50	median Lethal Dose
LEV	Local Exhaust Ventilation
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
LOED	Lowest Observed Effect Dose
LOEL	Lowest Observed Effect Level
MAC	Maximum Allowable Concentration
MATC	Maximum Acceptable Toxic Concentration
MC	Main Category
MITI	Ministry of International Trade and Industry, Japan
MOE	Margin of Exposure
MOS	Margin of Safety
MW	Molecular Weight
N	Dangerous for the environment (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
NAEL	No Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NOEC	No Observed Effect Concentration
NTP	National Toxicology Program (USA)
O	Oxidizing (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational Exposure Limit
OJ	Official Journal
OSPAR	Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic
P	Persistent
PAPS	3'-phosphoadenosine 5'-phosphosulfate
PBT	Persistent, Bioaccumulative and Toxic
PBPK	Physiologically Based Pharmacokinetic modelling
PBTK	Physiologically Based Toxicokinetic modelling

PEC	Predicted Environmental Concentration
pH	logarithm (to the base 10) (of the hydrogen ion concentration {H ⁺ })
pKa	logarithm (to the base 10) of the acid dissociation constant
pKb	logarithm (to the base 10) of the base dissociation constant
PNEC	Predicted No Effect Concentration
POP	Persistent Organic Pollutant
PPE	Personal Protective Equipment
QSAR	(Quantitative) Structure-Activity Relationship
R phrases	Risk phrases according to Annex III of Directive 67/548/EEC
RAR	Risk Assessment Report
RC	Risk Characterisation
RfC	Reference Concentration
RfD	Reference Dose
RNA	RiboNucleic Acid
RPE	Respiratory Protective Equipment
RWC	Reasonable Worst Case
S phrases	Safety phrases according to Annex III of Directive 67/548/EEC
SAR	Structure-Activity Relationships
SBR	Standardised birth ratio
SCE	Sister Chromatic Exchange
SDS	Safety Data Sheet
SETAC	Society of Environmental Toxicology And Chemistry
SNIF	Summary Notification Interchange Format (new substances)
SSD	Species Sensitivity Distribution
STP	Sewage Treatment Plant
T(+)	(Very) Toxic (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
TDI	Tolerable Daily Intake
TG	Test Guideline
TGD	Technical Guidance Document ¹
TNsG	Technical Notes for Guidance (for Biocides)
TNO	The Netherlands Organisation for Applied Scientific Research
UC	Use Category
UDS	Unscheduled DNA Synthesis
UN	United Nations
UNEP	United Nations Environment Programme
US EPA	Environmental Protection Agency, USA
UV	Ultraviolet Region of Spectrum

UVCB	Unknown or Variable composition, Complex reaction products of Biological material
vB	very Bioaccumulative
vP	very Persistent
vPvB	very Persistent and very Bioaccumulative
v/v	volume per volume ratio
w/w	weight per weight ratio
WHO	World Health Organization
WWTP	Waste Water Treatment Plant
Xn	Harmful (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
Xi	Irritant (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)

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The report provides the comprehensive risk assessment of the substance 2-nitrotoluene. It has been prepared by Spain in the frame of Council Regulation (EEC) No. 793/93 on the evaluation and control of the risks of existing substances, following the principles for assessment of the risks to man and the environment, laid down in Commission Regulation (EC) No. 1488/94.

Part I - Environment

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

The environmental risk assessment concludes that there is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Part II – Human Health

This part of the evaluation considers the emissions and the resulting exposure to human populations in all life cycle steps. The scenarios for occupational exposure, consumer exposure and humans exposed via the environment have been examined and the possible risks have been identified.

The human health risk assessment concludes that there is concern for workers with regard to mutagenicity and carcinogenicity as a consequence of inhalation and dermal exposure and with regard to repeated dose toxicity and toxicity for reproduction (fertility and development) as a consequence of dermal exposure. There is also concern for humans exposed via the environment with regard to carcinogenicity and mutagenicity as a consequence of inhalation and oral exposure arising from one and all local sites respectively. For consumers and for human health (physico-chemical properties) there is no concern.

The conclusions of this report will lead to risk reduction measures to be proposed by the Commission's committee on risk reduction strategies set up in support of Council Regulation (EEC) N. 793/93.