Screening Assessment for the Challenge

Benzene, 1-methyl-2-nitro-
(2-Nitrotoluene)

Chemical Abstracts Service Registry Number
88-72-2

Environment Canada
Health Canada

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Synopsis

Pursuant to section 74 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999), the Ministers of the Environment and of Health have conducted a screening assessment on benzene, 1-methyl-2-nitro- (2-nitrotoluene), Chemical Abstracts Service Registry Number 88-72-2. This substance was identified in the categorization of the *Domestic Substances List* (DSL) as a high priority for action under the Challenge. 2-Nitrotoluene was identified as presenting an intermediate potential for exposure of individuals in Canada and had been classified by other agencies on the basis of carcinogenicity and genotoxicity. The substance met the ecological categorization criteria for persistence, but did not meet the ecological categorization criteria for bioaccumulation potential or inherent toxicity to aquatic organisms. Although an ecological risk assessment has been prepared, the focus of this assessment of 2-nitrotoluene relates primarily to human health risks.

2-Nitrotoluene does not occur naturally in the environment. It is an organic substance that is found in Canada and elsewhere primarily as a chemical intermediate in a variety of industries. Under information reported pursuant to section 71 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999), the total quantity of 2-nitrotoluene imported into and used in Canada in 2006 was between 100 and 1000 kg, while no manufacturing was reported. The general population is not likely to be exposed to 2-nitrotoluene, since it is used only in industrial applications in Canada. The only reported Canadian use was in the explosives industry and the related products are not intended for the general public.

Exposures of the general population to 2-nitrotoluene through environmental media (air, drinking water and soil) are expected to be negligible. 2-Nitrotoluene is not expected to be found in food or beverages. Based upon the information obtained on current uses of 2-nitrotoluene in Canada, exposure of the general population is expected to be negligible.

As 2-nitrotoluene was classified on the basis of carcinogenicity by other national and international agencies, carcinogenicity was a key focus of this screening assessment. An increased incidence of tumours was reported in multiple tissues, such as the mesothelial tissues (tunica vaginalis of the testis, epididymis, abdominal wall or surface of abdominal organs), skin (subcutaneous tissues), mammary gland, liver and lungs, in rats exposed via the diet. Tumours in the circulatory system, large intestine and liver were reported in mice exposed via the dietary route as well. 2-Nitrotoluene was genotoxic in a range of *in vitro* and *in vivo* assays, was notably clastogenic in human peripheral lymphocytes and formed DNA adducts in exposed rodents. While the mode of induction of tumours has not been fully elucidated, based on the genotoxicity of 2-nitrotoluene, the tumours observed in the experimental animals are considered to have resulted from direct interaction with genetic material.

Exposure to 2-nitrotoluene has also been associated with non-cancer effects in experimental animals, including developmental and reproductive effects as well as effects

in the lungs, liver, spleen, bone marrow and the hematopoietic system. Margins of exposure were not calculated for non-cancer effects in this assessment since non-cancer effects occurred at a dose at which tumours were observed and because the information available indicates that exposures of the general Canadian population to 2-nitrotoluene from either environmental media or consumer products are expected to be negligible.

On the basis of the carcinogenic potential of 2-nitrotoluene, for which there may be a probability of harm at any exposure level, it is concluded that 2-nitrotoluene is a substance that may be entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

Based on available empirical data and modelling results, 2-nitrotoluene is expected to be persistent in air, water, soil and sediments but is not expected to bioaccumulate. The substance therefore meets the persistence criteria but not the bioaccumulation criteria as set out in the *Persistence and Bioaccumulation Regulations*. In addition, available empirical data suggest that the substance poses a moderate hazard to aquatic organisms. Based on a comparison of predicted no-effect concentrations with estimated realistic worst-case environmental exposure concentrations, it is considered unlikely that 2-nitrotoluene is causing ecological harm in Canada.

Based on the information available, it is concluded that 2-nitrotoluene is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

Based on the information available, it is concluded that 2-nitrotoluene meets one or more of the criteria set out in section 64 of CEPA 1999.

This substance will be considered for inclusion in the *Domestic Substances List* inventory update initiative. In addition and where relevant, research and monitoring will support verification of assumptions used during the screening assessment and, where appropriate, the performance of potential control measures identified during the risk management phase.

Introduction

The *Canadian Environmental Protection Act, 1999* (CEPA 1999) (Canada 1999) requires the Minister of the Environment and the Minister of Health to conduct screening assessments of substances that have met the categorization criteria set out in the Act to determine whether these substances present or may present a risk to the environment or to human health.

Based on the information obtained through the categorization process, the Ministers identified a number of substances as high priorities for action. These include substances that

- met all of the ecological categorization criteria, including persistence (P), bioaccumulation potential (B) and inherent toxicity to aquatic organisms (iT), and were believed to be in commerce in Canada; and/or
- met the categorization criteria for greatest potential for exposure (GPE) or presented an intermediate potential for exposure (IPE) and had been identified as posing a high hazard to human health based on classifications by other national or international agencies for carcinogenicity, genotoxicity, developmental toxicity or reproductive toxicity.

The Ministers therefore published a notice of intent in the *Canada Gazette*, Part I, on December 9, 2006 (Canada 2006), that challenged industry and other interested stakeholders to submit, within specified timelines, specific information that may be used to inform risk assessment, and to develop and benchmark best practices for the risk management and product stewardship of those substances identified as high priorities.

The substance 2-nitrotoluene was identified as a high priority for assessment of human health risk because it was considered to present IPE and had been classified by other agencies on the basis of carcinogenicity and genotoxicity. The Challenge for 2-nitrotoluene was published in the *Canada Gazette* on January 31, 2009 (Canada 2009). A substance profile was released at the same time. The substance profile presented the technical information available prior to December 2005 that formed the basis for categorization of this substance. As a result of the Challenge, submissions of information were received.

Although 2-nitrotoluene was determined to be a high priority for assessment with respect to human health and it also met the ecological categorization criteria for persistence, it did not meet the criteria for bioaccumulation potential or inherent toxicity to aquatic organisms. Therefore, this assessment focuses principally on information relevant to the evaluation of risks to human health.

Screening assessments focus on information critical to determining whether a substance meets the criteria for defining a chemical as toxic as set out in section 64 of CEPA 1999.

Screening assessments examine scientific information and develop conclusions by incorporating a weight of evidence approach and precaution.[1]

This final screening assessment includes consideration of information on chemical properties, hazards, uses and exposure, including the additional information submitted under the Challenge. Data relevant to the screening assessment of this substance were identified in original literature, review and assessment documents, stakeholder research reports and from recent literature searches, up to May 2009 for the exposure, human health effects and ecological sections of the document. Key studies were critically evaluated; modelling results may have been used to reach conclusions. Evaluation of risk to human health involves consideration of data relevant to estimation of exposure (non-occupational) of the general population, as well as information on health hazards (based principally on the weight of evidence assessments of other agencies that were used for prioritization of the substance). Decisions for human health are based on the nature of the critical effect and/or margins between conservative effect levels and estimates of exposure, taking into account confidence in the completeness of the identified databases on both exposure and effects, within a screening context. The final screening assessment does not represent an exhaustive or critical review of all available data. Rather, it presents a summary of the critical information upon which the conclusion is based.

This final screening assessment was prepared by staff in the Existing Substances Programs at Health Canada and Environment Canada and incorporates input from other programs within these departments. Both the ecological and human health portions of this assessment have undergone external written peer review/consultation. Comments on the technical portions relevant to human health were received from scientific experts selected and directed by Toxicology Excellence for Risk Assessment (TERA), including Michael Dourson (TERA), John Christopher (California Office of Environmental Health Hazard Assessment) and Michael Jayjock (The Lifeline Group).

Additionally, the draft of this screening assessment was subject to a 60-day public comment period. While external comments were taken into consideration, the final content and outcome of the screening assessment remain the responsibility of Health Canada and Environment Canada. Approaches used in the screening assessments under the Challenge have been reviewed by an independent Challenge Advisory Panel.

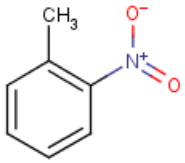
The critical information and considerations upon which the assessment is based are summarized below.

[1] A determination of whether one or more of the criteria of section 64 are met is based upon an assessment of potential risks to the environment and/or to human health associated with exposures in the general environment. For humans, this includes, but is not limited to, exposures from ambient and indoor air, drinking water, foodstuffs, and the use of consumer products A conclusion under CEPA 1999 on the substances in the Chemicals Management Plan (CMP) Challenge Batches 1-12 is not relevant to, nor does it preclude, an assessment against the hazard criteria specified in the *Controlled Products Regulations*, which is part of regulatory framework for the Workplace Hazardous Materials Information System [WHMIS] for products intended for workplace use.

Substance Identity

For the purposes of this document, this substance will be referred to as 2-nitrotoluene, a common name for this substance. Information on the identity of 2-nitrotoluene is summarized in Table 1.

Table 1. Substance identity

CAS RN	88-72-2
DSL name	Benzene, 1-methyl-2-nitro-
NCI names	Benzene, 1-methyl-2-nitro- (AICS, ASIA-PAC, DSL, ENCS, NZIoC, PICCS, SWISS, TSCA) 1-Methyl-2-nitrobenzene (ECL) 2-Nitrotoluene (EINECS) *o*-Nitrotoluene (PICCS) Toluene, 2-nitro- (PICCS)
Other names	Benzene, 1-methyl-2-nitro; 2-Methyl-1-nitrobenzene; 2-Methylnitrobenzene; 1,2-Methylnitrobenzol; 1-Methyl-2-nitrobenzol; 2-Methylnitrobenzol; alpha-Methylnitrobenzene; *o*-Methylnitrobenzene; *o*-Methylnitrobenzol; *o*-Mononitrotoluene; Mononitrotoluole; 2-Nitro-1-methyl-benzol; 2-Nitro-1-methylbenzol; *o*-Nitrotoluene; 2-Nitrotoluol; *o*-Nitrotoluol; *o*-Nitrotoluol D; NSC 9577; ONT; UN 1664; UN 1664 (DOT) [1]
Chemical group (DSL stream)	Discrete organics
Major chemical class or use	Benzene compounds
Major chemical subclass	Nitrobenzenes
Chemical formula	$C_7H_7NO_2$
Chemical structure	
SMILES	O=[N+]([O−])c1ccccc1C
Molecular mass	137.14 g/mol

Abbreviations: AICS, Australian Inventory of Chemical Substances; ASIA-PAC, Asia-Pacific Substances Lists; CAS RN, Chemical Abstracts Service Registry Number; DSL, Domestic Substances List; ECL, Korean Existing Chemicals List; EINECS, European Inventory of Existing Commercial Chemical Substances; ENCS, Japanese Existing and New Chemical Substances; NCI, National Chemical Inventories; NZIoC, New Zealand Inventory of Chemicals; PICCS, Philippine Inventory of Chemicals and Chemical Substances; SMILES, simplified molecular input line entry specification; SWISS, Swiss Giftliste 1 and Inventory of Notified New Substances; TSCA, *Toxic Substances Control Act* Chemical Substance Inventory.

[1]DOT = Department of Transportation

Source: NCI (2006)

Physical and Chemical Properties

Table 2 contains experimental and modelled physical and chemical properties of 2-nitrotoluene that are relevant to its environmental fate.

Table 2. Physical and chemical properties of 2-nitrotoluene

Property	Type	Value[1]	Temperature (°C)	Reference
Melting point (ºC)	Experimental[2]	−9.5[3], −2.9		Weast 1989
	Modelled	38.16		MPBPWIN 2008
Boiling point (ºC)	Experimental	222[3]		O'Neil 2001
	Modelled	225.86		MPBPWIN 2008
Density (kg/m^3)	Experimental	1162		O'Neil 2001
Vapour pressure (Pa)	Experimental	25[3] (0.188 mmHg)	20	Perry and Green 1984
	Modelled	15.7 (0.118 mmHg)	25	MPBPWIN 2008
Henry's Law constant (Pa·m^3/mol)	Experimental	1.27[3] (1.25×10^{-5} atm·m^3/mol)	25	Altschuh et al. 1999
	Modelled	2.83[3] (2.35×10^{-5} atm·m^3/mol) (bond method)	25	HENRYWIN 2008
		4.83 (4.77×10^{-5} atm·m^3/mol) (group method)	25	HENRYWIN 2008
Log K_{ow} (dimensionless)	Experimental	2.3[3]		Hansch et al. 1995
	Modelled	2.36		KOWWIN 2008
Log K_{oc} (dimensionless)	Modelled	2.50		KOCWIN 2009
Water solubility (mg/L)	Experimental	650	30	Yalkowsky and He 2003
	Modelled	380.7	25	WSKOWWIN 2008
pKa	modelled	Does not ionize in water		ACD 2005

Abbreviations: K_{oc}, organic carbon–water partition coefficient; K_{ow}, octanol–water partition coefficient.

[1] Values and units in parentheses represent those originally reported by the authors or estimated by the models.

[2] Alpha and beta crystallized forms, respectively.

[3] Value used for modelling.

Sources

2-Nitrotoluene is an anthropogenic substance and does not occur naturally in the environment (IARC 1996). It can be made by the nitration of toluene with mixed acids in either a batch or a continuous process (IARC 1996; Dugal 2005). The product of a typical batch process is a mixture containing 55–60% 2-nitrotoluene, 3–4% 3-nitrotoluene and 35–40% 4-nitrotoluene (Dugal 2005). 2-Nitrotoluene can also be formed as a breakdown product of dinitrotoluenes or trinitrotoluene (TNT) and may be released to the environment by facilities manufacturing these chemicals (NTP 2008). It has also been reported to be present in unfiltered cigarette smoke at a level of 21.4 ng/cigarette (Hoffmann and Rathkamp 1970). No other report of 2-nitrotoluene in cigarette smoke has been identified.

Based on information collected pursuant to a notice published under section 71 of CEPA 1999, 100–1000 kg of 2-nitrotoluene were imported into Canada in 2006. The substance was not reported to be manufactured in Canada (Environment Canada 2009a).

Previously received information from the Domestic Substances List nomination (1984–1986) showed that the total quantity of 2-nitrotoluene reported as imported into, manufactured in or in commerce in Canada during the calendar year 1986 was in the range from 10 million to 100 million kilograms (Environment Canada 1988). 2-Nitrotoluene production in and import into Canada have decreased significantly since the 1980s. Outside of Canada, 2-nitrotoluene has been identified as an Organisation for Economic Co-operation and Development (OECD) high production volume (HPV) chemical (OECD 1994) and as a US HPV chemical (US EPA 1986–2002). A total of 87 344 560 kg of 2-nitrotoluene was reported to be used in western Europe in 2000 (EURAR 2008). Recent trends have shown an overall decrease in use of 2-nitrotoluene in Europe (EURAR 2008).

Uses

According to submissions made under section 71 of CEPA 1999 and from the Challenge questionnaire submissions (Environment Canada 2009a), 100–1000 kg of 2-nitrotoluene were used in Canada in 2006. The majority of 2-nitrotoluene used in Canada is in the manufacturing of explosives (Environment Canada 2009a). 2-Nitrotoluene uses in Canada are expected to be limited to industrial applications.

The use of 2-nitrotoluene to make intermediates for the manufacture of explosives, such as dinitrotoluenes and TNT, has also been reported outside of Canada (OECD 1994; EURAR 2008). The production of TNT from 2-nitrotoluene has decreased considerably in western Europe in recent years, and it is now considered to be very infrequent (EURAR 2008).

From the literature, a number of other uses of 2-nitrotoluene have been reported outside of Canada. 2-Nitrotoluene has been used as an intermediate in the manufacture of agricultural and rubber chemicals, petrochemicals, colorants, pesticides and pharmaceuticals (OECD 1994).

Although 2-nitrotoluene can be used as an intermediate in the production of pesticides (IARC 1996; Dugal 2005), it has never been registered for use in pesticides in Canada (2009 email from Pest Management Regulatory Agency, Health Canada, to Existing Substances Risk Assessment Bureau, Health Canada; unreferenced).

In Canada, since 2-nitrotoluene is not listed in the Drug Products Database, the Natural Health Products Ingredients Database or the Licensed Natural Health Products Database, it is not expected that this substance would be present in pharmaceutical products, natural health products or veterinary drugs manufactured in Canada (2009 emails from Therapeutic Products Directorate, Natural Health Products Directorate and Veterinary Drugs Directorate, Health Canada, to Existing Substances Division, Health Canada; unreferenced). However, 2-nitrotoluene may be present in trace amounts in pharmaceutical products imported into Canada, as it has been reported to be used as a chemical intermediate in the manufacture of pharmaceutical products outside of Canada (OECD 1994).

Historical (1984–1986) Canadian uses identified for 2-nitrotoluene include use as a site-limited substance, chemical intermediate (organic), organic chemicals (specialty); as a fuel or fuel additive, explosive materials; and in refined petroleum and coal products (Environment Canada 1988). 2-Nitrotoluene use in Canada has decreased significantly since the 1980s.

Releases to the Environment

The low importation volumes of 2-nitrotoluene into Canada, along with information on its uses, indicate a low potential for release into the Canadian environment. In addition, release from consumer products is not expected, given the lack of evidence that 2-nitrotoluene is present in consumer products.

Based on information collected through a survey conducted pursuant to section 71 of CEPA 1999, one Canadian facility involved in explosives production reported that releases of 2-nitrotoluene to air were not detected. In addition, this facility reported no releases to water or soil (Environment Canada 2009a). This facility uses a treatment system to minimize releases and limit environmental exposure (Environment Canada 2009a). 2-Nitrotoluene has been reported to be used in a non-dispersive manner, in closed systems (ECB 2000).

2-Nitrotoluene is not reportable to the National Pollutant Release Inventory (NPRI 2007) or to the US Toxics Release Inventory Program (TRI 2006). Therefore, no release information is available from these sources.

Environmental Fate

Based on its physical and chemical properties (Table 2), the results of Level III fugacity modelling (Table 3) suggest that 2-nitrotoluene will reside predominantly in air, water or soil, depending on the compartment of release. The results from the model pKadB (ACD 2005) indicates that the substance is not expected to ionize in water.

Table 3. Results of Level III fugacity modelling for 2-nitrotoluene (EQC 2003)

Substance released to:	Fraction of substance partitioning to each medium (%)			
	Air	Water	Soil	Sediment
Air (100%)	77	15	8	0.08
Water (100%)	3	96	0.4	0.6
Soil (100%)	1	5.0	94.0	0.0

Persistence and Bioaccumulation Potential

Environmental Persistence

The reaction of 2-nitrotoluene with hydroxyl radicals has been measured experimentally, resulting in a transformation half-life of 23 days (Meylan and Howard 1993). With the ultraviolet absorption of 2-nitrotoluene in the visible light range (>295 nm), there is a possibility of direct photolysis under tropospheric conditions (BUA 1989). Nojima and Kanno (1977) found that 79% of 2-nitrotoluene was degraded in air when exposed to light (>300 nm) over a 5-hour exposure period. The photoreaction of 2-nitrotoluene in air resulted in the products 2-methyl-6-nitrophenol and 2-methyl-4-nitrophenol.

Table 4a presents empirical biodegradation data (MITI 1992) that show 0.5% biodegradation of 2-nitrotoluene over 14 days in a ready biodegradation test. This test suggests that the half-life in water is likely to be longer than 182 days (6 months) and that the substance is therefore likely to persist in that environmental compartment.

Table 4a. Empirical data for degradation of 2-nitrotoluene

Medium	Fate process	Degradation value	Degradation endpoint (units)	Reference
Water	Biodegradation	0.50	Biodegradation (%, in 14 days)	MITI 1992
Water	Biodegradation	18	Biodegradation in sterile microcosm (%, in 36 days)	Toze and Zappia 1999

		58	Biodegradation in non-sterile microcosm (%, in 36 days)	
Water	Biodegradation	>>4	Non-adapted inocula (half-life; weeks)	Canton et al. 1985
		1–2	Adapted inoculum (halflife; weeks)	
Sludge	Biodegradation	2	Adapted organisms, complete biodegradation (weeks)	Struijs and Stoltenkamp 1986
Sludge	Biodegradation	42.8 82.7	BOD_{10d} (%) BOD_{20d} (%)	BUA 1989

BOD_x, x-day biological oxygen demand

In a microcosm study to determine the ability of microorganisms to degrade compounds such as 2-nitrotoluene, Toze and Zappia (1999) reported an 18% decrease in concentration of the substance in a sterile microcosm compared with a 58% decrease in concentration in a non-sterile microcosm over a 36-day incubation period. The substance was not completely removed from both microcosms by day 35 and had reached a stable concentration by day 20 in both the sterile and non-sterile microcosms, after which little loss was observed. It was suggested that a repressive secondary metabolite may have reached inhibitory concentrations or that depletion of an essential nutrient had occurred.

Some biodegradation test results indicate that 2-nitrotoluene undergoes biodegradation after a period of acclimation. Canton et al. (1985) carried out a biodegradability study of 2-nitrotoluene following the approach developed by Pitter (1976), with both non-adapted and adapted inocula, resulting in a half-life much greater than 4 weeks and between 1 and 2 weeks, respectively. The results indicated that the test compound cannot be biodegraded in this type of test without adaptation of the inoculum.

Struijs and Stoltenkamp (1986) carried out a variation of the test method described by Pitter (1976) using a mixture of domestic sewage treatment plant (STP) and river mud material. The sludge was exposed to an increasing amount of the substance over a 21-day period; after 2 weeks, the biodegradation was almost complete.

Other test results from BUA (1989) show that 2-nitrotoluene is biodegradable with an adapted bacterial mixed culture taken from the sludge of an experimental purification plant for industrial and communal sewage. They showed a 10- and 20-day biological oxygen demand (BOD_{10d} and BOD_{20d}) of 42.8% and 82.7%, respectively, based on a theoretical chemical oxygen demand of 1635.04 mg/g nitrotoluene.

Robertson et al. (1992) demonstrated that toluene-grown cells of *Pseudomonas* sp. strain JS150 and *P. putida* F1 transformed 2-nitrotoluene into 2-nitrobenzyl alcohol. *Pseudomonas* sp. JS42 was shown to grow on 2-nitrotoluene as the sole source of carbon, nitrogen and energy, with nitrite release (Haigler et al. 1994; Parales et al. 1996, 1998).

There is also experimental evidence that (abiotic) photodegradation of 2-nitrotoluene can occur rapidly (half-life <<1 day) in natural waters in the presence of humic substances (Simmons and Zepp 1986). In the risk assessment report of the European Union (EURAR 2008), an average water column photolysis half-life of approximately 24 days was estimated, taking into account the fact that sunlight penetrates only the top few metres of surface waters. As for the air compartment, the products are likely to be 2-methyl-6-nitrophenol and 2-methyl-4-nitrophenol.

To augment available experimental data on the degradation of 2-nitrotoluene, a quantitative structure–activity relationship (QSAR)-based weight of evidence approach (Environment Canada 2007) was also applied using the degradation models shown in Table 4b. Given the ecological importance of the water compartment, the fact that most of the available models apply to water and the fact that 2-nitrotoluene is expected to be released to this compartment, biodegradation primarily in water was examined. 2-Nitrotoluene does not contain functional groups expected to undergo hydrolysis. Table 4b summarizes the results of available QSAR models for degradation in various environmental media.

Table 4b. Modelled data for degradation of 2-nitrotoluene

Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (days)
Air			
Atmospheric oxidation	AOPWIN 2000	$t_{½}$ = 13.85 days	>2
Ozone reaction	AOPWIN 2000	n/a[1]	n/a
Water			
Hydrolysis	HYDROWIN 2000	n/a[1]	n/a
Biodegradation (aerobic)	BIOWIN 2000 Submodel 3: Expert Survey (ultimate biodegradation)	2.65[2] "biodegrades fast"	<182
Biodegradation (aerobic)	BIOWIN 2000 Submodel 4: Expert Survey (primary biodegradation)	3.47[2] "biodegrades fast"	<182
Biodegradation (aerobic)	BIOWIN 2000 Submodel 5: MITI linear probability	0.19[3] "biodegrades slowly"	≥182
Biodegradation (aerobic)	BIOWIN 2000 Submodel 6: MITI non-linear probability	0.04[3] "biodegrades very slowly"	≥182
Biodegradation (aerobic)	TOPKAT 2004 Probability	0.021[3] "biodegrades very slowly"	≥182
Biodegradation (aerobic)	CATABOL ©2004–2008 % BOD	% BOD = 0 "biodegrades very slowly"	≥182

BOD, biological oxygen demand; MITI, Ministry of International Trade & Industry, Japan; n/a, not applicable; $t_{½}$, half-life.
[1]Model does not provide an estimate for this type of structure.
[2]Output is a numerical score from 0 to 5.
[3]Output is a probability score.

In air, a predicted atmospheric oxidation half-life value of 13.85 days (Table 4b) and an experimental value of 23 days (Meylan and Howard 1993) demonstrate that this substance is likely to be slowly oxidized. The substance is not expected to react with other photo-oxidative species in the atmosphere, such as ozone. However, it has been shown to degrade by photolytic reactions in sunlight (half-life <5 hours). With a photolysis half-life of less than 5 hours, 2-nitrotoluene is considered not persistent in air.

Due to the lack of reactive functional groups, the hydrolysis of 2-nitrotoluene is unlikely (half-life >50 years) (Rippen 1989).

The results of BIOWIN (2000) submodels 5 and 6 (MITI linear and non-linear probability) indicate that biodegradation is slow and that the half-life in water would be ≥182 days. The results of BIOWIN (2000) submodels 3 and 4 (ultimate and primary degradation) suggest that the substance has a half-life of <182 days. The predictions of a half-life of ≥182 days for CATABOL (©2004–2008) and TOPKAT (2004) are in all the domains of both models and are thus considered to be reliable; they suggest a very slow rate of biodegradation. Therefore, considering that BIOWIN (2000) submodels 5 and 6, CATABOL (©2004–2008) and TOPKAT (2004) all indicate that 2-nitrotoluene is persistent, the majority of model evidence suggests that the biodegradation half-life of 2-nitrotoluene is ≥182 days in water. The presence of the *N*-nitroso structure in the substance provides additional evidence, as these structural features are associated with chemicals that are persistent.

The results of laboratory biodegradation tests show that 2-nitrotoluene is able to undergo biodegradation only after a period of acclimation; that is, 2-nitrotoluene cannot be biodegraded without prolonged exposure to the substance and the adaptation of the inoculum. Because prolonged exposure of microbial populations is unlikely under the variable conditions in ambient surface waters (Environment Canada 2008), the empirical data suggest that biodegradation is likely to be slow (half-life ≥182 days) under environmental conditions.

Although there is evidence of rapid photolytic degradation in water, the methylated nitrophenol degradation products are expected to be not ready biodegradable and exhibit a moderate potential for toxicity to aquatic organisms - like 2-nitrotoluene itself (see for example, OECD 1994). Consequently when evaluating the persistence of 2-nitrotoluene, greatest weight has been placed on the evidence for slow biodegradation of the substance itself.

Using an extrapolation ratio of 1:1:4 for water:soil:sediment biodegradation half-lives (Boethling et al. 1995), the half-life in soil is also ≥182 days and the half-life in sediments is ≥365 days. This indicates that 2-nitrotoluene is expected to be persistent in soil and sediment.

The Transport and Persistence Level III Model (TaPL3) (TaPL3 2000) was used to estimate the Characteristic Travel Distance (CTD) defined as the maximum distance

traveled in air by 63% of the substance. Beyer et al. (2000) have proposed CTD's of >2000 km as representing high long-range atmospheric transport potential (LRATP), 700-2000 km as moderate LRATP, and <700 km as low LRATP. Based on the CTD estimate of 3198 km, the long-range atmospheric transport potential of 2-nitrotoluene is considered to be high. This means that based on this model, 2-nitrotoluene is subject to atmospheric transport to remote regions such as the Arctic.

The OECD POPs Screening Model can also be used to help identify chemicals with high persistence and long-range transport potential (Scheringer et al. 2006). The OECD model is a global model that compartmentalizes the earth into air, water and soil. This model is "transport-oriented" rather than "target-oriented" as it simply identifies the CTD without indicating specifically where a substance may be transported to (Fenner et al. 2005). Klasmeier et al. (2006) have suggested that a threshold of 5098 km, based on the model's CTD estimate for PCB-180, can be used to identify substances with high long-range-transport potential. PCB-180 is empirically known to be found in remote regions. The CTD calculated for 2-nitrotoluene using the OECD model is 2279 km indicating that 2-nitrotoluene still has a significant potential for transport in air, but this is below the boundary suggested for global pollutants by Klasmeier et al. (2006). The OECD POPs Screening Model also calculates the transfer efficiency (TE), which is the percentage of emission flux to air that is deposited to the surface (water and soil) in a remote region (TE % = D/E x 100, where E is the emission flux to air and D = the deposition flux to surface media in a target region). The TE for 2-nitrotoluene was calculated to be 0.00106 %, which is above the boundary of 0.000465% (PCB-28) established based on the model's reference substances empirically known to be deposited from air to soil or water. The low TE means that although 2-nitrotoluene has the potential for long-range travel in the atmosphere it is unlikely to be deposited to Earth's surface in any remote region.

In addition, the log K_{oa} (5.3) and log K_{aw} (-3.29) values for 2-nitrotoluene also suggest that it will have a low Arctic contamination potential (ACP) when examined using chemical partitioning space plots as described by Wania (2003, 2006).

Based on the empirical and modelled data (see Tables 4a and 4b), 2-nitrotoluene meets the persistence criteria in air, water, soil and sediment (half-life in air ≥2 days, half-lives in soil and water ≥182 days and half-life in sediment ≥365 days), as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Potential for Bioaccumulation

Experimental and modelled log K_{ow} values for 2-nitrotoluene (see Table 2 above) suggest that this chemical has low potential to bioaccumulate in biota.

Table 5a presents the empirical bioconcentration factor (BCF) values in fish.The BCF of 2-nitrotoluene has been experimentally determined to be between 4.4 and 29.9 (Table 5a).

Table 5a. Empirical data for bioaccumulation of 2-nitrotoluene

Test organism	Endpoint	Value (L/kg wet weight)	Reference
Carp (*Cyprinus carpio*)	BCF	6.6–29.9	MITI 1992
Guppy (*Poecilia reticulata*)	BCF	19	Deneer et al. 1987
		20	Canton et al 1985
Goldfish (*Carassius auratus*)	BCF	4.4	Wang et al. 1999

Although a number of experimental BCF data for 2-nitrotoluene were available, a predictive approach was also applied using available bioaccumulation factor (BAF) and BCF models, as shown in Table 5b. The Arnot and Gobas kinetic modelled bioaccumulation values did not take into account the metabolic potential of the substance. According to the *Persistence and Bioaccumulation Regulations* (Canada 2000), a substance is bioaccumulative if its BCF or BAF is ≥5000; however, measures of BAF are the preferred metric for assessing bioaccumulation potential of substances. This is because BCF may not adequately account for the bioaccumulation potential of substances via the diet, which predominates for substances with log K_{ow} >~4.0 (Arnot and Gobas 2003).

Table 5b. Fish BAF and BCF predictions for 2-nitrotoluene.

Test organism	Endpoint	Value (L/kg wet weight)	Reference
Fish	BAF	13.3	Arnot and Gobas 2003 (Gobas BAF middle trophic level)
Fish	BCF	11.1	Arnot and Gobas 2003 (Gobas BCF middle trophic level)
Fish	BCF	81.7	OASIS Forecast 2005
Fish	BCF	15.3	BCFWIN 2000

The modelled bioaccumulation values do not need to take into account the metabolic potential for substances with a log K_{ow} of less than 4.5, as uptake is mainly via the gills, and thus metabolism via the gut is not important. The experimental log K_{ow} of 2-nitrotoluene is 2.3. Therefore, although metabolism was not considered for 2-nitrotoluene, this would not greatly influence the bioaccumulation conclusion.

The available empirical evidence indicates that 2-nitrotoluene is expected to have low bioaccumulation potential. The modified Gobas BAF middle trophic level model for fish predicted a BAF of 13.3 L/kg, indicating that 2-nitrotoluene does not have the potential to bioconcentrate in fish and to biomagnify in food webs. The results of BCF model calculations provide additional evidence supporting the low bioconcentration potential of this substance.

Based on the available empirical and kinetic-based modelled values, 2-nitrotoluene does not meet the bioaccumulation criteria (BAF or BCF ≥5000) as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Potential to Cause Ecological Harm

The approach taken in this assessment was to examine the available scientific information and develop conclusions based on a weight of evidence approach and using precaution as required under CEPA 1999. Lines of evidence considered include results from conservative risk quotient calculations as well as information on the persistence, bioaccumulation, toxicity, sources and fate of the substance.

As indicated previously, 2-nitrotoluene is expected to persist in water, soil and sediment but does not meet the bioaccumulation criteria as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000). The low importation volumes of 2-nitrotoluene into Canada, along with information on its uses, indicate a low potential for release into the Canadian environment. However, if released into the environment, it will be found mainly in water and air.

Experimental and modelled ecological effects data are summarized in Tables 6a and 6b, respectively. The toxicity data indicate that 2-nitrotoluene typically exhibits moderate hazard to aquatic organisms.

Table 6a. Empirical data for toxicity of 2-nitrotoluene in aquatic organisms

Test organism	Test type	Endpoint	Value (mg/L)	Reference
Guppy (*Poecilia reticulata*)	Acute (96 h)	LC_{50}	30.1	Ramos et al. 1998
	Acute (24 h)	LC_{50}	29	Canton et al. 1985
	Chronic (14 days)	LC_{50}	32.9	Deneer et al. 1987
Fathead minnow (*Pimephales promelas*)	Acute (96 h)	LC_{50}	37.1	Bailey and Spanggord 1983; Liu et al. 1983
Medaka (*Oryzias latipes*)	Acute (48–96 h)	LC_{50}	7.0	Canton et al. 1985
	Acute (48 h)	LC_{50}	86	Yoshioka et al. 1986
	Chronic (28 days)	LC_{50}	9.4	Canton et al. 1985
	Chronic (28 days)	NOEC (mortality and behaviour)	1.9	

Test organism	Test type	Endpoint	Value (mg/L)	Reference
Daphnia magna	Acute (48 h)	LC_{50}	5.4	Canton et al. 1985
	Acute (24 h)	EC_{50}	16	Bringmann and Kuhn 1977
	Semichronic (length of study not reported)	NOEC	0.5	Canton et al. 1985
	Chronic (21 days)	LOEC (length and growth)	9.9	Deneer et al. 1989
	Acute (48 h)	EC_{50}	10.9	
	Acute (24 h)	EC_{50}	13.2	Ramos et al. 1998
	Acute (48 h)	EC_{50}	12.3	Ramos et al. 1998
Alga (*Chlorella pyrenoidosa*)	Acute (96 h)	EC_{50}	47.5	Deneer et al. 1987
	Acute (72 h)	EC_{50}	22	Ramos et al. 1999
	Chronic (72 h)	NOEC	4.4	Ramos et al. 1999
	Chronic (72 h)	LOEC	8.7	
Microorganism	Acute (24 h)	EC_{50} (inhibition of cell multiplication)	100	Bringmann and Kuhn 1977
Microorganism (*Tetrahymena pyriformis*)	Acute (40 h)	IGC_{50}	1.82	Schultz 1999

Abbreviations: EC_{50} (median effective concentration), the concentration of a substance that is estimated to cause some toxic sublethal effect on 50% of the test organisms; IGC_{50} (median impairment growth concentration), the concentration of a substance that is estimated to inhibit the growth of 50% of the test cells; LC_{50} (median lethal concentration), the concentration of a substance that is estimated to be lethal to 50% of the test organisms; LOEC (lowest-observed-effect concentration), the lowest concentration in a toxicity test that caused a statistically significant effect in comparison with the controls; NOEC (no-observed-effect concentration), the highest concentration in a toxicity test not causing a statistically significant effect in comparison with the controls.

Table 6b. Modelled data for toxicity of 2-nitrotoluene in aquatic organisms

Test organism	Type of test	Endpoint	Value (mg/L)	Reference
Fish	Acute (96 h)	LC_{50}	6.6	TOPKAT 2004
			51.2	ECOSAR 2004
			18.8	AIES 2003–2005
			53.3	ASTER 1999
Fish	Chronic (14 days)	LC_{50}	52.04	ECOSAR 2004
Mysid shrimp	Acute (96 h)	LC_{50}	39.5	ECOSAR 2004
Daphnia	Acute (48 h)	EC_{50}	30.4	ECOSAR 2004

Test organism	Type of test	Endpoint	Value (mg/L)	Reference
			27.3	TOPKAT 2004
Daphnia	Chronic (16 days)	EC_{50}	3.7	ECOSAR 2004
Alga	Acute (96 h)	EC_{50}	17	ECOSAR 2004
Microorganism	Acute	IGC_{50}	1.95–3.45	Castillo-Garit et al. 2008
Microorganism	Acute (15 min)	EC_{50}	6.16–10.0	Duchowicz et al. 2008

Abbreviations: EC_{50} (median effective concentration), the concentration of a substance that is estimated to cause some toxic sublethal effect on 50% of the test organisms; IGC_{50} (median impairment growth concentration), the concentration of a substance that is estimated to inhibit the growth of 50% of the test cells; LC_{50} (median lethal concentration), the concentration of a substance that is estimated to be lethal to 50% of the test organisms.

No suitable ecological effects studies were found for 2-nitrotoluene in media other than in water and sediment.

No data concerning concentrations of this substance in Canada have been identified; therefore, a modeling approach was applied to estimate environmental concentrations.

As 2-nitrotoluene is used industrially and is expected to be released to water, a worst-case industrial release scenario is used to estimate the aquatic concentration of the substance with the help of Environment Canada's (2009b) Industrial Generic Exposure Tool – Aquatic (IGETA). The scenario is made conservative by assuming that the total quantity of the substance used by Canadian industry is used by a single industrial facility at a small, hypothetical site and that the loss to sewers is high, at 5% of the total quantity resulting from the cleaning of chemical containers and process equipment. The scenario also assumes that the release occurs 250 days/year, typical for small and medium-sized facilities, and is sent to a local STP with a zero removal rate for the substance. Upon combining with the sewage treatment plant (STP) effluent, the receiving water at such a small site normally has an actual or equivalent flow of 0.4 m^3/second. Based on the above assumptions, industrial use of a total quantity of 1000 kg/year of the substance yields an aquatic concentration of 0.006 mg/L (Environment Canada 2009b). Details regarding the inputs used to estimate this concentration and the output of the model are described in Environment Canada (2009b).

A conservative predicted no-effect concentration (PNEC) was also derived from the lowest acute toxicity value identified from the measured values for fish, invertebrates, algae and microorganisms in Table 6a: a 40-hour LC_{50} for the microorganism (*Tetrahymena pyriformis*) of 1.82 mg/L. This value was selected as the critical toxicity value and divided by an assessment factor of 100 (to account for uncertainties related to extrapolation from acute to chronic values and from a laboratory EC_{50} to a no-effect value in the field). This yielded a PNEC of 0.018 mg/L, which is at least 10-fold lower than reported empirical chronic effect values.

The resulting conservative risk quotient (PEC/PNEC) (0.006/0.018) of 0.32 indicates that that exposures are unlikely to be high enough to cause harm to aquatic organisms. Since the most environmentally significant releases of this substance are likely to water and results of fugacity modelling indicate that most of the substance discharged to water will remain in that compartment, significant exposure of organisms at other types of locations or in media other than water are unlikely.

2-Nitrotoluene is thus unlikely to be causing ecological harm in Canada.

Uncertainties in Evaluation of Ecological Risk

It should be noted that the risk quotient conclusion was reached despite the conservative assumptions that were made in response to uncertainties encountered in the assessment. A key uncertainty relates to the lack of empirical data on environmental concentrations in Canada, which was addressed by predicting a realistic worst-case concentration in water using an industrial exposure model.

There are uncertainties associated with the use of QSAR models to estimate persistence and bioaccumulation. However, 2-nitrotoluene has a relatively simple structure, which reduces the uncertainty in predictions. Because of these uncertainties, greater reliance was placed on available empirical data when concluding about persistence and bioaccumulation potential.

Interpretation of the empirical data for degradation in water was not straightforward. Since the hazard posed by photolysis degradation products is expected to be similar to that of 2-nitrotoluene itself and since a prolonged period of acclimation is required before significant biodegradation is likely to occur, it was concluded that 2-nitrotoluene is persistent in water as well as in soil and sediment.

Also regarding ecotoxicity, based on the predicted partitioning behaviour of this chemical, the significance of soil and sediment as important media of exposure is not well addressed by the effects data available.

Potential to Cause Harm to Human Health in Canada

Exposure Assessment

Environmental Media and Food

Any potential exposure in Canada is expected to be limited to industrial applications (Environment Canada 2009a). In the published literature, there were no empirical data identified regarding measured concentrations of 2-nitrotoluene in environmental media (air, water, soil and sediment) in Canada. In response to the section 71 survey, one company indicated that 2-nitrotoluene was not detected in releases to air from their

facility (Environment Canada 2009a). In addition, this facility reported no releases to water or soil (Environment Canada 2009a).

Outside of Canada, 2-nitrotoluene has been detected mainly in the vicinity of chemical manufacturing plants where it is used or manufactured. It has historically been detected in ambient air (Pellizzari 1978; Japan Ministry of Environment 2004), drinking water (Zoeteman 1980), surface waters (Meijers and van Der Leer 1976; Zoeteman et al. 1980; Van De Meent et al. 1986; Feltes et al. 1990; Mussmann et al. 1994; Götz et al. 1998; Japan Ministry of Environment 2004), groundwater (Duguet et al. 1988; Mussmann et al. 1994; Hilmi et al. 1999; Best et al. 2001), soil (Hilmi et al. 1999), sediments (Japan Ministry of Environment 2004) and industrial waters (Howard et al. 1976; Spanggord et al. 1982a; Swaminathan et al. 1987; Kozawa et al. 1992; Mussmann et al. 1994; OECD 1994; Stangroom et al. 1998). Monitoring data indicate that the general population may be exposed to 2-nitrotoluene via inhalation of ambient air and ingestion of drinking water in the vicinity of production sites (HSDB 2009).

These monitoring studies have limited relevance because they are older, not based in Canada and primarily based on point sources. ChemCAN, a Canadian-specific environmental exposure model, was used to predict concentrations in environmental media based on the amount of the substance used in Canada (ChemCAN 2003). Contributions to total intake from air, drinking water and soil were negligible. In addition, the use of 2-nitrotoluene has decreased in recent years in Canada and Europe (Environment Canada 1988, 2009a; EURAR 2008).

No data were identified for 2-nitrotoluene in food and beverages from Canada or elsewhere. 2-Nitrotoluene is not expected to be found in food and beverages. It is not present in formulations for food packaging materials (2009 email from Foods Directorate, Health Canada, to Existing Substances Division, Health Canada; unreferenced).

The primary use of 2-nitrotoluene in Canada is in the explosives manufacturing industry. Given the use patterns and the lack of relevant monitoring data available, intake estimates for environmental media were not determined, but are expected to be negligible.

Confidence in the exposure characterization for environmental media is considered to be moderate to high. There is uncertainty due to limited information available with respect to the concentrations of 2-nitrotoluene in Canadian environmental media; however, based on the volumes of use and the associated use pattern, exposure to 2-nitrotoluene from environmental media and food is not expected.

Consumer Products Exposure

The use of consumer products is not likely to be a source of exposure to 2-nitrotoluene. No submission reported under section 71 of CEPA 1999 indicated that 2-nitrotoluene would be present in consumer products in Canada (Environment Canada 2009a). In Europe, 2-nitrotoluene has not been detected in consumer products, and thus there is no anticipated consumer exposure for this compound (EURAR 2008). From a single source

in the literature, 2-nitrotoluene may be present as an unintended manufacturing residual in art materials, putty, glazing, wood preservatives and brush cleaners (SRD 2004). This information could not be confirmed from other sources.

Due to the limited potential of 2-nitrotoluene's presence in consumer products, exposure was not characterized from use of consumer products and it is not expected to be a source of general population exposure in Canada.

Health Effects Assessment

An overview of the toxicological database for 2-nitrotoluene is presented in Appendix 1.

On the basis of investigations in experimental animals, 2-nitrotoluene has been classified by the European Commission as a Category 2 carcinogen ("May cause cancer") (ESIS 2008; EURAR 2008). Under the new European Commission regulation (CLP-Regulation (EC) No 1272/2008) on classification, labelling and packaging (European Commission 2009), 2-nitrotoluene was classified as a Category 1B carcinogen ("May cause cancer"). The European Commission classifications (ESIS 2008; European Commission 2009) were based upon cancer bioassays published by the National Toxicology Program (NTP 2002). On the basis of the same cancer bioassays, the Report on Carcinogens expert panel listed 2-nitrotoluene as "reasonably anticipated to be a human carcinogen" (NTP 2007). The NTP concluded from their 2-year cancer bioassays that there was "clear evidence of carcinogenic activity" of 2-nitrotoluene in rats and mice of both sexes (NTP 2002, 2008). Prior to the publication of the 2-year cancer bioassays by the NTP (2002), the International Agency for Research on Cancer (IARC) classified nitrotoluenes (including 2-nitrotoluene) as Group 3 ("not classifiable as to their carcinogenicity in humans") on the basis of "inadequate evidence" for the carcinogenicity of nitrotoluenes in humans, "limited evidence" of carcinogenicity of 2-nitrotoluene in experimental animals and "inadequate evidence" of carcinogenicity of 3- and 4-nitrotoluenes in experimental animals (IARC 1996). The available studies for 2-nitrotoluene that have been considered in this assessment are summarized below and are presented in more detail in Appendix 1.

Tumours were observed in the lungs, mesothelial tissues, skin (subcutaneous tissues), mammary glands, liver, large intestine and the circulatory system in rodents exposed to 2-nitrotoluene via the oral (dietary) route. In addition, 2-nitrotoluene has been reported to be a weak skin tumour initiator in the mouse skin painting model. No inhalation carcinogenicity bioassays have been identified.

The NTP conducted a 2-year cancer bioassay in rodents (NTP 2002) after 13- or 26-week exposure studies (NTP 1992, 1996) confirmed carcinogenic effects of 2-nitrotoluene in rats (NTP 2008). In the NTP (1992) 13-week study, male and female F344/N rats were administered 2-nitrotoluene at 0, 625, 1250, 2500, 5000 or 10 000 mg/kg (0, 56, 98, 178, 383 or 696 mg/kg body weight [kg-bw] per day in males; 0, 55, 102, 190, 382 or 779 mg/kg-bw per day in females) by diets *ad libitum* (EURAR 2008; NTP 2008). There were no exposure-related effects on survival. An increased incidence of mesothelioma and mesothelial cell hyperplasia of the tunica vaginalis of the testis was reported in male

rats in the 178 and 696 mg/kg-bw per day groups, respectively. Since mesotheliomas had not been previously identified in exposed or control rats from any of the subchronic bioassays conducted by the NTP, the investigators concluded that 2-nitrotoluene was carcinogenic in male rats (NTP 2008). In the subsequent study (NTP 1996), male F344/N rats were administered 2-nitrotoluene at 0 or 5000 mg/kg (0 or 292–296 mg/kg-bw per day) in the diet for 13 or 26 weeks (EURAR 2008; NTP 2008). The 13-week exposure (stop exposure) group was given the control diet (after 13 weeks) until necropsy at 26 weeks. There were no exposure-related effects on survival. No tumours were reported after 13 weeks of exposure. After 26 weeks of exposure, significant increases in the incidence of mesothelioma of the tunica vaginalis of the testis and epididymis were reported in both exposure groups. An increased incidence of cholangiocarcinoma was also reported in the exposed rats. The investigators concluded that these studies confirmed the carcinogenicity of 2-nitrotoluene based on the high incidence of mesothelioma and the occurrence of cholangiocarcinoma in male rats after short-term exposure (NTP 2008).

In the subsequent 2-year cancer bioassay (NTP 2002), male and female F344/N rats were administered 2-nitrotoluene at 0, 625, 1250 or 2000 mg/kg (0, 25, 50 or 90 mg/kg-bw per day in males; 0, 30, 60 or 100 mg/kg-bw per day in females) in the diet for 105 weeks (EURAR 2008; NTP 2008). Groups of male F344/N rats in a parallel stop exposure study were administered 2-nitrotoluene at 0, 2000 or 5000 mg/kg diet (0, 125 or 315 mg/kg-bw per day) for 13 weeks followed by control feed for the remainder of the 2 years of the study. Poor survival was reported for exposed male rats in both the stop exposure study and the core chronic exposure study. Survival was also reduced in the high-dose female rats. The reduced survival rates were attributed to the early development of tumours (Dunnick et al. 2003; NTP 2008). Similar tumour profiles were reported for the stop exposure groups of male rats and the chronically exposed animals. Significant increases in the incidences of malignant mesotheliomas, subcutaneous skin lipoma, subcutaneous fibroma, subcutaneous skin fibrosarcoma and subcutaneous fibroma or fibrosarcoma combined were reported in all male rat exposure groups. The incidence of mammary gland fibroadenomas was significantly increased in male rats except in the high dose group in the chronic exposure study. Significant increases in the incidence of hepatocellular adenoma and hepatocellular adenoma or carcinoma in the high-dose males and increases in the incidence of hepatocholangiocarcinoma, alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma in the high-dose males in the stop exposure group were also reported. In female rats, significant increases in the incidence of subcutaneous skin fibroma and fibrosarcoma were reported in the two highest exposure groups. The incidences of mammary gland fibroadenomas and hepatocellular adenoma in the high-dose female rats were also significantly increased. The NTP (2002) concluded that there was clear evidence of carcinogenic activity of 2-nitrotoluene in male and female rats, based on increased incidences of malignant mesothelioma (in males only), subcutaneous skin tumours, mammary gland fibroadenoma and liver tumours (in males only). Increased incidences of lung tumours in male rats and hepatocellular adenoma in female rats were also considered to be exposure related (NTP 2002, 2008).

Male and female B6C3F1 mice were administered 2-nitrotoluene at 0, 1250, 2000 or 5000 mg/kg diet (0, 165, 360 or 700 mg/kg-bw per day in males; 0, 150, 320 or 710 mg/kg-bw per day in females) for 105 weeks (NTP 2002). Poor survival was reported for the exposed male mice (EURAR 2008; NTP 2008). Survival was also reduced in the high-dose female mice. The reduced survival rates were attributed to the early development of tumours (Dunnick et al. 2003; NTP 2008). Significant increases in the incidences of hemangiosarcoma in all exposure groups and cecum carcinoma in the low and middle exposure groups were reported in male mice. The incidence of hemangiosarcomas was also significantly increased in high-dose female mice. While the increase in the incidence of hepatocellular carcinomas was significant only in the high-dose female mice, significant increases in the incidence of hepatocellular adenomas and hepatocellular adenomas or carcinomas combined were reported for both the middle and high exposure groups. Furthermore, exposure-related increases in the incidence of cecum carcinomas were also reported in the female mice. The NTP (2002) concluded that there was "clear evidence of carcinogenic activity" of 2-nitrotoluene in male and female mice based on the increases in the incidence of cecum carcinomas in male mice, liver tumours in female mice and hemangiosarcomas in all exposed animals (NTP 2008).

Slaga et al. (1985) examined the carcinogenic potential of 2-nitrotoluene in SENCAR mice exposed via dermal application. In the initiation–promotion study, animals were exposed to a single application of 2-nitrotoluene at 24, 120 or 240 mg (1200, 6000 or 12 000 mg/kg-bw) followed by single applications of the promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) at 4 µg every week for 30 weeks. The incidence of skin papillomas and carcinomas in mice in the highest dose group was slightly but not significantly increased (DFG 2002).

2-Nitrotoluene has been demonstrated to be genotoxic in multiple *in vitro* and *in vivo* assays. 2-Nitrotoluene has been classified by the European Commission as a Mutagen Category 2 ("May cause heritable genetic damage") (ESIS 2008; EURAR 2008). Under CLP-Regulation (EC) No 1272/2008, 2-nitrotoluene was reclassified as a Category 1B mutagen ("May cause heritable genetic damage") (European Commission 2009). After the review of available genotoxicity studies, the European Commission concluded that 2-nitrotoluene is a somatic cell mutagen with the potential to cause mutations in germ cells (EURAR 2008).

2-Nitrotoluene was clastogenic *in vitro* in both human and other mammalian cell lines (Ishidate et al. 1988; Huang et al. 1996; Matsushima et al. 1999). It also caused deoxyribonucleic acid (DNA) damage and repair in mammalian cell lines (Parton et al. 1995; Lee et al. 2007). In addition, 2-nitrotoluene induced sister chromatid exchange in Chinese hamster ovary cells (Galloway et al. 1987). In prokaryotic systems, 2-nitrotoluene consistently tested negative in a range of assays for mutagenicity (Chiu et al. 1978; Miyata et al. 1981; Tokiwa et al. 1981; Spanggord et al. 1982b; Haworth et al. 1983; Suzuki et al. 1983; Shimizu and Yano 1986; Lee et al. 2007). In *in vivo* investigations, 2-nitrotoluene was not clastogenic in rodents (NTP 2002), but induced chromosomal aberrations in the ovaries of the mosquito *Culex fatigans* (Sharma et al. 1989). In the same study, 2-nitrotoluene did not cause dominant lethal mutations in *Culex*

fatigans (Sharma et al. 1989). Exposure to 2-nitrotoluene has also induced unscheduled DNA synthesis and DNA repair in the hepatocytes of rats (Butterworth et al. 1982; Doolittle et al. 1983; NTP 1992). Furthermore, 2-nitrotoluene induced DNA adducts in rat liver and blood cells (Jones et al. 2003). Covalent binding of 2-nitrotoluene to hepatic DNA was also reported (Rickert et al. 1984). In humans, clastogenic effects were observed in workers occupationally exposed to nitrotoluenes (Sabbioni et al. 2006). In addition, mutations in proto-oncogenes *K-ras* and *β-catenin* as well as the tumour suppressor gene *p53* were found in 2-nitrotoluene-induced hemangiosarcomas and colon carcinomas in mice (NTP 2008). These gene mutations were considered to be induced by the mutagenic intermediates of 2-nitrotoluene *in vivo* and associated with changes in related protein levels in favour of tumour formation and growth (Hong et al. 2003; Sills et al. 2004). The genotoxicity of 2-nitrotoluene *in vivo* was postulated to be mediated by its active metabolites, which helped to explain the lack of mutagenicity of 2-nitrotoluene in bacterial mutagenicity assays (EURAR 2008; NTP 2008).

Information on the potential carcinogenicity of 2-nitrotoluene in humans is very limited. No epidemiological studies regarding the carcinogenicity of 2-nitrotoluene were identified in the literature; however, studies in which workers were co-exposed to 2-nitrotoluene and other chemicals are described in Appendix 1.

The fully elucidated mode of action for the induction of the various tumour types by 2-nitrotoluene has not been developed However, the NTP (2008) concluded that the gene mutations of *p53*, *β-catenin* and *K-ras* observed in hemangiosarcomas and colon carcinomas in mice exposed to 2-nitrotoluene are the result of the genotoxic effects of 2-nitrotoluene. Based on the information available, the tumors observed in the experimental animals are considered to have resulted from direct interaction with genetic material.

In experimental animals, exposure to 2-nitrotoluene has also been associated with a range of non-cancer effects, including developmental and reproductive effects as well as effects in the lungs, liver, spleen, bone marrow and the hematopoietic system.

Adverse effects in the lungs, liver, spleen, bone marrow and the hematopoietic system have been reported in rodents administered 2-nitrotoluene following chronic, subchronic and short-term oral exposures. The lowest reported lowest-observed-effect level (LOEL) for chronic exposures was 25 mg/kg-bw per day. At this dose, increased incidences of non-neoplastic lesions in the liver, bone marrow, spleen and lungs were reported in male and female rats exposed to 2-nitrotoluene in the diet for 105 weeks (NTP 2002). Increases in the incidence of non-neoplastic lesions in the mammary gland and mandibular lymph node were also reported in female rats under the same exposure conditions and at higher exposure levels (NTP 2002). The lowest LOEL for subchronic exposures was 89 mg/kg-bw per day. At this dose, non-neoplastic lesions in the kidneys and spleen were reported in male rats exposed to 2-nitrotoluene in the diet for 13 weeks (NTP 1992). Discrepancies in hematopoietic parameters, decreased body weight gains and olfactory epithelium degeneration were reported in other animal studies at higher exposure levels (Kovalenko 1973; Ciss 1978; Ciss et al. 1980a; Ton et al. 1995; NTP 1996). The lowest LOEL for short-term exposure was 90 mg/kg-bw per day. At this dose,

non-neoplastic lesions in the hematopoietic system and spleen were reported after rats were exposed to 2-nitrotoluene for 28 days via intragastric administration (Kaneko et al. 1993). At higher exposure levels, decreased body weight gains, non-neoplastic lesions in the liver and other hematological effects were reported (Kovalenko 1973; Ciss 1978; Ciss et al. 1980a; Lysy et al. 1988; NTP 1992).

Evidence of reproductive effects of 2-nitrotoluene has been demonstrated in mice and rats following oral exposures. The lowest LOEL was 25 mg/kg-bw per day, which was associated with atrophy of the germinal epithelium and interstitial cell hyperplasia of the testes in male rats exposed to 2-nitrotoluene in the diet for 105 weeks (NTP 2002). Degeneration of the testes with reduction in sperm count and motility in male rats and a prolongation of the menstrual cycle in female rats were observed at higher exposure levels (NTP 1992, 1996; Huntingdon Research Centre 1994). Developmental effects have also been reported in rats following oral exposures. The lowest LOEL was 50 mg/kg-bw per day for dose-related retardation in pup growth after the rat dams were exposed to 2-nitrotoluene for 41 days (Huntingdon Research Centre 1994). No other developmental effects were identified in the literature.

The confidence in the toxicity database in experimental animals is considered to be moderate to high, as data were identified for acute, repeated-dose, reproductive and developmental toxicity, carcinogenicity and genotoxicity. However, the level of detail reported in some of the repeated-dose studies is limited, and, although there was notable mortality in the critical cancer bioassay, this mortality was consistent with the development of tumours. In addition, data on both cancer and non-cancer effects associated with inhalation or dermal exposure are very limited. As well, no epidemiological studies specific to 2-nitrotoluene were identified.

Characterization of Risk to Human Health

As 2-nitrotoluene was classified on the basis of carcinogenicity by other national and international agencies (e.g., European Commission and National Toxicology Program), carcinogenicity was a key focus for this screening assessment. An increased incidence of tumours was reported in multiple tissues, such as the mesothelium, skin, mammary gland, liver or lungs, in rats exposed in the diet. Tumours in the circulatory system, large intestine and liver were reported in mice exposed via the dietary route. Furthermore, 2-nitrotoluene has been reported to be a weak skin tumour initiator in the mouse skin painting model. 2-Nitrotoluene was genotoxic in a range of *in vitro* and *in vivo* assays, was notably clastogenic in human peripheral lymphocytes and formed DNA adducts in exposed rodents. Although the mode of induction of tumours has not been fully elucidated, based on the genotoxicity of 2-nitrotoluene, the tumours observed in the experimental animals are considered to have resulted from direct interaction with genetic material.

Exposure to 2-nitrotoluene has also been associated with a range of non-cancer effects in experimental animals. Non-neoplastic lesions in multiple target tissues have been observed in rats chronically exposed to relatively low doses of 2-nitrotoluene. A dose of

25 mg/kg-bw per day was the lowest dose tested in these studies. At the same dose and under the same exposure conditions, reproductive changes, such as atrophy of the germinal epithelium and interstitial cell hyperplasia of the testes, were observed in male rats. The most sensitive non-cancer effects occurred at 25 mg/kg-bw per day. Margins of exposures were not calculated for non-cancer effects in this assessment since non-cancer effects occurred at a dose level at which tumours were observed and because the available information indicates that exposures to 2-nitrotoluene in the Canadian general population from either environmental media or consumer products is expected to be negligible.

Uncertainties in Evaluation of Risk to Human Health

The scope of this screening assessment does not take into account possible differences between humans and experimental species in sensitivity to effects induced by 2-nitrotoluene, although it is noteworthy that similar metabolic pathways do exist in humans and laboratory animals. In addition, the available oral carcinogenicity studies are limited, since no carcinogenicity bioassay exposed animals via inhalation, the potentially most relevant route of exposure for humans.

There is some uncertainty in the estimation of environmental exposure levels, since Canadian monitoring data were not identified. However, based on the volumes and uses identified, environmental exposure of the general population in Canada is not expected. This is supported by conservative environmental modelling.

Conclusion

Based on the information presented in this screening assessment, it is concluded that 2-nitrotoluene is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitutes or may constitute a danger to the environment on which life depends.

On the basis of the carcinogenicity of 2-nitrotoluene, for which there may be a probability of harm at any level of exposure, it is concluded that 2-nitrotoluene is a substance that may be entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

It is therefore concluded that 2-nitrotoluene meets one or more of the criteria under section 64 of CEPA 1999. 2- nitrotoluene meets the criteria for persistence but does not meet the criteria for bioaccumulation potential as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

This substance will be considered for inclusion in the in the *Domestic Substances List* inventory update initiative. In addition and where relevant, research and monitoring will support verification of assumptions used during the screening assessment and, where

appropriate, the performance of potential control measures identified during the risk management phase.

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Appendix 1: Summary of health effects information for 2-nitrotoluene

Endpoint	Lowest effect levels[1,2]/Results
Laboratory animals and *in vitro* assays	
Acute toxicity	**Lowest oral LD_{50}** = 110 mg/kg-bw in female cats (Hollander and Weigand 1975d) [additional studies: Back et al. 1972; Hollander and Weigand 1975c; Vasilenko and Kovalenko 1976; Vernot et al. 1977; Ciss 1978; Ciss et al. 1980a] **Lowest inhalation LD_{50}** = 1086 mg/m^3 (1.086 mg/L) in male SPF Wistar rats (Hollander and Weigand 1975a) [additional studies: Brown and Reinhardt 1972; Kinkead et al. 1977] **Lowest dermal LD_{50}** = 200 mg/kg-bw in male albino rabbits (McDonnell and Reinhardt 1972) [additional studies: Hollander and Weigand 1975b; Kinkead et al. 1977] **Other effects:** EURAR (2008) reported that 2-nitrotoluene is not irritating to the skin, eye or respiratory tract. EURAR (2008) also reported that 2-nitrotoluene is not corrosive to the skin, eye or respiratory tract. No data were identified for 2-nitrotoluene regarding skin and respiratory sensitization.
Short-term repeated-dose toxicity	**Lowest oral LOEL** = 90 mg/kg-bw per day. Lesions in the hematopoietic system and spleen were reported after Wistar rats (6 per sex per group) were exposed to 2-nitrotoluene (0, 3.6, 18, 90 or 450 mg/kg-bw per day, 28 days, intragastric administration) in corn oil (Kaneko et al. 1993). [additional studies: Kovalenko 1973; Ciss 1978; Ciss et al. 1980a; Lysy et al. 1988; NTP 1992] No short-term inhalation or dermal toxicity studies were identified.
Subchronic toxicity	**Lowest oral LOEL** = 89 mg/kg-bw per day. Lesions in the kidneys and spleen of males were reported after F344/N rats (10 per sex per group, 6 weeks of age) were exposed to 2-nitrotoluene (0, 45, 89, 179, 353 or 694 mg/kg-bw per day for males; 0, 44, 87, 178, 340 or 675 mg/kg-bw per day for females) in the diet for 13 weeks after acclimation periods of 13–15 days (NTP 1992). [additional studies: Kovalenko 1973; Ciss 1978; Ciss et al. 1980a; Ton et al. 1995; NTP 1996] No subchronic inhalation or dermal toxicity studies were identified.
Chronic toxicity/ carcinogenicity	**Non-neoplastic endpoints:** **Lowest oral LOEL** = 25 mg/kg-bw per day. Increased incidences of non-neoplastic lesions in the liver (eosinophilic focus, mixed cell focus, mixed cell cellular infiltration), bone marrow (hyperplasia), spleen (hematopoietic cell proliferation) and lungs (alveolar epithelial hyperplasia) were reported in male and female F344/N rats (60 per sex per group, 6–7 weeks of age) exposed to 2-nitrotoluene at 0, 625, 1250

Endpoint	Lowest effect levels[1,2]/Results
	or 2000 mg/kg (0, 25, 50 or 90 mg/kg-bw per day in males; 0, 30, 60 or 100 mg/kg-bw per day in females) in the diet for 105 weeks after acclimation periods of 12–14 days (NTP 2002). Additionally, atrophy of the germinal epithelium and interstitial cell hyperplasia of the testes were reported in the exposed male rats (NTP 2002). No other non-neoplastic endpoints were identified in the available chronic toxicity/carcinogenicity studies. **Neoplastic endpoints:** F344/N rats (10 per sex per group, 6 weeks of age) were administered 2-nitrotoluene at 0, 625, 1250, 2500, 5000 or 10 000 mg/kg (0, 56, 98, 178, 383 or 696 mg/kg-bw per day in males; 0, 55, 102, 190, 382 or 779 mg/kg-bw per day in females) by diets *ad libitum* for 13 weeks after acclimation periods of 10–15 days (NTP 1992, 2008; EURAR 2008). There were no exposure-related effects on survival. Increased incidence of mesothelioma and mesothelial cell hyperplasia of the tunica vaginalis of the testis was reported in male rats in the 178 mg/kg-bw per day group (3/10) and in the 696 mg/kg-bw per day group (2/10), respectively. Since mesothelioma had not been previously identified in exposed or control rats from any of the 13-week bioassays conducted by the NTP, the investigators concluded that 2-nitrotoluene was carcinogenic in male rats (NTP 2008). F344/N rats (male, 10–20 per group, 45 days of age) were administered 2-nitrotoluene at 0 or 5000 mg/kg (0 or 292–296 mg/kg-bw per day) in the diet for 13 or 26 weeks after an acclimation period of 9 days (NTP 1996, 2008; EURAR 2008). The 13-week exposure (stop exposure) group was given the control diet (after 13 weeks) until necropsy at 26 weeks. There were no exposure-related effects on survival. No tumours were reported after 13 weeks of exposure. After 26 weeks, significant increases in the incidence of mesothelioma of the tunica vaginalis of the testis and epididymis (5/20, stop exposure group; 7/20, 26-week exposure group) were reported in both exposure groups. Increased incidence of cholangiocarcinoma (2/20, stop exposure group; 1/20, 26-week exposure group) was also reported in the exposed rats. The investigators concluded that these studies confirmed the carcinogenicity of 2-nitrotoluene based on the high incidence of mesothelioma and the occurrence of cholangiocarcinoma in male rats after short-term exposures (NTP 2008). In a concurrent study, F344/N rats (altered flora: rats received a single gavage dose of an antibiotic 6 days before the start of the study and 13 weeks later) were administered 2-nitrotoluene at 0 or 5000 mg/kg (0 or 292–296 mg/kg-bw per day) in the diet for 13 weeks (NTP 1996, 2008; EURAR 2008). There were no exposure-related effects on survival. Exposure-related increases in the incidence of mesotheliomas (2/20) were reported (NTP 1996). In another parallel stop exposure study,

Endpoint	Lowest effect levels[1,2]/Results
	F344/N rats (altered flora: rats received a single gavage dose of an antibiotic 6 days before the start of the study and 13 weeks later) were administered 2-nitrotoluene at 0 or 5000 mg/kg (0 or 292–296 mg/kg-bw per day) in the diet for 13 weeks followed by the control diet for the remainder (26 weeks) of the study (NTP 1996, 2008; EURAR 2008). There were no exposure-related effects on survival. Exposure-related increases in the incidence of mesotheliomas (8/20) were reported (NTP 1996). No cholangiocarcinomas were reported in rats with altered intestinal flora. The NTP (1996) was not able to draw any conclusions by comparing results from normal and altered flora experiments in rats because of the low effectiveness of the antibiotic mixture against obligate anaerobic bacteria and the possible development of resistant aerobic bacteria after 1 week of antibiotic administration (NTP 2008). F344/N rats (60 per sex per group, 6–7 weeks of age) were administered 2-nitrotoluene at 0, 625, 1250 or 2000 mg/kg (0, 25, 50 or 90 mg/kg-bw per day in males; 0, 30, 60 or 100 mg/kg-bw per day in females) in the diet for 105 weeks after acclimation periods of 12–14 days (NTP 2002, 2008; EURAR 2008). Groups of F344/N rats (male, 70 per group) in a concurrent stop exposure study were administered 2-nitrotoluene at 0, 2000 or 5000 mg/kg diet (0, 125 or 315 mg/kg-bw per day) for 13 weeks followed by control feed for the remainder of the 2 years of the study. Poor survival was reported for exposed male rats in both the stop exposure study and the core chronic exposure study. Survival was also reduced in the high-dose female rats. The reduced survival rates were attributed to the early development of tumours (Dunnick et al. 2003; NTP 2008). In the chronic exposure study, significant increases in the incidences of malignant mesotheliomas (2/60, 20/60, 29/60, 44/60; associated with tunica vaginalis of the testis, epididymis, abdominal wall or surface abdominal organs), subcutaneous skin lipoma (0/60, 4/60, 13/60, 13/60), subcutaneous fibroma (5/60, 46/60, 52/60, 59/60), subcutaneous skin fibrosarcoma (0/60, 7/60, 17/60, 20/60) and subcutaneous fibroma or fibrosarcoma combined (5/60, 47/60, 55/60, 59/60) were reported in male rats. The incidence of mammary gland fibroadenomas was also significantly increased in male rats except in the highest dose group (0/60, 7/60, 10/60, 2/60). Significant increases in the incidence of hepatocellular adenoma (2/60, 3/60, 3/60, 7/60) and hepatocellular adenoma or carcinoma (3/60, 3/60, 3/60, 8/60) and increases in the incidence of hepatocholangiocarcinoma (0/60, 1/60, 0/60, 1/60), alveolar/bronchiolar adenoma (1/60, 5/60, 1/60, 2/60) and alveolar/bronchiolar adenoma or carcinoma (2/60, 5/60, 1/60, 2/60) in male rats were also reported. In female rats, significant increases in the incidence of subcutaneous skin fibroma (3/60, 3/60, 18/60, 20/60) and fibrosarcoma (3/60, 3/60, 21/60, 22/60) were reported in the two highest exposure groups. The incidences of mammary gland fibroadenomas (23/60, 47/60, 52/60, 56/60) and hepatocellular adenoma (1/60, 0/59, 1/60, 6/60) in the high-dose female rats were also significantly increased. Similar tumour profiles were reported for the stop exposure groups of male rats and the chronically exposed animals. Significant

Endpoint	Lowest effect levels[1,2]/Results
	increases in the incidence of malignant mesotheliomas (2/60, 44/60, 54/60), subcutaneous skin lipoma (0/60, 10/60, 12/60), subcutaneous fibroma (5/60, 45/60, 52/60), subcutaneous skin fibrosarcoma (0/60, 8/60, 12/60) and subcutaneous fibroma or fibrosarcoma combined (5/60, 47/60, 53/60) were reported. The incidence of mammary gland fibroadenomas was also significantly increased (0/60, 13/60, 20/60). Significant increases in the incidence of hepatocellular adenoma (2/60, 3/60, 4/60) and hepatocellular adenoma or carcinoma (3/60, 3/60, 6/60) and increases in the incidence of liver cholangiocarcinoma (0/60, 0/60, 3/60), alveolar/bronchiolar adenoma (1/60, 3/60, 8/60) and alveolar/bronchiolar adenoma or carcinoma (2/60, 3/60, 11/60) were also reported. The NTP concluded that there was clear evidence of carcinogenic activity of 2-nitrotoluene in male and female rats, based on increased incidences of malignant mesothelioma (in males only), subcutaneous skin tumours, mammary gland fibroadenoma and liver tumours (in males only). Increased incidences of lung tumours in male rats and hepatocellular adenoma in female rats were also considered to be exposure related (NTP 2002, 2008). B6C3F1 mice (60 per sex per group, 6 weeks of age) were administered 2-nitrotoluene at 0, 1250, 2000 or 5000 mg/kg diet (0, 165, 360 or 700 mg/kg-bw per day in males; 0, 150, 320 or 710 mg/kg-bw per day in females) for 105 weeks after acclimation periods of 12 days. Poor survival was reported for the exposed male mice (NTP 2002, 2008; EURAR 2008). Survival was also reduced in the high-dose female mice. The reduced survival rates were attributed to the early development of tumours (Dunnick et al. 2003; NTP 2008). Significant increases in the incidences of hemangiosarcoma (4/60, 17/60, 55/60, 60/60) in all exposure groups and cecum carcinoma (0/60, 12/60, 9/60, 0/60) in the low and middle exposure groups were reported in male mice. The incidence of hemangiosarcomas (0/60, 2/60, 3/60, 50/60) was also significantly increased in high-dose female mice. While the increase in the incidence of hepatocellular carcinomas (2/60, 4/59, 6/59, 16/60) was significant only in the high-dose female mice, significant increases in the incidence of hepatocellular adenomas (7/60, 5/59, 19/59, 29/60) and hepatocellular adenomas or carcinomas combined (9/60, 9/59, 24/59, 39/60) were reported for both the middle and high exposure groups. Furthermore, exposure-related increases in the incidence of cecum carcinomas (0/60, 1/60, 4/60, 3/60) were also reported in the female mice. The NTP concluded that there was "clear evidence of carcinogenic activity" of 2-nitrotoluene in male and female mice based on the increases in the incidence of cecum carcinomas in male mice, liver tumours in female mice and hemangiosarcomas in all exposed animals (NTP 2002, 2008). SENCAR mice (unspecified number of animals per sex per group) were exposed to a single dermal application of 2-nitrotoluene at 24, 120 or 240 mg (1200, 6000 or 12 000 mg/kg-bw) followed by single applications of the promoter 12-*O*-tetradecanoylphorbol-13-acetate

Endpoint	Lowest effect levels[1,2]/Results
	(TPA) at 4 µg every week for 30 weeks. The incidence of skin papillomas (13%, 2.5%, 10%, 16% of the animals) and carcinomas (2.5%, 0%, 2.5%, 5.0% of the animals) in mice in the highest dose group was slightly but not significantly increased (Slaga et al. 1985; DFG 2002). A/Jax mice (male, 30 per group) were administered 2-nitrotoluene (in corn oil) at 0, 1200, 3000 or 6000 mg/kg-bw via the intraperitoneal route, 3 times a week for 8 weeks. Exposed animals were sacrificed 16 weeks after the last injection. Dose-dependent but non-significant increases in lung tumours were reported (Slaga et al. 1985; EURAR 2008).
Reproductive toxicity	**Lowest oral LOEL** = 25 mg/kg-bw per day. Atrophy of the germinal epithelium and interstitial cell hyperplasia of the testes were reported in male F344/N rats (60 per group, 6–7 weeks of age) exposed to 2-nitrotoluene at 625, 1250 or 2000 mg/kg (25, 50 or 90 mg/kg-bw per day) in the diet for 105 weeks after acclimation periods of 12–14 days (NTP 2002). [additional studies: Ciss 1978; Ciss et al. 1980b; NTP 1992, 1996; Huntingdon Research Centre 1994] No inhalation or dermal reproductive toxicity studies were identified.
Developmental toxicity	**Lowest oral LOEL** = 50 mg/kg-bw per day. Dose-related retardation in pup growth was reported in CD rats (male/female unspecified number of animals per sex per group) exposed to 2-nitrotoluene at 0, 50, 150 or 450 mg/kg-bw per day for 20 days via dams and 21 days during lactation period, for a total of 41 days (Huntingdon Research Centre 1994). [additional studies: Ciss 1978; Ciss et al. 1980b] No inhalation or dermal developmental toxicity studies were identified.
Genotoxicity and related endpoints: *in vitro*	**Chromosomal aberrations:** Positive, without metabolic activation (S9), in human peripheral lymphocytes for chromosomal aberration (Huang et al. 1996). Positive, without metabolic activation (S9), in Chinese hamster lung cells for chromosomal aberration (Ishidate et al. 1988). Negative, with or without metabolic activation (S9), in Chinese hamster ovary cells for chromosomal aberration (Galloway et al. 1987). **Micronuclei induction:** Positive, without metabolic activation (S9), in Chinese hamster lung cells for micronuclei induction (Matsushima et al. 1999). Negative, with or without metabolic activation (S9), in Chinese hamster ovary cells for micronuclei induction (Lee et al. 2007).

Endpoint	Lowest effect levels[1,2]/Results
	Unscheduled DNA synthesis, DNA damage and Sister Chromatid Exchange: Positive, without metabolic activation (S9), for unscheduled DNA synthesis in F344/N rat hepatocytes (Parton et al. 1995). Positive, with and without metabolic activation (S9), for DNA damage (comet assay) in L5178Y mouse lymphoma cells (Lee et al. 2007). Weakly positive, with and without metabolic activation (S9), for sister chromatid exchanges in Chinese hamster ovary cells (Galloway et al. 1987). Negative, without metabolic activation (S9), for unscheduled DNA synthesis in rat spermatocytes, rat spermatids, rat hepatocytes or human hepatocytes (Doolittle et al. 1983; Working and Butterworth 1984; Furihata and Matsushima 1987; Butterworth et al. 1989; Brambilla and Martelli 1990). **Mutagenicity in bacteria:** Negative, with or without metabolic activation (S9), in *Salmonella typhimurium* strains TA92, TA94, TA98, TA100, TA102, TA104, TA1535, TA1537 and TA1538 for mutations (Chiu et al. 1978; Miyata et al. 1981; Tokiwa et al. 1981; Spanggord et al. 1982b; Haworth et al. 1983; Suzuki et al. 1983; Nahmi et al. 1984; Sundvall et al. 1984; Shimizu and Yano 1986; Gupta et al. 1987; Kawai et al. 1987; JETOC 1996). Positive, with metabolic activation and norharman, in *Salmonella typhimurium* strain TA98 for mutations (Suzuki et al. 1983). Negative, with or without metabolic activation (S9), in *Escherichia coli* strains WP2uvra and WP2uvra/PKM101 for reverse mutations (JETOC 1996). **Other:** Weakly positive, with metabolic activation, in *Bacillus subtilis* strains H17 and M45 for genetic recombination (Shimizu and Yano 1986). Negative, with metabolic activation, in *Bacillus subtilis* for loss of transforming DNA activity (Nahmi et al. 1984).
Genotoxicity and related endpoints: *in vivo*	**Chromosomal aberrations:** Positive in *Culex fatigans* for chromosomal aberrations. Larvae of *Culex fatigans* were treated with 2-nitrotoluene (dissolved in dimethylsulfoxide) at 0.01 µg/mL. Chromosomal breaks, translocations,

Endpoint	Lowest effect levels[1,2]/Results
	fragments and aneuploids were reported in the chromosomal preparations made from ovaries of adult females 12–15 h old. No polyploid cells were observed (Sharma et al. 1989). **Micronuclei induction:** Negative for micronuclei induction in the bone marrow cells of male F344/N rats exposed to 2-nitrotoluene via single intraperitoneal injections (625, 1250 or 2500 mg/kg-bw, corn oil vehicle) after 24 h or via single intraperitoneal injections (625 or 2500 mg/kg-bw, corn oil vehicle) after 48 h (NTP 2002). Negative for micronuclei induction in the bone marrow cells of male B6C3F1 mice exposed to 2-nitrotoluene via intraperitoneal injections (100, 200, 300 or 400 mg/kg-bw, 3 times at 24 h intervals) after 72 h (NTP 2002). Negative for micronuclei induction in peripheral blood cells of male and female B6C3F1 mice exposed to 2-nitrotoluene in the diet (625, 1250, 2500, 5000 or 10 000 mg/kg) for 13 weeks (NTP 2002). **Mutagenicity in germ cells:** Negative in *Culex fatigans* for heritable dominant lethal mutations. Treated males were crossed with normal females. The dominant lethality was determined in terms of the percentage frequency of the unhatched eggs (Sharma et al. 1989). **DNA adducts:** Hepatic DNA adducts (2′-deoxyguanosine and 2′-deoxyadenosine adducts of 2-methylaniline) were reported in male WELS Fohm rats (12 per group, 4 months of age) exposed to 2-nitrotoluene (40, 96 or 250 mg/kg-bw per day) in the diet (dissolved in sunflower oil and administered into water bottle) for 12 weeks (Jones and Sabbioni 2003). **Unscheduled DNA synthesis:** Positive for unscheduled DNA synthesis in hepatocytes of F344/N rats (male/female, unspecified number of animals per sex per group, 11–12 weeks of age) exposed to 2-nitrotoluene (single administration: 0, 100, 200 or 500 mg/kg-bw for males; 0, 200, 500 or 750 mg/kg-bw for females) via oral gavage (NTP 1992). Positive for unscheduled DNA synthesis in hepatocytes of F344/N rats (male, unspecified number of animals per group) exposed to 2-nitrotoluene (single administration: 200 or 500 mg/kg-bw in corn oil) via oral gavage (Butterworth et al. 1982; Doolittle et al. 1983).

Endpoint	Lowest effect levels[1,2]/Results
	Negative for unscheduled DNA synthesis in hepatocytes of B6C3F1 mice (male/female, unspecified number of animals per sex per group, 11–12 weeks of age) exposed to 2-nitrotoluene (single administration: 0, 200, 500 or 750 mg/kg-bw) via oral gavage (NTP 1992). Negative for unscheduled DNA synthesis in hepatocytes of F344/N rats (male, germ free, unspecified number of animals per group) exposed to 2-nitrotoluene (single administration: 200 mg/kg-bw) via oral gavage (Butterworth et al. 1982). Positive results were obtained in a separate group of germ-free rats who were pretreated with Charles River Associated Flora with similar dosing regimens (Butterworth et al. 1982). Negative for unscheduled DNA synthesis in hepatocytes of F344/N rats (male/female, germ free, unspecified number of animals per group) exposed to 2-nitrotoluene (single administration: 200 or 500 mg/kg-bw) via oral gavage (Butterworth et al. 1982). Positive results were obtained in a separate group of germ-free male rats who were pretreated with Charles River Associated Flora with similar dosing regimens (Butterworth et al. 1982). However, negative results were obtained in another separate group of germ-free female rats who were pretreated with Charles River Associated Flora with similar dosing regimens. The investigators concluded that this may indicate a sex difference in genotoxicity of 2-nitrotoluene that is independent of the intestinal bacterial metabolism. **Gene mutations:** Mutations in the tumour suppressor gene *p53* or proto-oncogene *β-catenin* was reported in the 2-nitrotoluene-induced hemangiosarcomas and colon tumours in B6C3F1 mice (60 per sex per group, 6 weeks of age) that were administered 2-nitrotoluene at 0, 1250, 2000 or 5000 mg/kg (0, 165, 360 or 700 mg/kg-bw per day in males; 0, 150, 320 or 710 mg/kg-bw per day in females) for 105 weeks after acclimation periods of 12 days (NTP 2002). Furthermore, *K-ras* gene mutations and cyclin D1 protein production were also identified in the colon tumours. The NTP (2008) concluded that the gene mutations of *p53*, *β-catenin* and *K-ras* are the result of the genotoxic effects of 2-nitrotoluene.
Humans	
Epidemiological studies	No epidemiological studies were identified in the literature regarding the toxic effects of 2-nitrotoluene exposures. Exposure limits were derived for occupational exposures to protect workers from 2-nitrotoluene. For short-term (60 min) exposures to 2-nitrotoluene vapours, a concentration of 200 ppm (1140 mg/m^3) was reported to cause severe toxic effects in workers (Goldblatt 1955). With respect to the exposure limit, 40 ppm (228 mg/m^3) was considered to be the not tolerated concentration for workers exposed to 2-nitrotoluene vapours, with 1 ppm (5.7 mg/m^3) as the upper limit to protect for longer periods of exposure (Goldblatt 1955). These values were derived by

Endpoint	Lowest effect levels[1,2]/Results
	taking into account data on human effects derived from clinical and experimental records (EURAR 2008). 2-Nitrotoluene can be used for the manufacture of magenta. Workers who are involved with the production of magenta may be exposed to 2-nitrotoluene (IARC 1987, 1993). IARC (1987, 1993) reviewed the manufacturing of magenta and concluded that there is "sufficient evidence" that magenta manufacturing entailed exposures that are carcinogenic (Group 1) in humans (NTP 2008). IARC's conclusion was based on a case–control study (Vineis and Magnani 1985) and two cohort studies (Case and Pearson 1954; Rubino et al. 1982). These studies are not considered viable for the current assessment but were included for the completeness of the database. Nine hundred and six workers from a dyestuff factory in northern Italy who worked at least 1 month between 1922 and 1970 were included in a cohort study conducted from 1946 to 1976 (Rubino et al. 1982). Mortality rates from the study were compared with national rates of 1951–1976. Significant increases in the incidence of mortality due to bladder cancer were reported in 53 magenta and safranin T manufacturing workers who were directly exposed to 2-nitrotoluene (standardized mortality ratio [SMR] = 62.5; $P < 0.001$; five deaths). However, the workers were also currently exposed to *o*-toluidine and 2-methylaniline (NTP 2008). A cohort study of male workers who were employed for at least 6 months in the manufacture of auramine and magenta in Britain was conducted between 1910 and 1952 (Case and Pearson 1954). Significant increases (SMR = 23.8; $P < 0.005$; three observed cases) in the incidence of death as the result of bladder cancers were reported among 85 magenta manufacture workers not involved in the production of auramine. There was no information reported regarding the potential exposure to 2-nitrotoluene or other chemicals in the magenta manufacturing process (NTP 2008). A case–control study conducted in Italy between 1978 and 1983 examined 512 cases of bladder cancer in males and 596 hospital controls (Vineis and Magnani 1985). The chemicals to which the subjects were exposed were assessed using job titles, job activities and knowledge of industrial use of chemicals from the published literature. Seventy-four chemicals were examined as part of a job exposure matrix. Increases in the risk (relative risk [RR] = 1.8, 95% confidence interval [CI] = 1.1–2.9, when calculated using industrial branches; RR = 3.0, 95% CI = 0.4–20.0, when calculated from job titles) of bladder cancer were reported in workers exposed to magenta. No information was available to determine whether the subjects were exposed to 2-nitrotoluene (NTP 2008). Occupational exposures and the associated health effects were investigated in workers of a Chinese trinitrotoluene (TNT) factory

Endpoint	Lowest effect levels[1,2]/Results
	(Jones et al. 2005). The production of TNT involves the continual batch nitration of nitrotoluenes (NT) and then dinitrotoluenes (DNT) with sulfuric acid and nitric acid. Hence, the workers were predisposed to be exposed to high levels of volatile intermediate products such as NT and DNT. For the study, the workers were grouped according to their job description and work location: group leader, NT-tank, DNT-tank, TNT-tank, laboratory of chemical analyses, transportation of TNT to packaging, packaging, control room, disposal of waste acid, disposal of wastewater and non-exposed control workers. The health of the participating workers was checked. A questionnaire was also required. Blood samples were collected from 99 exposed workers and from 61 non-matched, non-exposed controls working in the same factory. Exposure to mixed nitrotoluenes in workers was determined by measurement of the level of arylamine cleavage products released from hemoglobin following mild base hydrolysis. It was reported that hemoglobin adducts for 2-nitrotoluene were found at the highest concentration compared with the other NT. The investigators reported that 2-nitrotoluene or its metabolites were capable of forming hemoglobin adducts in humans (NTP 2008). The investigators also concluded that quantification of hemoglobin adducts provided an effective biomarker of exposure to the NT (NTP 2008). Occupational exposures and the associated health effects were investigated in workers who were exposed to high levels of nitrotoluenes, such as 2-nitrotoluene, 2,4-dinitrotoluene (24DNT) and 2,6-dinitrotoluene (26DNT). The exposed (n = 104) and control (n = 72) workers were employed in a factory manufacturing dinitrotoluenes (DNT) and 2,4,6-trinitrotoluene (TNT), situated in Liaoning, China. The industrial synthesis of DNT and TNT was achieved by continual batch nitration of mononitrotoluenes (NT) into DNT and then treating DNT with sulfuric acid and nitric acid to produce TNT. In this study, the workers were grouped according to their job description and work location, such as group leader, NT-tank, DNT-tank, TNT-tank, laboratory of chemical analyses, transportation of TNT to packaging, packaging, control room, disposal of waste acid and disposal of wastewater. The control workers performed tasks that included no chemical exposure. The median age (range) was 34.5 years (22.4–54.7 years) for the exposed and 36.8 years (15.9–53.2 years) for the controls. The median number of work years was 10.5 years (3.6–38.0 years) in the exposed and 17.6 years (4.9–39.4 years) in the control group. Males constituted 71% of the exposed workers and 82% of the factory controls. The external dose (air levels), the internal dose (urine metabolites), the biologically effective dose (hemoglobin [Hb] adducts and urine mutagenicity) and biological effects (chromosomal aberrations and health effects) of the nitrotoluenes were determined in this study. Individual susceptibility to nitrotoluene exposures was assessed by determining genetic polymorphisms of enzymes assumed to function in nitrotoluene metabolism. From exposure to 2-nitrotoluene levels of 759 ± 836 μg/m^3 (8-h time weighted average), corresponding Hb adduct 2-

Endpoint	Lowest effect levels[1,2]/Results
	methylaniline levels were measured at 7.54 ± 9.07 pmol/g Hb. The urine of the exposed workers was reported to be mutagenic. Non-specific health effects such as inertia, somnolence, nausea and dizziness were found to correlate with the levels of various nitrotoluene–Hb adducts. Clinical blood and urine variables were also significantly associated with the various Hb adduct levels. In addition, chromosomal aberrations in human peripheral lymphocytes were increased in the exposed workers and significantly correlated to nitrotoluene exposures. The effect of nitrotoluene exposure on the level of chromosomal aberrations was also found to be dependent on the SULT1A1, SULT1A2, NAT1, GSTT1 and GSTP1 genotypes. The authors suggested that these polymorphisms can potentially affect the genotoxic effects of nitrotoluenes (Sabbioni et al. 2006).

[1] LC_{50}, median lethal concentration; LD_{50}, median lethal dose; LOEC, lowest-observed-effect concentration; LOEL, lowest-observed-effect level.