JRC Scientific and Technical Reports

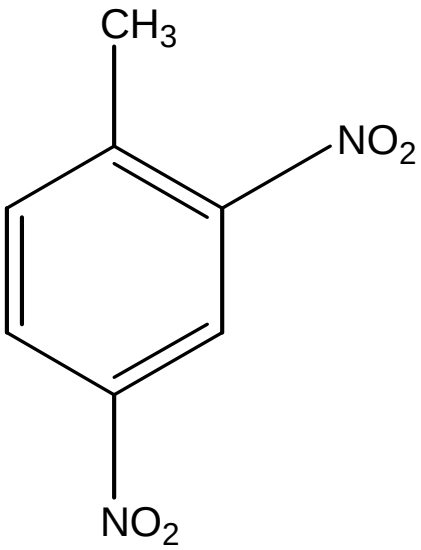
# European Union

## Summary Risk Assessment Report

### Existing Substances – 4th Priority List

**CAS: 121-14-2 EINECS No: 204-450-0**

**2,4-dinitrotoluene**

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# 2,4-DINITROTOLUENE

CAS No: 121-14-2

EINECS No: 204-450-0

# SUMMARY RISK ASSESSMENT REPORT

*Final report, 2008*

Spain

Rapporteur for the risk assessment of 2,4-dinitrotoluene is Spain: Ministry of Environment and Ministry of Health.

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| | |
|---|---|
| **Date of Last Literature Search:** | **2005** |
| **Review of report by MS Technical Experts finalised:** | **February 2007** |
| **Final report:** | **2008** |

# PREFACE

This report provides a summary, with conclusions, of the risk assessment report of the substance 2,4-dinitrotoluene that has been prepared by the Spanish Competent Authorities in the context of Council Regulation (EEC) No. 793/93 on the evaluation and control of existing substances.

For detailed information on the risk assessment principles and procedures followed, the underlying data and the literature references the reader is referred to the comprehensive Final Risk Assessment Report (Final RAR) that can be obtained from the JRC-IHCP website[1]. The Final RAR should be used for citation purposes rather than this present Summary Report.

[1] Former - European Chemicals Bureau – Existing Chemicals – http://ecb.jrc.ec.europa.eu/

# CONTENTS

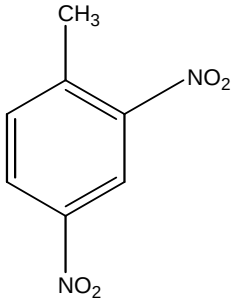


# TABLES

# 1 GENERAL SUBSTANCE INFORMATION

## 1.1 IDENTIFICATION OF THE SUBSTANCE

CAS Number: 121-14-2
EINECS Number: 204-450-0
IUPAC Name: 1,3-Dinitro-4-methylbezene
Synonyms: 1-Methyl-2,4-dinitro-benzene
1-Methyl-2,4-dinitro-benzol
2,4-dinitro-1-methylbenzene
2,4-dinitrotoluene
2,4-dinitrotoluol
Benzene, 1-methyl-2,4-dinitro
2,4-DNT
DNT
Dinitrotoluole
m-dinitrotoluol
Toluene, 2,4-dinitro-
Molecular weight: 182.14
Molecular formula: $C_7H_6N_2O_4$
Structural formula:




## 1.2 PURITY/IMPURITIES, ADDITIVES

Purity: ≥ 99%
Impurity There are no data available.

## 1.3 PHYSICO-CHEMICAL PROPERTIES

2,4-dinitrotoluene is a yellow solid with slight odour which appears in rhombic needles or monoclinic prisms. It is stable under normal laboratory conditions but it may react violently in the presence of a base, oxidant and reducing agent or when heated to the boiling point. It is slightly soluble in water, soluble in ether, benzene and acetone, and very soluble in chloroform and toluene.

Table 1.1 Summary of physico-chemical properties

| Property | Value | Comments |
|---|---|---|
| Physical state | Solid | - |
| Melting point | 69.9ºC | Bayer AG |
| Boiling point | 319.5ºC | Bayer AG |
| Relative density | 1.286 g/cm$^3$ at 110ºC<br>1.3206 g/cm$^3$ at 70ºC | Bayer AG<br>Roembke J, (1995). |
| Vapour pressure | 7.9 10-3 Pa at 20ºC | Bayer AG |
| Water solubility | 166 mg/l at room temperature | Bayer AG |
| Partition coefficient n-octanol/water (log value) | 1.98 | Chemical Information Systems, Inc. Irvine CA USA (1991) |
| Granulometry | - | - |
| Conversion factors | 1 ppm = 7.45 mg/m$^3$ at 25ºC | EPA (1999) |
| Flash point | 169ºC | Bayer AG |
| Autoflammability | Auto ignition temperature:<br>c.a. 400º C | Bayer AG |
| Flammability | - | - |
| Explosive properties | Under 150º C does not show a transmission of detonation | Bayer AG - |
| Oxidizing properties | - | - |
| Viscosity | Not applicable (solid) | - |
| Henry's constant | 5.45·10$^{-3}$ Pa-m$^3$/mol* | Calculated from an experimental dimensionless Henry's law constant value reported by Altschuh, J. (1999) |
| Surface tension | - | - |

## 1.4 CLASSIFICATION

### 1.4.1 Current classification

The classification of 2,4-dinitrotoluene is included in Annex I Directive 67/548/EEC (29$^{th}$ ATP):

Carc. Cat. 2; R45: May cause cancer.

Muta. Cat. 3; R68: Possible risk of irreversible effects.

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

T; R23/24/25: Toxic by inhalation, in contact with skin and if swallowed.

Xn; R48/22: Harmful: danger of serious damage to health by prolonged exposure if swallowed.

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

### 1.4.2 Proposed classification

It is proposed to keep the same classification as currently:

Carc. Cat. 2; R45: May cause cancer.

Muta. Cat. 3; R68: Possible risk of irreversible effects.

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

T; R23/24/25: Toxic by inhalation, in contact with skin and if swallowed.

Xn; R48/22: Harmful: danger of serious damage to health by prolonged exposure if swallowed.

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

# 2 GENERAL INFORMATION ON EXPOSURE

Production

The production of 2,4-dinitrotoluene, obtained as a mixture of isomers, c.a. 80% of 2,4-dinitrotoluene and c.a. 20% of 2,6-dinitrotoluene, is located in Italy, France and Germany. In addition, an amount from 11,025 to 50,025 kg/year is imported for use in explosives, according to data reported by a Spanish company. The resultant quantity of 2,4-dinitrotoluene produced and imported in the EU is 503,970 - 504,009 tons/year.

The production process consists of the reaction of a typical sulphuric acid/nitric acid nitrating mixture with toluene, which yields 78 wt% 2,4-dinitrotoluene, 18 wt% 2,6-dinitrotoluene, 2.5 wt% 3,4-dinitrotoluene, 1 wt% 2,3-dinitrotoluene and 0.5 w% 2,5-dinitrotoluene. If the single 2,4-isomer is required, the nitration is stopped at the mono-stage and pure *p*-nitrotoluene is obtained by crystallization. Subsequent nitration of the *p*-nitrotoluene yields only 2,4-dinitrotoluene. Furthermore 2,4-dinitrotoluene is also formed as an impurity during manufacture of 2,4,6-trinitrotoluene, by the dinitration of toluene, and must be removed by mechanical means.

Uses

The substance is largely used in closed systems as an intermediate for further synthesis of TDA at five different sites in EU. 2,4-dinitrotoluene has had other minor uses, for instance as an additive in the production of explosives, though very scarce information has been provided, specifically from an industry that imported an amount of 50 tons of 2,4-dinitrotoluene.

Table 2.1 Site specific information of the volume used

| Site | Scenario | Volume (metric tons/year) |
|---|---|---|
| A | Synthesis of TDA | 87,159 |
| B | Synthesis of TDA | 67,200 |
| C* | Synthesis of TDA | 45,600 |
| D | Synthesis of TDA | 304,000 |
| E | Additive in the production of explosives | 50 |

* This site will not be considered in the calculations because it has been closed in December 2005.

Trends

The IUCLID Data Set of 2001 from a German industry indicates that its production volume in 1992 was 14,000 tons, which were used as additive in explosive preparations (3%) and as an intermediate for the production of toluylen-diisociante. In 2001, precise information about 2,4-dinitrotoluene production volume in 2000 was provided. In that year, three plants were in operation, two of them in Germany (with a production of 48,000 and 75,200 tons, respectively) and another in Belgium (30,400 tons). Recently, the situation has changed. The up to now existing production facilities have been closed and a new world-scale unit replaced them since 2003 in Germany. The new production site has started its orderly production in 2005, with a final production volume capacity of 304,000 tons. Regarding to the breakdown of uses, current information indicates that all 2,4-dinitrotoluene is used as an intermediate in the production of toluylenediamine and further toluylene diisocianate.

Regarding industry of site A, information on the production is available only for two years: 113,300 tons in 2001 and 87,159 tons in 2002.

Concerning site C, recent information from industry states than the plant placed there has been closed the 31st of December 2005.

Finally, a Spanish industry has supplied information from a 2,4-dinitrotoluene processing site. The amount imported has followed a decreasing trend from 50,025 kg in 1998 to 11,025 kg in 2000. Since no current data have been provided, the amount of 50 tons has been taken as worst case processing volume for site E.

Legislative controls

There are several legal regulations related to 2,4-dinitrotoluene in different countries. In Germany, the Administrative Rules Concerning Substances Hazardous to Water of 1990 (Verwaltungsvorschrift Wassergefaehrdende Stoffe) sets up a classification as the basis for water-protection requirements for industrial plants in which water-hazardous substances are handled. 2,4-dinitrotoluene is classified as very hazardous to water.

In Czech Republic 2,4-dinitrotoluene was classified as a poison in the Government Provision No. 192 on Poisons and Another Substances Harmful to Human Health. Besides, a TWA of 1.0 mg/m$^3$ and a CLV of 2.0 mg/m$^3$ are given by the Hygienic Regulations of Ministry of Health

In India, the Manufacture, Storage and Import of Hazardous Chemicals Rules, of 1989, applies to 2,4-dinitrotoluene.

In Japan, the Poisonous and Deleterious Substances Control Law of 1991 designates 2,4-dinitrotoluene and its preparations as deleterious.

In Canada, the Occupational Safety and Health Regulations prescribes a Time Weighted Average (TWA) of 1.5 mg/m3 for skin absorption. The Workplace Hazardous Materials Information System (WHMIS), which imposes standards on employers for the use, storage and handling of controlled products, states in the Ingredient Disclosure List that a concentration equal to or greater than 1% weight/weight of 2,4-dinitrotoluene must be disclosed in the Safety Data Sheet.

In the USA, the Clean Air Act, 112 National Emission Standards For Hazardous Air Pollutants of 1985 establish a list of pollutants judged to be hazardous for which emission standards will be developed. This list includes 2,4-dinitrotoluene. This substance is as well included on a list required by the Clean Water Act section 304 of conventional pollutants requiring maximum daily effluent limitations. Releases of this hazardous substance, in quantities equal to or greater than 10 LBS (4.54 kg), are subject to reporting to the national response centre under the comprehensive environmental response, compensation, and liability act, and for purposes of section 311 of the Clean Water Act 2,4-dinitrotoluene shall not be discharged into or upon the navigable waters of the United States or adjoining shorelines, waters of the contiguous zone, or outer deep waters which may affect natural resources belonging to the United States.

Finally, two other international legal references can be mentioned. The International Maritime Dangerous Goods Code of 1991 includes 2,4-dinitrotoluene in the Hazard Class 6.1, as a poisonous substance, and in the packing group II. In the Recommendations on the Transport

of Dangerous Goods, of 1993, the Hazard Class 6.1, toxic substance, the packing group II, medium danger, and the packing method M, apply to 2,4-dinitrotoluene.

# 3 ENVIRONMENT

## 3.1 ENVIRONMENTAL EXPOSURE

Environmental releases

Site-specific information on releases of 2,4-dinitrotoluene to water during production has been provided by manufacturers and processors of sites A, B and C, while the emission to water in site D has been calculated from the information on the effluent concentration supplied by the industry. In addition, information provided by industry from site A indicates that this site is located in a coastal zone. For site E, the available information on emissions to waste water is related to the concentration in the effluent of the plant. The releases have been estimated from that value considering the information on the effluent flow of the plant provided by industry.

On the other hand, the information provided by industry indicates that there is no release of 2,4-dinitrotoluene to air in any of the sites.

Table 3.1 Summary of local release estimates (kg/day)

| Scale | | Air | Water |
|---|---|---|---|
| Local | Site A | 0 | 0.22 [a] |
| | Site B | 0 [a] | 16 [a] |
| | Site D | 0 [a] | 0.64 [b] |
| | Site E | 0 [a] | 0.02 [c] |

[a] Emission data provided by industry

[b] Value calculated from data provided by industry for the new production plant

[c] Value calculated from data provided by industry

Environmental fate

2,4-dinitrotoluene may be released into the environment during its production, processing and formulation and emissions to water are expected to be the most important entry routes of 2,4-dinitrotoluene to the environment.

The available biodegradation data show that 2,4-dinitrotoluene can undergo primary biodegradation to form several products. Furthermore, on the light of the available information 2,4-dinitrotoluene should be degraded by biological sewage treatment when suitable acclimation is provided to the cultures, so it can be classified as inherent biodegradable with adapted inoculum (non-ready biodegradable).

Final conclusions on degradability would have to be done on a weight of evidence basis, since, under certain anaerobic conditions, may pose the formation of recalcitrant amino derivatives and the production of more recalcitrant azoxytoluenes, among other compounds. The relevance of formation of recalcitrant metabolites under realistic environmental conditions is not known.

According to previous information, 2,4-dinitrotoluene was considered no-biodegradable in water, on the one hand, and inherent biodegradable in sediment and soil, on the other hand,

because of the different residence times in the different compartments. The adaptation of the microbial population is expected for sediment and soil. However, in running waters, the water column microbial population has a very high spatial and temporal variability, and the condition of continuous exposure of the same population allowing its acclimatization cannot be guaranteed even for continuous point emissions.

According to the TGD procedures, the biodegradation rate of 2,4-dinitrotoluene by soil microorganisms would correspond to a half-life of 10 months in soil and more than 8 years in sediments. There are additional data indicating a much more rapid degradation in soil, including soils preexposed to TNT, however, as these data are based on dissipation or primary degradation, the conservative value estimated by the TGD will be used.

Related to abiotic degradation, the gas-phase reaction with photochemically produced hydroxyl radicals has an estimated rate constant of $2.253 \cdot 10^{-13}$ $cm^3$/molecule/sec, which corresponds to a half-life of 71 days. In water, the log octanol-water partition coefficient is sufficiently large to indicate adsorption to soil organic matter. The relatively low volatility and high solubility of 2,4-dinitrotoluene indicate that it will tend to remain in water for long periods of time, unless acted upon by light, oxygen or biota. As a result, 2,4-dinitrotoluene can be transported to groundwater or surface waters. Photolysis is probably the most significant removal process, and the half-life estimated from the information of bibliography is 29 days.

The ultimate biodegradation rate constants and half-lives that will be used in the environmental modelling are summarized in Table 3.2:

Table 3.2 Environmental degradation

| Compartment | | Half-life |
|---|---|---|
| Atmospheric | | 71 days |
| Aquatic | Abiotic degradation | 29 days |
| | Biodegradation | ∞ days |
| Sediment | | 3,014 days |
| Soil | | 300 days |

Values utilised in EUSES calculations

Environmental concentrations

Only a $PEC_{local}$ for production and processing is calculated with EUSES for sites A, B and D, because they happen one just after the other in the same place. Industry of site A has indicated that about 40% of the waste water is treated in a WWTP, which has a discharge flow of 37,152 $m^3$/d. After treatment, waste waters are discharged into a lagoon, and a default factor of 10 given in the TGD has been used. Due to the fact that the place is located at a costal zone, the PEC in the aquatic compartment has been calculated for both fresh and marine water. Concerning site B, information on the flow of the effluent after physico-chemical treatment has been provided; the values are 8,115.35 $m^3$/h in site B. The 10% of the river flow has been supplied as well, being 34,7 $m^3$/s and 2 $m^3$/s, respectively. These values give a dilution factor of 15.4 for site B. In site D, the information on the flows on the WWTP effluent (0.74 $m^3$/s) and the receiving river (1,050 $m^3$/s) make it possible to estimate a dilution factor of 1,418. As indicated in the TGD, 1000 has been set as dilution factor for site D.

Table 3.3 PEClocal calculated for different aquatic compartments (incl. sediment, ground water, STP)

| Site | $PEC_{local,water}$ (µg/l) | $PEC_{local,sediment}$ (µg/kgwwt) | $PEC_{local\ ground\ water}$ (µg/l) | $PEC_{local\ STP}$ (µg/l) |
|---|---|---|---|---|
| A | 0.363 | 1.22 | $1.63 \cdot 10^{-3}$ | 3.46 |
| B | 5.28 | 17.8 | 0.14 | -* |
| D | 0.022 | 0.08 | $1.63 \cdot 10^{-3}$ | 5.85 |
| E | 0.142 | 0.479 | $1.63 \cdot 10^{-3}$ | 28 |

* There is only physico-chemical treatment

Regarding the marine environment, the default emission factors proposed in the TGD have been applied for site A, which is located at a coastal zone, and the resulting PEC in sea water is 0.06 µg/l and in marine sediment 0.201 µg/kg.

For agricultural soil and grassland, PECs have been calculated with EUSES considering only the atmospheric deposition, since no spreading of sludge from industrial sewage treatment plants has been assumed in any site.

Table 3.4 PEC local calculated for terrestrial compartment

| Compartment | Site A | Site B | Site D | Site E |
|---|---|---|---|---|
| Soil (total) averaged over 30 days (µg/kgwwt) | $3.61 \cdot 10^{-3}$ | $3.61 \cdot 10^{-3}$ | $3.61 \cdot 10^{-3}$ | $3.61 \cdot 10^{-3}$ |
| Agricultural soil (total) averaged over 180 days (µg/kgwwt) | $3.61 \cdot 10^{-3}$ | $3.61 \cdot 10^{-3}$ | $3.61 \cdot 10^{-3}$ | $3.61 \cdot 10^{-3}$ |
| Grassland (total) averaged over 180 days (µg/kgwwt) | $3.61 \cdot 10^{-3}$ | 0.504 | $3.61 \cdot 10^{-3}$ | $3.61 \cdot 10^{-3}$ |
| Pore water of agricultural soil (µg /l) | $1.63 \cdot 10^{-3}$ | $1.63 \cdot 10^{-3}$ | $1.63 \cdot 10^{-3}$ | $1.63 \cdot 10^{-3}$ |
| Pore water of grassland (µg /l) | $1.63 \cdot 10^{-3}$ | $1.63 \cdot 10^{-3}$ | $1.63 \cdot 10^{-3}$ | $1.63 \cdot 10^{-3}$ |

Regarding the atmosphere, no emissions to air are reported by industry and therefore only releases from WWTP are considered to obtain $C_{local,air}$, except for site B, where there is not a WWTP. The regional PEC is then taken as a background concentration and added to the local concentration to give the $PEC_{local}$.

Table 3.5 PEClocal calculated for atmosphere

| Site | Annual average total deposition flux (µg/m²/d) | $C_{local,air}$ (µg /m³) | $PEC_{local,air}$ (µg /m³) |
|---|---|---|---|
| A | $6.5 \cdot 10^{-6}$ | $4.15 \cdot 10^{-6}$ | $6.16 \cdot 10^{-6}$ |
| B | 0 | 0 | $2.75 \cdot 10^{-6}$ |
| D | $1.89 \cdot 10^{-5}$ | $1.2 \cdot 10^{-5}$ | $1.26 \cdot 10^{-5}$ |
| E | $5.95 \cdot 10^{-7}$ | $3.8 \cdot 10^{-7}$ | $3.07 \cdot 10^{-6}$ |

Finally, the calculations of PECs at a regional and continental scale were done using the EUSES model and the values obtained for the worst-case scenario of a regional production of 2,4-dinitrotoluene of 304,000 tonnes and the associated emissions, estimated with default values from EUSES.

Table 3.6: Regional and continental PEC

| Compartment | Regional | Continental |
|---|---|---|
| Surface water (total) (μg/l) | 0.0178 | $2.22 \cdot 10^{-4}$ |
| Surface water (dissolved) (μg/l) | 0.0178 | $2.22 \cdot 10^{-4}$ |
| Air (μg/l) | $2.75 \cdot 10^{-6}$ | $9.14 \cdot 10^{-8}$ |
| Agricultural soil (total) (μg/kg wet wt) | $2.4 \cdot 10^{-3}$ | $6.65 \cdot 10^{-5}$ |
| Pore water of agricultural soils (μg/l) | $1.09 \cdot 10^{-3}$ | $3.01 \cdot 10^{-5}$ |
| Natural soil (total) (μg/kg wet wt) | $3.6 \cdot 10^{-3}$ | $1.2 \cdot 10^{-4}$ |
| Industrial soil (total) (μg/kg wet wt) | 3.21 | 0.0241 |
| Sediment (total) (μg/kg wet wt) | 0.0542 | $7 \cdot 10^{-4}$ |

## 3.2 EFFECTS ASSESSMENT

Aquatic compartment (incl. sediment)

The provided information includes a set of data on the toxicity of 2,4-dinitrotoluene only for fresh water organisms. No information has been provided regarding toxicity on marine organisms.

Regarding the aquatic vertebrates there are available acute and long-term data. Lowest data validated for the assessment is a 90d-LOEC for fry growth of *Oncorhynchus mykiss* minor than 0.05 mg/l, which derivates a NOEC < 0.025 mg/l for fry growth. In relation to the aquatic invertebrates a 21d-NOEC for reproduction of *Daphnia magna* of 0.02 mg/l has been selected for the assessment. In the case of algae, a 8d-Toxicity threshold of 0.13 mg/l on *M. aeruginosa* has been validated. This value is considered a NOEC but although it was divided by a factor of 2, to extrapolate the LOEC to a NOEC equal to 0.065 mg/l, it would not be either the most sensitive group. This value is in the same order of magnitude as the rest of the other aquatic groups.

There are long-term information on aquatic vertebrates, invertebrate and algae. The 21d-NOEC for reproduction on *Daphnia magna*, has been selected for the PNEC derivation. This value, which is more reliable than the 90d-LOEC for fry grown of *O. mykiss*, is quite similar to the concentration validated for the aquatic vertebrates. Therefore, according to the TGD, an assessment factor of 10 is applied: $PNEC_{aquatic\ organisms}$ = lowest end chronic toxicity range / 10 = 0.02 /10 = 2 μg/l. **$PNEC_{aquatic\ organisms}$ = 2 μg/l**.

For the estimation of the PNEC for the marine compartment following points have been taken into account: Chronic toxicity data for fresh water compartment are similar when comparing fish to aquatic invertebrates; regarding aquatic invertebrates acute toxicity data are similar among different species, and, toxicity information on marine fish is included in the same range of acute and chronic toxicity for fresh water fish.

In rapporteur's opinion, the sound PNEC for the seawater organisms can not be derived appropriately with the available information. However, following the agreement adopted by the TC NES II 2004, the PNEC for the marine environment has been derived according to the Technical Guidance Document, by applying an assessment factor of 100 to the lowest chronic toxicity data for fresh water. $PNEC_{sea\_water}$ < lowest end chronic toxicity range / 100 = 0.02 /100 = 0.2 μg/l. **$PNEC_{sea\_water}$ = 0.2 μg/l.**

No data have been provided regarding toxicity on freshwater nor marine sediment organisms. Taken into account the lack of data, and according to the Technical Guidance Document, the equilibrium partitioning method can be applied as a conservative calculation method to identify a potential risk to the soil compartment. Thus, the $PNEC_{sed}$ has been calculated using the equilibrium partitioning method with the PNEC for aquatic organisms (where $K_{susp-water}$ = suspended sediment-water coefficient = 3.87 $m^3/m^3$ for 2,4-dinitrotoluene; $RHO_{susp}$ = bulk density of suspended sediment = 1150 $kg/m^3$). $PNEC_{sed}$ = 3.87 $m^3/m^3$ x 2 µg/l x 1000 /1150 $kg/m^3$ = 6.73 µg/kg. **$PNEC_{sed}$ = 6.73 µg/kg ww**.

Following the same approach the PNEC for the marine sediment using the equilibrium partitioning method: **$PNEC_{marine\_sed}$ = 0.673 µg/kg ww.**

A datum on *U. parduczi* has been used for calculations. The toxicity threshold of 0.55 mg/l is an $EC_5$. This effect percentile is within the range of variation of controls, and so it has been considered as a NOEC. According to TGD an assessment factor of 1 is applied: $PNEC_{microorganisms}$ = cell reproduction / 1= 0.55 / 1 = 0.55 mg/l. **$PNEC_{microorganisms}$ = 0.55 mg/l.**

Terrestrial compartment

The provided information includes a set of data on the toxicity on 2,4-dinitrotoluene on terrestrial compartment, including plants, soil invertebrates and soil microorganisms.

Taking into account the existing information, it has been selected a 14d-$EC_{50}$ of 4.9 mg/kg dw on the tomato *Lipersicum esculentum*. It has also been provided information on acute toxicity on earthworms ($LC_{50}$ =497-584 mg/kg dw). Therefore, accounting the information provided, and according to the TGD an assessment factor of 1000 is applied: $PNEC_{soil\ organisms}$= lowest end acute toxicity range / 1000 = 4.9 /1000 = 4.9 µg/kg. **$PNEC_{soil\ organisms}$ = 4.9 µg/kg.**

It has also been presented some information regarding hydroponic toxicity tests on plants, following the same approach, it has been selected a lowest $EC_{50}$ of 2.1 mg/l, resulting a PNEC of 2.1µg/l that has been compared with the $PEC_{pore-water}$. Following the same approach: $PNEC_{terrestrial\ plants\ pore-water}$= lowest end acute toxicity range / 1000 = 2.1 /1000 = 2.1 µg/l. **$PNEC_{terrestrial\ plants\ pore-water}$ = 2.1 µg/l.**

Taken into account the lack of data, and according to the Technical Guidance Document, the equilibrium partitioning method can be applied as a conservative calculation method to identify a potential risk to the soil compartment. Thus, the PNEC has been calculated using the equilibrium partitioning method with the PNEC for aquatic organisms (where: $K_{soil-water}$ = soil-water partition coefficient = 3.76 $m^3/m^3$ for 2,4-dinitrotoluene; $RHO_{soil}$ = bulk density of wet soil = 1700 $kg/m^3$). $PNEC_{soil}$ = 3.76 $m^3/m^3$ x 2.5 µg/l x 1000 / 1700 $kg/m^3$ = 5.52 µg/kg. **$PNEC_{soil}$ = 5.52 µg/kg.**

Atmosphere

No information is available for 2,4-dinitrotoluene to plants and other organisms exposed via air. The very low vapour pressure of the substance means that volatilisation to the atmosphere is likely to be limited and the resulting concentrations are likely to be very low. This means that the possibility of 2,4-dinitrotoluene contributing to atmospheric effects such as global warming, ozone depletion and acid rain are likely to be very small.

Secondary poisoning

According to the low bioaccumulation potential and the rapid elimination of this compound in fish and mammals, no biomagnification potential in top predators is expected from this substance. The possibility of high exposure concentrations in invertebrates feeding on contaminated algae has been considered. Nevertheless, the elimination rate in invertebrates is still enough for assuming low risk of secondary poisoning.

## 3.3 RISK CHARACTERISATION

Aquatic compartment (incl. STP and sediment)

Local, regional and continental PECs for aquatic and sediment compartments have been compared with the $PNEC_{aquatic}$ = 2 µg/l, derived from available information, and the $PNEC_{sediment}$ = 6.73 µg/kg ww, calculated using the equilibrium partitioning method according to the TGD. For the assessment of the Sewage Treatment Plants, a $PNEC_{microorganisms}$ of 550 µg/l, will be used. The $PNEC_{sea\_water}$ has been estimated as 0. 2 µg/l and the $PNEC_{marine\_seds}$ = 0.673 µg/kg ww. PEC/PNEC assessments are summarized in Table 3.7:

Table 3.7 Risk characterisation for aquatic compartment. Site-specific (sites A, B and D) local assessments for production and processing; assessment for formulation (site E); regional and continental assessments.

| | SITE | $PEC/PNEC_{surface\ water}$ | $PEC/PNEC_{STP}$ | $PEC/PNEC_{sediment}$ | $PEC/PNEC_{sea\ water}$ | $PEC/PNEC_{marine\ sediment}$ |
|---|---|---|---|---|---|---|
| LOCAL COMPARTMENT | A | 0.181 | $6.29 \cdot 10^{-3}$ | 0.18 | 0.3 | 0.3 |
| | B | 2.64 | - | 2.64 | | |
| | D | 0.011 | 0.01 | 0.011 | | |
| | E | 0.071 | 0.05 | 0.07 | | |

| REGIONAL COMPARTMENT | PEC/PNEC | CONTINENTAL COMPARTMENT | PEC/PNEC |
|---|---|---|---|
| Surface water (total) (µg/l) | 0.0089 | Surface water (total) (µg/l) | $1.11 \cdot 10^{-4}$ |
| Surface water (dissolved) (µg/l) | 0.0089 | Surface water (dissolved) (µg/l) | $1.11 \cdot 10^{-4}$ |
| Sediment (total) (µg/kg wet wt) | 0.008 | Sediment (total) (µg/kg wet wt) | $1.04 \cdot 10^{-4}$ |

Conclusions to the risk assessment for the aquatic compartment (including STP and sediments):

Conclusion (ii) applies to the aquatic and sediment compartment at continental and regional level and for sites A, D and E.

**Conclusion (ii)** applies to the marine compartment.

Conclusion (ii) applies to the STP compartment.

**Conclusion (iii)** applies to the need of risk reduction measures for the aquatic compartment and for sediment-dwelling organisms at local level site B. It is expected that any risk reduction measure for surface water would also reduce the risks for sediments.

Terrestrial compartment

Local, regional and continental PECs for the terrestrial compartment have been compared with a $PNEC_{soil}$ of 4.9 µg/kg. The pore water concentration will be compared with the PNEC for terrestrial plants exposed from pore water obtained from the hydroponic tests (2.1 µg/l)

Table 3.8 Predicted levels in terrestrial compartment

| LOCAL COMPARTMENT | SITE | PEC/PNEC |
|---|---|---|
| Agricultural soil (total) averaged over 30 days (µg/kg wet wt) | A | $7.37 \cdot 10^{-4}$ |
| | B | $7.37 \cdot 10^{-4}$ |
| | D | $7.37 \cdot 10^{-4}$ |
| | E | $7.37 \cdot 10^{-4}$ |
| Agricultural soil (total) averaged over 180 days (µg/kg wet wt) | A | $7.37 \cdot 10^{-4}$ |
| | B | $7.37 \cdot 10^{-4}$ |
| | D | $7.37 \cdot 10^{-4}$ |
| | E | $7.37 \cdot 10^{-4}$ |
| Grassland (total) averaged over 180 days (µg/kg wet wt) | A | $7.37 \cdot 10^{-4}$ |
| | B | $7.37 \cdot 10^{-4}$ |
| | D | $7.37 \cdot 10^{-4}$ |
| | E | $7.37 \cdot 10^{-4}$ |

Table 3.9 Risk characterisation for terrestrial plants exposed from pore water

| LOCAL COMPARTMENT | SITE | PEC/PNEC |
|---|---|---|
| Pore water of agricultural soil | A | $7.76 \cdot 10^{-3}$ |
| | B | $7.76 \cdot 10^{-3}$ |
| | D | $7.76 \cdot 10^{-3}$ |
| | E | $7.76 \cdot 10^{-3}$ |
| Pore water of grassland | A | $7.76 \cdot 10^{-3}$ |
| | B | $7.76 \cdot 10^{-3}$ |
| | D | $7.76 \cdot 10^{-3}$ |
| | E | $7.76 \cdot 10^{-3}$ |

| REGIONAL COMPARTMENT | PEC/PNEC |
|---|---|
| Agricultural soil (total) (μg/kg wet wt) | $4.89 \cdot 10^{-4}$ |
| Natural soil (total) (μg/kg wet wt) | $7.34 \cdot 10^{-4}$ |
| Industrial soil (total) (μg/kg wet wt) | 0.65 |
| Pore water of agricultural soil | $5.2 \cdot 10^{-4}$ |
| **CONTINENTAL COMPARTMENT** | **PEC/PNEC** |
| Agricultural soil (total) (μg/kg wet wt) | $1.36 \cdot 10^{-4}$ |
| Natural soil (total) (μg/kg wet wt) | $2.45 \cdot 10^{-5}$ |
| Industrial soil (total) (μg/kg wet wt) | 0.005 |
| Pore water of agricultural soil | $1.43 \cdot 10^{-5}$ |

Conclusions to the risk assessment for the terrestrial compartment:

**Conclusion (ii)** applies to the terrestrial compartment.

Atmosphere

No effects on the atmosphere are likely in the regional and continental scenarios, because of the low predicted environmental concentrations of 2,4-dinitrotoluene.

Table 3.10 Predicted levels in air compartment

| Scale | | PEC (μg/m³) |
|---|---|---|
| Local | Site A | $6.16 \cdot 10^{-6}$ |
| | Site B | $2.75 \cdot 10^{-6}$ |
| | Site D | $1.26 \cdot 10^{-5}$ |
| | Site E | $3.07 \cdot 10^{-6}$ |
| Regional | | $2.75 \cdot 10^{-6}$ |
| Continental | | $9.14 \cdot 10^{-8}$ |

Conclusions to the risk assessment for the atmosphere:

**Conclusion** (**ii)** applies to the atmospheric compartment.

Secondary poisoning

According to the low bioaccumulation potential and the rapid elimination of this compound in fish and mammals, a low risk for secondary poisoning on birds and mammals is expected from this substance.

Conclusions to the risk assessment for secondary poisoning:

**Conclusion** (**ii)** applies to the secondary poisoning.

# 4 HUMAN HEALTH

## 4.1 HUMAN HEALTH (TOXICITY)

### 4.1.1 Exposure assessment

Occupational exposure

2,4-dinitrotoluene is an orange-yellow crystalline solid with a very low vapour pressure.
It is commercially available as a purified isomer or as a component of DNT mixtures.
Three scenarios have been considered:

Scenario 1: Production and further processing of 2,4–DNT.
2,4-dinitrotoluene is produced in Europe in closed system as a captive intermediate for obtaining TDA (toluene-2,4-diamine) and TDI (toluene diisocyanate). Exposure is expected in those activities where the system is breached. The 95$^{th}$ percentile of 0.009 mg/m$^3$ derived from a quality dataset is considered as the reasonable worst case inhalation exposure value. Typical exposure values can be in the order of 0.0007 mg/m$^3$ (the 50$^{th}$ percentile). A reasonable worst case short-term value of twice as high as the full shift value is assumed: up to 0.018 mg/m$^3$. Dermal exposure is estimated by EASE as 0 - 2.1 mg/day. The upper value, considered as the reasonable worst case, seems an overestimation compared with limited reliability dermal exposure data available from the literature.

Scenario 2: Explosives Manufacture.
Small amounts of pure 2,4- DNT is imported by the explosives industry in order to use it as an additive (less than 1%) in the production of explosives (solid gunpowder formulations). Workers exposure would mainly occur during tasks such as weighing and charging.
For inhalation exposure, a reasonable worst case of 0.15 mg/m$^3$ has been estimated by EASE. Due to limited information dilution ventilation was chosen as the pattern of control. A reasonable worst case short-term value of twice as high as the full shift value is assumed: up to 0.3 mg/m$^3$. Typical exposure is assessed as half the reasonable worst case or 0.075 mg/m$^3$.

According with RISKOFDERM model, the reasonable worst case potential dermal exposure to hands is estimated to be 3 mg/day. The assessment has been carried out without taking into account the use of PPE. When PPE is used in accordance with Directive 89/656/EEC (this is, in fact, obligatory around the EU) dermal exposure will be reduced considerably.

Scenario 3: Use of explosives.
The recipients of the explosive formulation are companies dedicated to the manufacture of cartridges and munitions. Automatic filling devices are used. Potential for exposure could only be possible during charging of the system.

For inhalation exposure, a reasonable worst case of 0.006 mg/m$^3$ has been estimated by EASE. Due to limited information dilution ventilation was chosen as the pattern of control. A reasonable worst case short-term value of twice as high as the full shift value is assumed: up to 0.012 mg/m$^3$. Typical exposure is assessed as half the reasonable worst case or 0.003 mg/m$^3$.

Dermal exposure has been estimated by EASE as 0.42-4.2 mg/day. The upper value is considered as the RWC exposure level. The assessment has been carried out without taking

into account the use of PPE. When PPE is used in accordance with Directive 89/656/EEC (this is, in fact, obligatory around the EU), dermal exposure will be considerably reduced.

Consumer exposure

2,4-Dinitrotoluene is primarily used as a chemical intermediate in the production of toluene diisocyanate. This application uses about 99% of 2,4-dinitrotoluene production. Other minor uses are as gelatinizing-plasticizing agent in both commercial and military explosive compositions.

There is no information about 2,4-dinitrotoluene in consumer products. Nevertheless, according to Directive 2003/34/EC, since 15 January 2005, the use of this substance and preparations containing it should not be placed on the market for use by the general public.

Humans exposed via the environment

Indirect exposure via the environment is calculated using data for oral intake via food, drinking water and air for local (A, B, D and E sites) and regional scales. The resultant daily doses, for the uptake of 2,4-dinitrotoluene, are in the table below. These values have been obtained with EUSES, using the environmental concentrations calculated before for the local and regional scenarios and a standard consumption pattern of food.

Table 4.1: Daily Human Doses (mg/kg/d)

| Intake route | Local site A | Local site B | Local site D | Local site E | Regional |
|---|---|---|---|---|---|
| Drinking water | $8.62 \cdot 10^{-6}$ | $1.24 \cdot 10^{-4}$ | $6.46 \cdot 10^{-7}$ | $3.43 \cdot 10^{-6}$ | $5.1 \cdot 10^{-7}$ |
| Fish | $4.77 \cdot 10^{-6}$ | $6.87 \cdot 10^{-5}$ | $3.57 \cdot 10^{-7}$ | $2 \cdot 10^{-6}$ | $2.8 \cdot 10^{-7}$ |
| Leaf crops | $1.24 \cdot 10^{-7}$ | $7.71 \cdot 10^{-8}$ | $2.12 \cdot 10^{-7}$ | $8.15 \cdot 10^{-8}$ | $6.4 \cdot 10^{-8}$ |
| Root crops | $1.8 \cdot 10^{-8}$ | $1.8 \cdot 10^{-8}$ | $1.81 \cdot 10^{-8}$ | $1.8 \cdot 10^{-8}$ | $1.2 \cdot 10^{-8}$ |
| Meat | $1.76 \cdot 10^{-10}$ | $2.47 \cdot 10^{-9}$ | $2.15 \cdot 10^{-11}$ | $7.15 \cdot 10^{-11}$ | $1.3 \cdot 10^{-11}$ |
| Milk | $1.09 \cdot 10^{-9}$ | $1.52 \cdot 10^{-8}$ | $1.33 \cdot 10^{-10}$ | $4.41 \cdot 10^{-10}$ | $7.9 \cdot 10^{-11}$ |
| Air | $1.76 \cdot 10^{-9}$ | $5.89 \cdot 10^{-10}$ | $3.61 \cdot 10^{-9}$ | $8.76 \cdot 10^{-10}$ | $5.9 \cdot 10^{-10}$ |
| Total | $1.35 \cdot 10^{-5}$ | $1.93 \cdot 10^{-4}$ | $1.24 \cdot 10^{-6}$ | $5.53 \cdot 10^{-6}$ | $8.7 \cdot 10^{-7}$ |

Combined exposure

Exposure to 2,4-dinitrotoluene may reasonably be predicted to arise as a result of combined exposure from workplace and environmental sources.

### 4.1.2 Effects assessment

Toxicokinetics, metabolism and distribution

Data on toxicokinetics of 2,4-DNT (2,4-dinitrotoluene) in humans are limited to three *in vivo* studies, based on analyses of the urinary metabolites of workers exposed to technical-grade DNT in DNT production plants, and two *in vitro* studies that investigated the metabolism of 2,4-DNT by the human intestinal microflora. However, a lot of studies following oral administration have been performed in experimental animals, mainly in rats; *in vitro* experiments provided additional information.

The most important information for determining the oral absorption derives from studies carried out in rats, which showed that 2,4-DNT is rapidly absorbed after a single administration, the absorption is complete within 24 hours; radioactivity level in blood reaches a peak at 6 h and gradually declines over the first 9 h; its half-life in plasma is about 22 h; the urine is the major route of excretion for 2,4-DNT but the bile is relevant; metabolites excreted in bile can be absorbed from the intestine (enterohepatic cycling); and there are no major differences between sexes, dose groups or feeding periods. On the basis of this information, the oral absorption was considered to be 100% within 24 hours in rats and by extrapolation, regarding studies done on other species, 100% in rabbits, dogs and monkeys. For humans the worst case was assumed and oral absorption of 2,4-DNT was considered as 100% too.

No data are available for inhalation exposure route. Therefore, based on oral absorption data, the worst case inhalation absorption (i.e. 100%) should be assumed for both animals and humans.

No data are available for dermal exposure route. However, the absorption of 2,6-DNT was considered to be nearly 100% by both oral and inhalation routes of administration, and 5-7% following dermal application. The isomers 2,4- and 2,6-DNT have identical molecular weights and show nearly identical physical chemical properties. Therefore, it seems appropriate to extrapolate 2,4-DNT dermal absorption from that of 2,6-DNT. Moreover, the US-EPA computer model Dermwin v1.42, computed nearly identical results for the dermal penetration rates of the two isomers. Thus, a dermal penetration rate of 10% of 2,4-DNT is considered acceptable for both animals and humans.

No studies were located regarding distribution in experimental animals following inhalation or dermal exposure to 2,4-DNT. However, the distribution of 2,4-DNT and its metabolites was determined based on data from tissue distribution and toxicity studies carried out in experimental animals following oral administration.

Once absorbed 2,4-DNT and its metabolites are well distributed in all animal species showing a similar pattern of distribution with radioactivity concentrated in liver and kidneys. There is not sufficient evidence to warrant accumulation in these organs. Considering information from the toxicodynamic studies, it appears to be some suggestive evidence of common target organs following exposure of animals and humans by different routes (oral in animals; inhalatory and dermal in humans). Correlation of toxic effects between humans and animals with regard to hematologic and neurological effects has been noted but insufficient data are available to state definitively whether the other toxic effects observed in animals would also occur in humans. Furthermore, qualitative similarities in metabolism of humans and animals have been noted. Since for systemic effects it is assumed that the tissue distribution pattern will be the same irrespective of the administration route, the distribution pattern of 2,4-DNT

for animals orally exposed is considered applicable for both humans and animals exposed by dermal route and inhalation.

The routes of excretion were similar in rats, rabbits, dogs and monkeys, with the predominant route being via urine. By 24 hours after a single oral dose, the radioactivity recovered in the urine was 75-81%, and in less extension in faeces (3-9%); no radioactivity was found in the expired air.

Several metabolites have been identified in the urine of rats. The major urinary metabolite was 2,4-dinitrobenzyl alcohol glucuronide; 2,4-dinitrobenzoic acid, 4-(N-acetyl)amino-2-nitrobenzoic acid, 2-amino-4-nitrobenzoic acid, 4-(N-acetyl)amino-2-aminobenzoic acid, 4-amino-2-nitrobenzoic acid, 2-4-dinitrobenzyl alcohol, and 4-amino-2-nitrotoluene were also detected among others. The metabolite profile following a single oral radiolabel dose was not altered by feeding 2,4-DNT in the diet and there were no major differences between dose groups, between sexes or between feeding periods.

The bile is an important route of excretion for 2,4-DNT and its metabolites in rats. The major biliary metabolite of 2,4-DNT was 2,4-dinitrobenzyl alcohol glucuronide; 2,4-dinitrobenzoic acid, 4-amino-2-nitro(2-amino-4-nitro)benzyl alcohol sulphate, 2-amino-4-nitrobenzoic acid, 2,4-dinitrobenzaldehyde, 2,4-dinitrobenzyl alcohol, 2-4-diacetylaminobenzoic acid and 2-acetylamino-4-nitrotoluene were also detected.

In rats, sex differences in the excretion of 2,4-DNT metabolites have been observed. For male and female rats similarly treated, the urine excretion predominates in females while in males biliary excretion is the most important route; in addition, females excreted a greater percentage of the dose in the urine as 2,4-dinitrobenzyl alcohol glucuronide than did males. Biliary excretion measured after 24 h was greater for males (25%) than for females (18%), essentially complete within 24 hours for males and 12 hours for females, and there were no significant differences in the rates of biliary excretion between sexes; mean half times of excretion ranged from 3.3 to 5.3 h. Regarding urinary excretion, radioactivity excreted in the urine of rats which bile was not collected (60-90%) was higher than in the urine of rats which bile was collected (20-60%), indicating that biliary metabolites were absorbed from the intestine (enterohepatic cycling). Whether or not bile was collected, females excreted more radioactivity in urine than males.

The gut bacteria are important in the metabolism of 2,4-DNT *in vivo.* The four major metabolites identified in the urine of conventional rats (2,4-dinitrobenzyl alcohol glucuronide, 2,4-dinitrobenzoic acid, 4-(N-acetyl)amino-2-nitrobenzoic acid and 2-amino-4-nitrobenzoic acid) were also present in the urine of axenic rats. However, in axenic rats the amounts of 4-(N-acetyl)amino-2-nitrobenzoic and 2-amino-2-nitrobenzoic excreted in the urine are markedly reduced by comparison with conventional animals. In addition, a less radioactivity is covalently bound to hepatic macromolecules in axenic rats compared with conventional rats.

These observations indicate that the relationship between liver and intestinal microflora in the metabolism of 2,4-DNT is a complex one. The intestinal microflora is apparently an important site for the metabolism of biliary metabolites and metabolism by intestinal microflora appears to be essential for the production of metabolites that bind covalently to liver macromolecules.

The following pathway has been proposed for the bioactivation of 2,4-DNT in the whole animal. After an oral administration, 2,4-DNT is oxidated in the liver to 2,4-dinitrobenzyl alcohol, which undergoes a Phase II reaction being conjugated with glucuronide acid. The glucuronic conjugate can be either eliminated via urine or excreted to bile. The conjugate

excreted in bile is absorbed in intestine where the glucuronic acid is hydrolysed by glucuronidase, yielding again 2,4-dinitrobenzyl alcohol. This benzyl alcohol is further reduced in intestine at position 4, being the generated 4-amino-2-nitrobenzyl alcohol carried out again to the liver. Once in the liver, 4-amino-2-nitrobenzyl alcohol can be conjugated with sulphate at the hydroxyl group. The sulfoconjugated is unstable and quickly decomposes to electrophilic species with high capability to form covalent binding with DNA. Another possible route for bioactivation of 4-amino-2-nitrobenzyl alcohol re-entered in the liver is its oxidation of the amino group to form 4-hydroxylamino-2-nitrobenzyl alcohol. This compound can be the target of reaction of Phase II, especially conjugation with sulphate. The generated molecule is also very unstable and spontaneously changes to other electrophilic species, which can be covalently bound to DNA.

The metabolism and excretion of 2,4-DNT in workers exposed to technical-grade DNT by inhalation and dermal routes has been studied by the analysis of urinary metabolites. The major 2,4-DNT metabolite detected in the urine of workers was 2,4-dinitrobenzoic acid, although lesser amounts of 2-amino-4-nitrobenzoic acid, 2,4-dinitrobenzyl glucuronide, 2-(N-acetyl)amino-4-nitrobenzoic acid and traces of 4-amino-2-nitrobenzoic acid and 4-(N-acetyl)amino-2-nitrobenzoic were also found. In addition, the urine contained unchanged 2,4-DNT.

As seen in rats, female subjects excreted a higher proportion of urinary metabolites as dinitrobenzyl alcohol glucuronides than did males.

The appearance of reduced metabolites suggest either that human hepatic enzymes are capable of reduction of the nitro group of 2,4-DNT or that 2,4-DNT (or its metabolites) gain access to the intestinal microflora which is capable of reduction, after which the metabolites are reabsorbed and excreted into urine. In support of the last suggestion, it was observed that the metabolites produced by incubation of 2,4-DNT with human gut were the same as those produced by analogous samples from rats and mice.

The half-life for excretion of 2,4-DNT metabolites in urine of workers ranged from 0.8 to 4.5 hours. The half-lives for 2,4-dinitrobenzoic acid and 2,4-dinitrobenzyl alcohol glucuronide tended to be shorter than those for the metabolites that resulted from both oxidative and reductive. The highest rates of excretion of 2,4-dinitrobenzoic acid occurred near the end of the work shift. The half-life for urinary excretion of 2,4-dinitrobenzoic acid was calculated to be 2-5 hours. This estimate appears to be the initial phase of a biphasic elimination profile since even 3 days after the exposure, detectable levels of 2,4-dinitrobenzoic acid were present in urine. These data support that enterohepatic recycling occurred in humans.

In summary, 2,4-DNT metabolism and excretion seem to be qualitatively similar in both humans and rats, but the proportions of nitro-reduced metabolites were lower relative to oxidized metabolites in the urine from humans. These differences may be due more to the particular routes of exposure (inhalation and dermal for humans, oral for rats) than differences in species.

There are no studies on whether 2,4-DNT or its metabolites can cross the placenta or be excreted in breast milk, so it cannot be determined if foetuses may be exposed in uterus of if infants may be exposed via breast milk ingestion. There are also no data to show if 2,4-DNT and its metabolites are stored in maternal tissues and thus might be later mobilized during gestation or lactation; however, 2,4-DNT and its metabolites are not likely to be stored because of their low octanol-water partition coefficient.

Acute toxicity

No information is available on the effects of a single exposure to 2,4-dinitrotoluene in humans. However, acute toxicity studies have been carried out in rats, mice and cats.

The rodent $LD_{50}$, following oral administration, ranged from180 to 893 mg/kg b.w. in rats, and from 1,340 to 1,954 mg/kg b.w. in mice. However, most studies on rodent acute oral toxicity have limited quality and do not mention which was the purity of the test substance. In the only study performed according modern test guidelines and where purity (98%) was reported, the acute oral $LD_{50}$ (95% confidence limits) was 568 (434–705) mg/kg b.w. and 650 (520–743) mg/kg b.w. in male and female CD rats, respectively, and 1,954 (1,848–2,178) mg/kg b.w. and 1,340 (1,205–1,500) mg/kg b.w. in male and female albino Swiss mice, respectively. Accordingly, 2,4-DNT should be classified as harmful (Xn; R22) in both rats and mice.

With respect to the dermal route of exposure, the only available data are from a rat acute toxicity study. All rats treated with 2500 mg 2,4-DNT/kg b.w. survived over the 14-day post-exposure observation period without any toxic symptoms. On the basis of these data, 2,4-DNT should not be classified for dermal acute toxicity.

Regarding inhalation there are no available rodent data on acute toxicity. However, it seems reasonable to extrapolate acute toxicity by inhalation from data obtained in oral studies since both oral and inhalation absorption values were estimated to be 100%. Accordingly, 2,4-DNT should be classified as harmful (Xn; R20) in both rats and mice.

Nevertheless, in the cat acute toxicity study carried out with 2,4-DNT (99.9% purity) increased levels on both methaemoglobin and Heinz bodies were observed at 50 mg/kg b.w. but not at 10 mg/kg b.w. following a single oral administration. The increased levels on methaemoglobin were also observed from 20 to 40 mg/kg b.w. when 2,4-DNT was administered i.p. In addition, one of the two cats administered the highest dose either oral or i.p. died.

In rats, mice and dogs, methaemoglobinemia and other haemolytic anaemia related effects were induced by 2,4-DNT following oral repeated dosing. However, these effects were not reported in the acute toxicity studies performed in rats and mice, and there are no acute toxicity studies in dogs available.

It is known that, in general, the rat, mouse, rabbit, guinea pig and monkey seem to be significantly less sensitive to the formation of MetHb than humans and dogs. On the other hand, the cat is known to be particularly sensitive to the formation of MetHb. In consequence, 2,4-DNT poses a hazard to humans which may be either underestimated if based on rodent data or overestimated if based on cat data.

We consider from a precautionary start point that the cat data are appropriate to be used for hazard identification and C&L proposal. Therefore, based on cat data (death following oral administration of 50 mg/kg b.w.) 2,4-DNT is considered to be classified for acute toxicity as toxic by oral exposure. Since oral and inhalation absorption values were estimated to be 100% in experimental animals and humans, it seems reasonable to extrapolate toxicity by inhalation from oral toxicity data and to classify 2,4-DNT as toxic by inhalation. Finally, taking into account that the dermal absorption value was estimated to be 10% in rodent and by extrapolation in experimental animals and humans, 2,4-DNT should be considered as borderline toxic by dermal exposure. Overall, it seems justified to classify 2,4-DNT for acute toxicity as toxic (T; R23/24/25) by inhalation, dermal and oral routes of exposure.

Irritation/Corrosivity

According to available animal studies, 2,4-DNT is considered not irritant for skin and eye, and consequently no corrosive.

Sensitisation

No dermal sensitivity was observed in the Guinea-Pig Maximisation Test. Thus, 2,4-DNT is not classified as sensitising substance in accordance with the EU criteria.

Repeated dose toxicity

The most common adverse health effect in workers exposed to DNT is related to the ability of DNT to induce MetHb, the secondary effects of which were non-specific health effects such as headache, dizziness, nausea and drowsiness. Although an excess of mortality due to ischemic heart disease and residual diseases of the circulatory system have been reported for exposed workers, the usefulness of these data is limited because exposure measurements were not performed. Therefore, information on repeated dose toxicity has been derived from animal data.

Several studies have been investigated the toxicity of 2,4-DNT following repeated oral administration to rats, mice and dogs. Beagle dogs appear to be the most sensitive species of the ones tested for sub-acute and sub-chronic-toxicity of 2,4-DNT. For sub-acute toxicity, 1 mg/kg b.w./day was determined as the LOAEL in males on the basis of increased reticulocytosis observed in the dog 4-week study. However, 1 mg/kg b.w./day was also determined as the NOAEL in males for sub-chronic toxicity on the basis of increased methaemoglobin observed at 5 mg/kg b.w./day in the dog 13-week study. Since a high inter-individual variability was found in both 4-week and 13-week dog studies, the LOAEL of 1 mg/kg b.w./day derived from the dog 4-week study was considered the relevant value for both sub-acute and subchronic toxicity. Regarding chronic toxicity, both dogs and rats appear to be sensitive species to 2,4-DNT toxicity. In Beagle dogs, the NOAEL for males and females was 0.2 mg/kg b.w./day based on neurotoxicity (incoordination ad paralysis) observed in the 24-month study. In CD rats, the LOAEL in males was considered to be 0.57 mg/kg b.w./day on the basis of the hyperplastic foci incidence in the liver and atrophy of seminiferous tubules observed in the 24-month study. Nevertheless, because of the duration of both studies was the same (up to 24 month) the LOAEL of 0.57 mg/kg b.w derived from the rat study was considered the relevant value for chronic toxicity.

Mutagenicity

2,4-DNT is clearly mutagenic in *Salmonella typhimurium* strains both in the presence and absence of a rat liver metabolic activation system. The highest mutagenic activity was observed in strains with elevated levels of both nitroreductase and *O*-acetyltransferase activities, and extracellular nitro reduction was necessary for optimal detection of 2,4-DNT mutagenicity in standard strains. Aminohydroxylamino dimethylazoxybenzenes or aminohydroxylamino dimethylazobenzenes produced either by reduction of hydroxylaminonitrotoluenes or of dimethyl dinitroazoxybenzenes, could be considered the active metabolites responsible for the 2,4-DNT mutagenic activity in bacteria. With respect to DNA damage, 2,4-DNT was genotoxic in the *Salmonella typhimurium umu* test. As occurred in mutation tests, the most sensitive strain was NM3009, which has high *O*-acetyltransferase and nitroreductase activities.

2,4-DNT was not mutagenic in the CHO/HGPRT system, when tested in the presence of rat liver S9 under usual (aerobic) assay conditions. However, it was mutagenic either when CHO cells were incubated with rat liver S9 under anaerobic (reduced oxygen tension) conditions or when primary rat hepatocytes were used as metabolic activation system. In addition, 2,4-DNT was a direct mutagen in the P388 mouse lymphoma/TK system. Results on clastogenicity were contradictory. Thus, in one study, 2,4-DNT of 99% purity did not induce chromosomal aberrations in CHO cells (with or without S9), whereas in two other studies, 2,4-DNT of unknown purity was reported to be clastogenic in human lymphocytes (without S9) and in CHL cells (with and without S9). With respect to DNA damage, 2,4-DNT induced sister chromatid exchanges in CHO cells when tested in the presence of rat liver S9. There was no evidence of unscheduled DNA synthesis in rat (hepatocytes, spermatocytes and spermatids) or human (hepatocytes) cells incubated with 2,4-DNT. This lack of 2,4-DNT genotoxic activity is accord with the concept that reduction by intestinal flora is required in addition to hepatic metabolism for activation. Finally, 2,4-DNT of unknown purity was reported to cause DNA damage (single-strand breaks) when tested at cytotoxic concentrations in the alkaline elution/rat hepatocyte assay.

Dogs administered 2,4-DNT (10 mg/kg b.w./day) in hard gelatine capsules for 2 years had no chromosomal aberrations in their bone marrow or kidney. 2,4-DNT when fed to male and female rats at up to 45 mg/kg b.w./day for 2 years did not induce either chromosomal aberrations in the bone marrow or kidney. Nevertheless, 2,4-DNT when fed to male rats at 93 mg/kg b.w./day for 19 weeks induced chromosomal aberrations (chromatid breaks) in lymphocytes; in addition, significant increases in the number of chromatid breaks were also observed after treatment for 5 and 13 weeks in kidney cultures, being the number of breaks increased with the duration of treatment. With respect to DNA damage, unscheduled DNA synthesis was induced by 2,4-DNT when administered at up to 200 mg/kg by gavage to male rats. DNA covalent binding was observed in several organs (liver, kidney, lung and mammary glands) of rats administered i.p. a single dose of 150 mg/kg of 2,4-DNT, the binding being highest in the liver. Moreover, when rats were administered 2,4-DNT by either gavage or i.p., no differences in liver DNA covalent binding were noted. The sulfotransferase inhibitors decreased the covalent binding to DNA, indicating that sulfation is important in the biotransformation of 2,4-DNT to reactive metabolites.

In *Drosophila melanogaster*, 2,4-DNT induced sex-linked recessive lethal mutation after injection, but failed to induced lethal mutations after feeding and translocations after injection.

2,4-DNT did not induce dominant lethal mutations when administered to rats for 5 days (by gavage) or up to 13 weeks (by feeding). Negative results were also obtained for dominant lethal mutations in mice administered 2,4-DNT for 2 days (by gavage) or up to 13 weeks (by feeding). In addition, 2,4-DNT did not induce sperm abnormalities in mice.

In conclusion, the weight of evidence indicates that 2,4-DNT is an *in vivo* mutagenic agent for somatic cells. Therefore, 2,4-DNT is classified as mutagenic category 3 (Xn, R68).

Carcinogenicity

Regarding carcinogenicity in rats, there are two studies considered adequate: a carcinogenicity study in Fischer 3444 rats and a chronic toxicity study in CD rats. The same signs of carcinogenicity (i.e. skin/subcutaneous tissue fibromas in males, mammary gland fibroadenomas in females and hepatocarcinomas in both sexes) were found in both studies. The dose that produced tumours in females in the chronic study was in the dose-level range of

the carcinogenicity study. On the other hand, the high-dose of the chronic study (34/45 mg/kg b.w./day for males/females) reduced significantly survival and showed a high toxicity. With respect to females, the incidence of mammary gland fibroadenoma in Fischer 344 rats treated with 15.7 mg/kg b.w./day, and CD rats treated from 5.1 mg/kg b.w./day was significantly higher than in controls. In males, significant increased incidences of subcutaneous tissue or skin fibroma were found in CD rats administered 34 mg/kg b.w./day and in Fischer 344 rats treated from 4.7 mg/kg b.w./day. In addition, there was a sporadic occurrence of squamous-cell papillomas, basal-cell carcinoma, fibrosarcomas, and lipomas in Fischer 344 male rats. The incidence of hepatocellular carcinoma in liver of Fischer 344 males treated with 4.7 and 11.8 mg/kg b.w./day for 18 months was higher ($p > 0.05$) than in controls. Despite of the not significant excess, the same tumour occurred with a significant increased incidence in male and female CD rats given 34 and 45 mg/kg b.w./day, respectively. At that dose, most of the treated CD rats showed neoplastic nodules in liver after 12-month treatment

Regarding carcinogenicity in mice, there are two studies considered adequate, one of them being a chronic toxicity study. In the carcinogenicity study, carried out with B6C3F1 mice, no carcinogenic effect was reported. Data of the chronic toxicity study, carried out with CD-1 mice, were considered relevant for carcinogenicity, since renal tumours were observed in males. In males the incidence of kidney tumours (both benign and malignant) was significantly elevated in groups treated with 13.3 and 96.9 mg/kg b.w./day for more than 12 months. In addition, carcinomas in liver of males and females were found at 885 and 911 mg/kg b.w./day respectively for 12 months (1/4 and 1/4, respectively), and in male livers at 885 mg/kg b.w./day for 12 months and allowed to recover for 1 month (2/4) vs. none in controls.

In summary, there is a good evidence of an increase in the tumour incidence in rats and mice. These observations are consistent with genotoxic aetiology, which is consistent with the findings from the genotoxicity studies. In addition, two studies support the hypothesis that occupational exposure to DNT may be carcinogenic. Those studies associated an excess of hepatobiliary cancer and both urothelial cancer and renal cell cancer with jobs where workers were supposedly exposed to purified 2,4-DNT and miners supposedly exposed to technical grade DNT, respectively. Therefore, according to EU criteria, 2,4-DNT is considered carcinogenic category 2 and then classified as T; R45.

Toxicity for reproduction

In sub-acute, sub-chronic and chronic studies, effects on reproduction such as decrease of testis weights, decrease in spermatogenesis or atrophy of the seminiferous tubules have been observed in males from different species (rats, mice, dogs) and these effects are the most commonly reported. After a short term feeding study (5 days) in rats, a decrease of sperm positive females has been reported.

In a rat three generation study, the absence of the $F_2$ parental generation for the group given the high dose (34/45 mg/kg b.w./day) and the few animals mated in the $F_1$ indicated an adverse effect on fertility. Furthermore, a decreased viability index of high-dose $F_{1b}$ generation ($p < 0.05$) was found when compared with controls. None of the three high-dose females from $F_{1b}$ produced second litters.

In the mouse teratogenicity study, pregnant treated dams showed increased incidence of mortality when compared with that of controls. Teratogenic effects have not been observed at tested doses.

The data obtained from workers exposed to 2,4-DNT, although not conclusive, showed the same effects on reproduction as those obtained in sub-acute, sub-chronic and chronic studies in experimental animals, i.e. reduction of sperm counts.

Fertility effects in rats, mice, dogs, and also in humans support the classification of toxic for reproduction category 3 (Xn; R62) according to EU criteria. The LOAEL for impair fertility was considered to be 0.57 mg/kg b.w./day based on atrophy of seminiferous tubules observed in the 24-month rat study.

With respect to developmental toxicity, the effects observed are regarded as a secondary effect due to parental systemic toxicity, therefore, no need of developmental classification according to EU criteria.

### 4.1.3 Risk characterisation

Workers

When considering the risks to human health arising from occupational exposure to 2,4-dinitrotoluene, the key areas of concern are for repeated dose toxicity, carcinogenicity, mutagenicity and toxicity for reproduction (fertility).

The mutagenicity and carcinogenicity effects of 2,4-dinitrotoluene do not allow the identification of a threshold level of exposure below which there would be no risk for the development of these effects in humans. In addition, the life-time cancer risk calculated for workers indicate concern for mutagenicity and carcinogenicity as a consequence of inhalation and dermal exposure for all worker scenarios (1, 2 and 3). Therefore, it is considered that risk reduction measures are required and **conclusion (iii)** applies.

Regarding repeated dose toxicity and toxicity for reproduction (fertility), the calculated MOS are judged not to be enough for one worker scenario (2) as a consequence of inhalation, and for two worker scenarios (2 and 3) as a consequence of dermal exposure. Consequently, **conclusion (iii)** can be derived for inhalation for scenario 2, and for dermal exposure for scenarios 2 and 3.

On the other hand, there is no concern for the remaining end-points: acute toxicity by inhalation and dermal route; irritation/corrosivity for skin and eyes; sensitisation; repeated dose toxicity and toxicity for reproduction (fertility) for two worker scenarios (1 and 3) by inhalation and for one worker scenario (1) by dermal route. Therefore, **conclusion (ii)** applies.

Consumers

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

Humans exposed via the environment

When considering the risks to human health arising from indirect exposure to 2,4-dinitrotoluene via environment the key areas of concern are for mutagenicity and carcinogenicity.

The mutagenicity and carcinogenicity effects of 2,4-dinitrotoluene do not allow the identification of a threshold level of exposure below which there would be no risk for the development of these effects in humans. However, based on the calculated life time cancer

risk, the risk is judged to be tolerable for the regional scale and for local sites (A, D and E). Nevertheless, the calculated life-time cancer risk indicates concern for carcinogenicity and mutagenicity for the local site B. Therefore, **conclusion (iii)** is reached for both carcinogenicity and mutagenicity as a consequence of oral exposure arising from the local site B.

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

Combined exposure

Exposure to 2,4-dinitrotoluene may reasonably be predicted to arise as a result of combined exposure from workplace and environmental sources. The risk to human health under conditions of combined exposure is dominated by occupational exposure.

## 4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

There is no risk of concern in the industry setting, regarding its physico-chemical properties. Adequate safety measures are taken and information is provided on the label and safety data sheet. Therefore, since risk reduction measures already being applied are considered sufficient, conclusion (ii) is reached.

# 5 RESULTS

## 5.1 ENVIRONMENT

Aquatic compartment (incl. STP and sediment)

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

This conclusion applies to the aquatic and sediment compartment at continental and regional level and for sites A, D and E.

This conclusion applies to the marine compartment.

This conclusion (ii) applies to the STP compartment.

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

This conclusion applies to the need of risk reduction measures for the aquatic compartment and for sediment-dwelling organisms for one site at local level (site B). Nevertheless, it is expected that any risk reduction measure for surface water will also reduce the risks for sediments.

Terrestrial compartment

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

This conclusion applies to the terrestrial compartment.

Atmosphere

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

This conclusion applies to the atmospheric compartment.

Secondary poisoning

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

This conclusion applies to the secondary poisoning according to the low bioaccumulation potential and the rapid elimination of this compound in fish and mammals, a low risk for secondary poisoning on birds and mammals is expected from this substance.

## 5.2 HUMAN HEALTH

### 5.2.1 Human health (toxicity)

Workers

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

This conclusion is reached because of:

- concerns for carcinogenicity and mutagenicity as a consequence of inhalation and dermal exposure arising from all worker scenarios.
- concerns for repeated dose toxicity and toxicity for reproduction (fertility) as a consequence of dermal exposure arising from manufacture and use of explosives (worker scenarios 2 and 3).
- concerns for repeated dose toxicity and toxicity for reproduction (fertility) as a consequence of inhalation arising from manufacture of explosives (worker scenario 2).

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

This conclusion applies to acute toxicity by inhalation and dermal route; irritation/corrosivity for skin and eyes; sensitisation; repeated dose toxicity and toxicity for reproduction (fertility) for two worker scenarios (1 and 3) by inhalation and for one worker scenario (1) by dermal route, because these endpoints are of no concern.

Consumers

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

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

Humans exposed via the environment

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

This conclusion is reached because of:

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

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

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

Combined exposure

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

### 5.2.2 Human health (risks from physico-chemical properties)

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

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

European Commission

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**Abstract**

The report provides the summary of the comprehensive risk assessment of the substance 2,4-dinitrotoluene.

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

Part I - Environment
This part of the evaluation considers the emissions and the resulting exposure to the environment in all life cycle steps. Following the exposure assessment, the environmental risk characterisation for each protection goal in the aquatic, terrestrial and atmospheric compartment has been determined.
The environmental risk assessment concludes that there is concern for the aquatic compartment and sediment dwelling organisms at one local site. There is no concern for the atmosphere, the terrestrial ecosystem and micro-organisms in the sewage treatment plant

Part II – Human Health
This part of the evaluation considers the emissions and the resulting exposure to human populations in all life cycle steps. The scenarios for occupational exposure, consumer exposure and humans exposed via the environment have been examined and the possible risks have been identified.
The human health risk assessment concludes that there is concern for workers with regard to mutagenicity and carcinogenicity at all worker scenarios and with regard to repeated dose toxicity and toxicity for reproduction (fertility) from manufacture and use of explosives. There is also concern for humans exposed via the environment with regard to carcinogenicity and mutagenicity from one local site. For consumers and for human health (physico-chemical properties) there is no concern.

The conclusions of this report lead to risk reduction measures to be proposed by the Commission's committee on risk reduction strategies set up in support of Council Regulation (EEC) N. 793/93.

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