FOREWORD INTRODUCTION

Dinitrotoluene (isomers mixture)

CAS N°: 25321-14-6

SIDS Initial Assessment Report

For

SIAM 18

Paris, France, April 20 – 23, 2004

1. Chemical Name:	Dinitrotoluene (isomers mixture)
2. CAS Number:	25321-14-6
3. Sponsor Country:	Contact Point: BMU (Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit) Contact person: Prof. Dr. Ulrich Schlottmann Postfach 12 06 29 D- 53048 Bonn-Bad Godesberg
4. Shared Partnership with:	
5. Roles/Responsibilities of the Partners:	
• Name of industry sponsor /consortium	Bayer AG, Germany Contact person: Dr. Burkhardt Stock D-51368 Leverkusen Gebäude 9115
• Process used	OECD/ICCA - The BUA Peer Review Process: see next page
6. Sponsorship History	
• How was the chemical or category brought into the OECD HPV Chemicals Programme ?	by ICCA-Initiative
7. Review Process Prior to the SIAM:	last literature search (update): 30 July 2003 (Human Health): databases medline, toxline; search profile CAS-No. and special search terms 14 October 2003 (Ecotoxicology): databases CA, biosis; search profile CAS-No. And special search terms
8. Quality check process:	As basis for the SIDS-Dossier the IUCLID was used. All data have been checked and validated by BUA.
9. Date of Submission:	Deadline for circulation: 23 January 2004
10. Date of last Update:	Last literature search: IUCLID Chapters 1-2: 2002-07-19, Chapters 3-4: 2003-07-01, Chapter 5: 2003-04-01

11. Comments:

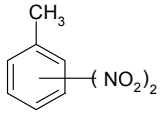
OECD/ICCA - The BUA* Peer Review Process

Qualified BUA personnel (toxicologists, ecotoxicologists) perform a quality control on the full SIDS dossier submitted by industry. This quality control process follows internal BUA guidelines/instructions for the OECD/ICCA peer review process and includes:

- a full (or update) literature search to verify completeness of data provided by industry in the IUCLID/HEDSET
- Review of data and assessment of the quality of data
- Review of data evaluation
- Check of adequacy of selection process for key studies for OECD endpoints, and, where relevant, for non-OECD endpoints by checking original reports/publications
- Review of key study description according robust summaries requirements; completeness and correctness is checked against original reports/publications (if original reports are missing: reliability (4), i.e. reliability not assignable)
- Review of validity of structure-activity relationships
- Review of full SIDS dossier (including SIAR, SIAP and proposal for conclusion and recommendation for further work)
- In case of data gaps, review of testing plan or rationale for not testing

* BUA (GDCh-Beratergremium für Altstoffe): Advisory Committee on Existing Chemicals of the Association of German Chemists (GDCh)

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	25321-14-6
Chemical Name	Dinitrotoluene (isomers mixture)
Structural Formula	

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

Dinitrotoluene (DNT) is a technical mixture containing approximately 80% 2,4-dinitrotoluene, appr. 20% 2,6-dinitrotoluene and < 5% 3,4-, 2,3- and 2,5-DNT. The toxicological profile of the DNT mixture well reflects the properties of the pure main isomers. Data on the pure 2,4- and 2,6-isomers are presented here only if they provide relevant additional information.

In humans Dinitrotoluene (DNT, technical grade) is absorbed following dermal and inhalative exposure and is rapidly metabolized and excreted in urine.

There are no acute inhalation studies on technical grade DNT and the 2,4-isomer available. LC_{50} of the 2,6-isomer, is reported to be 0.36 mg/l, however, this isomer accounts only for about 18% of technical grade DNT. No acute dermal studies on technical grade DNT and the 2,6-isomer are available. The acute dermal toxicity of 2,4-DNT, the main component of technical grade DNT, is relatively low with an LD_{50} greater than 2500 mg/kg bw in rats. Technical grade DNT is moderately toxic following oral administration to rats, with LD_{50} values of 268 to 660 mg/kg bw reported. After a 24-hour occlusive application DNT is not irritating to the skin (interior side of the ear) of rabbits. Although DNT has been reported to induce slight irritation to the eye of one rabbit, the effect is reversible within 7 days, and therefore DNT is not considered to be an eye irritant in humans.

There are no data available to evaluate the sensitizing potential of technical grade DNT. The 2,4-DNT isomer showed no sensitizing properties in a guinea pig maximization test, whereas 2,6-DNT gave a mild positive response. Patch tests or photo-patch tests in 10 or 5 healthy humans showed no allergic potential of DNT (unspecified isomer), whereas a single case of positive photo-patch test reaction was reported for a worker with skin problems.

There are no inhalative or dermal repeated dose studies on technical grade DNT or on the 2,4-/2,6-DNT isomers available. Chronic feeding of technical grade DNT to rats led to hematological changes (especially methemoglobinemia), and toxicity to liver, kidney, adrenal glands and testes in rats. At the lowest administered dose of 3.5 mg/kg bw/day signs of hepatotoxicity became obvious. No NOAEL can be derived for repeated dose toxicity.

Technical grade DNT is mutagenic in bacterial test systems in the presence and absence of metabolic activation, but it shows no mutagenic or genotoxic activity in mammalian cells *in vitro*. Technical grade DNT shows no mutagenic activity in the mouse bone marrow micronucleus assay and in mouse dominant lethal and spot tests. However, a distinct activity of DNT to induce DNA repair in the liver of rats is reported. Additionally, DNA binding properties in various rat organs, mainly rat liver were demonstrated for 2,4-DNT and 2,6-DNT isomers. Gut flora may play an important role in activation of DNT to reactive metabolites. Overall, technical grade DNT shows the potential to induce genotoxic changes *in vivo*.

Technical grade DNT shows hepatocarcinogenic properties in rats. In a long-term feeding study liver tumors were dose dependently induced in male rats from the lowest administered dose (3.5 mg/kg bw/day) and in female rats from 14 mg/kg bw/day. In an initiation-promotion liver foci assay DNT showed tumor promoting activity, however, only weak initiating properties. The pure 2,4- and 2,6-DNT isomers also induced liver tumors in rats. Additionally, the 2,4-isomer was shown to induce tumor formation in the renal tubular epithelium of male mice. After long-term feeding of technical grade DNT to rats (104 weeks) increased incidences of abnormally small testes and increased

ovary weights were observed at 14 mg/kg bw/day. A daily dose of 35 mg/kg bw DNT led to testicular degeneration and hypospermatogenesis after 52 weeks of exposure. The NOAEL for changes on reproductive organs was 3.5 mg/kg bw/day in this study. Technical grade DNT did not negatively affect fertility in dominant lethal assays. Overall, impairment of male rat fertility after chronic exposure of toxic doses cannot be excluded.

In pregnant rats, administration of technical grade DNT by gavage on gestation days 7-20 did not induce teratogenic/developmental effects even at dose levels, which produced significant maternal toxicity. The NOAEL for teratogenic/developmental toxicity can be determined to be 150 mg/kg bw/day.

In humans, heavy DNT exposure causes signs of methemoglobinemia, which are reversible 2-3 days after removal from exposure. Signs of disturbances in liver function and exposure-dependent nephrotoxic effects directed to the tubular system were additionally found in exposed workers. Single findings in studies without reliable exposure data and/or only small numbers of significantly exposed workers indicating increased incidences of hepatobiliary or urothelial cancer in occupationally DNT exposed workers do not permit a conclusion on the carcinogenicity of DNT in humans. Preliminary observations pointing to an increased risk of ischemic heart disease or to an adverse effect on the human male reproductive system could not be confirmed by further studies.

Environment

Dinitrotoluene (DNT) is a technical mixture containing approximately 80 % 2,4-dinitrotoluene (121-14-2) and 20 % 2,6-dinitrotoluene (606-20-2). To a lower content also the isomers 2,3-DNT (1.3 %), 2,5-DNT (0.5 %) and 3,4-DNT (2.4 %) are contained in the technical mixture. DNT is an orange-yellow substance of characteristic odour with a melting point of 56 - 59°C and boiling point of 250 °C. With a density of 1.52 g/cm³ at 15 °C DNT is heavier than water. The water solubility was determined to 166 mg/l for 2,4-DNT and 145 mg/l for 2,6-DNT at 25 °C. A vapour pressure of 0.016 Pa for 2,4-DNT and of 0.032 Pa for 2,6-DNT was measured at 25 °C. For the isomers mixture a log Kow of 2.00 was calculated.

In the atmosphere DNT is degraded by photochemically produced OH radicals. The half-life is calculated to be ca. 84 days. In surface waters, from photodegradation measurements a half-life of 1 day was derived for (predominantly) direct photolysis under the radiative conditions of latitude 40 °N. In surface waters, with regard to the geographical conditions in Germany and the low light intensity in natural water bodies, the half-life of 2,4-DNT for direct photolysis is calculated to be 20 days in a natural water body (surface layer: 6.5 days).

DNT is not expected to hydrolyze in the environment due to the lack of hydrolyzable groups.

Biodegradation was tested under aerobic and anaerobic conditions. In organic soil a DT50 for 2,4-DNT of 7 days and a DT90 of 191 days was determined. In an aquatic ready test (aerobic) according the OECD TG 301C, 0 % biodegradation was reported after 14 days. Thus DNT is not readily biodegradable. In a test system under anaerobic conditions, performed according to EPA-Guideline No. 796.3140, 0 % biodegradation was observed within 56 days. DNT can be primary biodegraded and also mineralized by selected adapted bacteria cultures under specific conditions. However, under environmental conditions where no adaptation of the microorganisms can be assumed, no biodegradation of DNT is expected.

According to the Mackay fugacity model level I, the favorite target compartment of DNT is water with 97 - 98 %. The calculated Henry's law constant (0.0094 Pa m^3/mol at 25 °C) proves a low potential for volatilization from surface waters.

DNT bioconcentration factors measured in fish are in the range of 0.6–21.2 indicating no significant bioaccumulation potential.

K_{oc} values were calculated with PCKOCWIN v1.66 (K_{oc} = 371) and with the TGD equation for nitrobenzenes (K_{oc} = 123). These results indicate a low to medium sorption potential of DNT onto the organic phase of soil or sediments. A test on leaching from three different type of soils is available for 2,4-DNT. After 2 days of leaching no 2,4-DNT was found in the leachates.

Concerning the acute toxicity of DNT towards aquatic species, experimental results for the trophic level fish are available. The acute toxicity determined for fish (*Oryzias latipes*) was 27 mg/l (48h-LC_{50}). Acute toxicity data available for the single isomers of the technical mixture show that the toxicity of 2,4- and 2,6-DNT is in the same order of the toxicity found for the technical mixture. However, the other isomers are about an order of magnitude more toxic to fish than the main isomers. With the model of concentration additivity a LC_{50} of about 17 mg/l can be estimated for the technical mixture which is in good agreement with the experimentally determined value.

With *Daphnia magna* acute tests with different DNT-isomers were performed according to standard procedures or similar methods. Also for *Daphnia* it is shown that the isomers 2,3-, 2,5- and 3,4-DNT are about an order of magnitude more toxic than the 2,4- and 2,6-isomers. Assuming that the toxicity of the technical mixture can be explained by the additive toxicity of the single isomers a 48h-EC_{50} of about 23 mg/l can be estimated for the technical mixture.

For algae, tests available for the different isomers show that the toxicities of the 2,4- and 2,3-isomers are more similar than the toxicities of the 2,4- and 2,6-DNT. As 2,4-DNT seems to be the most toxic isomer to algae, it can be concluded that the toxicity of the isomeric mixture for this trophic level can be described using the data for 2,4-DNT.

The effect values from short-term tests for the technical DNT are:
Oryzias latipes: 48 h-LC_{50} = 27 mg/l (measured)
Pimephales promelas: 96 h-LC_{50} = 17 mg/l (estimated from the toxicity of the single isomers)
Daphnia magna: 48 h-EC_{50} = 23 mg/l (estimated from the toxicity of the single isomers)
Selenastrum capricornutum: 96 h-E_rC_{50} = 2.6 mg/l (2,4-DNT, regarded as representative for technical mixture)
Chlorella pyrenoidosa: 96 h-E_rC_{50} = 0.9 mg/l (2,4-DNT, regarded as representative for technical mixture)
Microcystis aeruginosa: 96 h-EC_{50} = 0.08 mg/l (2,4-DNT, regarded as representative for technical mixture)

Reliable tests on chronic toxicity towards fish, *Daphnia,* algae and blue-green algae are available as well. The lowest effect values were obtained with the 2,4-DNT:
Oncorhynchus mykiss : 90 d-NOEC = 0.27 mg/l (e) (growth)
Daphnia magna : 21 d-NOEC = 0.02 mg/l (e) (reproduction)
Scenedesmus subspicatus : 48 h-E_rC_{10} = 1.9 mg/l (n)
Scenedesmus pannonicus): 96 h-E_rC_{10} = 0.32 mg/l (n)
Microcystis aeruginosa: 96 h-E_rC_{10} = 0.056 mg/l (n)

It can be concluded that the toxicity of the technical mixture is also covered by these data.

For terrestrial organisms reliable experimental data are available with plants and earthworms. The most sensitive plant species was *Brassica rapa* with a 14 d-EC_{50} of 6.5 mg/kg soil dry weight (nominal) using the proposed guideline of the German BBA. For the earthworm *Eisenia fetida* a 14 d-LC_{50} of 668 mg/kg soil dry weight (nominal) was determined with the OECD TG 207.

Following the EU Technical Guidance Document, for the derivation of the PNECaqua an assessment factor of 10 is chosen since long term tests for three trophic levels for DNT isomers are available. Using the lowest determined concentration, the *Daphnia magna*-NOEC of 0.02 mg/l (effective), a $PNEC_{aqua}$ of 2 µg/l is derived.

Exposure

DNT is manufactured by nitration of toluene (or nitrotoluenes) producing a mixture of approximately 80 % 2,4-DNT and 20 % 2,6-DNT. Several nitroaromatics, including DNT isomers, occur as byproducts of 2,4,6-trinitrotoluene (TNT) manufacturing. The global production capacity of DNT is about 1.6 Mio. t/a.

About 99 % of DNT are used for polyurethanes, as an intermediate for toluylenediamine (TDA) and toluylenediisocyanate (TDI). Bayer processes > 99 % of the DNT to TDA. About 1 % of the global DNT production is used in other applications: E.g. the manufacturing of TNT and propellants, as an intermediate in the production of dyes and for staining ofrefractory bricks.DNT is contained in preparations registered in Nordic countries but not in the Swiss product register. The new EU Directive 2003/34/EC (EU 2003) bans the use of DNT in consumer products in the EU market.

Manufacturing and processing (including filling) of DNT at the Sponsor company are executed in closed systems. Due to 2 step treatment of the exhausts from DNT manufacturing and processing, from the Bayer sites no relevant amount of DNT is emitted into the atmosphere. At the Bayer sites, waste from the manufacturing and processing of DNT is disposed off in an incinerator for hazardous wastes. The wastewater is treated to recover DNT and remove traces of DNT. The effluent concentrations of both isomers (2,4- and 2,6-DNT) were below the detection limit (< 85 µg/l and < 4 µg/l) (Σ for both isomers). No information is available from other sites.

In the river Elbe, the concentrations of 2,4-DNT and 2,6-DNT decrease significantly from the Czech border to the North Sea, indicating that there is no relevant DNT source in the corresponding catchment area. 2,4-DNT is not

thought to be relevant for the river Rhine. There are no data on DNT in the air. DNT occurs at former munitions manufacturing sites.

Surveys of the workplaces have been performed also according to German Technical Guidance TRGS 402. In Germany there is no workplace limit concentration for 2,4-DNT. For 2,6-DNT there is a TRK-value (Technical Guidance Concentration) of 0.05 mg/m3. At all Bayer plants the exposure of workers is well below this limit.

In humans, DNT isomers have a half-life time of 1-3 hours. 24 h after exposure DNT is completely eliminated. The levels of all DNT isomers in blood are measured at least once a year in each worker of the Bayer AG DNT manufacturing and processing plants as part of the Bayer health surveillance program. In 2000 – 2002, the DNT isomer levels in blood were below the limit of detection (5 µg/l blood).

DNT is used as an industrial intermediate e.g. for polymers, explosives, and some other chemicals. Since DNT is chemically converted in the production chain e.g. during hydrogenation and phase separation, final products are thought to be virtually free of DNT. Residual levels of DNT in the Bayer isocyanates are below the detection limit of 100 ppm. Due to these products, no exposure occurs in the consumer area.

At an emission of < 25 kg/year into the atmosphere, a quantitatively relevant human dose can be excluded. Exposure of the population via the hydrosphere is considered to be minimal. Based on the very low emissions of DNT into air and water by the Bayer manufacturing and processing plants, a significant indirect exposure of the general public via the environment or via the food chain is not expected.

RECOMMENDATION

Human Health: The chemical is currently of low priority for further work.

Environment: The chemical is a candidate for further work.

RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

Human Health:

Technical grade DNT possesses properties indicating a hazard for human health (moderate acute toxicity, toxic after prolonged exposure, mutagenic and carcinogenic properties, influences on fertility in toxic doses possible). Based on data presented by the Sponsor country, exposure from production and processing as chemical intermediate is well controlled in occupational settings and is anticipated to be low for consumers, therefore this chemical is currently of low priority for further work. Countries may desire to investigate any exposure scenarios that were not presented by the Sponsor country, e.g. indirect exposure of the general public from munitions dumps or former munitions sites.

Environment:

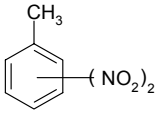
The chemical possesses properties indicating a hazard for the environment. Based on data presented by the Sponsor company, exposure from production and processing as chemical intermediate is low. However, in addition to the use as chemical intermediate some direct uses of DNT have been identified. Therefore, an exposure assessment and, if then indicated, an environmental risk assessment is recommended. Countries may desire to investigate any exposure scenario not presented by the Sponsor Country, e.g. exposure from munitions dumps or former munitions sites.

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number: 25321-14-6
IUPAC Name: Dinitrotoluene (isomers mixture)
Molecular Formula: $C_7H_6N_2O_4$
Structural Formula:



Molecular Weight: 182.14 g/mol
Synonyms: Dinitrotoluene (mixed isomers)
DNT
DNT 80/20
Dinitrotoluene (2,4 and 2,6 mix)
Dinitrotoluene, all isomers
Dinitrotoluene (mixed isomers)
Dinitrotoluene mixture
2,4/2,6-DNT
Methyl dinitrobenzene (mixed isomers)
Toluene, ar,ar-dinitro

1.2 Purity/Impurities/Additives

The technical isomers mixture DNT 80/20 derives its name from the approximate composition:

2,4-DNT (CAS-No. 121-14-2) ca. 80 %

2,6-DNT (CAS-No. 606-20-2) ca. 20 %

The composition is specified by Booth (2003)

2,3-DNT (CAS-No. 602-01-7) 1.3 %

2,4-DNT (CAS-No. 121-14-2) 78 %

2,5-DNT (CAS-No. 619-15-8) 0.5 %

2,6-DNT (CAS-No. 606-20-2) 18 %

3,4-DNT (CAS-No. 610-39-9) 2.4 %

The BUA reports the same composition for the industrial isomers mixture with the exception of 2,4-DNT (77.9 %) and 3,4-DNT (2.3 %).

1.3 Physico-Chemical properties

Table 1 Summary of physico-chemical properties of **technical isomers mixture DNT 80/20**

Property	Value	Reference	IUCLID
Substance type	Organic, aromatic, nitrocompound	Booth, 2003	1.1.1
Physical state	orange-yellow solid	Bayer AG, 2003a	1.1.1
Melting point	56 - 59 °C	MITI, 1992	2.1
Boiling point	250 °C	Clayton and Clayton, 1994	2.2
Relative density at 15 °C at 71 °C	 1.52 g/cm³ 1.32 g/cm^3	 Bayer AG, 2003a; Clayton and Clayton, 1994	2.3
Vapour pressure at 20 °C	2,4-DNT: 0.000079 hPa 2,5-DNT: 0.000338 hPa 2,6-DNT: 0.000149 hPa (measured)	Bayer AG, 1986a	2.4
Vapour pressure at 25 °C	2,4-DNT: 0.00016 hPa 2,5-DNT: 0.00065 hPa 2,6-DNT: 0.00032 hPa (measured)	Bayer AG, 1986a	2.4
Partition coefficient n-octanol/water (log K_{ow})	DNT 80/20: 2.00 (weighted average of the following values)	Bayer AG, 2003b	2.5
	2,4-DNT: 1.98 2,6-DNT: 2.1 (measured)	Hansch, Leo, and Hoekman, 1995	2.5
Water solubility at 25 °C Solubility in organic solvents at 25 °C	2,4-DNT: 166 mg/l 2,5-DNT: 258 mg/l 2,6-DNT: 145 mg/l (measured) Soluble in alcohol and ether	Bayer, 1986b Budavari, 1996	2.6.1
Surface tension	47.14 mN/m	Bayer AG, 2003c	2.6.2
Flash point	ca. 160 °C	Bayer AG, 2003c	2.7
Auto flammability (ignition temperature)	ca. 400 °C	Bayer AG, 2003a	2.8
Viscosity at 20 °C	0.654 mPa s (dynamic, liquid phase)	Bayer AG, 2003c	2.13
Conversion factors in air at 20 °C	1mg/m^3 = 0.13 ppm 1 ppm = 7.57 mg/m^3	Clayton and Clayton, 1994	2.14

2 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

Dinitrotoluene (DNT) is manufactured by nitrating toluene with 2 equivalents of nitric acid in the presence of concentrated sulfuric acid. This reaction produces a mixture which consists of

approximately 80 % of the 2,4-isomer and 20 % of the 2,6-isomer (exact composition see Table 2; Booth, 2003).

Another method is the nitration of nitrotoluenes. The composition of the product mixture depends on the starting material (Table 2; Booth, 2003). Although pure 2,4-DNT is obtained by nitration of 4-nitrotoluene, in general, 4-nitrotoluene is not used for the production of di- and trinitrotoluenes (e.g. 2,4,6-trinitrotoluene: Boileau, Fauquignon, and Hueber, 2002). Small concentrations of several nitroaromatics, including DNT isomers, occur as byproducts of 2,4,6-trinitrotoluene (TNT) manufacturing.

Table 2 DNT isomeric mixtures obtained by nitration (Booth, 2003)

Starting material	**DNT (% w/w)**					**Melting point (°C)**
	2,3-DNT	2,4-DNT	2,5-DNT	2,6-DNT	3,4-DNT	
Toluene	1.3	78	0.5	18	2.4	55 - 57
2-Nitrotoluene		67		33	< 0.5	50 - 51
3-Nitrotoluene	25		20		55	
4-Nitrotoluene		99				69

There are no data on the global DNT production. As DNT is nearly exclusively used for the manufacturing of TDI (toluylene diisocyanate), the production capacity of DNT can be calculated from the world-wide TDI production capacity and is about 1.6 Mio t (SRI, 2002; Table 3).

Table 3 TDI manufacturing capacities in 2001 (SRI, 2002)

Region	**TDI capacities (1000 t/a)**	**Estimated DNT capacity (% of total 1.6 Mio. t)**
Western Europe	488	31
North America	684	44
Eastern Europe	43	3
Japan	211	14
Korea	106	7
Other	20	1
Total	1552	100

Starting from toluene Bayer produced about 200,000 t/a DNT in 2001. In Europe, Bayer manufactures and processes DNT at 1 site (Dormagen). Small quantities of DNT were also processed at the Leverkusen site in 2003. In Dormagen a new worldscale unit started up in 2003 to replace the up to then existing units in Antwerpen, Brunsbüttel, and Dormagen (Bayer Polymers,

2003). As the new Dormagen plant is still in trial operation no current production data are available. The production capacity is 300,000 t/a.

About 99 % of DNT are used for polyurethanes, as an intermediate in the production of toluylenediamine (diaminotoluene, TDA) and further toluylene diisocyanate (TDI; BUA, 1987; ATSDR, 1998). Bayer processes all DNT to TDA (Bayer Polymers, 2003).

About 1 % of the global DNT production is used in other applications: E.g. in the manufacturing of TNT, and as a waterproofing, plasticizing, and gelatinizing agent in explosives (HSDB, 1998). DNT serves as a modifier for smokeless powders in the munitions industry (HSDB, 1998) and as a flash inhibitor in gun powder formulations (Northrop, 2001a). Small amounts of DNT are also used as an intermediate in the production of dyes (HSDB, 1998; OECD, 1997). DNT is also industrially used to stain refractory bricks and causes the prominent yellow of calcinated refractory bricks (Bayer Polymers, 2003).

ATSDR (1998) reports that 2,4 DNT is used in automotive airbags. Unfortunately the composition of airbag propellants is confidential, but according to ATSDR (pure) 2,4-DNT is used which is apparently not identical with the DNT mixture from DNT manufacturing (*cf.* Chapter 2.3.2).

DNT is contained in preparations registered in Nordic countries (SPIN, 2003). DNT is not recorded in the Danish product register. In the Finnish product register (in 2001) there is a confidential listing. In the Norwegian product register it is reported to be used in the production of basic metals (7 preparations with a consumption of 10.9 t in 2000 and 6 preparations with a consumption of 10.7 t in 2001). In the Swedish product register it is listed in consumer products in 1999 (7 preparations with a consumption of 11 t) and 2000 (5 preparations with a consumption of 3 t). DNT is not listed in the Swiss product register (2003). The new EU Directive 2003/34/EC (EU, 2003) bans the use of DNT in consumer products in the EU market.

2.2 Environmental Exposure and Fate

2.2.1 Sources of Environmental Exposure

Releases of DNT isomers into the environment may occur during manufacturing and processing as well as from munitions manufacturing and from the formulation and use of products containing DNT (*cf.* Nordic product registers).

Information on exposure from manufacturing and processing of DNT isomers is available for the Bayer manufacturing and processing plants in Germany (Bayer Polymers, 2003).

The Bayer DNT plants are dedicated systems in which only the isomers mixture is manufactured and processed (Bayer Polymers, 2003).

Manufacturing and processing (including filling) of DNT are executed in closed systems (e.g. transport via pipings, ISO-container [20 feet container]; sampling without dead volume, gas-shuttle pipe for filling processes). Cleaning of the reactors takes place only in the case of maintenance (*cf.* Chapter 2.2). No releases into the environment are expected during transport (Bayer Polymers, 2003).

The exhausts from manufacturing and processing of the DNT mixture are connected to air washing units and a thermal exhaust purification plant. Thus, from the Bayer production and processing sites no relevant amount of DNT is emitted into the atmosphere under normal operating conditions (Bayer Polymers, 2003).

At all Bayer sites, waste from the manufacturing and processing of DNT is disposed off in an incinerator for hazardous wastes (Bayer Polymers, 2003).

At the Bayer manufacturing plant, wastewater with significant organic load is separated from wastewater with minor load. The significantly loaded wastewater is extracted and the extract is recycled to recover DNT and other valued substances. The extracted wastewater is stripped and the remainder is lead to the Bayer industrial wastewater treatment plant, together with the wastewater with minor load (Bayer Polymers, 2003).

The concentrated sewage sludge is incinerated in hazardous waste incinerators especially dedicated to this sludge (Bayer Polymers, 2003).

24 h/d, 365 d/a, the air and water emissions of the production sites at Antwerpen, Brunsbüttel, and Dormagen, and at the Leverkusen processing site are monitored by an Environmental Surveillance Group which operates independently of any manufacturing unit. These groups are equipped with mobile detectors for various potential emissions. They also operate stations with measuring and sampling devices for air and water (Bayer Polymers, 2003).

The situation of the effluent from the Leverkusen site might serve as an example for the deduction of the Predicted Environmental Concentration (PEC_{local}). In 2002, in the effluent of the Leverkusen wastewater treatment plant, both 2,4- and 2,6-DNT were neither detectable by the daily monitoring with a detection limit of 20 µg/l nor in randomly selected fine monitoring samples with a detection limit of 2 µg/l (Bayer Polymers, 2003).

The effluent of the Bayer Leverkusen plant passes into the Rhine. Taking into account the 10 percentile of the river flow (1050 m^3/s), the dilution factor (700), and the detection limit (2 µg/l), for the receiving water a

PEC_{local} of < 0.0058 µg/l

is calculated for the DNT mixture. For the manufacturing and processing plant inDormagen a

PEC_{local} of < 0.085 µg/l

is derived.

For the Bayer manufacturing and processing sites, PEC values are compiled in Table 4 (Bayer Polymers, 2003).

Table 4 PEC_{local} for Bayer DNT manufacturing and processing sites

	Dormagen 2000	**Leverkusen 2002**
Wastewater outlet 2,4-DNT (mg/l)	< 0.035 (all data)	< 0.002 (all data)
Wastewater outlet 2,6-DNT (mg/l)	< 0.050 (all data)	< 0.002 (all data)
Water flow of wastewater treatment plant (ca. m^3/s)	0.74	1.5
River flow (m^3/s)	1050 (10-percentile)	1050 (10-percentile)
Dilution factor	1428*	700

PEC_{local} 2,4-DNT (µg/l)	< 0.035	< 0.0029
PEC_{local} 2,6-DNT (µg/l)	< 0.05	< 0.0029
PEC_{local} 2,4- + 2,6-DNT (µg/l)	< 0.085	< 0.0058

*for calculation 1000 was used

For 2,4-DNT, the OECD (1997) reported a PEC_{local} of 0.09 and 0.02 µg/l for two production sites on the river Rhine (ca. 1994), and a PEC_{local} of 1.66 µg/l for a processing site on the river Schwarze Elster, Germany. In the USA, the local 2,4-DNT concentration in surface water during the emission episodewere estimated to 0.064 to 56 µg/l for four production sites and 0.0002 to 0.1 µg/l for three processing sites (OECD, 1997).

In TNT production DNT is a byproduct. In the TNT manufacturing process, all TNT and DNT isomers and several other nitroaromatics are formed. Thus, although the DNT isomers are present, the composition of this nitroaromatics mixture does not reflect the composition of the DNT isomers mixture (CAS no. 25321-14-6) which is in the focus of this study. The major wastewater of the TNT production is the condensate wastewater (Liu, Bailey, and Pearson, 1983; Pearson et al., 1979; Toze and Zappia, 1999). The desired 2,4,6-trinitrotoluene is separated by a sellite process (reaction with sodium sulfite which converts the non-symmetrical TNT isomers to water-soluble sulfonate salts) from other TNT isomers, from byproducts and the process water. The process water is steam distilled to concentrate to 35 % water. The concentrate is recycled or incinerated. The distillate is condensed (condensate wastewater) and discharged into the environment (Pearson et al., 1979).

The major components of the condensate wastewater are 2,4 DNT (e.g. 15 mg/l, 43 % of organics) and 2,6-DNT (e.g. 7 mg/l, 22 %). The other DNT isomers and several other compounds are also present in this wastewater (Pearson et al., 1979).

DNT isomers have also repeatedly been reported to occur in leachates and groundwater from decommissioned munitions factory sites (Toze et al., 1999) and ammunition destruction sites (Wikström, Hägglund, and Forsman, 2000) (*cf.* Chapter 2.2.9). At the munitions factory sites DNT is a byproduct of munitions manufacturing. The postulate, that DNT is also a degradation intermediate of TNT, has not been supported by biodegradation studies (*cf.* Chapter 2.2.6).

2.2.2 Photodegradation

There are no experimental data on the stability of DNT in the atmosphere. DNT entering into the atmosphere is expected to be photodegraded by OH-radicals. The calculated half-life of DNT in air due to indirect photodegradation is $t_{½air}$ = 84 days, considering a mean OH-radicals concentration of 0.5 x 10^6 radicals per cm^3 (Bayer AG, 2003b). A half-life about 71 days is estimated for 2,4-DNT (OECD, 1997).

DNT isomers can rapidly be degraded in water in the presence of light. The photodegradation in surface waters was investigated by Simmons and Zepp (1986). A half-life of 1 day can be derived for (predominantly) direct photolysis under the radiative conditions of latitude 40 °N in surface waters. In the presence of humic compounds, which function as a photosensitizer for indirect photolysis, the half-life of the DNT isomers is reduced by approximately one order of magnitude.

Based on the same quantum yield as above (quantum yield = 0.002) with regard to the geographical conditions in Germany, and the low light intensity in natural water bodies, the half-life of 2,4-DNT is calculated to be 20 days in a natural water body (surface layer: 6.5 days) (OECD, 1997; BUA, 1987).

Since direct photolysis seems to contribute significantly to degradation in water, it is likely that direct photodegradation is also relevant in the atmosphere (exact data unknown). The data on photodegradation are listed in Table 5.

Table 5 Photodegradation of DNT (IUCLID 3.1.1)

Parameter	Method	Result	Reference
Indirect photodegradation in air (all DNT isomers)	Calculation 24 h-day; 0.5 * 10^6 OH/cm³	$t_{½}$ = 84 d	Bayer AG, 2003b
Direct photodegradation in water (5 DNT isomers measured)	Merry-go-round photoreactor, photodegradation calculated for surface waters at 40 °N	$t_{½}$ = 1 d	Simmons and Zepp, 1986
2,4-DNT in a natural water body	Calculation of direct photodegradation in Germany (surface layer)	$t_{½}$ = 20 d ($t_{½}$ = 6.5 d)	OECD, 1997; BUA, 1987

2.2.3 Stability in Water

DNT is not expected to undergo hydrolysis in the environment due to the lack of hydrolyzable functional groups (Harris, 1990). The same conclusion was drawn by the OECD (1997) for 2,4-DNT.

2.2.4 Stability in Soil

Roembke et al. (1995) reported a half-life of 7 days in a soil test system for 2,4-DNT (Table 6) (90 % degradation 191 days (mathematically extrapolated)). The test (GLP-study) was performed according to a German guideline proposal of the Biologische Bundesanstalt Braunschweig of 1986 (BBA). The test was conducted at 20 - 22 °C and at pH of 6.3 during 100 days. 5 mg/kg (dry weight [dw]) 2,4-DNT were amended in soil containing 48 % (w/w) dw of microbial biomass. No differentiation is possible whether abiotic or biotic degradation has occurred, since no sterile control was performed.

Table 6 Test on stability in soil of DNT (IUCLID 3.1.3)

Parameter	Method	Result	Reference
Stability in soil (2,4-DNT)	German BBA-Proposal	$t_{½}$ = 7 d	Roembke et al., 1995

2.2.5 Transport between Environmental Compartments

The distribution of DNT in a “unit world” was calculated according to the Mackay fugacity model level I (Table 7) using the data of the single isomers (2,4-DNT and 2,6-DNT) since the Mackay calculation is only reasonable for pure substances. The main target compartment for both isomers is water (97 - 98 %) (Bayer AG, 2003b).

Table 7 Input Parameters and Results of the Mackay Fugacity Model Level I for 2,4-DNT and 2,6-DNT (IUCLID 3.3.2)

Input Parameters	Value 2,4-DNT	Value 2,6-DNT
Temperature	25 °C	25 °C
Melting point	69.9°C	65.9 °C
Vapour pressure	0.016 Pa	0.032 Pa
Water solubility	166 mg/l	145 mg/l
log K_{ow}	1.98	2.1
Results Compartment	**Calculated distribution**	
Water	97.9 %	96.7 %
Air	0.6 %	1.3 %
Soil	0.7 %	1 %
Sediment	0.7 %	1 %
Susp. Sediment	< 0.01 %	< 0.01 %
Fish	< 0.01 %	< 0.01 %
Aerosol	< 0.01 %	< 0.01 %

The distribution coefficient (Table 8) of DNT between aqueous solutions and air was calculated using the bond-method which yields the same result for all isomers. The Henry's law constant (H) was 0.00938 Pa m^3/mol at 25 °C, which equals a dimensionless H of 3.79 x 10^{-6}. Using the group method leads to a Henry's law constant (H) of 0.040 Pa m^3/mol at 25 °C (Bayer AG, 2003b). This result indicates DNT being essentially nonvolatile respectively having a low volatility from aqueous solution according to the criteria of Thomas (1990).

Table 8 Distribution in water-air (IUCLID 3.3.2)

Property	Method	Value	Reference
Henry's law constant	bond-method (calculation)	$9.4*10^{-3}$ Pa*m^3/mol	Bayer AG 2003b
	group-method (calculation)	$40*10^{-3}$ Pa*m^3/mol	

2.2.6 Biodegradation

Based on the available biodegradation results, DNT is not readily biodegradable.

An aerobic ready test was performed according to a national Japanese standard method comparable to the OECD TG 301C. After two weeks 0 % biodegradation was observed (MITI, 1992).

In an anaerobic test conducted with anaerobic domestic sludge also no biodegradation was observed after 56 days. The test was conducted according the EPA Test Guidelines 796.3140. Toxicity controls showed no toxic effect on the inoculum (Bayer AG, 1991).

No standard tests on inherent biodegradation are available for DNT or the single isomers. However, primary degradation and also mineralization of 2,4-DNT and 2,6-DNT by adapted inoculum have been shown in several studies (Table 9).

In a respirometry test Davis et al. (1981) observed a primary degradation of 80 % for 2,4-DNT within 2 days by an industrial inoculum consisting of 4 bacteria and one yeast species. For 2,6-DNT a primary degradation of 50 % was found after 7 days.

Table 9 Tests on Biodegradation of DNT (IUCLID 3.5)

Inoculum	Procedure	Test substance	Biodegradation	Reference
Aerobic activated sludge	Comparable to OECD TG 301C	DNT 80/20	0 % after 14 d	MITI, 1992
Anaerobic activated sludge	EPA Test Guideline 796.3140	DNT 80/20	0 % after 56 d	Bayer AG, 1991
Acinetobacter, Alcaligenes, Flavobacterium, Pseudomonas (bacteria) and *Rhodotorula* (yeast), industrial source	Respirometry Test	2,4-DNT 2,6-DNT	80 % primary degradation in 2 d, 4-methyl-3-nitroaniline identified as metabolite 50 % primary degradation in 7 d , 2-methyl-5-nitroaniline identified as metabolite	Davis et al.,1981
Microcosms from explosives-contaminated sites	Degradation in microcosm	2,4-DNT 2,6-DNT	2,4-DNT and 2,6-DNT were mineralized (28 and 8 %, repectively) and biotransformed; 4-amino-2-nitrotoluene and 2-amino-4-nitrotoluene identified as metabolites from 2,4-DNT; 2-amino-6-nitrotoluene identified as metabolite from 2,6-DNT	Bradley et al., 1997
Selected bacteria strains isolated from contaminated soils at munitions sites	Shake flask studies	2,4-DNT 2,6-DNT	Within 2-3 d both 2,4-DNT and 2,6-DNT were completely primary degraded and to about 40 % mineralized concomitantly	Nishino et al., 1999
Munitions wastewater from aerated equalization basin of wastewater treatment plant	Degradation in batch reactors	2,4-DNT	Ca. 100 % primary degradation within 1-2 weeks, intermediates (4-amino-2-nitrotoluene and 2-amino-4-nitrotoluene) removed, ammonia released (mineralization?)	Christopher, Boardman, and Freedman, 2000
Domestic activated sludge, adapted to benzene	Aerobic or anaerobic cyclon fermentors	2,4-DNT	Aerobic conditions: no biodegradation. Anaerobic conditions: complete primary degradation within 2 weeks, nitroso and amino transformation products also degraded	Liu, Thomson, and Anderson, 1984
Mixed culture of nitroaromatics degrading bacteria	Aerobic fluidized-bed biofilm reactor	2,4-DNT 2,6-DNT	Simultaneous degradation (> 94 %) within 0.75-12.5 h; stoichiometric release of N, > 90 % in the form of nitrite, no aromatic reduction products found at detection limit of about 10 µg/l	Lendenmann, Spain, and Smets, 1998

Table 9 (cont.) Tests on Biodegradation of DNT (IUCLID 3.5)

Inoculum	Procedure	Test substance	Biodegradation	Reference
Wetland mesocosms containing sediments from non-contaminated site and plants	Incubation in wetland meso-cosms under field conditions (planted, non-planted, and non-planted UV-shielded wetlands)	Mixture of several nitro-aromatics 2,4-DNT 2,6-DNT	Removal of high levels of explosives by planted wetlands during 7 d of hydraulic retention time: 58 % 61 %	Best, Miller, and Larson 1999, 2001
Groundwater from contaminated site	Batch degradation experiments in shake flask	Mixtures of 2,4-DNT with other organics	Primary degradation > 99 % within 11 d	Wikström, Hägglund, and Forsman, 2000
Enrichment culture from natural surface water downstream from an ammunition plant	14C tracing in batch degradation experiments	2,4-DNT 2,6-DNT (different concentra-tions)	2,4-DNT: 45 – 64 % mineralization 2,6-DNT: 4 – 55 % mineralization after 35 days (dependent on concentration)	Bausum, Mitchell, and Major, 1992
Natural surface water (4 different sites)	Shake flask study	2,4-DNT 2,6-DNT	No degradation within 6 weeks	Bausum, Mitchell, and Major, 1992
Adapted industrial sewage	Batch degradation experiment	2,4-DNT	100 % primary degradation within 11 d (cometabolism in the presence of ethanol)	Noguera and Freedman, 1997
Domestic activated sludge	Batch experiments in cyclone fermenter, aerobic and anaerobic conditions,	2,4-DNT in the absence and prese-nce of co-metabolic substrate (benzene)	100 % primary degradation after 10 d under anaerobic cometabolic conditions, slow mineralization demonstrated; no degradation under aerobic conditions and under anaerobic conditions without cometabolic substrate	Liu et al., 2000
Contaminated soil and unspecified inoculum	Two step bioremediation process for soil	2,4-DNT in the pre-sence of several other nitro-aromatics	Ca. 100 % primary degradation within 11 months, several degradation intermediates also degraded	Lundgren, 2001
Burkholderia cepacia wildtype and genetically modified strain	Batch degradation experiments	2,4-DNT	Mineralization (not quantified)	Chung et al., 2001

In recent years, additional studies have been published on the removal of aromatic nitro compounds from wastewater of TNT manufacturing plants and on the remediation of groundwater and soil of decommissioned munitions plants. Studies which focus on biodegradation have been added to Table 9. Studies which focus on technical aspects of bioremediation were compiled in the *List of Publications on Removal of Dinitrotoluenes from Munitions Sites and Wastewater* (Bayer AG, 2003d).

Summarising the results from the available studies it can be concluded that DNT can be primary biodegraded and also mineralized by selected adapted bacteria cultures under specific conditions.

However, under environmental conditions where no adaptation of the microorganisms can be assumed, no biodegradation of DNT is expected. In industrial sewage treatment plants where a sufficient adaptation of the microorganisms has taken place, a significant removal of DNT is assumed.

From the available data no clear statement can be made on the biodegradation behaviour of the single DNT-isomers. Most studies examined only the degradation of the main isomers, 2,4- and 2,6-DNT. From some of these studies it could be concluded that 2,4-DNT is better degradable than 2,6-DNT. However, the outcome of the studies is strongly dependant on the origin and adaptation grade of the employed inoculum.

There is only one study available that examined the biodegradation of several DNT isomers (2,3-, 2,4-, 2,5-, 2,6-, 3,4- and 3,5-DNT) (Spanggord et al., 1981). They found no mineralisation during 6 weeks of incubation with natural local waters. After addition of 500 ppm yeast extract all DNT isomers decreased to non-detectable levels after 5 days of incubation. However, with an enriched mixed culture isolated from a natural local water, that was able to utilize 2,4-DNT as sole carbon and energy source, only the 2,4-DNT, but not the other DNT-isomers, could be mineralised.

For wastewater treatment plants of the chemical industry, the OECD (1997) reports removal rates for 2,4-DNT of > 88-99 % but cautions that these results cannot be extrapolated onto other treatment plants.

2.2.7 Bioaccumulation

Measured bioconcentration factors (BCF) determined for fish (*Cyprinus carpio*) according to OECD TG 305C, were in the range of 0.6 – 21.2 at DNT concentrations of 0.025 and 0.25 mg/. Thus no significant potential for bioaccumulation of DNT in aquatic organisms is indicated (MITI, 1992).

Liu, Bailey, and Pearson (1983) used ^{14}C-2,4-DNT to determine BCF in the viscera and in muscles of *Lepomis macrochirus*. They found BCF <= 84 after 4 d of uptake and a rapid clearence ((BCF = 1 after 3 d), even when 2,4-DNT was applied in synthetic wastewater containing TNT and other nitroaromatics. In the muscle, no significant bioaccumulation occured (BCF up to 4). From log K_{ow} these authors also calculated the BCF to be 32 for all DNT isomers (Table 10).

Liu, Bailey, and Pearson (1983) measured also 2,4-DNT-BCF of the water flea *Daphnia magna* (BCF = 13), of the aquatic sediment dwelling *Lumbriculus variegatus* (BCF = 58), and of the green alga *Selenastrum capricornutum* (BCF = 2507). It is not clear, whether 2,4-DNT has been accumulated or only absorbed to the algae cells (OECD, 1997).

Table 10 Bioaccumulative properties of DNT in fish (IUCLID 3.7)

Organism	Method	BCF	Reference
Cyprinus carpio	MITI (comparable to OECD TG 305C)	BCF = 0.6 – 20.9 after 56 d	MITI, 1992
Lepomis macrochirus	^{14}C-2,4-DNT alone or in complex mixture (munitions plant wastewater)	BCF in the viscera: - 2,4-DNT alone: uptake 2 d 53, 4 d 78; clearance 3 d 1, 10 d 0 - 2,4-DNT in synthetic wastewater: uptake 2 d 47, 4 d 84; clearance 3 d 0, 10 d 2 BCF in the muscle: - 2,4-DNT alone: uptake 2 d 4, 4 d 4; clearance 3 d 0, 10 d 2 - 2,4-DNT in synthetic wastewater: uptake 2 d 5, 4 d 5; clearance 3 d 0, 10 d 0	Liu, Bailey, and Pearson, 1983
	calculated from log K_{ow}	BCF = 32 for all six DNT isomers	Liu, Bailey, and Pearson, 1983

2.2.8 Geoaccumulation

The distribution between the organic phase of soil or sediment solids and porewater can be calculated by using QSAR. A K_{oc} value of 371 was calculated with PCKOCWIN v1.66 (Bayer AG, 2003b). Using a K_{ow} of 2, the TGD equation for nitrobenzenes

$\log K_{oc} = 0.77 \log K_{ow} + 0.55 = 2.09$

results in $K_{oc} = 123$.

No 2,4-DNT was found in the leachate from three different types of soils after 2 days of leaching. It was concluded that 2,4-DNT is not mobile in soil. The test was conducted according the German test guideline proposal of the BBA of 1986. The test was performed under laboratory conditions, at pH 5.9 – 6.6, in soil with an organic carbon content of 0.7 - 2.29 % (w/w). The applied test concentration was 5 mg 2,4-DNT per kg soil (Roembke et al., 1995).

These results indicate a low to medium sorption potential of DNT onto the organic phase of soil or sediments according to the criteria of Litz (1990). However, adsorption of nitroaromatic compounds to solid particles actually depends on their clay content (Haderlein, Weissmahr, and Schwarzenbach, 1996).

Table 11 Geoaccumulative properties of DNT (IUCLID 3.3.2)

Parameter	Method	Result	Source
Soil organic carbon–water distribution coefficient	Calculated with PCKOCWIN, v.1.60	$K_{oc} = 371$	Bayer AG, 2003b
Soil organic carbon–water distribution coefficient	$\log K_{oc} = 0.77 \log K_{ow} + 0.55$	$K_{oc} = 123$	Bayer AG, 2003b
Soil– water	German proposal of the BBA	no 2,4-DNT leached	Roembke et al., 1995

2.2.9 Environmental Monitoring

Actual monitoring data for 2,4-DNT and 2,6-DNT in the German part of the river Elbe have been published (ARGE Elbe, 2003; Table 12). The concentrations of DNT decrease significantly from the Czech border to the North Sea, indicating that there is no relevant DNT source in the corresponding Elbe catchment area.

Table 12 Summary of Elbe monitoring data 2000

Sampling points	Km	Samples	2,4-DNT max [µg/l]	2,4-DNT (90 % percentile) [µg/l]	2,6-DNT max [µg/l]	2,6-DNT (90 % percentile) [µg/l]
Schmilka (close to border of the Czech Republic, mixed samples)	4.1	51	0.55	0.20	0.32	0.056
Schmilka; left bank	3.9	13	1.0	0.11	0.58	0.14
Schmilka; right bank	3.9	13	0.88	0.095	0.52	0.11
Zehren (mixed samples)	89.6	35	(0.40)	(0.19)	(0.22)	(0.060)
Zehren; left bank	89.7	13	0.21	0.10	0.085	< 0.050
Zehren; right bank	89.7	13	0.23	0.11	0.088	< 0.050
Dommitzsch (mixed samples)	172.6	48	0.34	0.20	0.18	0.050
Dommitzsch; left bank	172.6	13	0.14	0.12	< 0.050	< 0.050
Dommitzsch; right bank	172.6	12	0.14	0.12	< 0.050	< 0.050
Schnackenburg (single samples)	474.5	6	< 0.01	(max < 0.01)	< 0.02	(max < 0.02)
Grauerort (Elbe estuary, single samples)	660.5	6	< 0.01	(max < 0.01)	< 0.02	(max < 0.02)

Some historical data representing background concentrations and contaminated environmental waters are listed in Table 13. E.g. in 6 samples from the river Rhine near Düsseldorf Hambsch (2002) found no 2,4-DNT at a detection limit of 0.002µg/l in 1997/1998. Presently, 2,4-DNT is not thought to be relevant for the river Rhine (GKSS, 2001).

Although it is not the scope of this study to assess risks originating from former munitions sites, for comparison, several historical reports on the occurence of DNT in waters in the proximity of decommissioned TNT manufacturing sites are also listed in Table 13. At these sites DNT may occur in groundwater at considerable levels (Rippen, 1998).

Table 13 Historical data on 2,4-DNT and 2,6-DNT concentrations in environmental waters

Medium	Water	2,4-DNT [µg/l]	2,6-DNT [µg/l]	Year (ca.)
Rivers	Rhine		Maximum 3	1974
	Rhine (Netherlands)	0.3		1979
	Rhine	0.2 (sum of 2,4-DNT, 2,5-DNT, 2,6-DNT)	0.2 (sum of 2,4-DNT, 2,5-DNT, 2,6-DNT)	1984
	Rhine (Duesseldorf)		< 0.02-0.06	1986
	Rhine (Wesel)		< 0.02-0.07	1986
	Rhine (Netherlands)		3	Before 1987
	Main (tributary to the Rhine)*	0.52 µg/l (90 % percentile) 101 of 170 samples above the detection limit of 0.1 µg/l		1984-1987
	Rhine*		10 samples: < 0.02-0.2 µg/l	1987
	Rhine (Karlsruhe, Wahnbach, Cologne, Duesseldorf)**	< 0.002 (determination limit of 6 measurements for every sampling site)		03/1997 – 01/1998
	Wupper (tributary to the Rhine)**	< 0.002 (determination limit of 2 measurements)		02-03/ 1998
	Elbe (Lauenburg)	1.3	0.5	1989
	Elbe (Brunsbuettel)	0.1	0.04	1989
	Elbe (Schmilka, close to Czech border)**	Maximum 0.41 µg/l (6 of 8 samples above the determination limit)		04/1997
	Elbe (Schnackenburg)**	Maximum 0.19 µg/l (3 of 5 samples above the determination limit)		04/1997
	Mulde (tributary to the Elbe)**	< 0.002 (determination limit of 5 measurements)		02-12/ 1997
	2 small creeks in the proxity of a former TNT manufacturing site near Hirschenhagen, Germany	3 and 13	4 and 8	1989
Lakes	Pfauenteich in the proximity of a former TNT manufacturing site near Clausthal-Zellerfeld, Germany	0.7, 0.8, 1.3	0.07,0.3	1989
	Waconda Bay (Lake Chichamauga, TN, USA)	< 0.1-22	1.3-39 (average 19.4)	1980
Maritime waters	Dokai Bay, Japan	Maximum 210	Maximum 14.9	1981

Table 13 (cont.) Historical data on 2,4-DNT and 2,6-DNT concentrations in environmental waters

Medium	Water	2,4-DNT [µg/l]	2,6-DNT [µg/l]	Year (ca.)
Ground water	6 former TNT manufacturing sites close to Pasadena, TX, USA	2-91000	Maximum 77000, average16800	1985
	In the proximity of a former TNT manufacturing site near Stadtallendorf, Germany	<1-810	3-590	1982
	In the proximity of a former TNT manufacturing site near Stadtallendorf, Germany	<0.1-0.4	<0.1-0.7	1988
	TNT manufacturing site Umatilla, OR, USA	400	5	Before 1989
	TNT manufacturing site Milan, TN, USA	Maximum 100	Maximum 34	Before 1989

Data compiled by Rippen (1998), data labelled with an asterisk *added from OECD (1997), data labelled with two asterisks **added from Hambsch (2002)

There are no data on DNT in the air.

Although it is not the scope of this study to assess risks originating from former munitions sites, for comparison, several reports on the occurence of DNT at decommissioned TNT manufacturing sites are listed in Table 14. The composition of the condensate wastewater of TNT production is reported as well.

Table 14 DNT concentrations at TNT manufacturing sites

Site	Medium	2,4-DNT	2,6-DNT	Other substances	Reference
Swedish ammunition destruction site	Groundwater	12 mg/l	9 mg/l	Several nitroaromatics	Wikström, Hägglund, and Forsman, 2000
US Army Ammunition Plants (AAP): - Milan AAP - Iowa AAP - Volunteer AAP	Groundwater	 16.6 mg/l <0.002 mg/l <0.002 mg/l	 5.2 mg/l <0.002 mg/l 0.006 mg/l	TNT, byproducts of TNT synthesis and metabolites	Best, Miller, and Larson, 2001
US Volunteer Army Ammunition Plant (VAAP; Chattanooga, TN)	Wastewater (condensate wastewater)	14.7 mg/l	7.3 mg/l	2,3-DNT 0.4 mg/l 2,5-DNT 0.4 mg/l 3,4-DNT 0.5 mg/l 3,5-DNT 0.52 and several nitroaromatics	Pearson et al., 1979; Liu, Bailey, and Pearson, 1983; Liu et al., 1984
US Volunteer Army Ammunition Plant (VAAP; Chattanooga, TN) Badger Army Ammunition Plant (BAAP; Baraboo, WI)	Soil	19 g/kg 8.9 g/kg	1.38 g/kg 0.48 g/kg		Zhang et al., 2000
US Badger Army Ammunition Plant (BAAP; Baraboo, WI)	Soil	14 g/kg	0.55 g/kg		Nishino and Spain, 2001
Decommissioned TNT manufacturing plant in Hessisch Lichtenau, Germany	Soil	3.6 g /kg	2.5 g /kg		Nishino et al., 1999
Decommissioned explosives manufacturing facility Gyttorp, Sweden	Soil	4 g/kg		Total concentration of other nitrotoluene explosives 5.3 g/kg	Lundgren, 2001

It has to be considered that the composition of the DNT residue mixture found at former munition sites is different from the technical DNT isomers mixture [CAS 25321-14-6] which is the scope of this hazard assessment.

2.3 Human Exposure

2.3.1 Occupational Exposure

2.3.1.1 Workplaces

In principle, during manufacturing and processing of DNT workers may be exposed through the inhalational and dermal routes.

All DNT manufactured by Bayer Polymers is processed to TDA in closed installations. For processing, DNT is transported in pipelines and ISO containers (*cf.* Chapter 2.2.1) (Bayer Polymers, 2003).

Surveys of the workplaces have been performed also according to German Technical Guidance TRGS 402 (1997). This includes regular surveys in the working area for any possible exposure to a

dangerous substance at different work situations and appropriate control measures (Bayer Polymers, 2003).

To protect workers several precautionary and protective measures are taken. These measures include technical equipment like suction devices at filling and sampling stations as well as appropriate personal protection equipment which is prescribed in detail for different work situations e.g. during sampling, maintenance, and repair work. For sampling, devices without dead volume are used, and the persons involved have to wear goggles and gloves. Depending on the work to be done during maintenance, a gas filter mask or a respirator with independent air supply has to be used as well as full protective clothing (Bayer Polymers, 2003).

Workers in the DNT manufacturing and processing plants are informed also by way of a safety data sheet on the recommended safety measures (see above; Bayer Polymers, 2003).

2.3.1.2 Workplace Monitoring

In Germany there is no workplace limit concentration laid down neither for the DNT isomers mixtures [CAS 25321-14-6], nor for 2,4-DNT (TRGS 900, 2002). For 2,6-DNT there is a TRK-value (Technical Guidance Concentration) of 0.05 mg/m3 (TRGS 900, 2002), for 3,4-DNT of 1.5 mg/m^3 (DFG, 2003).

At all Bayer DNT manufacturing and processing plants the exposure of workers is well below this limit. Data of the 1999 routine monitoring of 2,4-DNT and 2,6-DNT have been supplied to the EU. In Antwerpen (former DNT manufacturing and processing site), all samples (n = 4) were below the detection limit of 0.002 - 0.003 mg/m^3. In Brunsbüttel (former DNT manufacturing and processing site), 13 samples were taken in 1999. The maxima were 0.03 mg/m^3 for 2,4-DNT and 0.005 mg/m^3 for 2,6-DNT. The 95 % percentile was < 0.005 for both isomers. In Dormagen, 2,4-DNT was determined once and the result was below the detection limit of 0.01 mg/m^3. For the Leverkusen DNT workplaces, there is one total shift measurement in 2003. The level of 2,6-DNT was below the detection limit of 0.007 mg/m^3 (Bayer Polymers, 2003).

2.3.1.3 Biological Monitoring

In humans DNT isomers have a half-life time of about 1 - 3 h. 24 h after exposure DNT is completely eliminated (Turner et al., 1985).

The levels of all DNT isomers (2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-DNT) in blood are measured at least once a year in each worker of the Bayer AG DNT manufacturing and processing plants as part of the Bayer health surveillance program. Furthermore, biological monitoring will be performed in case of intoxications (contact with DNT; Bayer Industry Services, 2003).

As representative values (including data from former DNT manufacturing and processing site),, data of 2000 - 2002 are described here. On the whole, 3012 workers (2000: 1056; 2001: 957; 2002: 999) from the manufacturing and processing plants have participated during this time in the biological monitoring investigations (Bayer Industry Services, 2003).

In the previous 3 years, in all workers (n = 3012) the concentrations of DNT isomers in blood were below the corresponding detection limits (DNT isomers in blood: 5 µg/l blood) (Bayer Industry Services, 2003).

2.3.2 Consumer Exposure

DNT is nearly exclusively used as an intermediate for chemical synthesis (*cf.* Chapter 2.1) (ATSDR, 1998). More than 99.9 % of the DNT production volume is used as an industrial intermediate e.g. for polymers, explosives, and some other chemicals (*cf.* Chapter 2.1). Since DNT is chemically converted in the production chain e.g. during hydrogenations, and phase separations, final Bayer products are thought to be virtually free of DNT. Residual levels of DNT in the Bayer isocyanates are below the detection limit of 100 ppm (Bayer Polymers, 2003).

DNT is used for the (industrial) staining of refractory bricks. DNT does not persist, but is oxidised during the calcination of the bricks (Bayer Polymers, 2003).

2,4-DNT can be detected in gunpowder (up to 0.5 %), ammunition reloading powder (up to 6 %), and gunshot residues (traces; Northrop, 2001a, b). ATSDR (1998) reports that 2,4 DNT is used in automotive airbags. Unfortunately the composition of airbag propellants is confidential, but according to ATSDR pure 2,4-DNT is used (which is apparently not identical with the DNT mixture from DNT manufacturing). In Germany, airbag propellants are treated like explosives and are not deposited into the environment. Airbags are removed from wrecked cars only by licensed experts, and these propellants are disposed of like explosives. It is unlikely that there is a relevant consumer or environmental exposure due to airbag propellants.

DNT is listed in industrial preparations in the Norwegian product register, confidentially in the Finnish product register, and in consumer products (5 preparations with a consumption of 3 t in 2000) in the Swedish product register. There are no other informations available from these product registers (SPIN database, 2003). As the EU Directive 2003/34/EC (EU, 2003) bans the use of DNT in consumer products, it is expected that DNT will not be used consumer products.

Since 2,4-DNT and the DNT isomers mixture are used in the same fields of applications, 2,4-DNT can be used as a model for exposure assessment of DNT isomer mixtures. The OECD (1997) reports that 2,4-dinitrotoluenes are used exclusively as intermediates or precursors. They thus undergo complete chemical conversion and cannot be further cleaved from products. No exposure occurs in the consumer area (OECD, 1997).

At an introduction of < 25 kg/year into the atmosphere, a quantitatively relevant human dose can be excluded (OECD, 1997). Exposure of the population via the hydrosphere is considered to be minimal (OECD, 1997).

DNT occurs at decommissioned TNT manufacturing sites (*cf.* Chapter 2.2.9). However, the situation is comprehensively examined and controlled in the Sponsor country.

Based on the very low emissions of DNT into air and water by the Bayer manufacturing and processing plants (*cf.* Chapter 2.1), a significant indirect exposure of the general public via the environment or via the food chain is not expected.

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

Dinitrotoluene (DNT) is a technical mixture containing approximately 80 % 2,4-dinitrotoluene, app. 20 % 2,6-dinitrotoluene and < 5 % 3,4-, 2,3- and 2,5-DNT. The toxicological profile of the DNT mixture well reflects the properties of its main components, the pure 2,4- and 2,6-isomer. Therefore, toxicological data of the pure 2,4- and 2,6-DNT are presented here only for completeness, especially if they provide additional relevant information.

3.1.1 Toxicokinetics, Metabolism and Distribution

Studies in Animals

In vivo Studies

The predominant route of uptake of dinitrotoluenes in rats, shown for the pure 2,4- and 2,6- isomer, is via the gastro-intestinal tract and the respiratory system. For 2,6-DNT it was shown that dermal resorption plays a minor role (BUA, 1987; 1993). Dinitrotoluenes (2,4-DNT, 2,6-DNT and technical grade DNT) are believed to be metabolized in the liver to dinitrobenzylalcohol, which is then conjugated to form a glucuronide conjugate that is excreted in bile or urine. This conjugate is thought to be hydrolyzed by intestinal microflora to aminonitrobenzyl alcohol. Such bacteria are present in the gastrointestinal flora of rodents and humans. Aminonitrobenzyl alcohol is thought to be reabsorbed and returned to the liver where it is further metabolized to an unidentified toxic metabolite or to the precursor of a toxic metabolite (NIOSH, 1985; Klassen, Amdur, and Doull, 1995). In male rats given 2,4-DNT the major metabolites excreted in urine were 2,4-dinitrobenzoid acid (44 %) and 2,4-dinitrobenzyl glucuronide (27 %). Females excreted equal amounts of the two metabolites (39 %) (Turner et al., 1985).

Studies in Humans

In vivo Studies

Urine of 17 dinitrotoluene (technical grade DNT consisting of 76.4 % 2,4-DNT, 18.8 % 2,6-DNT and 4.8 % other isomers) exposed workers (14 males and 3 females) was collected over 72 hours. The main metabolites found in urine were 2,4-dinitrobenzoic acid (52.5 % in men and 28.8 % in woman) and 2-amino-4-nitrobenzoic acid (about 37 % in men and woman). Furthermore, 2,4- and 2,6-dinitrobenzyl glucuronide (9.5 % in men and 33.3 % in woman) and 2(N-acetyl)amino-4-nitrobenzoic acid (0.8 % in men and 0.3 % in woman) were found. Men appeared to excrete relatively more dinitrobenzoic acid than women whereas women appeared to excrete relatively more dinitrobenzyl glucuronides than men. In all, the amount by which men exceeded women with respect to the dinitrobenzoic acids is almost exactly that by which women exceeded men in regard to the dinitrobenzylglucuronides (Levine et al., 1985). The calculated half-times for elimination of total DNT-related metabolites detected in urine of 3 of the above mentioned workers (2 men, 1 woman) ranged from 0.9 to 2.7 hours (men: 0.88 and 2.63 hours, woman, two measures at separate periods: 2.29 and 2.76 hours), and those of individual metabolites from 0.8 to 4.5 hours (Turner et al., 1985). During disposal of military waste (containing technical grade DNT, mainly consisting of about 71 - 77 % 2,4-DNT and 18 - 20 % 2,6-DNT) air analyses yielded maximum concentrations of 20 µg/m³ 2,4-DNT. The maximum concentrations in the urine of workers regularly exposed amounted to 2.1 µg/l of 2,4-DNT, 95.0 µg/l of 2,4-dinitrobenzoic acid, and 3.6 µg/l of 2,6-DNT (Letzel et al., 2003).

In an US DNT manufacturing plant (technical grade DNT consisting of 76.4 % 2,4-DNT, 18.8 % 2,6-DNT and 4.8 % other isomers) absorption was measured by quantification of excreted DNT metabolites in the urine. The maximum daily DNT absorption in a participant in this study ranged from 0.24 - 1.0 mg/kg bw. The highest concentrations in urine were found in post-shift samples (Levine et al., 1985). The urine of the workers contained more metabolites than would have resulted from the dinitrotoluene present in the inhaled air, indicating dermal absorption (Levine et al., 1985; Woollen et al., 1985). Wiping of skin suspected of being contaminated (mainly hands and forehead) showed levels of “not detected” to 179.5 µg 2,4-DNT (Levine et al., 1985).

Conclusion

In humans DNT is absorbed following dermal and inhalative exposure and is rapidly metabolized and excreted in urine.

3.1.2 Acute Toxicity

Inhalation

There are no acute inhalation studies on technical grade DNT or the main isomer component 2,4-DNT available. The 2,6-isomer, which accounts for about 18 % of technical grade DNT, is reported to be toxic in an acute inhalation study on rats (4h-LC_{50}= 0.36 mg/l)(BUA, 1993).

Dermal

There are no acute dermal toxicity studies with technical grade DNT available. Since technical grade DNT contains about 75 - 80 % of the 2,4-isomer, the acute dermal toxicity evaluation is based on data with pure 2,4-DNT. No acute dermal toxicity study is available for the 2,6-isomer.

The acute dermal toxicity of 2,4-DNT is low. 5 Wistar rats per sex were exposed dermally to 2500 mg pure 2,4-DNT/kg bw. All animals survived without any signs of intoxication (Loeser, 1982).

Oral

No guideline studies are available.

Gavage treatment of male rats with 0.1, 0.4, 0.8, 1.5 and 2.0 g DNT/kg in lutrol (DNT 80/20, consisting of ca. 80 % 2,4-DNT and ca. 20 % 2,6-DNT) and further isomers see chapter 1.2) resulted in death of 0, 4, 5, 7 and 10 rats out of 10 each. Observation period was 14 days. Diuresis, diarrhea, weight loss, shaggy fur and loss of hair were reported as clinical signs. The oral LD_{50} of DNT 80/20 in male rats was calculated with 660 mg/kg (Loeser, 1978). An LD_{50} of 268 mg/kg bw was reported for technical grade DNT (consisting of 75.8 % 2,4-DNT, 19.8 % 2,6-DNT, 2.5 % 3,4-DNT and < 2.3 % 2,3-, 2,5-, and 3,5-DNT) in corn oil orally applied to rats (number and sex of animals not given). Observation period was limited to 48 hours (Soares and Lock, 1980).

The acute oral toxicities of the pure 2,4- or 2,6-isomers are comparable to that of technical grade DNT, with LD_{50} values of 268 - 790 and 177 - 535 mg/kg bw for rats, respectively (BUA, 1987).

Male mice were given 500 mg/kg bw technical grade DNT (consisting of 75.8% 2,4-DNT, 19.8% 2,6-DNT, 2.5% 3,4-DNT and < 2.3 % 2,3-, 2,5-, and 3,5-DNT) in two consecutive daily doses of 250 mg/kg. No mortality occurred up to 48 hours post treatment (no further details) (Soares and Lock, 1980). After oral application of technical grade DNT (highest purity available) to mice (10/sex and dose) LD_{50} values of 750 mg/kg bw were obtained for female mice and 1100 mg/kg for male mice. Clonic convulsions and dyspnea were reported as signs of intoxication (Hasegawa et al., 1989).

Conclusion

There are no acute inhalation studies on technical grade DNT and the 2,4-isomer available. LC_{50} of the 2,6-isomer is reported to be 0.36 mg/l, however, this isomers accounts only for about 18 % of technical grade DNT. No acute dermal studies on technical grade DNT and the 2,6-isomer are available. The acute dermal toxicity of 2,4-DNT, the main component of technical grade DNT, is relatively low with an LD_{50} greater than 2500 mg/kg bw in rats. Technical grade DNT is moderately toxic following oral administration to rats, with LD_{50} values of 268 to 660 mg/kg bw reported.

3.1.3 Irritation

Skin Irritation

The skin irritating potential was examined in a study on 2 rabbits (male and female; no guideline study) with occlusive application of 500 mg DNT 80/20 pasted with water for 24 hours to the interior side of the ear. No signs of irritation were found up to 7 days post treatment (Thyssen, 1979).

2,4- and 2,6-DNT were also reported to be not irritating to the skin of rabbits (BUA, 1987).

Eye irritation

The eye irritating potential was examined in a study on 2 rabbits (male and female; no guideline study) with application of 50 mg DNT 80/20 pasted with water. No signs of irritation were found in one animal. The other animal showed slight erythema at the observation time point 24 hours. During the post observation period of 7 days the effect was reversible (Thyssen, 1979).

2,4- and 2,6-DNT were also reported to be not irritating to the eye of rabbits (BUA, 1987).

Conclusion

After a 24-hour occlusive application DNT is not irritating to the skin (interior side of the ear) of rabbits. Although DNT has been reported to induce slight irritation to the eye of one rabbit, the effect is reversible within 7 days, and therefore DNT is not considered to be an eye irritant in humans.

3.1.4 Sensitisation

Studies in Animals

Skin

There are no studies with application of technical grade DNT available. There is one limited study reporting a mild sensitizing effect of 2,6-DNT (2/10 animals responded) in the guinea pig maximization test. However, no sensitizing effects were observed for 2,4-DNT and 4 other isomers in this study. No further details on test procedure and test results available (Ellis et al., 1980).

Studies in Humans

Skin

In tests following the standard photo-patch test procedure of the Scandinavian Photodermatitis Research Group, 10 and 5 healthy control persons showed no reaction in patch tests or photo-patch tests with 0.5 and 1.0 % DNT (isomers not specified), respectively. However, one case of photosensitization to DNT was reported for a rock-blaster occupationally exposed to dynamite for 10 years and showing dermatological lesions. This man reacted strongly in photo-patch tests but not in patch tests with 0.5 and 1.0 % DNT in peanut oil (Emtestam and Forsbeck, 1985).

Conclusion

There are no data available to evaluate the sensitizing potential of technical grade DNT. The 2,4-DNT isomer showed no sensitizing properties in a guinea pig maximization test, whereas 2,6-DNT gave a mild positive response. Patch tests or photo-patch tests in 10 or 5 healthy humans showed no allergic potential of DNT (unspecified isomer), whereas a single case of positive photo-patch test reaction was reported for a worker with skin problems.

3.1.5 Repeated Dose Toxicity

Studies in Animals

Inhalation

There are no repeated dose inhalation studies on technical grade DNT or on the 2,4-/2,6-DNT isomers available.

Dermal

There are no dermal repeated dose studies on technical grade DNT or on the 2,4-/2,6-DNT isomers available.

Oral

The repeated dose toxicity of technical grade DNT in rats was studied in subacute studies and in a chronic study, the last one being comparable to a guideline study.

In a 4 week pilot-study only a limited number of endpoints was evaluated after administration of technical grade DNT (consisting of 76.5 % 2,4-DNT, 18.8 % 2,6-DNT, 2.4 % 3,4-DNT and < 2.3% 2,3-, 2,5-, and 3,5-DNT) to Fischer 344 rats (10 males and females/dose group) in the food in dosages of about 37.5, 75 and 150 mg/kg bw/day. A dose-dependent decrease of body weight gain (m/f, low dose by: 22 % / 16 %, mid dose by: 58 % / 36 %, high dosed animals even lost weight during the study: -22.1 g / -1.8 g) was observed simultaneous to dose-dependent reductions of food intake (up to 50 % for high dosed animals). All animals survived without clinical signs of intoxication. Significant increases of reticulocytes (up to 6.2 or 2.8-times over control for males and females of the highest dose groups, respectively) and Heinz bodies (up to 5.25 % in males and 0.14 % in females of the highest dose group in comparison to 0 % in controls) were observed in all dose groups. Methemoglobin levels were significantly elevated for high-dose males (2.3-fold) and low- and high-dose females (up to 3.5-fold), whereas mid-dose females only showed a slight elevation. Gross pathology revealed mottled discolored areas in lungs, liver and kidney, rough or granular surface changes of liver and spleen besides enlargement and thickening of the spleen in males of all dose groups without dose-dependence, and in high-dose females. No examinations of organ weights or histopathological changes were performed. The lowest tested dose of 37.5 mg DNT/kg bw/day can be regarded as the LOAEL in this study (CIIT, 1977).

In a study of limited examinations with 4 male Fischer rats daily oral doses of 75 mg/kg technical DNT (consisting of 70 % 2,4-DNT, 25 % 2,6-DNT, 5 % other isomers) for 5 days led to increased relative liver weights (4.5 % versus 3.2 % in control) and some changes in enzyme activity in the liver (benzphetamine-N-demethylase decreased by ca. 50 %, DT-diaphorase increased by ca. 50 %, cytochrome P450 and ethoxycoumarin-O-deethylase decreased by ca. 25 %). The activity of epoxide hydrolase was enhanced to 300 - 350 % of control (Dent and Graichen, 1982).

In a chronic study 130 Fischer 344 rats/sex and group were exposed to technical grade DNT (consisting of 76.5 % 2,4-DNT, 18.8 % 2,6-DNT, 2.4 % 3,4-DNT and < 2.3 % 2,3-, 2,5-, and 3,5-DNT) in doses of about 3.5, 14 and 35 mg/kg bw/day via the food. Interim sacrifices of 10 rats/sex/dose were done at week 26 and 52. In the low- and mid-dose group further 20 rats/sex/dose were sacrificed at week 78, the terminal sacrifice of all surviving rats of these dose groups was at week 104. Excessive mortality was observed in mid-dose male rats (48 of 130 animals died). Because of histopathological findings noted at the week 52 interim sacrifice all rats in the high-dose group were sacrificed prematurely at week 55. DNT-exposure led to a dose-related decrease in mean body weight gain of all treated animals. In week 102/104 mean body weight gain was 92 % (m) and 86 % (f) for the low-dose group and 66 % (m) and 74 % (f) of control for the mid-dose group. In the high-dose group only 50 % (m) and 57 % (f) of control body weight gain was noted at

week 50. Food consumption was dose-dependently reduced in treated males predominantly during weeks 5 - 20 and in females during weeks 50 - 78. Hematological examinations demonstrated raised reticulocyte and leucocyte counts and decreased erythrocyte, hematocrit and hemoglobin values in the mid- and high-dose males up to sacrifice. For these parameters a similar response was found in high-dose females. A number of clinical-chemical parameters (serum glutamic pyruvic transaminase, blood urea nitrogen and alkaline phosphatase) were altered in the mid- and high-dose group, primarily in males. Absolute liver weights were dose-dependently enhanced in males (by 14 %, 62 %, 215 %) and enhanced in females (by 17 %, 65 %, 55 %). Absolute kidney weights were increased in high-dosed males (by 12 %) and in mid-dosed males (by 11 %) and females (by 16 %). Increased absolute weights of ovaries and testes occurred not dose-dependently and were observed only in the mid-dose group (ovaries by 57 %) and low-dose group (testes by 13 %). All other changes in relative organ weights did not correlate with absolute organ weight changes and can therefore be regarded as a consequence of reduced body weight of treated animals. Necropsy revealed gross alterations of the liver at all dosages including increased incidences of nodules and tissue masses in mid- and high-dose groups (see Section 3.2.9). Hepatotoxicity was also obvious in males of the low-dose group from microscopic changes (areas of cell alteration, hyperbasophilia and megalocytosis of hepatocytes, vacuolation and necrosis of individual hepatocytes). Further targets in high-dose males were the kidney (exacerbation of chronic interstitial nephritis), the pancreas (increased interstitial pigment) and hematopoiesis (increased red cell turnover indicated by hemosiderosis and extramedullary hematopoiesis). Additionally, an increased incidence and severity of testicular degeneration and hypospermatogenesis occurred. An exacerbation of spontaneous cardiomyopathy was observed at week 26 and at the week 55 terminal sacrifice in comparable severity, but not at the week 52 interim sacrifice, and was consequently interpreted as being in the process of resolving. In the mid-dose group besides hepatotoxicity an exacerbation of chronic interstitial nephritis and of degenerative changes in the adrenal glands existed in males and females, in males of the low-dose group signs of hepatotoxicity were present (CIIT, 1978; 1982a). Testicular changes became obvious in all dose groups and are described in detail in Section 3.1.8. No NOAEL can be derived from this study. The lowest tested dose of 3.5 mg/kg bw/day can be regarded as the LOAEL.

Studies in Humans

Experiences with human DNT exposure are presented in chapter 3.1.9.

Conclusion

There are no inhalative or dermal repeated dose studies on technical grade DNT or on the 2,4-/2,6-DNT isomers available. Chronic feeding of technical grade DNT to rats led to hematological changes (especially methemoglobinemia), and toxicity to liver, kidney, adrenal glands and testes in rats. At the lowest administered dose of 3.5 mg/kg bw/day signs of hepatotoxicity became obvious. No NOAEL can be derived for repeated dose toxicity.

3.1.6 Mutagenicity

In vitro Studies

The in vitro mutagenicity and genotoxicity of DNT was investigated in well-performed studies with bacterial and mammalian cell test systems.

In a bacterial reverse mutation assay (performed according to Ames, 1975) a concentration of 1000 µg/plate technical grade DNT (consisting of 76.5 % 2,4-DNT, 18.8 % 2,6-DNT, 2,4 % 3,4-DNT and < 2.5 % 2,3-, 2,5-, and 3,5-DNT) induced increased numbers of revertants in *Salmonella typhimurium* strains TA 1538 and TA 98 with and without metabolic activation (cytotoxic

concentration > 1000 µg/plate). These strains are preferentially responding to frame-shift mutagens. In TA 1538 a ca. 11-fold increase of revertants was recorded in the absence of S9-mix. With S9 a ca. 3-fold induction occurred. In TA 98 about a doubling of control mutation frequency was recorded after treatment. Testing TA 98 in suspension a positive and dose dependent mutagenic effect became obvious at ≥ 250 µg/ml. A concentration of 500 µg/ml technical grade DNT led to about 5-fold increases of revertants in *Salmonella typhimurium* TM 677 with and without S9 (cytotoxic concentration > 500 µg/ml). No mutagenic effects were detected in strains TA 1535, TA 1537, and TA 100 (Couch, Flowe, and Regan, 1979; Couch, Allen, and Abernethy, 1981).

Up to cytotoxic concentrations (≥ 1.6 mM) technical grade DNT (consisting of 76.5 % 2,4-DNT, 18.8 % 2,6-DNT, 2.4 % 3,4-DNT and < 2.5 % 2,3-, 2,5-, and 3,5-DNT) did not induce gene mutations in the HPGRT assay with CHO K1 cells in the presence and absence of rat liver S9-mix (Abernethy and Couch, 1982). In a poorly documented mammalian cell gene mutation assay in mouse lymphoma cells ($TK^{+/-}$), technical grade DNT (consisting of an 80:20 mixture of 2,4-DNT and 2,6-DNT) yielded negative test results with and without metabolic activation. Although the tested concentrations are not given for technical grade DNT it can be assumed from the data shown for other test substances that up to cytotoxic concentrations have been tested (Styles and Cross, 1983).

In an in vitro UDS test with primary rat hepatocytes, performed according to generally accepted scientific standards, technical grade DNT (consisting of 76.5 % 2,4-DNT, 18.8 % 2,6-DNT, 2.4 % 3,4-DNT and < 2.5 % 2,3-, 2,5-, and 3,5-DNT) did not induce DNA repair in concentrations of 0.01 or 0.1 mM (cytotoxic conc. > 0.1 mM) (Bermudez, Tillery, and Butterworth, 1979).

In vivo Studies

The in vivo mutagenicity and genotoxicity of DNT was investigated in several well-documented tests.

In a bone marrow micronucleus assay on 5 male mice per dose and sampling time, mainly performed according to OECD TG 474, single intraperitoneal doses of 200 or 400 mg technical grade DNT/kg bw (containing 20 % 2,6-DNT; presumably consisting of 71.1 % 2,4-DNT, 19.8 % 2,6-DNT, 4.3 % 2,3-DNT, 4.0 % 3,4-DNT and < 1 % other isomers) gave negative results at all sampling times (24, 48 or 72 hours). The highest tested dose is described as 80 % of MTD (MTD was defined in an earlier study with 4 day observation following a single i.p. injection; data not shown). No further information on toxicity is given (Ashby et al., 1985).

In mouse dominant lethal assays, performed according to generally accepted scientific standards, 20 males per group were treated with two consecutive daily oral or intraperitoneal doses of 250 mg DNT/kg bw (consisting of 75.8 % 2,4-DNT, 19.5 % 2,6-DNT, 2.5 % 3,4-DNT and < 2.5 % 2,3-, 2,5-, and 3,5-DNT). Dose selection was based on LD_{50} as MTD. 48 hours post treatment the males were mated with 3 females each for one week. This mating procedure was repeated for in total 7 weeks. Following both application routes, the number of implants, post-implantation deaths and fertile matings were not negatively influenced and therefore gave no indications for a mutagenic effect in this assay. In the same paper a mouse spot test with a single intraperitoneal application of 100 mg DNT/kg bw is described. However, this test was performed with only 2 females of each of 2 genotypes. No indication for a mutagenic effect of technical grade DNT was found (Soares and Lock, 1980).

A poorly documented study on sister chromatid exchange (SCE) induction in rat lymphocytes after gavage application with 100 mg DNT/kg bw (technical DNT, composition not specified) yielded a slight increase of SCEs (< 50 % over control) (Kligerman, Wilmer, and Erexson, 1982).

In several publications the induction of unscheduled DNA synthesis (UDS) in the liver of rats after in vivo treatment with technical grade DNT (mainly consisting of 71.1 % 2,4-DNT, 19.8 % 2,6-DNT, 4.3 % 2,3-DNT, 4.0 % 3,4-DNT and < 1 % other isomers) is described. All of the tests are performed according to generally accepted scientific standards with 2 - 6 animals (F344 or AP rats) per dose. DNT dose-dependently induced DNA repair in rat liver after oral application in dosages ranging from 10 - 250 mg/kg bw (Mirsalis and Butterworth, 1981; 1982; Mirsalis, 1982, Ashby et al., 1985; Hamilton and Mirsalis, 1987; Mirsalis et al., 1989). When germ-free reared rats were compared to those associated with a special mixture of anaerobic bacteria similar to the normal gut microflora, it was demonstrated that the presence of gut flora in rats with its metabolic capacity was a prerequisite for the induction of liver UDS by DNT in vivo (Mirsalis et al., 1981; 1982). This observation may give an explanation for the negative results obtained with the *in vitro* rat hepatocyte UDS test (see above).

The pure isomers 2,4-DNT and 2,6-DNT show mutagenic profiles similar to that of technical grade DNT. Additionally, DNA binding properties in various rat organs, mainly rat liver were demonstrated for both isomers (BUA, 1987).

Conclusion

Technical grade DNT is mutagenic in bacterial test systems in the presence and absence of metabolic activation, but it shows no mutagenic or genotoxic activity in mammalian cells in vitro. Technical grade DNT shows no mutagenic activity in the mouse bone marrow micronucleus assay and in mouse dominant lethal and spot tests. However, a distinct activity of DNT to induce DNA repair in the liver of rats is reported. Additionally, DNA binding properties in various rat organs, mainly rat liver were demonstrated for 2,4-DNT and 2,6-DNT isomers. Gut flora may play an important role in activation of DNT to reactive metabolites. Overall, technical grade DNT shows the potential to induce genotoxic changes in vivo.

3.1.7 Carcinogenicity

In vivo Studies in Animals

Oral

A chronic study, which was comparable to a guideline study, besides several short-term assays gives information on the carcinogenic action of technical grade DNT in animals. Experiences with cancer in humans, discussed in connection with DNT exposure, are described in Section 3.1.9.

Feeding of 130 Fischer 344 rats/sex and group for up to 104 weeks with interim sacrifices and daily doses of about 3.5, 14 and 35 mg technical DNT/kg bw (consisting of 76.5 % 2,4-DNT, 18.8 % 2,6-DNT, 2.4 % 3,4-DNT and < 2.3 % 2,3-, 2,5-, and 3,5-DNT) mainly resulted in liver carcinogenicity (study described in detail in Section 3.1.5). The hepatocarcinogenic effect increased dose dependently and was obvious from 3.5 mg/kg bw/day in males (7.7 % tumor bearing animals versus 0.8 % in control) and 14 mg/kg bw/day in females (34.6 % tumor bearing animals versus 0 % in control). In the mid dose group the incidences of skin fibromas (85 %) and fibrosarcomas (19 %) as well as of mammary gland fibroadenomas (28 %) were enhanced, predominantly in males. The frequencies of interstitial cell tumors of the testes were high throughout all groups, including controls (CIIT, 1978; 1982a).

In a 52-week feeding study with 28 male Fischer 344 rats per group, designed for the determination of lung and liver carcinogenicity, technical grade DNT (consisting of 76.5 % 2,4-DNT, 18.8 % 2,6-DNT, 2.4 % 3,4-DNT and < 2.3 % 2,3-, 2,5-, and 3,5-DNT) in a dose of about 35 mg/kg bw/day produced hepatocarcinomas in 9/19 and cholangiosarcoma in 2/19 animals compared to 0/20 control animals (Leonard, Graichen, and Popp, 1987; Popp and Leonard, 1983).

In an initiation-promotion liver foci assay technical grade DNT (consisting of 76.5 % 2,4-DNT, 18.8 % 2,6-DNT, 2.4 % 3,4-DNT and < 2.3 % 2,3-, 2,5-, and 3,5-DNT) had promoting activity on the development of DEN-initiated liver foci in Fischer 344 rats (Goldsworthy and Popp, 1986; Leonard and Popp, 1982; Leonard, Adams and Popp, 1986; Popp and Leonard, 1982). Otherwise DNT was a weak initiator in rat liver only when applied 12 hours after partial hepatectomy (Goldsworthy and Popp, 1986; Leonard and Popp, 1981; Leonard, Lyght and Popp, 1983).

No tumor initiating activity of a 2:1 mixture of 2,4-DNT and 2,6-DNT was determined in short-term skin initiation-promotion assays (dermal and i.p. application) with Sencar mice (Slaga et al., 1985). Likewise, a short-term lung tumor assay with multiple i.p. injections of the mixture (3 injections per week over 8 weeks; 16 weeks post exposure period) did not show any induction of lung tumors in Strain A mice (Slaga et al., 1985).

The pure 2,4-DNT isomer induced the same tumor spectrum in long term feeding studies in rats as was shown for the technical grade isomer mixture. Additionally, tumors of the renal tubular epithelium were observed in male mice after chronic 2,4-DNT feeding. 2,6-DNT showed hepato-carcinogenic properties in a 12-month feeding study on rats (BUA, 1987).

Studies in Humans

Experiences with human DNT exposure are presented in chapter 3.1.9.

Conclusion

Technical grade DNT shows hepatocarcinogenic properties in rats. In a long-term feeding study liver tumors were dose dependently induced in male rats from the lowest administered dose (3.5 mg/kg bw/day) and in female rats from 14 mg/kg bw/day. In an initiation-promotion liver foci assay DNT showed tumor promoting activity, however, only weak initiating properties. The pure 2,4- and 2,6-DNT isomers also induced liver tumors in rats. Additionally, the 2,4-isomer was shown to induce tumor formation in the renal tubular epithelium of male mice.

3.1.8 Toxicity for Reproduction

Studies in Animals

Effects on Fertility

There are no guideline studies for the reproductive toxicity of DNT available. Findings from dominant lethal tests and a chronic feeding study give information on effects on male fertility.

After two consecutive daily oral or i.p. doses of 250 mg DNT/kg bw (consisting of 75.8 % 2,4-DNT, 19.5 % 2,6-DNT, 2.5 % 3,4-DNT and < 2.5 % 2,3-, 2,5-, and 3,5-DNT) no adverse effects on male fertility became obvious in mouse dominant lethal assays (see also Section 3.1.6) with 20 males/group, performed according to generally accepted scientific standards. 48 hours posttreatment the males were mated with 3 females each for one week. This mating procedure was repeated for in total 7 weeks. Following both application routes, the number of implants, post-implantation deaths and fertile matings were not negatively influenced. However, in comparison to the control group the percentage of fertile matings was significantly increased in week 2 of mating and slightly increased in weeks 3 – 5 of mating (Soares and Lock, 1980).

In a chronic feeding study 130 Fischer 344 rats/sex and group were exposed to technical grade DNT (consisting of 76.5 % 2,4-DNT, 18.8 % 2,6-DNT, 2.4 % 3,4-DNT and < 2.3 % 2,3-, 2,5-, and 3,5-DNT) in doses of about 3.5, 14 and 35 mg/kg bw/day for up to 104 weeks with interim sacrifices at several timepoints. In the high-dose group, however, all surviving rats were sacrificed prematurely at week 55 because of toxicity. Excessive mortality was observed for mid-dose male rats and body

weight gain was reduced throughout all dose groups in a dose dependent manner (further details of the study in Section 3.1.5). Several effects on male reproductive tissues were reported. In the low-dose group statistically significant increases of the absolute (+13 %) and relative weights of the testes were observed. However, this effect was not seen in other dose groups and may therefore be regarded as a consequence of imprecise preparation. No other effects on testes or ovaries became obvious at this dose. Otherwise in 29 % of the mid- and 25 % of the high- dose group animals abnormally small testes were conspicuous at gross examination compared to 11.5 % in control animals. In the high-dose group absolute but not relative testicular weights were significantly reduced besides moderate to moderately severe testicular degeneration plus hypospermatogenesis (in 15 or 14/20 animals versus 2/25 in control) at histopathological examination. The observed changes point to a possible impairment of male fertility after chronic exposure with toxic doses. High incidences of interstitial cell tumors of the testes were found in control and treated animals. Only in the mid-dose group increases of the absolute (1.5-fold increase; not statistically significant) and relative (2-fold increase; statistically significant) weight of the ovaries at week 104 were observed (CIIT, 1982a). The NOAEL for changes at reproductive tissues can be determined with 3.5 mg/kg bw/day in this study.

The 2,4-DNT isomer induced adverse reproductive effects and anti-spermatogenic activity in male rats, however only at toxic doses. The NOAEL for reproductive performance was 60 mg/kg bw per day in a study with 5 daily gavage administrations (BUA, 1987). No data are available for 2,6-DNT.

Developmental Toxicity

DNT was examined for teratogenic effects and developmental retardations in a well performed study on rats.

Pregnant Fischer 344 rats were exposed to oral doses of 35, 75, or 150 mg technical grade DNT/kg bw/day (consisting of 76 % 2,4-DNT, 19 % 2.6-DNT, 2.4 % 3.4-DNT, 1.5 % 2.3-DNT and < 1 % of 2,5-DNT and 3,5-DNT) in corn oil on gestation days 7 – 20. One part of the dams was used for evaluating the teratogenic potential (sacrifice on gestation day 20 and examination of uterine contents for external, visceral, and sceletal malformations), the other part of the dams was subject of a developmental toxicity study evaluating the age of appearance of physical or neurobehavioural signs (sacrifice of dams on postnatal day 30, of offspring on postnatal day 60). The highest dose of 150 mg/kg turned out to be highly toxic for the dams with clinical signs of intoxication and a mortality rate of 46.2 % between day 11 and 18 of gestation. Therefore, in the second and third breeding the doses were reduced to 14, 37.5 and 100 mg/kg bw/day. As a consequence the number of animals per dose group (14, 35, 37.5, 75, 100, 150 mg/kg) was limited to 6 - 13. Hydroxyurea (200 mg/kg bw/day) served as positive control. In the 100 and 150 mg/kg group, the only groups examined for hematological parameters, signs of methemoglobinemia typical for DNT (compare Section 3.1.5) became obvious on gestation day 20 in dams and fetuses. At these doses and at 14 mg/kg, but not at 35 to 75 mg/kg, maternal weight gain was significantly reduced (14 mg/kg: by 29 %; 100 mg/kg: by 37 %, and 150 mg/kg: body weight loss). Significant increases of relative liver weights at ≥ 75 mg and relative spleen weights at ≥ 35 mg became obvious. There were no statistically significant differences among groups for the incidences of resorptions, or live or dead fetuses when these measures were expressed as a percentage of implants for each dam. Reproductive parameters were not affected and there were no indications for a teratogenic or embryo-/fetotoxic potential of DNT at any dose (CIIT, 1982b; Price et al., 1985; Wolkowski-Tyl et al., 1981). The NOAEL for teratogenicity/embryo- and fetotoxicity of DNT is 150 mg/kg bw/day, the NOAEL for maternal toxicity is 14 mg/kg bw/day in this study.

In the postnatal developmental toxicity part of the study described above, in total 53 litters (5 - 14 litters per treatment group) were observed from the day of birth through postnatal day 60. Signs of postnatal toxicity were reversed both for dams and pups by postnatal days 30 and 60, respectively.

Dams had reduced body weights on postnatal day 15 (100 mg/kg group) and reduced reticulocyte count on day 30 (75 mg/kg group). In the offspring single changes of litter size, crown-rump length, body weight and hematological parameters without dose- or time-dependency were recorded. During the postnatal period either statistically significant facilitation or retardation of growth or development were observed at single dosages (eye opening earlier at 14 mg and delayed at 35 and 75 mg; cliff avoidance delayed at 35 and 75 mg; wire grasping earlier at 14 mg and delayed at 35 mg; female pups: dose-related decrease of rearing in the open field; CIIT, 1982b). In view of the absence of a dose-relationship a connection of the observed effects with DNT exposure is unlikely. The NOAEL for developmental toxicity is 150 mg/kg bw/day in this study.

The 2,4-DNT isomer did not induce teratogenic effects in a three-generation study on rats (BUA, 1987). No data are available for 2,6-DNT.

Studies in Humans

Experiences with human DNT exposure are presented in chapter 3.1.9.

Conclusion

After long-term feeding of technical grade DNT to rats (104 weeks) increased incidences of abnormally small testes and increased ovary weights were observed at 14 mg/kg bw/day. A daily dose of 35 mg/kg bw DNT led to testicular degeneration and hypospermatogenesis after 52 weeks of exposure. The NOAEL for changes on reproductive organs was 3.5 mg/kg bw/day in this study. Technical grade DNT did not negatively affect fertility in dominant lethal assays. Overall, impairment of male rat fertility after chronic exposure of toxic doses cannot be excluded.

In pregnant rats, administration of technical grade DNT by gavage on gestation days 7 - 20 did not induce teratogenic/developmental effects even at dose levels, which produced significant maternal toxicity. The NOAEL for teratogenic/developmental toxicity can be determined with 150 mg/kg bw/day.

3.1.9 Experience with human exposure

A total of 154 workers in a military plant manufacturing powder containing technical DNT were followed for 12 months using medical examinations. During that period, 112 reported complaints and 84 had objective evidence of sickness. Among the complaints were unpleasant taste (62 %) and weakness (51 %), among the evidences of sickness were pallor (36 %), cyanosis (34 %), and anemia (23 %). The symptoms can be interpreted as signs of methemoglobinemia, which disappeared within 2 or 3 days after removal from the exposure (McGee et al., 1942). Similar findings were seen in 714 persons exposed to DNT between 1942 and 1945 (McGee et al., 1947). In both studies no details on exposure are given. In a study with 82 employees from a mechanical plant (dismantling of military waste) 63 showed TNT (trinitrotoluene) or DNT (not further specified) or metabolite concentrations above the analytical detection limit in urine (maximum air concentration was 20 $\mu g/m^3$ for 2,4-DNT). These persons reported more frequently symptoms like bitter taste, burning eyes, and discoloration of the skin and hair than persons (n = 19) without detectable exposure. Clinical laboratory examinations revealed findings outside the normal range, but no relation to the exposure could be found on a group basis (Letzel et al., 2003).

Hematological investigations were performed on 81 workers, exposed to time-weighted average concentrations of 1.64 mg/m^3 or 0.67 mg/m^3 of DNT (isomers not specified) in comparison to 30 unexposed persons. All DNT exposed workers showed significantly decreased erythrocyte counts, levels of hemoglobin, and GST-activity. Heinz body counts, Fe content, ALT- and SDH-activities were significantly increased. In the highly exposed workers additionally the amounts of methemoglobin were increased and CuZn-SOD-activity was decreased. These changes point to

hemolytic anemia and disturbances in the liver. However, since the study is reported in Chinese with English abstract only, the findings cannot be reliably assessed (Wu et al., 2000).

In a DNT manufacturing plant inhalative exposure was estimated to range from 50 - 590 µg DNT/m^3. The maximum daily DNT absorption in a participant in this study ranged from 0.24 - 1.0 mg/kg bw. Absorption was measured by quantification of excreted DNT metabolites in the urine (Levine et al., 1985). The urine of the workers contained more DNT metabolites than would have resulted from the dinitrotoluene present in the inhaled air, indicating dermal absorption (Levine et al., 1985; Woollen et al., 1985).

Two cohorts of 156 and 301 men who had worked a month or more during the 1940s and 1950s in ammunition plants with opportunity of substantial exposure to technical grade DNT (consisting of approximately 76 % 2,4-DNT, 19 % 2,6-DNT, and 5 % other isomers) or 2,4-DNT (consisting of 98 % 2,4-DNT and 1 % 2,6-DNT), but also many other materials (e.g. toluene, nitric and sulfuric acids), were followed through the end of 1980. In comparison with the mortality rates of US white males as the standard, no enhanced frequencies of malignant neoplasms as cause of death were found (standardized mortality rate: SMR 87), but an unsuspected excess of mortality from ischemic heart disease was noted: SMRs in the two cohorts were 131 and 143 (Kristensen, 1989; Levine et al., 1986a; 1986b). Since the workers were exposed to an undefined mixture of chemicals and no workplace measurements are available, this effect cannot be related to DNT.

A further retrospective cohort mortality study failed to detect an association between dinitrotoluene exposure and an increased risk of ischemic heart disease (IHD) or cerebrovascular disease mortality after examination of a total of 4989 workers with DNT exposure and 5636 unexposed workers (at least 5 months of occupation at the study facility between January 1949 and January 1980). No quantitative information on DNT exposure is given in this study (Stayner et al., 1992). Among these DNT-exposed workers an excess of hepatobiliary cancer was observed (6 cases, SMR 2.67; unexposed cohort with 7436 workers: 4 cases, SMR 0.81). A relation to the duration of exposure could not be demonstrated. Limitations of the study are the small number of workers with long duration of DNT exposure and the lack of detailed exposure data (Stayner et al., 1993).

6 cases of urothelial cancer and 14 cases of renal cancer occurred among 500 underground miners formerly (1984-1997) highly exposed to explosives containing 30 % technical DNT for periods of 7 - 37 years in a plant in the former German Democratic Republic. The composition of the remaining 70 % of the explosives is not given in the paper. Incidences of urothelial and renal cancer were increased by factors of 4.5 and 14.3 in comparison to the incidences anticipated from the local cancer registers. The cancer cases and a representative group of 183 formerly DNT-exposed minors were grouped into four exposure categories according to type and duration of professional contact. For renal cancer no exposure-response relationship could be determined. Of the 6 urothelial cancer cases 1 was found in the medium exposed group, 4 in the high and 1 in the very high exposed group (skin contact and inhalative exposure, no quantitative data). All of the 6 persons were genotyped and identified as slow acetylators (Brüning et al., 1998; 1999a; 1999b). 19 of the cancer cases and 161 representative underground miners that had all been highly exposed to the explosives containing DNT, were reinvestigated for signs of subclinical renal damage. Studied parameters were α1-microglobulin and GST α as biomarkers for damage of the proximal tubule and GST π for damage of the distal tubule. Results indicated exposure-dependent nephrotoxic effects directed to the tubular system (Brüning et al., 2001; Brüning, Thier, and Bolt, 2002).

Observations pointing to an adverse effect on the male reproductive system were not confirmed by further studies. The health hazard evaluation of workers exposed to DNT in an US chemical company yielded significant decreased sperm counts and an excess of spontaneous absorptions for wives of exposed workers, which was statistically not significant (Ahrenholz and Meyer, 1980; data taken from review). However, no conspicuous findings resulted when 84 workers exposed both to

dinitrotoluene (exposure level within OSHA threshold limit value of 1.5 mg/m^3, detailed exposure data not available; composition of DNT not given) and toluenediamine and 119 unexposed workers were the subjects of a physicians urogenital examination, a reproductive and fertility questionnaire, an estimation of testicular volume, an assessment of serum follicle-stimulating hormone, and an analysis of semen for sperm count and morphology (Hamill et al., 1982). In a further study reproductive and fertility questionnaires filled by about 670 workers in 3 US chemical plants, who were exposed to DNT (not further specified) but mostly also to toluene diamine and/or toluene diisocyanate, yielded no significant difference between the fertility of unexposed workers and of workers exposed to the chemicals (Levine, 1983; Levine, Dal Corso, and Blunden, 1985).

No further relevant data are available for experiences with human exposure to the pure isomers 2,4- or 2,6-DNT.

Conclusion

In humans, heavy DNT exposure causes signs of methemoglobinemia, which are reversible 2 - 3 days after removal from exposure. Signs of disturbances in liver function and exposure-dependent nephrotoxic effects directed to the tubular system were additionally found in exposed workers. Single findings in studies without reliable exposure data and/or only small numbers of significantly exposed workers indicating increased incidences of hepatobiliary or urothelial cancer in occupationally DNT exposed workers do not permit a conclusion on the carcinogenicity of DNT in humans. Preliminary observations pointing to an increased risk of ischemic heart disease or to an adverse effect on the human male reproductive system could not be confirmed by further studies.

3.2 Initial Assessment for Human Health

Dinitrotoluene (DNT) is a technical mixture containing approximately 80 % 2,4-dinitrotoluene, app. 20 % 2,6-dinitrotoluene and < 5 % 3,4-, 2,3- and 2,5-DNT. The toxicological profile of the DNT mixture well reflects the properties of the pure isomers. Data on the pure 2,4- and 2,6-isomers are presented here only if they provide relevant additional information.

In humans DNT (DNT, technical grade) is absorbed following dermal and inhalative exposure and is rapidly metabolized and excreted in urine.

There are no acute inhalation studies on technical grade DNT and the 2,4-isomer available. LC_{50} of the 2,6-isomer is reported to be 0.36 mg/l, however, this isomers accounts only for about 18% of technical grade DNT. No acute dermal studies on technical grade DNT and the 2,6-isomer are available. The acute dermal toxicity of 2,4-DNT, the main component of technical grade DNT, is relatively low with an LD_{50} greater than 2500 mg/kg bw in rats. Technical grade DNT is moderately toxic following oral administration to rats, with LD_{50} values of 268 to 660 mg/kg bw reported.

After a 24-hour occlusive application DNT is not irritating to the skin (interior side of the ear) of rabbits. Although DNT has been reported to induce slight irritation to the eye of one rabbit, the effect is reversible within 7 days, and therefore DNT is not considered to be an eye irritant in humans.

There are no data available to evaluate the sensitizing potential of technical grade DNT. The 2,4-DNT isomer showed no sensitizing properties in a guinea pig maximization test, whereas 2,6-DNT gave a mild positive response. Patch tests or photo-patch tests in 10 or 5 healthy humans showed no allergic potential of DNT (unspecified isomer), whereas a single case of positive photo-patch test reaction was reported for a worker with skin problems.

There are no inhalative or dermal repeated dose studies on technical grade DNT or on the 2,4-/2,6-DNT isomers available. Chronic feeding of technical grade DNT to rats led to hematological

changes (especially methemoglobinemia), and toxicity to liver, kidney, adrenal glands and testes in rats. At the lowest administered dose of 3.5 mg/kg bw/day signs of hepatotoxicity became obvious. No NOAEL can be derived for repeated dose toxicity.

Technical grade DNT is mutagenic in bacterial test systems in the presence and absence of metabolic activation, but it shows no mutagenic or genotoxic activity in mammalian cells in vitro. Technical grade DNT shows no mutagenic activity in the mouse bone marrow micronucleus assay and in mouse dominant lethal and spot tests. However, a distinct activity of DNT to induce DNA repair in the liver of rats is reported. Additionally, DNA binding properties in various rat organs, mainly rat liver were demonstrated for 2,4-DNT and 2,6-DNT isomers. Gut flora may play an important role in activation of DNT to reactive metabolites. Overall, technical grade DNT shows the potential to induce genotoxic changes in vivo.

Technical grade DNT shows hepatocarcinogenic properties in rats. In a long-term feeding study liver tumors were dose dependently induced in male rats from the lowest administered dose (3.5 mg/kg bw/day) and in female rats from 14 mg/kg bw/day. In an initiation-promotion liver foci assay DNT showed tumor promoting activity, however, only weak initiating properties. The pure 2,4- and 2,6-DNT isomers also induced liver tumors in rats. Additionally, the 2,4-isomer was shown to induce tumor formation in the renal tubular epithelium of male mice.

After long-term feeding of technical grade DNT to rats (104 weeks) increased incidences of abnormally small testes and increased ovary weights were observed at 14 mg/kg bw/day. A daily dose of 35 mg/kg bw DNT led to testicular degeneration and hypospermatogenesis after 52 weeks of exposure. The NOAEL for changes on reproductive organs was 3.5 mg/kg bw/day in this study. Technical grade DNT did not negatively affect fertility in dominant lethal assays. Overall, impairment of male rat fertility after chronic exposure of toxic doses cannot be excluded.

In pregnant rats, administration of technical grade DNT by gavage on gestation days 7 - 20 did not induce teratogenic/developmental effects even at dose levels, which produced significant maternal toxicity. The NOAEL for teratogenic/developmental toxicity can be determined with 150 mg/kg bw/day.

In humans, heavy DNT exposure causes signs of methemoglobinemia, which are reversible 2 - 3 days after removal from exposure. Signs of disturbances in liver function and exposure-dependent nephrotoxic effects directed to the tubular system were additionally found in exposed workers. Single findings in studies without reliable exposure data and/or only small numbers of significantly exposed workers indicating increased incidences of hepatobiliary or urothelial cancer in occupationally DNT exposed workers do not permit a conclusion on the carcinogenicity of DNT in humans. Preliminary observations pointing to an increased risk of ischemic heart disease or to an adverse effect on the human male reproductive system could not be confirmed by further studies.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Concerning the aquatic effects only one test for the DNT technical mixture is available. This is a short term toxicity test towards fish. Conversely, a lot of valid studies are available for the single components of the mixture (2,3-DNT, 2,4-DNT, 2,5-DNT,2,6-DNT and 3,4-DNT). Thus, the acute toxicity of DNT isomers to fish were compared with the toxicity of the technical-grade DNT mixture. The available data for fish show that the toxicity of the technical mixture can be explained by the additive toxicity of the single isomers.. With respect to the variability of the organisms and methods applied, it is concluded that for the trophic levels, for which data on the mixture are

lacking, the existing data for the single isomers can be used to interpret the aquatic effects for the whole mixture using the concept of additive toxicity.

Acute Toxicity Test Results

Acute toxicity to fish (*Brachydanio rerio*) has been tested in a test system according to the Japanese Industrial Standard (JIS) K 0101-1986-71. The reported 48 h EC_{50} was of 27 mg/l (MITI, 1992). This value is assumed to be a nominal concentration (Table 15).

Table 15 Tests on aquatic toxicity of DNT mixture and DNT isomers to fish (IUCLID 4.1)

Species	Test type	Parameter	Test substance	Effects [mg/l]	Reference
Oryzias latipes	static or semistatic	LC_{50} (48 h)	DNT technical mixture	27 (n)	MITI, 1992*
Poecilia reticulata	semistatic	LC_{50} (14 d)	2,4-DNT 2,6-DNT	12.6 (n) 17.8 (n)	Deneer et al., 1987
Pimephales promelas	static	LC_{50} (96 h)	2,3-DNT 2,4-DNT 2,5-DNT 2,6-DNT 3,4-DNT 3,5-DNT	1.9 (n) 32.5 (n) 1.3 (n) 19.8 (n) 1.5 (n) 22.0 (n)	Pearson et al., 1979*
Pimephales promelas	static	LC_{50} (96h)	2,3-DNT 2,4-DNT 2,5-DNT 2,6-DNT 3,4-DNT 3,5-DNT	1.8 (n) 32.8 (n) 1.3 (n) 18.5 (n) 1.5 (n) 22.6 (n)	Liu, Bailey, and Pearson, 1983
Pimephales promelas	static	LC_{50} (96h) NOEC	2.4-DNT	31 (e) 25 (e)	Liu, Spanggord, and Bailey, 1976
Gasterosteus aculeatus (estuary/marine)	semistatic	LC_{50} (96h)	2,4-DNT	6.3 (e)	Van den Dikkenberg et. al., 1989
Brachidanio rerio	semistatic	LC_{50} (4d)	2,4-DNT	> 16 (n)	Canton, Adema, and de Zwart, 1984
Oryzias latipes	semistatic	LC_{50} (4d)	2,4-DNT	> 16 (n)	Adema et. al., 1981
Jordanella floridae: (1-2 d old) (4-5 w old)		 LC_{50} (2d) LC_{50} (4d) LC_{50} (2+4d)		 25 (n) 22 (n) > 16 < 32 (n)	
Poecilia reticulata (4-5 w old): (tested by TNO) (tested by RIV)		 LC_{50} (2d) LC_{50} (4d) LC_{50} (2+4d)		 33 (n) 25 (n) > 16 (n)	

(n): nominal concentration
(e): effective concentration
(c): calculated
* : studies which are flagged as robust summary studies

Standard acute tests on fish toxicity of the main isomeric compounds 2,4-DNT and 2,6-DNT yielded toxicity values in the same range as the reported value of the DNT mixture. Tests with

Pimephales available for the other DNT isomers present in the technical mixture (2,3-, 2,5- and 3,4-DNT) show that these isomers are about an order of magnitude more toxic than the main isomers 2,4- and 2,6-DNT. With the model of concentration additivity a LC_{50} of about 17 mg/l can be estimated for the technical mixture using the data from Pearson et al. (1979) for the different isomers. This effect value is in good agreement with the value of 27 mg/l reported for the technical mixture.. Results of short-term tests for the other trophic levels *Daphnia*, algae and bacteria are presented in Tables 16, 17, and 19.

With *Daphnia magna* acute tests with different DNT-isomers were performed according to standard procedures or similar methods. Also for *Daphnia* it is shown that the isomes 2,3-, 2,5- and 3,4-DNT are about an order of magnitude more toxic than the 2,4- and 2,6-isomers. Assuming that the toxicity of the technical mixture can be explained by the additive toxicity of the single isomers a 48h-EC_{50} of about 23 mg/l can be estimated for the technical mixture from the data reported by Pearson et al. (1979).

Table 16 Tests on acute toxicity of DNT isomers to aquatic invertebrates (IUCLID 4.2)

Species	Test type	Parameter	Test substance	Effects [mg/l]	Reference
Daphnia magna (crustacea)	static	EC_{50} (48 h)	2,3-DNT 2,4-DNT 2,5-DNT 2,6-DNT 3,4-DNT 3,5-DNT	4.7 (n) 35.0 (n) 3.4 (n) 21.7 (n) 3.1 (n) 45.1 (n)	Pearson et al., 1979*
	flow through	EC_{50} (48 h)	2,4-DNT	30.4 (e)	Liu et al., 1984
	semistatic	EC_{50} (24 h)	2,4-DNT 2,6-DNT	38 (n) 20 (n)	Kuehn et al., 1988
	static	EC_{50} (24h)	2,3-DNT 2,4-DNT 2,6-DNT	3.2 (n) 22 (n) 14 (n)	Bringmann and Kuehn, 1977
Daphnia magna (crustacea)	static	EC_{50} (48 h)	2,3-DNT 2,4-DNT 2,5-DNT 2,6-DNT 3,4-DNT 3,5-DNT	4.7 (e) 47.5 (e) 3.1 (e) 21.8 (e) 3.7 (e) 45.2 (e)	Liu, Bailey, and Pearson, 1983
Daphnia magna (crustacea)	not specified	EC_{50} (48 h)	2,3-DNT 2,6-DNT	3.9 (n) 21 (n)	Bringmann and Kuehn, 1982
Daphnia magna (crustacea)	static	IC_{50} (48 h)	2,3-DNT 2,4-DNT 2,6-DNT 3,4-DNT	5.6 (n) 34 (n) 34 (n) 5.6 (n)	Deneer et al., 1988
Daphnia magna (crustacea)	static	EC_{50} (48 h)	2,4-DNT	26.2 (n)	Randall and Knopp, 1980
Daphnia magna (crustacea)	static	EC_{50} (48 h)	2,4-DNT	< 16 (n)	Adema et al., 1981
Daphnia magna (crustacea)	static	EC_{50} (48 h)	2,4-DNT	35 (n)	Liu, Spanggord, and Bailey, 1976
Lumbriculus variegatus (aquatic worm)	flow through	EC_{50} (48 h)	2,4-DNT	80.9 (e)	Liu et al., 1984

Also for algae, tests are available for the different isomers (Table 17). The results show that the toxicities of the 2,4- and 2,3-isomers are more similar than the toxicities of the 2,4- and 2,6-DNT. As the 2,4-DNT seems to be the most toxic isomer to algae, it can be concluded that the toxicity of the isomeric mixture for this trophic level can be described using the data for the 2,4-DNT.

Table 17 Tests on acute toxicity of DNT isomers to aquatic plants and blue-green algae (IUCLID 4.3)

Species	Endpoint	Parameter	Test substance	Effects [mg/l]	Reference
Scenesmus subspicatus (Algae)	growth rate	EC_{10} (48h) EC_{50} (48 h) EC_{10} (48h) EC_{50} (48 h)	2,4-DNT 2,6-DNT	1.9 (n) 6.3 (n) 10 (n) 22 (n)	Kuehn and Pattard, 1990
Selenastrum capricornutum (Algae)	growth rate	EC_{50} (96 h)	2,4-DNT 2,6-DNT	2.6 (e) 16.4 (e)	Dodard et al., 1999*
	growth rate	$EC_{37,4}$ (4d) $EC_{>98}$ (4d) $EC_{13.5}$ (14d) $EC_{>99}$ (14d)	2,4-DNT	0.9 (n) ≥ 4.7 (n) 0.9 (n) ≥ 9.4 (n)	Liu et al., 1984
Chlorella pyrenoidosa (Algae)	growth rate	EC_{50} (96 h)	2,3-DNT 2,4-DNT 2,6-DNT 3,4-DNT	0.9 (n) 0.9 (n) 6.7 (n) 0.7 (n)	Deneer et al., 1988*
Scenesmus subspicatus (Algae)	growth rate	TT (7 days)	2,4-DNT	1.4 (n)	Trénel and Kuehn, 1982
Scenedesmus quadricauda (Algae)	biomass	TT (8 days)	2,3-DNT 2,4-DNT 2,6-DNT	0.83 (n) 2.7 (n) 12 (n)	Bringmann and Kuehn, 1977
Scenedesmus obliquus (Algae)	growth rate	EC_{50} (48 h)	2,4-DNT	6.3 (n)	Liu and Lang, 2000
	growth rate	EC_{50} (48 h)	2,4-DNT 2,6-DNT	5.5 (n) 15.9 (n)	Liu and Lang, 1995
	growth rate	EC_{50} (48 h)	2,4-DNT 2,6-DNT	6.2 (n) 7.3 (n)	Lu, Yuan, and Zhao, 2001
Microcystis aeruginosa (Blue-green algae)	biomass	TT (8 days)	2,3-DNT 2,4-DNT 2,6-DNT	0.22 (n) 0.13 (n) 0.50 (n)	Bringmann, 1975
Potamogeton pectinatus (Magnoliophytina)	growth rate	LOEC (21 d)	2,4-DNT 2,6-DNT	5 (e)	Best et al., 2001
Heteranthera dubia (Magnoliophytina)					
Phragmites australis (Magnoliophytina)					
Phalaris arundinacea (Magnoliophytina)					
Lemna perpusilla (Magnoliophytina)	biomass	LOEC (11 d)	2,4-DNT	0.1 – 5.0 (n)	Schott and Worthley, 1974
Lemna minor (Magnoliophytina)	growth rate	EC_{50} (7-10d)	2,4-DNT	1.6 (n)	Adema and de Zwart, 1984
Scenedesmus pannonicus (Algae)	growth rate	EC_{50} (4 d)	2,4-DNT	1.6 – 2.3 (n)	Adema et al., 1981*
Chlorella pyrenoidosa (Algae)				3.8 (n)	
Selenastrum capricornutum (Algae)				1.6 (n)	
Microcystis aeruginosa (Blue-green algae)				0.08 (n)	
Stephanodiscus hatzschii (Diatom)				6.2 (n)	
Euglena gracilis (Flagellatae)				9.6 (n)	

Table 17 (cont.) Tests on acute toxicity of DNT isomers to aquatic plants and blue-green algae (IUCLID 4.3)

Species	Endpoint	Parameter	Test substance	Effects [mg/l]	Reference
Scenedesmus pannonicus (Algae)	growth rate	EC_{10} (4 d)	2,4-DNT	0.32 (n)	Adema et al., 1981*
Chlorella pyrenoidosa (Algae)				0.56 (n)	
Selenastrum capricornutum (Algae)				0.56 (n)	
Microcystis aeruginosa (Blue-green algae)				0.056 (n)	
Stephanodiscus hatzschii (Diatom)				1.0 (n)	
Euglena gracilis (Flagellatae)				1.0 (n)	

Chronic Toxicity Test Results

Several chronic toxicity tests towards fish and *Daphnia* are available for different DNT-isomers (Table 18).

In a chronic early life stage test (90d) with *Oncorhynchus mykiss* a NOEC of 0.27mg/l was measured for 2,4-DNT for the endpoint growth (Bailey et al., 1984). For *Daphnia magna* long-term tests with different isomers are available. A comparison of the effect values measured by Deneer et al. (1988) for the isomers 2,3-, 2,4-, 2,6- and 3,4-DNT shows that for the endpoint immobilisation the 2,4-DNT was most toxic. For the endpoint population growth and length of *Daphnia* the 3,4-DNT was the most toxic isomer, however only with a factor of 3 compared to the 2,4-DNT. The lowest 21d-NOEC of 0.02 mg/l was determined by Kuehn et al. (1988) in a reproduction test according to the German proposal of the Federal Environmental Agency (UBA) of 1984 for 2,4-DNT. This value is an effective concentration that was estimated based on the observed recoveries of higher tested concentrations, since the detection limit was at 0.05 mg/l. It is assumed that this NOEC covers also the toxicity of the technical mixture, as 2,4-DNT is the main component of the mixture and exhibited the highest toxicity in *Daphnia* long-term tests. For photothropic organisms, the EC_{10} (96 h) of 0.056 mg/l (nominal) determined by Adema et al. (1981) for *Microcystis aeruginosa* is used as long-term value.

Determination of $PNEC_{aqua}$

Chronic tests on three trophic levels are available for 2,4-DNT. For the other isomers that are components of the technical mixture, only for 2 trophic levels long-term tests are available (*Daphnia* and algae). However, from the available data it can be concluded that the toxicity of the technical mixture is covered by the toxicity of the 2,4-DNT, an assessment factor of 10 can be applied for the derivation of the $PNEC_{aqua}$ for DNT according to EU Technical Guidance Document. From the effect value for the most sensitive species, *Daphnia magna* (Kuehn et al., 1988), a

$PNEC_{aqua}$ of 2 µg/l

is obtained.

Table 18 Tests on chronic toxicity of DNT isomers to fish and *Daphnia* (IUCLID 4.5.1 & 4.5.2)

Species	Endpoint	Parameter	Test substance	Effects [mg/l]	Reference
Oncorhynchus mykiss (Fish, fresh water)	fry growth (length, weight)	NOEC (90 d)	2,4-DNT	0.27 (e)	Bailey et al., 1984*
Pimephales promelas (Fish, fresh water)	reproduction rate	LOEC (179 d)	2,4-DNT	0.28 (e)	Bailey et al., 1984
Gasterosteus aculeatus (Fish, estuary, marine)	growth (length, weight)	NOEC (35 d)	2,4-DNT	0.77 (e)	van den Dikkenberg et al., 1989
Daphnia magna (Crustacea)	immobilization, mortality, reproduction	LC_{50} (21 d)	2,4-DNT	19 (n)	Adema et al., 1981
Daphnia magna (Crustacea)	immobilization	IC_{50} (21 d)	2,3-DNT 2,4-DNT 2,6-DNT 3,4-DNT	1.8 (n) 0.6 (n) 9.6 (n) 1.1 (n)	Deneer et al., 1988
	Growth of population	LRCT* (21 d)	2,3-DNT 2,4-DNT 2,6-DNT 3,4-DNT	3.2 (n) 1.0 (n) 10.0 (n) 0.3 (n)	
		NOEC (21 d)**	2,3-DNT 2,4-DNT 2,6-DNT 3,4-DNT	1 (n) 0.3 (n) 3 (n) 0.09 (n)	
	Length of *Daphnia*	LRCT* (21 d)	2,3-DNT 2,4-DNT 2,6-DNT 3,4-DNT	1.0 (n) 1.0 (n) 1.0 (n) 0.3 (n)	
		NOEC (21 d)**	2,3-DNT 2,4-DNT 2,6-DNT 3,4-DNT	0.3 (n) 0.3 (n) 0.3 (n) 0.09 (n)	
Daphnia magna (Crustacea)	reproduction rate	NOEC (21 d)	2,4-DNT 2,6-DNT	0.02 (e) 0.06 (e)	Kuehn et al., 1988*
Daphnia magna (Crustacea)	reproduction rate	NOEC (21 d)	2,4-DNT	<0.5 (e)	Bayer AG, 1986c

* LRCT = Lowest rejected concentration tested with effect (similar to LOEC)
** derived from LOEC using a factor of 3.2 (spacing of test concentrations)

Toxicity to Microorganisms

There are data available for the different DNT isomers (Table 19).

Table 19 Tests on acute toxicity of DNT isomers to microorganisms (IUCLID 4.4)

Species	Endpoint	Parameter	Test substance	Effects [mg/l]	Reference
Pseudomonas putida (Bacteria)	cell multipl.	TT (16 h)	2,3-DNT 2,4-DNT 2,6-DNT	9 (n) 57 (n) 26 (n)	Bringmann and Kuehn, 1977*
Pseudomonas putida (Bacteria)	O_2-consumption	EC0	2,4-DNT	100	Bayer AG, 2003c
Pseudomonas putida (Bacteria)		EC_{10} (10 h)	2,4-DNT	38 (n)	Trénel and Kuehn, 1982
Sandy and loamy natural soil organisms	dehydrogenase activity	sandy soil: EC_{26-46} loamy soil: EC_{32} (28 d) ist chronisch	2,4-DNT 2,4-DNT	5, 50 **(n) 50** (n)	Roembke et al., 1995*
Tetrahymena pyriformis (Protozoa)	cell multipl	EC_{50} (24 h)	2,6-DNT	100 (n)	Yoshioka, 1985
Chilomonas paramaecium (Protozoa)	cell multipl.	TT (48 h)	2,3-DNT 2,4-DNT 2,6-DNT	1.8 (n) 13 (n) > 20 (n)	Bringmann and Kuehn, 1981
Uronema parduzci (Protozoa)	cell multipl.	TT (20 h)	2,3-DNT 2,4-DNT 2,6-DNT	1.6 (n) 0.55 (n) 23 (n)	Bringmann and Kuehn, 1981
Entosiphon sulcatum (Protozoa)	cell multipl.	TT (72 h)	2,3-DNT 2,4-DNT 2,6-DNT	5.9 (n) 0.98 (n) 11 (n)	Bringmann and Kuehn, 1981

** mg/kg soil (dw)

From the available data it can be concluded that the toxicity of the technical mixture is covered by the toxicity of the 2,4-DNT.

4.2 Terrestrial Effects

Tests on toxicity of DNT were performed according the principles of Good Laboratory Praxis (GLP).

DNT technical mixture was tested with *Bassica rapa* and with *Avena sativa*. An EC_{50} of 6.5 mg/kg dw after 14 days for *Bassica rapa* was derived from the effects of the tested nominal concentrations. The test was conducted according the BBA-proposed guideline of 1984, using a soil with 60 % humidity and a temperature of 20 - 21 °C and a photoperiod of 16 h light / 8 h dark. The same test was perfomed with *Avena sativa* and an EC_{50} of 65 mg/kg dw was found.

The results are presented in Table 20.

Table 20 Tests on toxicity of DNT isomers to terrestrial organisms (IUCLID 4.6.2 & 4.6.3)

Species	Endpoint	Parameter	Test substance	Effects	Reference
Brassica rapa (terrestrial plant)	growth	EC_{50} (14 d)	2,4-/2,6-DNT (80/20)	6.5 mg/kg soil (dw) (n)	Bayer AG 1990b*
Avena sativa (terrestrial plant)	growth	EC_{50} (14 d)	2,4-/2,6-DNT (80/20)	65 mg/kg soil (dw) (n)	
Avena sativa (terrestrial plant)	growth	EC_{50} (16 d)	2,4-DNT	61 mg/l (n)	Roembke et al. 1995
Eisenia fetida (soil dwelling organism)	mortality	LC_{50} (14 d)	2,4-/2,6-DNT (80/20)	668 mg/kg soil (dw) (n)	Bayer AG 1990c*
Eisenia fetida (soil dwelling organism	mortality	LC_{50} (14 d)	2,4-DNT	536 mg/kg dw	OECD 1997
Lycopersicum esculentum	growth	EC_{50} (14 d)	2,4-DNT	4.9 mg/kg dw (n)	EU 2004
Folsomia candida	Mortality Reproduction Mortality of parental org.	LC_{50} (24 h) EC_{10} (34 d) EC_{10} (34d)	2,4-DNT	42.8 mg/kg dw 3.2 mg/kg dw 2.8 mg/kg dw	OECD 1997

The tests available for *Avena sativa* indicate that the toxicity of the technical mixture is covered by the toxicity of the pure 2,4-DNT. Also for earthworms a comparison of available tests with the technical mixture and with pure 2,4-DNT indicates that the toxicity of the technical mixture to earthworms is more or less covered by the toxicity of the 2,4-DNT.

4.3 Other Environmental Effects

No data available.

4.4 Initial Assessment for the Environment

Environmental behaviour:

In the atmosphere DNT is degraded by photochemically produced OH radicals. The half-life is calculated to be ca. 84 days. In surface waters, from photodegradation measurements a half-life of 1 day was derived for (predominantly) direct photolysis under the radiative conditions of latitude 40 °N. In surface waters, with regard to the geographical conditions in Germany and the low light intensity in natural water bodies, the half-life of 2,4-DNT for direct photolysis is calculated to be 20 days in a natural water body (surface layer: 6.5 days).

DNT is not expected to hydrolyse in the environment due to the lack of hydrolyzable groups.

Biodegradation was tested under aerobic and anaerobic conditions. In organic soil a DT_{50} for 2,4-DNT of 7 days and a DT_{90} of 191 days was found. In an aquatic ready test (aerobic) according the OECD TG 301C, 0 % biodegradation was reported after 14 days. Thus dinitrotoluene is not readily biodegradable In a test system under anaerobic conditions, performed according the EPA-Guideline No. 796.3140, 0 % biodegradation was observed within 56 days. DNT can be primary biodegraded and also mineralized by selected adapted bacteria cultures under specific conditions. However, under environmental conditions where no adaptation of the microorganisms can be assumed, no biodegradation of DNT is expected.

According to the Mackay fugacity model level I, the favourite target compartment of DNT is water with 97 - 98 %. The calculated Henry's law constant (0.01-0.04 Pa m^3/mol at 25 °C) proves a low potential for volatilization from surface waters.

DNT bioconcentration factors measured in fish are in the range of 0.6–21.2 indicating no significant bioaccumulation potential.

K_{oc} values were calculated with PCKOCWIN v1.66 (K_{oc} = 371) and with the TGD equation for nitrobenzenes (K_{oc} = 123). These results indicate a low to medium sorption potential of DNT onto the organic phase of soil or sediments. A test on leaching from three different type of soils is available for 2,4-DNT. After 2 days of leaching still no 2,4-DNT was found in the leachates.

Concerning the acute toxicity of DNT towards aquatic species, experimental results for the trophic level fish are available. The acute toxicity determined for fish (*Oryzias latipes)* was 27 mg/l (48 h-LC_{50}). Acute toxicity data available for the single isomers of the technical mixture show that the toxicity of 2,4- and 2,6-DNT is in the same order of the toxicity found for the technical mixture. However, the other isomers are about an order of magnitude more toxic to fish than the main isomers. With the model of concentration additivity a LC_{50} of about 17 mg/l can be estimated for the technical mixture which is in good agreement with the experimentally determined value.

With *Daphnia magna* acute tests with different DNT-isomers were performed according to standard procedures or similar methods. Also for *Daphnia* it is shown that the isomers 2,3-, 2,5- and 3,4-DNT are about an order of magnitude more toxic than the 2,4- and 2,6-isomers. Assuming that the toxicity of the technical mixture can be explained by the additive toxicity of the single isomers a 48h-EC_{50} of about 23 mg/l can be estimated for the technical mixture.

For algae, tests available for the different isomers show that the toxicities of the 2,4- and 2,3-isomers are more similar than the toxicities of the 2,4- and 2,6-DNT. As the 2,4-DNT seems to be the most toxic isomer to algae, it can be concluded that the toxicity of the isomeric mixture for this trophic level can be described using the data for the 2,4-DNT.

The effect values from short-term tests for the technical DNT are:

Oryzias latipes:	48 h-LC_{50} = 27 mg/l (measured)
Pimephales promelas:	96 h-LC_{50} = 17 mg/l (estimated from the toxicity of the single isomers)
Daphnia magna:	48 h-EC_{50} = 23 mg/l (estimated from the toxicity of the single isomers)
Selenastrum capricornutum:	96 h-E_rC_{50} = 2.6 mg/l (e) (2,4-DNT, regarded as representative for technical mixture)
Chlorella pyrenoidosa:	96 h-E_rC_{50} = 0.9 mg/l (n) (2,4-DNT, regarded as representative for technical mixture)
Microcystis aeruginosa:	96 h-EC_{50} = 0.08 mg/l (n) (2,4-DNT, regarded as representative for technical mixture)

Reliable tests on chronic toxicity towards fish, *Daphnia,* algae and blue-green algae are available as well. The lowest effect values were obtained with 2,4-DNT:

Oncorhynchus mykiss :	90 d-NOEC = 0.27 mg/l (e) (growth)
Daphnia magna:	21 d-NOEC = 0.02 mg/l (e) (reproduction)
Scenedesmus subspicatus:	48 h-E_rC_{10} = 1.9 mg/l (n)
Scenedesmus pannonicus:	96 h-E_rC_{10} = 0.32 mg/l (n)
Microcystis aeruginosa:	96 h-E_rC_{10} = 0.056 mg/l (n)

It can be concluded that the toxicity of the technical mixture is also covered by these data.

For terrestrial organisms reliable experimental data are available with plants and earthworms. The most sensitive plant species was *Brassica rapa* with a 14 d-EC_{50} of 6.5 mg/kg soil dry weight (nominal) using the proposed guideline of the German BBA. For the earthworm *Eisenia fetida* a 14d-LC_{50} of 668 mg/kg soil dry weight (nominal) was determined with the OECD TG 207.

Following the EU Technical Guidance Document, for the derivation of the $PNEC_{aqua}$ an assessment factor of 10 is chosen since long term tests for three trophic levels for DNT isomers are available. Using the lowest determined concentration, the *Daphnia magna*-NOEC of 0.02 mg/l (effective), a

$$\mathbf{PNEC_{aqua} = 2\ \mu g/l}$$

is derived.

5 RECOMMENDATIONS

Environment:

The chemical is a candidate for further work.

The chemical possesses properties indicating a hazard for the environment. Based on data presented by the Sponsor company, exposure from production and processing as chemical intermediate is low. However, in addition to the use as chemical intermediate some direct uses of DNT have been identified. Therefore, an exposure assessment and, if then indicated, an environmental risk assessment is recommended. Countries may desire to investigate any exposure scenario not presented by the Sponsor Country, e.g. exposure from munitions dumps and former munitions sites.

Human Health:

The chemical is currently of low priority for further work.

Technical grade DNT possesses properties indicating a hazard for human health (moderate acute toxicity, toxic after prolonged exposure, mutagenic and carcinogenic properties, influences on fertility in toxic doses possible). Based on data presented by the Sponsor country, exposure is well controlled in occupational settings and is anticipated to be low for consumers, therefore this chemical is currently of low priority for further work. Countries may desire to investigate any exposure scenarios that were not presented by the Sponsor country.

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I U C L I D

Data Set

Existing Chemical	:	ID: 25321-14-6
CAS No.	:	25321-14-6
EINECS Name	:	dinitrotoluene
EC No.	:	246-836-1
Molecular Formula	:	C7H6N2O4

Producer related part

Company	:	Bayer AG
Creation date	:	14.07.1994

Substance related part

Company	:	Bayer AG
Creation date	:	14.07.1994
Status	:	
Memo	:	X AKTUELL / ICCA EG-ABGABE JUNI 1995
Printing date	:	08.09.2004
Revision date	:	06.07.1995
Date of last update	:	08.09.2004
Number of pages	:	235
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

IUPAC Name : Dinitrotoluene (isomers mixture)
Smiles Code : Cc1cccc(N(=O)=O)c1N(=O)=O
Molecular formula : C7H6N2O4
Molecular weight : 182.14
Petrol class :

Remark : Smiles code (Cc1cccc(N(=O)=O)c1N(=O)=O) is from EPIWIN program and represents 2,3-DNT

17.11.2003 (1)

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type : typical for marketed substance
Substance type : organic
Physical status : solid
Purity :
Colour :
Odour :

Result : Composition of industrial product is given by Booth (2003)
2,3-DNT (CAS-No. 602-01-7) 1.3 %
2,4-DNT (CAS-No. 121-14-2) 78 %
2,5-DNT (CAS-No. 619-15-8) 0.5 %
2,6-DNT (CAS-No. 606-20-2) 18 %
3,4-DNT (CAS-No. 610-39-9) 2.4 %
Flag : Critical study for SIDS endpoint
31.03.2004 (2)

Purity type : measured for specific batch
Substance type : organic
Physical status : solid
Purity :
Colour : yellow to brown
Odour : characteristic odour

Result : Composition of technical isomers mixture (e.g. DNT 80/20)
2,4-DNT (CAS-No. 121-14-2) ca. 80 %
2,6-DNT (CAS-No. 606-20-2) ca. 20 %
Flag : Critical study for SIDS endpoint
03.10.2003 (3)

Purity type : typical for marketed substance
Substance type : organic
Physical status : solid
Purity :
Colour :
Odour :

Test substance : Composition of industrial product is given by BUA (1987)
2,4-DNT (CAS-No. 121-14-2) 77.9 %
2,6-DNT (CAS-No. 606-20-2) 18 %
3,4-DNT (CAS-No. 610-39-9) 2.3 %
2,3-DNT (CAS-No. 602-01-7) 1.3 %
2,5-DNT (CAS-No. 619-15-8) 0.5 %

17.11.2003 (1)

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

Benzene, methyldinitro-

Remark : CAS-name
20.08.2003 (4)

Dinitrotoluene (isomers mixture)

02.08.2003

Dinitrotoluene 80/20

Remark : Synonym is derived from the composition of the technical isomers mixture. It consists of approximately 80 % of 2,4-DNT and 20 % of 2,6-DNT. The following isomers are also present: 2,3-DNT 1.3 %, 2,5-DNT 0.5 %, and 3,4-DNT 2.4 %.

19.01.2004 (3) (2)

Dinitrotoluene, mixture of isomers

Source : Information entered in IUCLID 1994-09-27

Dinitrotoluenes

02.08.2003 (1)

Dinitrotoluol-Gemisch

Source : Information entered in IUCLID 1994-09-27

Dinitrotoluol-Isomerengemisch

Source : Information entered in IUCLID 1994-09-27

DNT

Remark : The isomers are abreviated as follows: 2,4-DNT, 2,6-DNT...

01.08.2003 (1)

Methyldinitrobenzenes

02.08.2003 (1)

1.3 IMPURITIES

Purity : typical for marketed substance
CAS-No :
EC-No :
EINECS-Name :
Molecular formula :
Value :

Remark : See 1.1.1
17.11.2003

1.4 ADDITIVES

1.5 TOTAL QUANTITY

Remark : TDI manufacturing capacity distribution reflects DNT manufacturing capacity distribution since DNT is nearly exclusively used for TDI manufacturing in the same region.

Result : TDI (toluylene diisocyanate) production capacities (1000 t/a) and manufacturing capacities distribution

Region	1000 t/a	% of total
Western Europe	488	31
North America	684	44
Eastern Europe	43	3
Japan	211	14
Korea	106	7
Other	20	1
Total	1552	100

Flag : Critical study for SIDS endpoint
17.11.2003 (5)

Quantity : 200000 - tonnes produced in 2001

Result : Starting from toluene Bayer produced about 200,000 t/a DNT in 2001
Flag : Critical study for SIDS endpoint
18.08.2003 (6)

Quantity : ca. 125000 - tonnes produced in 1987

Remark : Estimated annual production volume in Sponsor country in the 1980s
18.08.2003 (1)

Quantity : ca. 850000 - tonnes produced in 1987

Remark : Estimated worldwide annual production volume in the 1980s
18.08.2003 (1)

Quantity : 100000 - 500000 tonnes produced in 2003

26.05.2004

1.6.1 LABELLING

Labelling	:	as in Directive 67/548/EEC
Specific limits	:	
Symbols	:	T, N, ,
Nota	:	, ,
R-Phrases	:	(45) May cause cancer (23/24/25) Toxic by inhalation, in contact with skin and if swallowed (48/22) Harmful: danger of serious damage to health by prolonged exposure if swallowed (51/53) Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment (62) Possible risk of impaired fertility
S-Phrases	:	(53) Avoid exposure - obtain special instructions before use (45) In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible) (61) Avoid release to the environment. Refer to special instructions/Safety data sets
Flag	:	Critical study for SIDS endpoint

30.04.2003

1.6.2 CLASSIFICATION

Classified	:	as in Directive 67/548/EEC
Class of danger	:	carcinogenic, category 2
R-Phrases	:	(45) May cause cancer
Specific limits	:	

31.03.2004

Classified	:	as in Directive 67/548/EEC
Class of danger	:	dangerous for the environment
R-Phrases	:	(51/53) Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
Specific limits	:	

27.02.2003

Classified	:	as in Directive 67/548/EEC
Class of danger	:	harmful
R-Phrases	:	(48/22) Harmful: danger of serious damage to health by prolonged exposure if swallowed
Specific limits	:	

27.02.2003

Classified : as in Directive 67/548/EEC

Class of danger : mutagenic, category 3
R-Phrases : (68) Possible risks of irreversible effects
Specific limits :

27.02.2003

Classified : as in Directive 67/548/EEC
Class of danger : toxic
R-Phrases : (23/24/25) Toxic by inhalation, in contact with skin and if swallowed
Specific limits :

27.02.2003

Classified : as in Directive 67/548/EEC
Class of danger : toxic for reproduction, category 3
R-Phrases : (62) Possible risk of impaired fertility
Specific limits :

27.02.2003

1.6.3 PACKAGING

1.7 USE PATTERN

Type of use : type
Category : Non dispersive use

Remark : 1 %
industrial: explosives industry
use: additive in explosive preparations

09.03.1999

Type of use : type
Category : Use in closed system

Remark : 99 %
industrial: used in synthesis
use: intermediates

1.7.1 DETAILED USE PATTERN

1.7.2 METHODS OF MANUFACTURE

1.8 REGULATORY MEASURES

Type of measure : Banned
Legal basis : other: EU Directive 2003/34/EC

Remark : The EU Directive 2003/34/EC bans the use of DNT in consumer products.
27.11.2003 (7)

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

Type of limit : TRK (DE)
Limit value : .05 mg/m3

Remark : 2,6-DNT
26.05.2004

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

Classified by : KBwS (DE)
Labelled by :
Class of danger : 3 (strongly water polluting)

Remark : classification for 2,4-dinitrotoluene

1.8.4 MAJOR ACCIDENT HAZARDS

Legislation : Stoerfallverordnung (DE)
Substance listed : yes
No. In Seveso directive : App 2, No. 4 c

1.8.5 AIR POLLUTION

Classified by : TA-Luft (DE)
Labelled by :
Number : 2.3 (carcinogenic substances)
Class of danger : other: class not fixed

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

Type of search : Internal and External
Chapters covered : 1
Date of search : 24.07.2002

Flag : Critical study for SIDS endpoint
18.08.2003

Type of search : Internal and External
Chapters covered : 2
Date of search : 24.07.2002

Flag : Critical study for SIDS endpoint
18.08.2003

Type of search : Internal and External
Chapters covered : 3, 4
Date of search : 01.07.2003

Flag : Critical study for SIDS endpoint
18.08.2003

Type of search : Internal and External
Chapters covered : 5
Date of search : 01.04.2003

Remark : Human health: last literature search April 2003: CAS number search in external and internal databases, e.g. Biosis, Embase, Toxline, Scisearch.
Flag : Critical study for SIDS endpoint
18.08.2003

1.13 REVIEWS

Memo : BUA Report

Reliability : (2) valid with restrictions
collection of data
Flag : Critical study for SIDS endpoint
20.11.2003 (1)

Memo : BUA Supplementary Report

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
23.03.2004 (8)

Memo : IARC Report

Reliability : (2) valid with restrictions
20.11.2003 (9) (10)

Memo : US Department for Health and Human Services ATSDR

Reliability : (2) valid with restrictions
collection of data
20.11.2003 (11)

Memo : Current Intelligence Bulletin "Dinitrotoluene" (NIOSH)

Remark : Dinitrotoluenes (2,4-DNT, 2,6-DNT and technical grade DNT) are believed to be metabolized in the liver to dinitrobenzylalkohol, which is then conjugated to form a glucuronide conjugate that is excreted in bile or urine. This conjugate is thought to be hydrolyzed by intestinal microflora to aminonitrobenzyl alkohol. These bacteria are present in the gastrointestinal flora of rodents and humans. Aminonitrobenzyl alkohol is thought to be reabsorbed and returned to the liver where it is further metabolized to an unidentified toxic metabolite or to the precursor of a toxic metabolite.

Reliability : (2) valid with restrictions
collection of data

Flag : Critical study for SIDS endpoint
25.11.2003 (12)

Memo : Bibra Toxicity Profile

Reliability : (2) valid with restrictions
collection of data
20.11.2003 (13)

Memo : MAK

Reliability : (2) valid with restrictions
collection of data
20.11.2003 (14)

Memo : review

Reliability : (2) valid with restrictions
collection of data
20.11.2003 (15)

2.1 MELTING POINT

Value : 56 - 59 °C
Sublimation :
Method : other: not given
Year : 1992
GLP : no data
Test substance : other TS: ca. 80 % 2,4-DNT and 20 % 2,6-DNT

Reliability : (2) valid with restrictions
Reliable source
Flag : Critical study for SIDS endpoint
19.08.2003 (16)

Value : 55 - 57 °C
Sublimation :
Method : other: not given
Year : 2003
GLP : no data
Test substance : other TS: ca. 80 % 2,4-DNT and 20 % 2,6-DNT

Remark : solidifying temperature (dried)
Reliability : (4) not assignable
Not assignable/manufacturer data without proof
19.08.2003 (3)

Value : 60 °C
Sublimation :
Method : other: experimental, not specified
Year : 2000
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data
11.06.2003 (17)

Value : 70 °C
Sublimation :
Method : other: not given
Year : 2003
GLP : no data
Test substance : other TS: presumably pure 2,4-DNT

Remark : data presumably refers to 2,4-DNT but this assumption cannot be verified by the information supplied
Reliability : (3) invalid
Documentation insuffcient for assessment
17.11.2003 (18)

Value : 69.4 °C
Sublimation :
Method : other: not given
Year : 2003
GLP : no data
Test substance : other TS: presumably pure 2,4-DNT

Reliability : (2) valid with restrictions

Data from handbook or collection of data
18.08.2003 (19)

Value : 48.5 - 50 °C
Sublimation :
Method : other: not given
Year : 2001
GLP : no data
Test substance : other TS: 65 % 2,4-DNTand 35 % 2,6-DNT

Remark : Data for dinitrotoluene 65/35 for comparison
Reliability : (4) not assignable
Not assignable/manufacturer data without proof
18.08.2003 (20)

Sublimation :
Method : other: not specified
Year : 1991
GLP :
Test substance : other TS: 2,4-DNT and 2,6-DNT, no purity given

Result : 2,4-DNT 72 °C
2,6-DNT 66 °C
Reliability : (2) valid with restrictions
Data from handbook or collection of data
30.10.2003 (21)

2.2 BOILING POINT

Value : 250 °C at
Decomposition :
Method : other: not given
Year : 1994
GLP : no data
Test substance : other TS: technical-grade DNT

Reliability : (2) valid with restrictions
Data from handbook or collection of data
Flag : Critical study for SIDS endpoint
20.08.2003 (22)

Value : 284 °C at
Decomposition :
Method : other: reported from database of calculation program
Year : 2000
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data
29.08.2003 (17)

Value : 323.3 °C at 1013 hPa
Decomposition :
Method : other: not given
Year : 2003
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data
29.08.2003 (19)

2.3 DENSITY

Type : density
Value : 1.3208 g/cm³ at 71 °C
Method : other: not given
Year : 1994
GLP : no data
Test substance : other TS: technical-grade DNT

Reliability : (2) valid with restrictions
Data from handbook or collection of data
Flag : Critical study for SIDS endpoint
01.09.2003 (22)

Type : density
Value : ca. 1.52 g/cm³ at 15 °C
Method : other: not given
Year : 2003
GLP : no data
Test substance : other TS: 80 % 2,4-DNT and 20 % 2,6-DNT

Reliability : (4) not assignable
Not assignable/manufacturer data without proof
Flag : Critical study for SIDS endpoint
01.09.2003 (3)

Type : density
Value : 1.521 - 1.538 g/cm³ at °C
Method :
Year : 1991
GLP :
Test substance : other TS: 2,4-DNT and 2,6-DNT

Result : 2,4-DNT 1.521 kg/l
2,6-DNT 1.538 kg/l
Reliability : (2) valid with restrictions
Data from handbook or collection of data
17.11.2003 (21)

Type : density
Value : 1.3208 at °C
Method : other: not given
Year : 2003
GLP : no data
Test substance : no data

Remark : No temperature reported, no information on substance supplied
Reliability : (4) not assignable
Not assignable: Data without proof
17.11.2003 (18)

Type : density
Value : 1.3206 at 71 °C

Method : other: not given
Year : 1995
GLP : no data
Test substance : other TS: ambiguous whether pure 2,4-DNT (99 % 2,4-DNT) or technical 2,4-DNT isomers mixture (78 % 2,4-DNT) is meant

Reliability : (3) invalid
Documentation insufficient for assessment
17.11.2003 (23)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value : .00016 - .00065 hPa at 25 °C
Decomposition : no
Method : other (measured): vapour pressure weighing machine
Year : 1986
GLP : no
Test substance : other TS: 2,4-DNT, 2,5-DNT, 2,6-DNT, all purities > 99.5 %

Result : Measured values (hPa):

Temp	2,4-DNT	2,5-DNT	2,6-DNT
20 °C	0.788E-04	3.380E-04	1.486E-04
25 °C	1.593E-04	6.481E-04	3.182E-04
30 °C	3.147E-04	1.216E-03	6.647E-04
35 °C	6.083E-04	2.236E-03	1.356E-03
40 °C	1.151E-03	4.033E-03	2.703E-03
45 °C	2.135E-03	7.139E-03	5.273E-03
50 °C	3.885E-03	1.241E-02	1.008E-02
55 °C	6.942E-03	2.123E-02	1.888E-02
60 °C	1.219E-02	3.573E-02	3.472E-02
65 °C	2.105E-02	0.059*	0.063*
70 °C	3.578E-02	0.097*	0.111*
75 °C	5.990E-02		
80 °C	0.099*		

*extrapolated

Test condition : 2 replicate measurements
Reliability : (2) valid with restrictions
Basic data given
Flag : Critical study for SIDS endpoint
20.11.2003 (24)

Value : .0029 hPa at 25 °C
Decomposition :
Method : other (calculated): with MPBPWIN, v.1.40
Year : 2003
GLP :
Test substance : other TS: 2,3-DNT

Result : EPIWIN: Vapor Pressure Estimations (25 deg C):
VP: 0.00188 mm Hg (Antoine Method) = 0,0025 hPa
VP: 0.00215 mm Hg (Modified Grain Method)= 0,0029 hPa
VP: 0.00379 mm Hg (Mackay Method) = 0,0051 hPa
Selected VP: 0.00215 mm Hg (Modified Grain Method)
Reliability : (2) valid with restrictions

Accepted calculation method

24.11.2003 (25)

Value : .00028 - .00076 hPa at 20 °C
Decomposition :
Method : other (measured):electron-capture gas-chromatographic method
Year : 1977
GLP : no
Test substance : other TS: 2,4-DNT and 2,6-DNT, purity not specified

Result : Temperature dependence is given by the (Clausius -Clapeyron) equations
2,4-DNT: log10(p[Torr])=(13.08 +/- 0.19)- (4992 +/- 59) K/T
2,6-DNT: log10(p[Torr])=(13.99 +/- 0.18)- (5139 +/- 52) K/T
The values of vapor pressure at 20 °C can be calculated according to the equation:
2,4-DNT: 0.00028 hPa
2,6-DNT: 0.00076 hPa
Test condition : Temperature range of the measurements: 277.15 to 344.15 K
Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles

19.11.2003 (26)

Value : 1 hPa at 20 °C
Decomposition :
Method : other (measured): not specified
Year : 2003
GLP : no data
Test substance : other TS: DNT 80/20

Result : Other results: 0.1 hPa at 50 °C.
Thus, the reported data are not consistant (typing error?)
Reliability : (3) invalid
Documentation insufficient for assessment

17.11.2003 (19)

Value : 1.33 hPa at 20 °C
Decomposition :
Method : other (measured): not specified
Year : 1994
GLP : no data
Test substance : other TS: technical-grade DNT

Remark : Data is not consistent with the existing database
Reliability : (4) not assignable
Documentation insufficient for assessment

19.11.2003 (22)

Value : 1.33 hPa at 20 °C
Decomposition :
Method : other (measured): not specified
Year : 1991
GLP : no data
Test substance : other TS: 2,4-DNT

Test substance : Although the CAS no. of 2,4-DNT is reported, it is stated that the result is obtained from a liquid isomers mixture. Thus, it is not clear which data was reported
Reliability : (3) invalid
Documentation insuffcient for assessment

20.11.2003 (27)

2.5 PARTITION COEFFICIENT

Partition coefficient : octanol-water
Log pow : 1.98 at °C
pH value :
Method : other (measured)
Year : 1995
GLP : no data
Test substance : other TS: 2,4-DNT

Reliability : (2) valid with restrictions
Data from handbook or collection of data
Flag : Critical study for SIDS endpoint
18.08.2003 (28) (29)

Partition coefficient : octanol-water
Log pow : 2.1 at °C
pH value :
Method : other (measured)
Year : 1995
GLP : no data
Test substance : other TS: 2,6-DNT

Reliability : (2) valid with restrictions
Data from handbook or collection of data
Flag : Critical study for SIDS endpoint
18.08.2003 (28)

Partition coefficient : octanol-water
Log pow : 2.18 at 25 °C
pH value :
Method : other (calculated): with KOWWIN, v.1.66
Year : 2003
GLP :
Test substance :

Reliability : (2) valid with restrictions
Accepted calculation method
19.11.2003 (25)

Partition coefficient : octanol-water
Log pow : 1.72 at °C
pH value :
Method : other (calculated)
Year : 1987
GLP :
Test substance : other TS: 2,6-DNT

Remark : BUA report (peer-reviewed) cites unpublished data of Bayer 1986 (this report is not available)
Reliability : (2) valid with restrictions
Data from peer-reviewed handbook or collection of data
19.11.2003 (1)

Partition coefficient : octanol-water
Log pow : 2.02 - 2.04 at °C

pH value :
Method : other (measured)
Year : 1987
GLP : no data
Test substance : other TS: 2,4-DNT and 2,6-DNT

Method : Determination of partition coefficients by measuring the capacity factor of the compounds on a reversed phase HPLC column following the recommendations by Hammers et al. (1982). HPLC consisting of a Pye Unicam 4010 double piston pump and a Pye Unicam 4020 UV-detector.
Result : logKow
2,4-DNT 2.04
2,6-DNT 2.02
logKow was calculated from experimental data obtained with CH3OH als mobile phase.
Test condition : Measurements were carried out at room temperature on a reversed phase C-18 column (Merck Lichrosorb, particle size 10 µm, length 10 cm), mobile phase: CH3OH/H2O
Reliability : (2) valid with restrictions
Basic data given

01.09.2003 (30)

Partition coefficient : octanol-water
Log pow : 2.28 at °C
pH value :
Method : other (calculated)
Year : 1983
GLP :
Test substance : other TS: all DNT isomers

Method : log Kow (octanol water partition coefficient) values were calculated according to the method of Leo, Hansch, and Elkins (1971) [Leo A, Hansch C, and Elkins D (1971). Partition coefficients and their uses. Chem. Rev. 71, 525-616)]
Result : Result is valid for all DNT isomers
Reliability : (2) valid with restrictions
Accepted calculation method

17.11.2003 (31)

Partition coefficient : octanol-water
Log pow : 2.02 at °C
pH value :
Method : other (measured): not specified
Year : 1991
GLP :
Test substance : other TS: 2,6-DNT, no purity given

Reliability : (2) valid with restrictions
Data from handbook or collection of data

30.10.2003 (32) (21)

Partition coefficient : octanol-water
Log pow : 1.89 at °C
pH value :
Method : other (measured): not specified
Year : 1983
GLP :
Test substance : other TS: 2,4-DNT, no purity given

Remark	:	Rosenblatt et al. (1991) cite a an unpublished report of Major MA (1989). US Army Biomed. Res. Develop. Lab., Fort Detrick, Frederick, MD (unpublished data)
Reliability	:	(4) not assignable Secondary literature

30.10.2003 (21)

Partition coefficient	:	octanol-water
Log pow	:	2 at °C
pH value	:	
Method	:	
Year	:	1986
GLP	:	
Test substance	:	other TS: 2,4-DNT
Remark	:	BUA report (peer-reviewed) cites unpublished data of Bayer 1986 (this report is not available)
Reliability	:	(2) valid with restrictions Data from peer-reviewed handbook or collection of data

19.11.2003 (1)

Partition coefficient	:	octanol-water
Log pow	:	2 at °C
pH value	:	
Method	:	other (calculated)
Year	:	1986
GLP	:	
Test substance	:	other TS: 2,5-DNT
Remark	:	BUA report (peer-reviewed) cites unpublished data of Bayer 1986 (this report is not available)
Reliability	:	(2) valid with restrictions Data from peer-reviewed handbook or collection of data

19.11.2003 (1)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	at °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
PKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	other: see test conditions
Year	:	1986
GLP	:	no
Test substance	:	other TS: 2,4-, 2,5-, 2,6-DNT >99.5 % purity
Result	:	At room temperature (22 °C) 2,4-DNT: 166 mg/l 2,5-DNT: 258 mg/l 2,6-DNT: 145 mg/l
Test condition	:	Amount of substance was stirred during 48h in bidistilled water at 22 °C and then filtered.

Analytical method: HPLC
Reliability : (2) valid with restrictions
Basic data given
Flag : Critical study for SIDS endpoint
21.11.2003 (33)

Solubility in : Water
Value : 270 mg/l at 22 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
PKa : at 25 °C
Description :
Stable :
Deg. product :
Method : other: not given
Year : 1925
GLP : no
Test substance : other TS: presumably 2,4-DNT

Source : Seidell (1941) is cited in the HSDB internet database and in the EPIWIN-Software database [Seidell A (1941). Solubilities of Organic Compounds. New York, D. Van Norstrand Co. Inc., p. 532.]. Desvergnes (1925) is cited by Seidell without further information available [Desvergnes, Monit. scient., <5> 15, 158, Chem.Zentralbl., 1925, 2052].
Reliability : (2) valid with restrictions
Data from handbook or collection of data
Flag : Critical study for SIDS endpoint
21.11.2003 (34)

Solubility in : Water
Value : 370 mg/l at 50 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
PKa : at 25 °C
Description :
Stable :
Deg. product :
Method : other: not given
Year : 1925
GLP : no
Test substance : other TS: presumably 2,4-DNT

Source : Seidell (1941) is cited in the HSDB internet database and in the EPIWIN-Software database [Seidell A (1941). Solubilities of Organic Compounds. New York, D. Van Norstrand Co. Inc., p. 532.]. Desvergnes (1925) is cited by Seidell without further information available [Desvergnes, Monit. scient., <5> 15, 158, Chem.Zentralbl., 1925, 2052].
Reliability : (2) valid with restrictions
Data from handbook or collection of data
20.11.2003 (34)

Solubility in : Water
Value : 2540 mg/l at 100 °C
pH value :
concentration : at °C

Temperature effects	:	
Examine different pol.	:	
PKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	other: not given
Year	:	1925
GLP	:	no
Test substance	:	other TS: presumably 2,4-DNT
Source	:	Seidell (1941) is cited in the HSDB internet database and in the EPIWIN-Software database [Seidell A (1941). Solubilities of Organic Compounds. New York, D. Van Norstrand Co. Inc., p. 532.]. Desvergnes (1925) is cited by Seidell without further information available [Desvergnes, Monit. scient., <5> 15, 158, Chem.Zentralbl., 1925, 2052].
Reliability	:	(2) valid with restrictions Data from handbook or collection of data

20.11.2003 (34)

Solubility in	:	Water
Value	:	208 - 270 mg/l at °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
PKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	other: not specified
Year	:	1986
GLP	:	
Test substance	:	other TS: 2,4-DNT and 2,6-DNT, no purity given
Result	:	2,4-DNT: 270 mg/l 2,6-DNT: 208 mg/l
Reliability	:	(2) valid with restrictions Data from handbook or collection of data

20.11.2003 (29)

Solubility in	:	Water
Value	:	208 - 280 mg/l at 25 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
PKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	other: not specified
Year	:	1991
GLP	:	no data
Test substance	:	other TS: 2,4-DNT and 2,6-DNT
Result	:	aqueous solubility (mg/l at 25 °C): 2,4-DNT 280 mg/l 2,6-DNT 208 mg/l

Reliability : (2) valid with restrictions
Data from handbook or collection of data
20.11.2003 (21)

Solubility in : Organic Solvents
Value : at °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
PKa : at 25 °C
Description :
Stable :
Deg. product :
Method :
Year : 2003
GLP :
Test substance :

Result : Soluble in alcohol and ether
Reliability : (2) valid with restrictions
Data from handbook or collection of data
Flag : Critical study for SIDS endpoint
20.11.2003 (35)

Solubility in : Water
Value : 300 mg/l at °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
PKa : at 25 °C
Description :
Stable :
Deg. product :
Method : other: not given
Year : 2003
GLP : no data
Test substance : no data

Reliability : (3) invalid
Documentation insufficient for assessment
20.11.2003 (18)

Solubility in : Organic Solvents
Value : at °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
PKa : at 25 °C
Description : of high solubility
Stable :
Deg. product :
Method : other: not given
Year : 1941
GLP : no
Test substance : other TS: presumably 2,4-DNT

Result	:	Solubility of DNT in organic solvents at 15 °C 579.3 g/kg ethylacetat 819 g/kg acetone 50.1 g/kg methanol 19.2 g/kg ethanol (96 %) 30.4 g/kg ethanol (100 %) 606.4 g/kg benzene 650.8 g/kg trichlormethane 24.3 g/kg tetrachlormethane 23.1 g/kg carbon disulfide 454.7 g/kg toluene 768.1 g/kg pyridine
Source	:	Seidell (1941) is cited in the HSDB internet database and in the EPIWIN-Software database [Seidell A (1941). Solubilities of Organic Compounds. New York, D. Van Norstrand Co. Inc., p. 532.]. Desvergnes (1925) is cited by Seidell without further information available [Desvergnes, Monit. scient., <5> 15, 158, Chem.Zentralbl., 1925, 2052].
Reliability	:	(2) valid with restrictions Data from handbook or collection of data

20.11.2003 (34)

2.6.2 SURFACE TENSION

Test type	:	other: not given
Value	:	47.135 mN/m at 25 °C
Concentration	:	
Method	:	other: not given. At saturated pressure
Year	:	2003
GLP	:	no data
Test substance	:	other TS: DNT 80/20
Result	:	Other results: 0.047713 N/m at 15.56 °C 0.047426 N/m at 20 °C
Reliability	:	(2) valid with restrictions Data from handbook or collection of data
Flag	:	Critical study for SIDS endpoint

18.08.2003 (19)

2.7 FLASH POINT

Value	:	ca. 160 °C
Type	:	
Method	:	other: not given
Year	:	2003
GLP	:	no data
Test substance	:	other TS: ca. 80 % 2,4-DNT and 20 % 2,6-DNT
Reliability	:	(2) valid with restrictions Data from handbook or collection of data
Flag	:	Critical study for SIDS endpoint

01.09.2003 (19)

Value	:	207 °C
Type	:	
Method	:	other: no data

Year : 1994
GLP : no data
Test substance : other TS: technical-grade DNT

Reliability : (2) valid with restrictions
Data from handbook or collection of data
19.11.2003 (22)

Value : ca. 163 °C
Type :
Method : other: DIN 51758
Year : 2003
GLP : no data
Test substance : other TS: 80 % 2,4-DNT and 20 % 2,6-DNT

Reliability : (4) not assignable
Not assignable/manufacturer data without proof
01.09.2003 (3)

2.8 AUTO FLAMMABILITY

Value : ca. 400 °C at
Method :
Year : 2003
GLP : no data
Test substance : other TS: 80 % 2,4-DNT and 20 % 2,6-DNT

Remark : Ignition temperature
Reliability : (2) valid with restrictions
Data from handbook or collection of data
Flag : Critical study for SIDS endpoint
19.11.2003 (19)

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

Value : .654 - mPa s (dynamic) at 20 °C
Result :
Method : other: not given
Year : 2003
GLP : no data
Test substance : no data

Result : Viscosity of the liquid phase at saturated pressure:
0.000654 Pas at 20 °C
0.000519 Pas at 50 °C
0.000384 Pas at 100 °C
Viscosity of the gas phase at normal pressure:
0.000006 Pas at 25 °C
0.000007 Pas at 100 °C
0.000009 Pas at 200 °C
Reliability : (2) valid with restrictions
Data from handbook or collection of data
Flag : Critical study for SIDS endpoint
12.06.2003 (19)

2.14 ADDITIONAL REMARKS

Memo : Conversion factors in air at 20 °C

Result : 1mg/m3 = 0.13 ppm
1 ppm = 7.57 mg/m3
Reliability : (2) valid with restrictions
Data from handbook or collection of data
Flag : Critical study for SIDS endpoint
17.11.2003 (22)

3.1.1 PHOTODEGRADATION

Type : water
Light source : other: see test condition
Light spectrum : nm
Relative intensity : based on intensity of sunlight
Deg. product :
Method : other (measured)
Year : 1986
GLP : no
Test substance : no data

Result :

	Quantum yields	Wavelength (nm)
2,3-DNT	(7.0 +/- 1.2) x 10E-4	366
2,4-DNT	(2.0 +/- 0.47) x 10E-3	313
2,5-DNT	(1.0 +/- 0.16) x 10E-2	366
2,6-DNT	(1.2 +/- 0.2) x 10E-3	366
3,4-DNT	(7.2 +/- 1.8) x 10E-5	366

	ka (per d)	quantum yield (per d)
2,3-DNT	506	0.35
2,4-DNT	358	0.72
2,5-DNT	896	9.0
2,6-DNT	557	0.67
3,4-DNT	723	0.052

ka = light absorption rate. Typical near surface ka are reported here

Calculated photolysis half lifes: about 1 d (full exposure to sunlight; near surface conditions at latitude 40 °N, averaged annual rate constants). The presence of humic substances enhanced the sunlight induced photodegradation rates of 2,4-DNT and 2,6-DNT by an factor of 2-5 and 11-17, respectively.

Test condition : - Merry-go-round photoreactor
- Monochromatic light: 313 nm (2,4-DNT: potassium chromate in potassium carbonate), 366 nm (2,6-DNT: mercury lamp)
- Concentrations: 10E-6 to 10E-5 M
- Distilled water pH 5.5
- Dark controls in each run
- Analytical monitoring

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles
Flag : Critical study for SIDS endpoint
18.08.2003 (36)

Type : air
Light source :
Light spectrum : nm
Relative intensity : based on intensity of sunlight
INDIRECT PHOTOLYSIS
Sensitizer :
Conc. of sensitizer : 500000 molecule/cm³
Rate constant : .00000000000001916 cm³/(molecule*sec)
Degradation : 50 % after 83.7 day(s)
Deg. product :
Method : other (calculated): with SRC-AOPWIN v. 1.90 (2000)
Year : 2003
GLP : no

Test substance	:	
Remark	:	The calculated half-life is based on a mean OH radical concentration of 500000 OH radicals/cm3 as 24 h average.
Reliability	:	(2) valid with restrictions Accepted calculation method
Flag	:	Critical study for SIDS endpoint
18.08.2003		(25)
Type	:	water
Light source	:	Sun light
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
Deg. product	:	not measured
Method	:	other (measured): Incubation in wetland mesocosms under field conditions
Year	:	2001
GLP	:	no
Test substance	:	other TS: mixture of nitrotoluenes including 2,4-DNT and 2,6-DNT
Method	:	Effects of 2,4-DNT and 2,6-DNT were investigated in wetland mesocosms under field conditions in small-scale 4-months field study as a surface-flow, modular system. The groundwater of a well located at the border of a TNT manufactury valley was used as influent. The influent contained 16.7 mg/l of the 2,4-DNT and 5.2 mg/l of the 2,6-DNT. The effect of 3 treatments were compared: planted, non planted and UV-shielded in three different lagoons (three replicates). Explosives-contaminated groundwater was continuously pumped into the lagoons and a 7-day hydraulic retention time was maintained.
Result	:	The initial concentration of nitrocompounds in effluent decreased exponentially with time due to biodegradation and photodegradation under field conditions. The average level of nitroaromatics was 94.6 mg/l in the influent. In the effluents of the lagoons they were 14 mg/l in the planted, 28.1 in the non-planted and 34.4 mg/l in the UV-shielded lagoons. 58 % of 2,4-DNT and 61 % of 2,6-DNT were eliminated in constructed wetland lagoons during 7 d of hydraulic retention time. The contribution of photodegradation to the removal rates was 60 % for 2,4-DNT and 59 % for 2,6-DNT in the planted and in the non-planted treatments.
Test substance	:	The groundwater of a well located at the border of a TNT manufactury valley was used as influent (= incubation medium). The influent contained 16.7 mg/l of the 2,4-DNT and 5.2 mg/l of the 2,6-DNT (and several other nitroaromatics and other substances)
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles
Flag	:	Critical study for SIDS endpoint
01.09.2003		(37)
Type	:	water
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
DIRECT PHOTOLYSIS		
Halflife t1/2	:	6.5 - 20 day(s)
Degradation	:	% after
Quantum yield	:	
Deg. product	:	
Method	:	other (calculated)

Year : 1987
GLP :
Test substance :

Remark : Calculation by the German Environmental protection agency (UBA), cited according to BUA (1987), used by the OECD (1997) in the Screening Information Data Set SIDS for High Production Volume Chemicals (http://www.chem.unep.ch/irptc/sids/volume4/part1/dinitrotoluene/sids_rpt.html).
Result : Based on the quantum yield = 0.002, with regard to the geographical conditions in Germany, and the low light intensity in natural water bodies, the half-life of 2,4-DNT is calculated to be 20 days in a natural water body (surface layer: 6.5 days)
Reliability : (2) valid with restrictions
Accepted calculation method
Flag : Critical study for SIDS endpoint
17.01.2004 (1)

Type : water
Light source : Sun light
Light spectrum : nm
Relative intensity : based on intensity of sunlight
Conc. of substance : 1 mg/l at °C
DIRECT PHOTOLYSIS
Halflife t1/2 : 2.7 hour(s)
Degradation : % after
Quantum yield :
Deg. product :
Method : other (measured)
Year : 1981
GLP : no data
Test substance : other TS: 2,4-DNT

Result : 1.0 ppm of 2,4-dinitrotoluene had sunlight photolysis half-lives of 43 hr in distilled water and 2.7, 9.6, and 3.7 hrs in river, bay, and pond waters, respectively.
Source : HSDB Database
Reliability : (4) not assignable
Reference not available
01.09.2003 (38)

Type : water
Light source :
Light spectrum : nm
Relative intensity : based on intensity of sunlight
INDIRECT PHOTOLYSIS
Sensitizer : water with additives
Conc. of sensitizer :
Rate constant : $cm^3/(molecule*sec)$
Degradation : % after
Deg. product :
Method :
Year : 1994
GLP : no data
Test substance : other TS: 2,4-DNT, no purity reported

Remark : Results from this test are not relevant for environment.
Result : A first order rate constant of 1.8 hour-1 (which corresponds to a half-life of about 9 days) was determined for photodegradation in water.

Test condition : TiO2 is added to the water. TiO2 is photocatalytically active.
Reliability : (4) not assignable
Reference not available

10.08.2004 (39)

3.1.2 STABILITY IN WATER

Type : abiotic
t1/2 pH4 : at °C
t1/2 pH7 : at °C
t1/2 pH9 : at °C
t1/2 pH 12 : .3 - 2 day(s) at °C
Degradation : > 7 - 29 % after 14 day(s) at pH 11 and °C
Deg. product : yes
Method : other: Alkaline hydrolysis
Year : 2001
GLP : no data
Test substance : other TS: Mixture of nitroaromatics including 2,4-DNT and 2,6-DNT

Method : 5 g soil stirred with 50 ml Ca(OH)2 at pH 11 and pH 12, respectively, at room temperature; 5 g soil in destilled water as reference, electrolyte concentration adjusted with KCl

Result : Concentration [mg/kg] of 2,4-DNT and 2,6-DNT in untreated HTNT2 and ELBP2 soils and total amounts [mg/kg] after alkaline treatment at pH 12 and pH 11 (a)

	HTNT2			ELBP2		
substance	untreat.	pH 12	pH 11	untreat.	pH 12	pH 11
2,4-DNT	289.2	98.6	208.6	142.7	56.2	98.5
2,6-DNT	70.5	31.0	41.7	58.0	34.4	43.5

(a) total amounts are mean values of amounts measured at the 7th and 14th day
HTNT2 and ELBP2: sites from which contaminated soil was taken

Reaction Rates (k) of 2,4-DNT using pseudo-first-order model

	HTNT2		ELBP2	
	pH 12	pH 11	pH 12	pH 11
k (h-1)	0.082	0.004	0.017	<0.001
t1/2 (d)	0.3	7	2	>29

HTNT2: 66% 2,4-DNT hydrolysed in 4 days, subsequently no further reaction
DNT increased during hydrolysis of 2,4,6-TNT. The authors assume that this is not due to formation of DNT but might be due to desorption effects.

Test condition : Extraction of samples: Neutralisation of soil slurries with HCl and subsequent centrifugation, triplicate extraction of supernatant with 25 ml ethyl acetate

Analytical prodedure: Analysis of nitro compounds by GC/ECD

Evaluation of results: quantities of nitroaromatic compounds measured in untreated soil were used as reference values (100 %); reaction rates of 2,4-DNT were calculated using a pseudo-first-order model.

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
19.11.2003 (40)

Type : abiotic
t1/2 pH4 : at °C

t1/2 pH7	:	at °C
t1/2 pH9	:	at °C
Deg. product	:	
Method	:	
Year	:	1990
GLP	:	
Test substance	:	
Result	:	DNT is not expected to undergo hydrolysis in the environment due to the lack of hydrolyzable functional groups
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

17.01.2004 (41)

3.1.3 STABILITY IN SOIL

Type	:	laboratory
Radiolabel	:	no
Concentration	:	5 mg/kg
Soil temperature	:	20 °C
Soil humidity	:	
Soil classification	:	
Year	:	
Content of clay	:	5.1 %
Content of silt	:	13 %
Content of sand	:	82 %
Organic carbon	:	2.3 %
Ph	:	5.8 - 6.3
Cation exch. capacity	:	9.7 meq/100 g soil dry weight
Microbial biomass	:	48 mg biomass/100 g soil dry weight
Dissipation time		
DT50	:	7 day(s)
DT90	:	191 day(s)
Dissipation	:	% after
Deg. product	:	
Method	:	other: BBA Nr. IV-4-1 part 2 adopted Dec. 1986 "Biodegradation in soil"
Year	:	1995
GLP	:	yes
Test substance	:	other TS: 2,4-DNT, purity 99 %
Method	:	Method developed by the Biologische Bundesanstalt für Land- und Forstwirtschaft, Berlin und Braunschweig (Germany)
Remark	:	Microbial biomass: 48.0-51.6 mg/100 g at day 0; 24.0-30.8 mg/100 g soil at the end of the test. No differentiation is possible whether abiotic or biotic degradation has occurred, since no sterile control was performed.
Result	:	DT90 (191.4 days) was mathematically extrapolated.
Test condition	:	- Soil organisms were not pre-adapted to DNT - Soil water: 40 % of maximum water capacity - Test duration 100 days - Substance specific analysis method - Sampling rate: 0, 1, 2, 4, 8, 16, 32, 64, 100 day - Sterile control was not performed - Degradation calculated, based on the extractable part (with acetone) of the compound at the beginning of the test
Reliability	:	(2) valid with restrictions Study meets generally accepted scientific principles. Basic data given
Flag	:	Critical study for SIDS endpoint

10.08.2004 (23)

3.2.1 MONITORING DATA

Type of measurement : background concentration
Media : surface water
Concentration : .0001 - .00051 mg/l
Method :

Result : DNT concentrations: 90 % percentiles [µg/l]

Sampling Points	no samples	2,4-DNT	2-6 DNT
Schmilka (mixed samples)	51	0.20	0.056
Schmilka; left bank	13	0.11	0.14
Schmilka; right bank	13	0.095	0.11
Zehren (mixed samples)	35	(0.19)	(0,060)
Zehren; left bank	13	0.10	< 0.050
Zehren; right bank	13	0.11	< 0.050
Dommitzsch (mixed samples)	48	0.20	0.050
Dommitzsch; left bank	13	0.12	< 0.050
Dommitzsch; right bank	12	0.12	< 0.050
Schnackenburg	6	(max < 0.01)	(max < 0.02)
Grauerort (single samples)	6	(max < 0.01)	(max < 0.02)

DNT concentrations: maxima [µg/l]

Sampling Points	no samples	2,4-DNT	2-6 DNT
Schmilka (mixed samples)	51	0.55	0.32
Schmilka; left bank	13	1.0	0.58
Schmilka; right bank	13	0.88	0.52
Zehren (mixed samples)	35	(0.40)	(0,22)
Zehren; left bank	13	0.21	0.085
Zehren; right bank	13	0.23	0.088
Dommitzsch (mixed samples)	48	0.34	0.18
Dommitzsch; left bank	13	0.14	< 0.050
Dommitzsch; right bank	12	0.14	< 0.050
Schnackenburg	6	< 0.01	< 0.02
Grauerort (single samples)	6	< 0.01	< 0.02

Reliability : (2) valid with restrictions
Data from handbook or collection of data
Flag : Critical study for SIDS endpoint
19.08.2003 (42)

Type of measurement : background concentration
Media : surface water
Concentration : < .002 µg/l
Method : GC/MS

Method : GC/MS after sorption and concentration on RP-C18 material according to the method of Lenz et al. (1998) [Lenz S, Sacher F, Brauch H-J, Hambsch B (1998). Entwicklung chemisch-analytischer Verfahren zur Erfassung gentoxischer Substanzen in Waessern. Vom Wasser 91, 47-60]
Result : - The river Rhine was sampled at 4 sites 6 times (every second month) between March 1997 and January 1998:
Karlsruhe, Wahnbachtalsperre, Cologne, and Duesseldorf.
- The river Wupper, which flows into the Rhine, was sampled at the Kohlfurter Bridge twice, in February and March 1998.
- The river Mulde, which flows into the Elbe, was sampled 5 times at Dessau between February and December 1997.
2,5-DNT could not be determined in any of the samples of the rivers Rhine,

Wupper and Mulde with a determination limit of 0.002 µg/l.
- The river Elbe was sampled at Schmilka (Czech border) 8 times between February 1997 and May 1998. The 2,5-DNT concentration was below the limit of determination (0.002 µg/l) in 2 samples and in the other 6 samples, reached up to 0.41 µg/l in April 1997.
- The river Elbe was sampled also downstream at Schnackenburg 5 times between February and December 1997. The 2,5-DNT concentration was below the limit of determination (0.002 µg/l) in 2 samples and in the other 3 samples, reached up to 0.19 µg/l in April 1997.

Reliability : (2) valid with restrictions
Basic data given
Flag : Critical study for SIDS endpoint
27.11.2003 (43)

Type of measurement : concentration at contaminated site
Media : ground water
Concentration : ca. 9 - 12 mg/l
Method : GC/MS

Method : Groundwater screening:
- Extraction with CH2Cl2 for 10 min
- o-Terphenyl used as internal standard
- Organic phase analyzed by GC/MS (Hewlett Packard 6890/5973) using PE-5MS column (Perkin Elmer, Norwalk, CT)

Remark : The groundwater originated from a Swedish location where ammunition destruction by open burning has been performed for more than 40 years
Result : Concentration in ground water:
2,4-DNT 0.067 mM = 12 mg/l
2,6-DNT 0.050 mM = 9 mg/l
Several other nitroaromatics detected
Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles. Basic data given
Flag : Critical study for SIDS endpoint
19.08.2003 (44)

Type of measurement : concentration at contaminated site
Media : ground water
Concentration : < .004 - 22 mg/l
Method : HPLC

Method : According to US EPA (1992) Test Methods for Evaluating Solid wastes, proposed update II, Method 8330. Rep. SW-846 (3rd ed), Office of Solid Waste and Emergency Response, Washington, DC
Remark : 3 US Army Ammunition Plants (AAP):
- Milan AAP (TN, USA)
- Iowa AAP
- Volunteer AAP (Chattanooga, TN; National Cleanup Technology Demonstration Site)

Result : Groundwater contained TNT and several byproducts of TNT synthesis and degradation including DNT isomers:

mg/l	2,4-DNT	2,6-DNT
VAAP	16.6	5.2
MAAP	<0.002	<0.002
IAAP	<0.002	0.006

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles
Flag : Critical study for SIDS endpoint
19.08.2003 (37)

Type of measurement : concentration at contaminated site
Media : other: wastewater from US army ammunition plants
Concentration :
Method : HPLC, GC

Result : Average concentration of DNT isomers in authentic wastewater from US Volunteer Army Ammunition Plants
2,3-DNT 0.4 mg/l
2,4-DNT 14.7 mg/l
2,5-DNT 0.4 mg/l
2,6-DNT 7.3 mg/l
3,4-DNT 0.5 mg/l
Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles
Flag : Critical study for SIDS endpoint
19.11.2003 (31)

Type of measurement : concentration at contaminated site
Media : other: condensate wastewater from US Army TNT production facility
Concentration : .4 - 14.7
Method : GC/electron capture detector or GC/MS

Method : - 79 Condensate wastewater samples analyzed separately
- Extraction: for 2,4-DNT and 2,6-DNT: 1 ml condensate wastewater extracted with 10 ml benzene, and used for analysis by GC with electron capture detector; for the other components 50 ml of wastewater extracted with equal volume of benzene, extract concentrated to 50 % volume and used for GC/MS
- GC: Column packing: 3.5 % Dexsil 300GC on Chromosorb WAWDMCS 80-100 mesh. Carrier: Argon/methane (95 % : 5 % v/v). Detector: electron capture at 180 °C
- Detection limit 0.001 mg/l
Remark : DNT is a byproduct of the 2,4,6-trinitrotoluene (TNT) production. In the TNT manufacturing process, all TNT and DNT isomers and several other nitroaromatics are produced. The major wastewater of this production is the condensate wastewater. The desired 2,4,6-trinitrotoluene is separated by a sellite process (reaction with sodium sulfite which converts the non-symmetrical TNT isomers to water-soluble sulfonate salts) from byproducts and the process water. The process water is steam distilled to concentrate to 35 % water. The concentrate is recycled or incinerated. The distillate is condensed (condensate wastewater) and discharged into the enviromnent. The major components of the condensate wastewater are 2,4-DNT (43 % of organics), 2,6-DNT (22 %), and 1,3-dinitrobenzene (11 %).
Result : The following concentrations of DNT isomers (mg/l) were measured in condensate waste water:
2,3-DNT 0.40
2,4-DNT 14.70
2,5-DNT 0.40
2,6-DNT 7.30
3,4-DNT 0.50
3,5-DNT 0.52
This condensate wastewater composition is also reported by Liu et al. (1984) [Liu DHW, Spanggord RJ, Bailey HC, Javitz HS, Jones DCL (1984). Toxicity of TNT Wastewaters to Aquatic Organisms, Volume II. SRI International, Report LSU-4262, Menlo Park California.]. The condensate wastewater contains also several other nitroaromatics
Reliability : (2) valid with restrictions
Test procedure in accordance with national standard methods

Flag : Critical study for SIDS endpoint
22.08.2003 (45)

Type of measurement : concentration at contaminated site
Media : soil
Concentration :
Method : HPLC UV-detector

Method : Determination of DNT concentrations by HPLC analysis using a Hewlett-Packard series 1050 HPLC equipped with an UV detector
Result : - Volunteer Army Ammunition Plant (VAAP; Chattanooga, TN)
- Badger Army Ammunition Plant (BAAP; Baraboo, WI)
Concentrations of 2,4-DNT and 2,6-DNT were 19 and 1.38 g/kg in VAAP soil and 8.9 and 0.48 g/kg in BAAP soil.
Reliability : (2) valid with restrictions
Study well documented; meets generally accepted scientific principles
Flag : Critical study for SIDS endpoint
19.08.2003 (46)

Type of measurement : concentration at contaminated site
Media : soil
Concentration :
Method : HPLC with UV-VIS detector; GC-MS

Result : The vadose zones beneath waste pits at Badger Army Ammunition Plant (BAAP, Baraboo, Wisconsin) are heavily contaminated with 2,4-DNT and 2,6-DNT. At BAAP, in the presence of other nitroaromatics, soil contents of 14 g/kg and 0.55 g/kg of 2,4-DNT and 2,6-DNT, respectively, were measured.
Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles
Flag : Critical study for SIDS endpoint
19.11.2003 (47)

Type of measurement : concentration at contaminated site
Media : soil
Concentration :
Method : HPLC

Method : HPLC was performed with a Hypercarb porous graphite column (5 µm x 150 mm, Hypersil, U. K.) with a mobile phase of acetonitrile/water (90 : 10) containing trifluoroacetic acid. UV detection at 230 mm.
Result : Dried soil of the decommissioned TNT manufacturing plant in Hessisch Lichtenau contained 3.6 g 2,4-DNT/kg soil and 2.5 g 2,6-DNT/kg soil
Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles
Flag : Critical study for SIDS endpoint
31.03.2004 (48)

Type of measurement : concentration at contaminated site
Media : soil
Concentration :
Method :

Remark : Soil stems from Gyttorp facility in Sweden, which was used for explosives manufacturing from 1864 to 1995.
Result : Concentration of 2,4-DNT in soil: 4 g/kg. The total concentration of other nitrotoluene explosives was 5.3 g/kg
Reliability : (2) valid with restrictions

Basic data given
Flag : Critical study for SIDS endpoint
06.10.2003 (49)

Type of measurement : other: forensic examination of gunshot residues
Media : other: gunpowder and gunshot residues
Concentration :
Method : Micellar electrokinetic capillary electrophoresis

Result : 2,4-DNT was found in ammunition gunpowder up to 0.5 % (reloading powder up to 6%). During firing, the 2,4-DNT decreases to about 0.3 % of the total detectable gunpowder.
Traces of 2,4-DNT can be detected in gunshot residues (detection limit in picogram range)
Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles
Flag : Critical study for SIDS endpoint
19.11.2003 (50) (51)

Type of measurement : other: untreated wastewater from munition production facility
Media : other: wastewater
Concentration :
Method : GC/MS

Result : Untreated wastewater from ADI Mulwala Munition Production Facility (Australia) contains
2,4-DNT 50 mg/l
2,6-DNT 10 mg/l
and other nitroaromatic compounds
Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles
19.11.2003 (52)

Type of measurement : other: background and contaminated sites
Media : other: water, sediment, soil
Concentration :
Method :

Result : Historical data on 2,4-DNT and 2,6-DNT concentrations in environmental waters (Data are presented in the following order:

Water	2,4-DNT [µg/l]	2,6-DNT [µg/l]	Year (ca.))
Rhine	-	Maximum 3	1974
Rhine (Netherlands)	0.3	-	1979
Rhine	0.2 (sum of 2,4-DNT, 2,5-DNT, 2,6-DNT)	0.2 (sum of 2,4-DNT, 2,5-DNT, 2,6-DNT)	1984
Rhine (Duesseldorf)	-	< 0.02-0.06	1986
Rhine (Wesel)	-	< 0.02-0.07	1986
Rhine (Netherlands)	-	3	Before 1987
Elbe (Lauenburg)	1.3	0.5	1989
Elbe (Brunsbuettel)	0.1	0.04	1989
2 small creeks in the proxity of a former TNT manufacturing site near Hirschenhagen, Germany	3 and 13	4 and 8	1989
Pfauenteich in the proximity of a former TNT manufacturing site near Clausthal-Zellerfeld, Germany	0.7-1.3	0.07-0.3	1989
Waconda Bay (Lake Chichamauga, TN, USA)	< 0.1-22	1.3-39 (average 19.4)	1980
Dokai Bay, Japan	Maximum 210	Maximum 14.9	1981
6 former TNT manufacturing sites close to Pasadena, TX, USA	2-91000	Maximum 77000, average16800	1985

In the proximity of a former TNT manufacturing site near Stadtallendorf, Germany	<1-810	3-590	1982	
In the proximity of a former TNT manufacturing site near Stadtallendorf, Germany	<0.1-0.4	<0.1-0.7	1988	
TNT manufacturing site Umatilla, OR, USA	400	5	Before 1989	
TNT manufacturing site Milan, TN, USA	100	Maximum 34	Before 1989	

Reliability : (4) not assignable
Secondary literature
Flag : Critical study for SIDS endpoint
11.11.2003 (53)

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : adsorption
Media : water - soil
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method : other: BBA Nr. IV-4-1 part 2 adopted Dec. 1986 "Leaching in soil"
Year : 1992

Method : Method developed by Biologische Bundesanstalt, Braunschweig (Germany)
Remark : Soil classification:
Clay: 3.5-8.3 %
Silt: 9.1-27.8 %
Sand: 64.1-87.4 %
Organic carbon: 0.7-1.34 %
pH: 5.8-6.6
Cation exchange capacity: 4.9-9.7 meq/100 g soil dw
Result : Applied amount: 5 mg 2,4-DNT/kg soil = 0.98 mg 2,4-DNT/column
In the leachate of all 3 soil columns the 2,4-DNT concentration was < 6.0 µg/l (< 0.3 % of 2,4-DNT applied).
2,4-DNT is not mobile in soil according to test guideline.
Test condition : The leaching behaviour of 2,4-DNT was determined in three different soils under laboratory conditions. The substance was applied on soil columns (350 mm long, 50 mm diameter) which were then eluated for 2 days with water (393 ml/column/2 days). The concentration of 2,4-DNT was determined in the leachate.
Applied amount: 5 mg 2,4-DNT/kg soil = 0.98 mg 2,4-DNT/column
Test substance : 2,4-DNT, purity 99 %
Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles. Basic data given
Flag : Critical study for SIDS endpoint
27.11.2003 (23)

Type : adsorption
Media : water - soil
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)

Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method : other: as described by Patterson (1996)
Year : 1999

Method : Stainless steel columns containing weathered basalt were used for sorption studies to estimate the mobility of munition residues (e.g. nitrotoluenes, dinitrotoluenes, 2,4,6-trinitrotoluene) in the aquifer material according to Patterson (1996).
Result : Sorption coefficients Kd (l/kg) were determined for weathered basalt originally by Toze et al. (1977) (This publication is not available). The sorption coefficients in Montmorillonite clay of Haderlein, Weissmahr and Schwarzenbach (1996) are used for comparison.
2,4-DNT Kd = 4.10 l/kg (weathered basalt); Kd = 7400 l/kg (montmorillonite clay)
2,6-DNT Kd = 0.50 l/kg (weathered basalt); Kd = 125 l/kg (montmorillonite clay)
Reliability : (2) valid with restrictions
Basic data given
Flag : Critical study for SIDS endpoint
11.08.2003 (54)

3.3.2 DISTRIBUTION

Media : air - biota - sediment(s) - soil - water
Method : Calculation according Mackay, Level I
Year : 2003

Remark : Based on the model calculations (Mackay level I, V.2.11) the target compartment of the environmental distribution of
2,4-DNT and 2,6-DNT is the hydrosphere.
Result : Calculated distribution between environmental compartments:

	2,4-DNT (%)	2.6-DNT (%)
Water:	97.9	96.7
Sediment:	0.7	1.0
Soil:	0.7	1.0
Air:	0.6	1.3
Susp. Sediment:	<0.01	<0.01
Aerosol:	<0.01	<0.01
Fish:	<0.01	<0.01

Test condition : Data used in the calculation:

	2,4-DNT	2,6-DNT
temperature (°C):	25	25
molar mass (g/mol):	182.14	182.14
log Kow:	1.98	2.1
vapour pressure (pa):	0.016	0.032
water solubility (mg/l):	166	145
melting point (°C):	69.9	65.9

Unit world Modelling Data*

	Volumes (m3)	Organic C (g/g)	Density (kg/m³)
air:	6.0E+9		1.185
water:	7.0E+6		1000
soil:	4.5E+4	0.02	1500
sediment:	2.1E+4	0.05	1300
susp. sediment:	3.5E+1	0.167	1500
biota (fish):	7.0E+0		1000

		aerosol 1.2E-1 1500 *Compartment properties were based on the parameters from Mackay (1991), modified by the Federal Environmental Agency (UBA, Germany).
Reliability	:	(2) valid with restrictions Accepted calculation method Data for comparison
Flag	:	Critical study for SIDS endpoint
10.08.2004		(25)
Media	:	air - biota - sediment(s) - soil - water
Method	:	Calculation according Mackay, Level I
Year	:	2003
Remark	:	Based on the model calculations (Mackay level I, V.2.11) the target compartment of the environmental distribution of DNT (25321-14-6) is the hydrosphere.
Result	:	Calculated distribution between environmental compartments: Water: 98.1 % Sediment: 0.8 % Soil: 0.8 % Air: 0.4 % Susp. Sediment: < 0.01 % Aerosol: < 0.01 % Fish: < 0.01 %
Test condition	:	Data used in the calculation: temperature (°C): 25 molar mass (g/mol): 182.14 log Kow: 2.0 vapour pressure (Pa): 0.016 water solubility (mg/l): 270 melting point (°C): 56 Unit world Modelling Data* Volumes (m3) Organic C (g/g) Density (kg/m³) air: 6.0E+9 1.185 water: 7.0E+6 1000 soil: 4.5E+4 0.02 1500 sediment: 2.1E+4 0.05 1300 susp. sediment: 3.5E+1 0.167 1500 biota (fish): 7.0E+0 1000 aerosol 1.2E-1 1500 *Compartment properties were based on the parameters from the first publication of Mackay (1991), modified by the Federal Environmental Agency (UBA, Germany). log Kow is the mean value between the available experimental results for 2,4- and 2,6-DNT. Vapour pressure of 2,4-DNT was used.
Reliability	:	(2) valid with restrictions Accepted calculation method
20.11.2003		(25)
Media	:	water - air
Method	:	other (calculation): HENRYWIN v3.1, 2000
Year	:	2003
Result	:	Henry's law constant (H) = 0.00938 Pa m3 mol-1 at 25 °C (Bond method), which equals a dimensionless H of 0.00000379.

= 0.040 Pa m3 mol-1 at 25 °C (group-method).
Test condition : Temperature: 25 °C
Reliability : (2) valid with restrictions
Accepted calculation method
Flag : Critical study for SIDS endpoint
21.11.2003 (25)

Media : water - soil
Method : other (calculation): PCKOCWIN v1.66 (2000)
Year : 2003

Result : Koc = 371
Test condition : Temperature: 25 °C
Reliability : (2) valid with restrictions
Accepted calculation method
Flag : Critical study for SIDS endpoint
18.08.2003 (25)

Media : water - air
Method : other (measurement)
Year : 1981

Method : According to Mackay 1976: 2,4-DNT containing solution in bottle is sparged with watersaturated nitrogen (to prevent water loss from 2,4-DNT solution). The 2,4-DNT content of the nitrogen and the solution is determined by GC. 2,4-DNT detection at 254 nm.
Two measurements were made.
Result : Value torr M-1Pa m3 mol-1
1 0.45 ± 0.04 0.060 ± 0.005
2 0.34 ± 0.05 0.045 ± 0.007
Test substance : 2,4-DNT, purity >= 99 %
Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles
22.11.2003 (38)

Media : water - soil
Method : other (measurement)
Year : 1996

Result : Adsorption of nitroaromatic compounds to solid particles depends on their clay content
Adsorption of 2,4-DNT and 2,6-DNT (and other nitroaromatic compounds) to 3 homoionic kalium ion clay minerals was determined:
1. Kaolinite
- Distribution coefficient Kd (l/kg dry matter) 2,4-DNT: 690; 2,6-DNT: 10
2. Illite
- Distribution coefficient Kd (l/kg dry matter) 2,4-DNT: 3650; 2,6-DNT: 52
3. Montmorillonite
- Distribution coefficient Kd (l/kg dry matter) 2,4-DNT: 7400; 2,6-DNT: 125
Further results were:
- Adsorption of nitroaromatic compounds is high when the exchangeable cations at the clays include K+ or NH4+ but much smaller for homoionic clays containing Na+, Ca2+, Mg2+, and Al3+
- Highest adsorption coefficients are found for polynitroaromatic compounds
- Ionic strength (in the range of 0.0001 - 0.1 M) had no measurable effect on the adsorption
It is rationalized that electron donor-acceptor complex formation occurs with oxygene at the external siloxane surface of clay minerals (which

increases in the aforementioned order of the three minerals). The mobility of nitroaromatic compounds decreases with increasing degree of nitration. In general, bulky alkyl groups decrease the adsorption although some exceptions exist.

Test condition : - Solutions of test substances were prepared in methanol (or acetonitrile if not soluble in methanol), final concentration of organic solvent <= 0.5 %
- Solutions were spiked with known quantities of air-dried clay minerals (5 - 200 g/l)
- Equilibrium was reached after about 30 - 60 min on rotary shaker in the dark at 21 +- 1.5 °C
- Phase separation by centrifugation at 12,000 rpm for 1 min
- HPLC-UV analysis of solutes in the supernatant
- Cation analysis with ion chromatograph Metrohm Model 690, Herisau, CH, using Metrohm Super-Sep cation column

Test substance : 2,4-DNT, 2,6-DNT, minimum purity >= 97 % (obtained from Fluka AG, Buchs, CH), checked by HPLC-UV analysis

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles

Flag : Critical study for SIDS endpoint

10.08.2004 (55)

Media : water - soil
Method : other (measurement)
Year : 1989

Remark : Cited by Roembke et al. (1995) [Roembke J, Bauer C, Brodesser J, Brodsky J, Danneberg G, Heimann D, Renner I, Schallnass H-J (1993) Grundlagen fuer die Beurteilung des oekotoxikologischen Gefaehrdungspotentials von Altstoffen im Medium Boden - Entwicklung einer Teststrategie (Basis for the Assessment of the Ecotoxicological Potential of "Old Chemicals" in the Terrestrial Environment - Development of a Testing Strategy). Research Report UBA-FB 106 04 103, UBA Texte 53/95], Page 39 (Koc = 250). On Page 52 of the same publication, it is reported, that Koc = 192

Result : Koc = 250

Reliability : (4) not assignable
Original reference not yet available

27.11.2003 (56)

Media : water - soil
Method : other (calculation): no data given
Year : 1995

Result : Koc = 192 [cited from Page 52, on Page 39 it is reported, that Burrows et al. (1989) found 250]

Reliability : (3) invalid
Documentation insufficient for assessment

27.11.2003 (23)

Media : water - soil
Method :
Year : 1989

Result : Howard reports that the Koc of 2,4-DNT was measured by Spangord et al. (1980) [Environmental Fate Studies on Certain Munitions Wastewaters. Final Report Phase 2, Laboratory studies US NTIS ADA099256] to be 12 (measured with Holston River sediments after 10 d).
Conclusion of the author: 2,4-DNT is slightly mobile in soil. It has little tendency to adsorb to sediment.

Reliability : (4) not assignable
Original reference not available
19.11.2003 (57)

Media : water - soil
Method :
Year : 1991

Result : 2,4-DNT log Koc = 2.40 > Koc = 251
2,6-DNT log Koc = 1.89 > Koc = 78
Reliability : (2) valid with restrictions
Data from handbook or collection of data
06.11.2003 (21)

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type : aerobic
Inoculum : activated sludge
Concentration : 100 mg/l related to Test substance
related to
Contact time :
Degradation : 0 (±) % after 14 day(s)
Result : under test conditions no biodegradation observed
Deg. product :
Method : other: Japanese Guideline by MITI of 1974; corresponds to OECD 301C Modified MITI Test
Year : 1992
GLP : no data
Test substance : other TS: DNT isomers mixture (CAS 25321-14-6)

Method : "Biodegradation test of chemical substance by microorganisms etc." stipulated in the Order Prescribing the Items of the Test Relating to the New Chemical Substance (1974, Order of the Prime Minister, Minister of Health and Welfare, the MITI No. 1). This guideline corresponds to "301C, Ready Biodegradability: Modified MITI Test I", stipulated in the OECD Guidelines for Testing of Chemicals (May 12, 1981).
Result : Under test conditions no biodegradation observed. There is no explanation why the test period lasted only 14 days.
Test condition : sludge concentration: 30 mg/l
Reliability : (2) valid with restrictions
Test procedure according to national standards.
Flag : Critical study for SIDS endpoint
19.01.2004 (16)

Type : anaerobic
Inoculum : other: anaerobic sludge, domestic
Concentration : 20 mg/l related to DOC (Dissolved Organic Carbon)
related to
Contact time :
Degradation : 0 (±) % after 56 day(s)
Result : under test conditions no biodegradation observed
Deg. product :
Method : other: according to EPA Test Guideline (§ 796.3140 "Anaerobic biodegradability of organic chemicals")

Year : 1991
GLP : yes
Test substance : other TS: DNT 80/20 (= DNT isomers mixture)

Method : Federal Register (50 FR 39252, Sept. 27, 1985, as amended at 52 FR 19058, May 20, 1987)
Remark : Toxicity controls with DNT 80/20 (= DNT isomers mixture; 43 mg/l) showed no toxic effects on the inoculum
Result : analysis of DIC, TOC and TIC
Test condition : Sludge from the digester of a municipal sewage treatment plant; incubation in the dark; temperature 35 +/- 2 °C; measurement of CO2 and methane
Test substance : The CAS-No. of 2,4-DNT is incorrectly reported to be the CAS-No. of DNT 80/20 (DNT isomers mixture, CAS-No. 25321-14-6).
Reliability : (2) valid with restrictions
Test procedure in accordance with national standard methods with acceptable restrictions
Flag : Critical study for SIDS endpoint
19.01.2004 (58)

Type : aerobic
Inoculum : other: Inoculum from industrial wastewater treatment containing Acinetobacter, Alcaligenes, Flavobacterium, Pseudomonas (bacteria) and Rhodotorula (yeast)
Concentration : 50 mg/l related to Test substance
related to
Deg. product : yes
Method : other: according to Umbreit (1972)
Year : 1981
GLP : no
Test substance : other TS: 2,4-DNT and 2,6-DNT, purity not given
Deg. products : 119-32-4 204-314-0 3-nitro-p-toluidine
99-55-8 202-765-8 5-nitro-o-toluidine

Method : Primary degradation monitored by GC/MS after extraction
Remark : Municipal seed (inoculum with municipal activated sludge organisms) showed inhibition at all test concentrations(200 down to 10 mgl-1)
Result : 2,4-DNT:
Degradation: 80 % after 2 days.
In extracts taken after 2 days the only metabolite which could be identified was 4-methyl-3-nitroaniline [119-32-4]. From day 3 to 7 no further degradation occured. Respiration was stimulated at 200 mg 2,4-DNT/l, whereas at 100 mg 2,4-DNT/l no appreciable effect on respiration was observed.
2,6-DNT:
Degradation: 50 % after 7 days.
2-Methyl-5-nitroaniline [99-55-8] was detected by GC-MS analysis as a byproduct after 7 days. No inhibition was observed at any of the doses from 10 to 200 mg/l.
Test condition : Toxicity screening at 28 °C. Results not reported.
Temperature: 23 °C; inoculum density: 18x10E+8 cells/ml
Inocolum species: Acinetobacter, Alcaligenes, Flavobacterium, Pseudomonas (bacteria) and Rhodotorula (yeast)
Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles
Flag : Critical study for SIDS endpoint
22.01.2004 (59)

Type : aerobic
Inoculum : other: microcosms from explosives-contaminated sites

Contact time :
Degradation : ca. 33 - 80 (±) % after 28 day(s)
Result : other: 2,4-DNT and 2,6-DNT were mineralized (28 and 8 %, repectively) and biotransformed
Deg. product :
Method : other: Degradation in microcosm
Year : 1997
GLP : no data
Test substance : other TS: 2,4-DNT and 2,6-DNT >/= 99 %, uniformly ring labelled 2,4-DNT >98%, 2,6-DNT >97.4 %
Deg. products : 119-32-4 204-314-0 3-nitro-p-toluidine
124-38-9 204-696-9 carbon dioxide
603-83-8 210-059-6 3-nitro-o-toluidine
99-55-8 202-765-8 5-nitro-o-toluidine

Result : Approximately 28 % of the 2,4-DNT radiolabel was recovered as 14CO2. Approximately 20 % of added 2,4-DNT remained undegraded at the end of the incubation while approximately 22 % and 6 % were transformed to 4-amino-2-nitrotoluene (119-32-4) and 2-amino-4-nitrotoluene (99-55-8). In aquifer microcosms containing 2,6-DNT, approximately 67 % of the substrate remained undegraded and approximately 14 % was transformed to 2-amino-6-nitrotoluene (603-83-8). About 8 % of the 2,6-DNT was mineralized to CO2. Degradation of 2,6-DNT proceeded more slowly than degradation of 2,4-DNT.
Test condition : Indigenous microorganisms from aquifer water and sediment able to degrade DNT were amended with uniformly ring-labeled 2,4- and 2,6-DNT. Microcosms were incubated statically in the dark at room temperature. Abiological controls were prepared ny addinh HgCl2 and autoclaving the microcosms (121 °C for 1 h). Evolved 14CO2 was measured.
Reliability : (2) valid with restrictions
study well documented, meets generally accepted scientific principles
Flag : Critical study for SIDS endpoint
22.01.2004 (60)

Type : aerobic
Inoculum : other: soil from contaminated sites and several bacterial strains degrading 2,4-DNT and/or 2,6-DNT
Contact time :
Degradation : > 99 (±) % after 2 day(s)
Result : other: 2,4-DNT and 2,6-DNT mineralized by soil bacteria and several isolated bacterial strains
Deg. product : yes
Method : other: 14C tracing in batch degradation experiments
Year : 1999
GLP : no
Test substance : other TS: Ring labled 14C-2,4-DNT and 14C-2,6-DNT
Deg. products : nitrite
124-38-9 204-696-9 carbon dioxide

Method : Degradation (mineralization) was determined by removal of DNT and reduction products (HPLC), and from 14CO2 and nitrite release.
Result : Within 2 d more than 40 % of the initially present radiolabelled 2,4-DNT and 2,6-DNT was trapped as 14CO2 (degraded concomitantly by soil bacteria and several isolated bacterial strains).
In the draw and fill reactor experiments, 2,4-DNT (1 mM) and 2,6-DNT (0.2 mM) were added repeatedly into the reactor and degraded by soil bacteria within about 2-3 d. No accumulation of a DNT isomer occurred. Degradation of 2,4-DNT is faster than degradation of 2,6-DNT.

Test condition : Bacterial inocumlum:
Enrichment cultures were isolated from contaminated soil, groundwater, and activated sludge. 1 ml of water or activated sludge or 1 g of soil was inoculated into 100 ml of nitrogen-free minmal medium (BLK) containing 2,4-DNT (18 mg/l) or 2,6-DNT (9 mg/l) as sole source of nitrogen and organic carbon. Cultures were shaken at 30 °C. DNT was monitored by HPLC, and when decreasing, the cultures were transfered to fresh medium. After 2-14 months (several transfers) cultures of Burkholderia cepacia JS872 (degrading 2,4-DNT), B. cepacia JS922 (degrading 2,4-DNT and 2,6-DNT simultaneously), and Hydrogenophaga palleronii JS863 (degrading 2,6-DNT)were obtained.
Cultivation was at 30 °C on solid or in liquid defined media free of inorganic nitrogen and organic C supplemented with thew appropriate DNT isomer (550 mg/l, for JS922 275 mg/l of each isomer) as sole carbon and nitrogen source. Maintenance media contained sorptive XAD-7 beads (10 g/L; Sigma, St. Louis, MO) to maintain a sub-toxic, longterm supply of DNT
Draw and fill reactor experiments:
- 1.2 l working volume, two 1.5 cm diameter ports in the top of the reactor for air exchange and sampling. - Incubation temperature 30 °C, and reactors were stirred at 160 rpm
- Soil was from the former TNT-manufacturing plant at Hessisch Lichtenau, Germany: 50 % (w/w) dried, sieved clay plus silt, 50 % (w/w) sand; 3.6 g 2,4-DNT/kg soil and 2.5g 2,6-DNT/kg soil. The organic content was 7.8 % of dry weight.
- 600 ml of a 10 % (w/v) soil slurry in phosphate buffer (20 mM, pH 7.0), stirred for 12 h, and a suspension of induced cells was added to initiate the experiment.
- The inoculum consisted of a mixture of 5 ml each of strains JS872, JS863, and JS922 (2.5, 3.4, and 3.5 mg of protein ml-1, respectively)
- DNT concentration was monitored by HPLC. NaOH (50 % w/v) was used to maintain the pH between 6.75 and 7.25. Initial DNT concentrations were approximately 1 mM. When the concentrations of each isomer dropped below 20 µM, 90 % of the slurry was drained from the bottom of the reactor, and additional contaminated soil and phosphate buffer were added to the original level. No further additions of bacteria or nutrients were made during the 600 h experiment. An identical control reactor was operated without added bacteria.
Mineralization experiments:
Experiments with pure cultures and with 10 % (w/v) slurries of uncontaminated soil (from US sites) were conducted in duplicate 250 mL shake flasks containing 25 mL of nutrient medium without N and organic C. KOH used to trap CO2 to glass center wells, and the flasks were sealed with ground glass stoppers. For experiments with single isomers, 2,4-DNT was provided at 1 mM, and 2,6-DNT at 0.2 mM. Cultures receiving mixtures of DNT were provided 2,4-DNT at 0.8 mM and 2,6-DNT at 0.2 mM. Controls were treated with HgCl2 (2.5 mg/l).
Mineralization experiments with aged contaminated soil (Hessisch Lichtenau) were conducted in a 2 l slurry bioreactor with a 1.8 l working volume. Spent soil slurry (200 ml) from the draw and fill reactor was added as an inoculum to 1.6 l of 10 % (w/v) in 20 mM phosphate buffer, pH 7.0. The reactor contained approximately 570 mg of 2,4-DNT and 390 mg of 2,6-DNT from the soil and 40 µCi of radiolabeled DNT (0.43 mg of 2,4-DNT, 0.14 mg of 2,6-DNT, each isomer added individually in separate sequential experiments). 30 °C air was pumped across the surface of the slurry at 1 l h-1 and the CO2 was traped in a gas washing bottle containing NaOH (0.24 N).
Analytical methods:
HPLC was performed with a Hypercarb porous graphite column (5 µm x 150 mm, Hypersil, U. K.) with a mobile phase of acetonitrile/water (90 : 10) containing trifluoroacetic acid. UV detection at 230 mm. Nitrite and nitrate

analyses were performed using a colorimetric method or by ion chromatography.

Test substance : 2,4-Dinitro[ring-U-14C]toluene (16.6 mCi mmol-1) was from ChemSyn (Lenaxa, KS). It was purified by HPLC before use to > 98 % radiochemical purity. 2,6-Dinitro[ring-U-14C]toluene (51 mCi mmol-1) was from Amersham. It was 98% radiochemically pure as determined by HPLC and was used without further purification.

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles

Flag : Critical study for SIDS endpoint

22.01.2004 (48)

Type : aerobic
Inoculum : other: munitions wastewater from aerated equalization basin of WWTP
Concentration : 3 mg/l related to Test substance
related to
Contact time :
Degradation : 84 - 95 (±) % after 9 day(s)
Result : other: 2,4-DNT and 2 amino metabolites degraded, ammonia released
Deg. product : yes
Method : other: Degradation in batch reactors
Year : 2000
GLP : no data
Test substance : other TS: 2,4-DNT of highest purity commercially available
Deg. products : 119-32-4 204-314-0 3-nitro-p-toluidine
99-55-8 202-765-8 5-nitro-o-toluidine

Method : In batch reactors the effect of nutrient and co-substrate amendments on the rate and extent of DNT removal was studied

Result : 2,4-DNT was nearly completely degraded within 1-2 weeks. Adding ethanol (100-500 mg/l) and phosphate (0.8-3.3 mg/l) significantly accelerated the rate of aerobic 2,4-DNT (0.3-5.6 mg/l) biodegradation. Phosphate alone was less effective. Ethyl ether (commonly present in munitions plant wastewater) had little effect, too.
To check the effect of interruptions of DNT supply to adapted microbial communities in sewage, 2,4-DNT was added at varying intervals (from once every 3 days to once every 15 days). Under all conditions DNT removal resumed without a lag phase.
Under aerobic conditions 2,4-DNT was reduced to 4-amino-2- nitrotoluene [119-32-4] and 2-amino-4-nitrotoluene [99-55-8]. The highest level of aminonitrotoluene formation was 23 % of total 2,4-DNT degraded. Aminonitrotoluene isomers were consumed within 1 day after DNT disappeared in semi-continuously operated reactors. DNT nitrogen was partly recovered as ammonia.

Test condition : - 500 ml serum bottles, filled with wastewater from aerated equalization basin
- Incubation in the dark, aerated
- Analysis: DNT by HPLC (HP 1090) with a diode array detector at 246 nm, nitrite, nitrate, phosphate, sulfate by IC (Dionex chromatograph)

Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles

Flag : Critical study for SIDS endpoint

22.01.2004 (61)

Type : anaerobic
Inoculum : other: domestic activated sludge, adapted to benzene
Concentration : 5 mg/l related to Test substance
related to
Contact time :
Degradation : 100 (±) % after 14 day(s)

Result : other: During degradation of 2,4-DNT nitroso and amino intermediates are formed which are completely degraded within 2 weeks of incubation
Deg. product : yes
Method : other: anerobic test in cyclon fermentors
Year : 1984
GLP : no data
Test substance : other TS: 2,4-DNT, no purity given
Deg. products : 3-nitroso-p-toluidine
4-nitro-2-nitroso-toluene
119-32-4 204-314-0 3-nitro-p-toluidine
99-55-8 202-765-8 5-nitro-o-toluidine

Result : - No breakdown of 2,4-DNT in the aerobic fermentors was observed, even after 14 days of incubation.
- Under anerobic conditions and in the presence of methanol (1 ml/l) a 2,4-DNT concentration of 5 mg/l was completely degraded within the test period. The metabolic products disappeared with time.
- In biotransformation experiments with dimethyl sulfoxide as additionally energy source instead of methanol no
degradation was observed.
Test condition : - Inoculum from municipal activated sludge. Adapted to benzene as carbon source.
- Six cyclo fermentors (3 anaerobic, 3 aerobic). Two of them served as control without test substance and other two with the inhibitor HgCl2 together with DNT.
- Fermentors were continuously purged with nitrogen (anerobic) or with a flow of air (aerobic).
- Degradation was monitored analytically (GC-MS).
- 14 days exposure.
Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles
Flag : Critical study for SIDS endpoint
22.01.2004 (62)

Type : aerobic
Inoculum : other: mixed culture of simultaneously 2,4-DNT and 2,6-DNT degrading bacteria
Contact time :
Degradation : (±) % after
Result : other: 2,4-DNT and 2,6-DNT were mineralized simultaneously
Deg. product : yes
Method : other: aerobic degradation in fluidized-bed biofilm reactor
Year : 1998
GLP : no data
Test substance : other TS: mixture of 2,4-DNT and 2,6-DNT
Deg. products : nitrate
nitrite

Result : Removal efficiences higher than 98 % for 2,4-DNT and 94 % for 2,6-DNT were archieved at all loading rates. All nitrogen was released stoichiometrically from both DNT isomers. Due to presence of nitrite oxidizing bacteria, all nitrite released from DNT was oxidized to nitrate. No aromatic reduction products found at detection limit of about 10 µg/l.
Test condition : - bacterial culture: mixed culture of nitroaromatics degrading bacteria, constant feed concentrations of 2,4-DNT (35.7 +/- 3.3 mg/l) and 2,6-DNT (10 +/- 0.4 mg/l) were maintained for the majority of the operation (4 month)
- fluidized-bed reactor: volume 1.5 l, filled with 0.74 kg of acid-washed

Ottawa sand, 20 °C, pH 7 +/- 0.1, dissolved oxygen was maintained higher than 4.5 mg/l, reactor was operated at hydraulic retention times of 12.5, 6.3, 1.5 and 0.75 h in turn
- analysis: transformation products were analyzed on a HP 1050 HPLC system equipped with a diode array detector, nitrite and nitrate were analyzed with a Dionex DX-300 chromatography system, biomass concentration was measured as COD or protein per mass of sand

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles
Flag : Critical study for SIDS endpoint
22.01.2004 (63)

Type : aerobic
Inoculum : other: Bacterial consortium from TNT contaminated soil
Contact time :
Degradation : 58 - 61 (±) % after 7 day(s)
Result : other: biodegradation and photodegradation under field conditions observed
Deg. product :
Method : other: Incubation in wetland mesocosms under field conditions
Year : 2001
GLP : no data
Test substance : other TS: mixture of several nitroaromatics

Method : Degradation of 2,4-DNT and 2,6-DNT was investigated in wetland mesocosms under field conditions in small-scale 4-months field study as a surface-flow, modular system.
The groundwater of a well located at the border of a TNT manufactury valley was used as influent. The influent contained 16.7 mg/l 2,4-DNT and 5.2 mg/l 2,6-DNT.
The effect of 3 treatments were compared: planted, non planted and UV-shielded in three different lagoons (three replicates).
Explosives-contaminated groundwater was continuously pumped into the lagoons and a 7-day hydraulic retention time was maintained.

Result : 58 % of 2,4-DNT and 61 % of 2,6-DNT were eliminated in constructed wetland lagoons during 7 d of hydraulic retention time.
The initial concentration of nitrocompounds in effluent decreased exponentially with time.
The average level of nitroaromatics was 94.6 mg/l in the influent. In the effluents of the lagoons they were 14 mg/l in the planted, 28.1 in the non-planted and 34.4 mg/l in the UV-shielded lagoons.
The contribution of photodegradation to the removal rates was 60 % for 2,4-DNT and 59 % for 2,6-DNT in the planted and in the non-planted treatments.

Test substance : The groundwater of a well located at the border of a TNT manufactury valley was used as influent (= incubation medium). The influent contained 16.7 mg/l of the 2,4-DNT and 5.2 mg/l of the 2,6-DNT (and several other nitroaromatics and other substances)

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles
Flag : Critical study for SIDS endpoint
22.01.2004 (37)

Type : aerobic
Inoculum : other: wetland mesocosms containing sediments from non-contaminated site and plants
Contact time : 7 day(s)
Degradation : (±) % after
Result : other: Removal of high levels of explosives by planted, non-planted, and non-planted UV-shielded wetlands

Deg. product :
Method : other: Incubation in wetland mesocosms under field conditions
Year : 1999
GLP : no data
Test substance : other TS: mixture of nitroaromatics including 2,4-DNT and 2,6-DNT

Method : Degradation of 2,4-DNT and 2,6-DNT was investigated in wetland mesocosms under field conditions in small-scale 4-months field study as a surface-flow, modular system.
The groundwater of an explosives-contaminated site was used as influent. The influent contained 16.7 mg/l 2,4-DNT and 5.2 mg/l 2,6-DNT.
The effect of 3 treatments were compared: planted, non planted and UV-shielded in three different lagoons (three replicates).
Explosives-contaminated groundwater was continuously pumped into the lagoons and a 7-day hydraulic retention time was maintained.

Result : Removal rates varied with isomer, treatmant modul, and time. The following results were obtained:

Removal rate/ period in days	2,4-DNT %	2,6-DNT %
planted		
0-17	60	82
17-31	63	77
31-45	64	79
45-61	85	76
61-73	45	49
73-87	89	81
87-101	31	52
101-115	58	61
average:	62	70
non-planted		
0-17	44	72
17-31	45	63
31-45	38	78
45-61	18	76
61-73	-20	33
73-87	32	72
87-101	- 3	53
101-115	3	53
average:	20	63
non-planted, UV-shielded		
0-17	33	41
17-31	50	78
31-45	15	34
45-61	-15	9
61-73	-32	-16
73-87	7	22
87-101	-21	- 9
101-115	-12	0
average:	3	20

Test substance : The groundwater of the Volunteer Army Ammunition Plant (Chattanooga, TN) was used as influent (= incubation medium). The influent contained 16.7 mg/l of 2,4-DNT and 5.2 mg/l 2,6-DNT (and several other nitroaromatics and other substances)
Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles
Flag : Critical study for SIDS endpoint
22.01.2004 (64)

Type	:	aerobic
Inoculum	:	other: microbial community from contaminated groundwater
Contact time	:	
Degradation	:	> 99 (±) % after 11 day(s)
Result	:	other: Primary degradation > 99 % within 11 d
Deg. product	:	no
Method	:	other: Batch degradation experiments in shake flask
Year	:	2000
GLP	:	no
Test substance	:	other TS: mixtures of 2,4-DNT with other organics
Result	:	Initial microbial community was able to degrade 2,4-DNT within 11 d (> 99 %). After the second addition of substrate (on day 43), 2,4-DNT degradation had an extended lag period, presumably due to accumulation of nitrite, depletion of background concentration of 2,4-DNT which is essential to maintain enzyme levels, or in combination with nitrite, accumulation or toxicity of an intermediate. Alternatively, protist might have diminished the bacterial community. To examine the origin of this lag phase, the activity of 2,3-dioxygenase, a key enzyme in a aromatic degradation pathway which catalyzes the meta cleavage of catechol. this level was extremely low in the bacterial community after about day 40 until the end of incubation. At day 63 of the experiment 2,4-DNT was degraded to 59 +/- 40 %.
Test condition	:	For the test sealed flasks, shaken and incubated at 25°C, containing contaminated groundwater (20 ml, 12 mg/l 2,4-DNT) and a solution of salts (20 ml) were amended with 2,4-DNT (2.5 mM) or other nitroaromatics at day 0 and day 43. Groundwater originated from a Swedish location where ammunition destruction by open burning had been performed for more than 40 years. Degradation was tracked (day 0, 3, 11, 43, 46, 63) with solid phase micro extraction (65 micrometer copolymer polydimethylsiloxane/divinyl benzene, 30 min at 20 °C) and immediate analysis on GC. A calibration curve (0.0025-2.5mM) was made for each analyzed compound. Analysis was performed with capillary GC-FID (SPME injection in splitless mode 2 min at 250 °C) and capillary GC-MS.
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles
Flag	:	Critical study for SIDS endpoint

22.01.2004 (44)

Type	:	aerobic
Inoculum	:	other: enrichment culture from natural surface water downstream from an ammunition site
Contact time	:	
Degradation	:	4 - 64 (±) % after
Result	:	other: 2,4-DNT and 2,6-DNT were mineralized (after adaption: half-life 2-4 d)
Deg. product	:	yes
Method	:	other: 14C tracing in batch degradation experiments
Year	:	1992
GLP	:	no data
Test substance	:	other TS: 2,4-DNT and 2,6-DNT, 14C-labled
Deg. products	:	124-38-9 204-696-9 carbon dioxide
Remark	:	After 2-3 days, biodegradation started at all concentrations tested (0.004-10 mg/l). Biodegradation increased with increasing substrate concentrations. Biodegradation was determined via 14CO2 measurement.
Result	:	Degradation of 2,4-DNT began at 2 to 3 days incubation, at all

concentrations tested and reached 64 % (10 mg/l test concentration) resp. 45 % (0.004 mg/l test concentration). Degradation of 2,6-DNT was not initiated until about 17 days of incubation and did not occur to any significant degree at substrate concentrations below 1.0 mg/l. 33 to 55 % of 2,6-DNT degraded at concentrations from 1.0 to 10 mg/l. Degradation rates of 32 %/day for 2,4-DNT and 14.5 %/day for 2,6-DNT (for 10 mg/l, the highest concentration tested), decreased with decreasing substrate concentration. At very low substrate concentrations, degrader populations did not increase.
For 2,4-DNT, mean second-order rate constant at 25°C was 3.9E-10/cell/min, while for 2,6-DNT this was 9.9E-10/cell/min.
In 4 tests in shake flasks with water from pristine environments no biodegradation was observed within 6 weeks.

Test condition : 2,4- and 2,6-DNT serve as sole sources of carbon and energy for microbial growth, with up to 60 percent of substrate carbon appearing as CO2. Mixed enrichment cultures were developed for each DNT isomer separately, by sequential transfer to increasing substrate concentrations. Maximal substrate concentrations tolerated were 130 mg/l. Material from river water was used. The flasks were incubated at 25°C and were monitored for the disappearance of DNT by UV spectrophotometry and gas chromatography.

Reliability : (2) valid with restrictions
Study well documented; meets generally accepted scientific principles

Flag : Critical study for SIDS endpoint

22.01.2004 (65)

Type : anaerobic
Inoculum : industrial sewage, adapted
Contact time :
Degradation : 100 (±) % after 11 day(s)
Result : other: 2,4-DNT was cometabolized but not mineralized
Deg. product :
Method : other: Batch degradation experiment
Year : 1997
GLP : no data
Test substance : other TS: 14C-2,4-DNT > 97.8%

Remark : A similar experiment was conducted with an acclimated denitrifying enrichment culture, able to treat munitions wastewater that contains DNT. Only in the presence of ethanol as primary substrate, 2,4-DNT was completely transformed within 11 days. The pricipal initial biotransformation pathway was reduction of DNT to aminonitrotoluenes. Subsequent transformations resulted in formation of 6-nitroindazole, 2- and 4-nitrotoluene. Acetylation was another important transformation pathway, resulting in 4-acetamide-2-nitrotoluene and 4-acetamidtoluene. Reduction of aminonitrotoluenes to 2,4-diaminotoluene also occurred.

Result : Only in the presence of ethanol as primary substrate a denitrifying enrichment culture transformed all of the applied 2,4-DNT within 11 days. Hydrophilic metabolites were found. Mineralization was negligible.

Test condition : Microorganisms were grown in mineral medium in the presence of nitrate and ethanol at room temperature (denitrifying enrichment culture); 2,4-DNT concentrations: 0.25-0.60 mM (ca. 45-110 mg/l)

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles

Flag : Critical study for SIDS endpoint

22.01.2004 (66)

Type : anaerobic

Inoculum : activated sludge, domestic
Concentration : 10 mg/l related to Test substance
related to
Contact time :
Degradation : 100 (±) % after 10 day(s)
Result : other: 2,4-DNT was degraded under cometabolic conditions
Deg. product : no
Method : other: Batch experiments in cyclone fermentor
Year : 2000
GLP : no data
Test substance : other TS: 2,4-DNT, purchased from Aldrich, no data on purity

Method : Batch experiments in cyclone fermentor under aerobic and anaerobic conditions, with and without co-metabolites, and with and without pre-exposure of microorganisms
Result : The authors report that 2,4-DNT was degraded by
- Aerobic metabolization: lag phase 0 d, t1/2 79 d
- Aerobic cometabolization (benzene): lag phase 0 d, t1/2 63 d
- Anaerobic metabolization: lag phase 0 d, t1/2 78 d
- Anaerobic cometabolization (benzene): lag phase 1.1 d, t1/2 1.7 d
Sodium acetate was not effective as an anaerobic cosubstrate suggesting that benzene induces growth of microorganisms with ring cleavage abilities under anaerobic conditions.
However, since most degradation rates are similar to the controls, it is assumed that 100 % primary degradation after 10 d occured under anaerobic cometabolic conditions, but no degradation occured under aerobic conditions and under anaerobic conditions without cometabolic substrate
Test condition : - Incubation in cyclone fermentor at 21-22 °C with steady supply of air (aerobic) or nitrogen (anaerobic)
- Controls with HgCl2 at 200 mg/l
- 2,4-DNT (10 mg/l) as the sole source of nitrogen and carbon in metablism experiments; benzene 1 ml/l or sodium acetate 0.5 g/l in cometabolism experiments
- Analysis by GC (HP 5890) equipped with DB5 capillary column (o.25 mm x 30 m) and FID
Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles
Flag : Critical study for SIDS endpoint
22.01.2004 (67)

Type : anaerobic
Inoculum : other: contaminated soil and unspecified inoculum
Contact time :
Degradation : ca. 100 (±) % after 11 month
Result : other: 2,4-DNT completely degraded, intermittantly observed intermediates not detectable at the end of treatment
Deg. product : yes
Method : other: Two step bioremediation process for soil
Year : 2001
GLP : no
Test substance : other TS: 2,4-DNT

Method : TOSS (= Two Step Static: sequential anaerobic and aerobic stages) bioremediation process was used for treating 200 t of 2,4-DNT contaminated soil. In the first stage 2,4-DNT contaminated soil was mixed with a carbon source (starch and glucose), an inoculum, and water to achieve anaerobic conditions. The mixture was maintained under anaerobic conditions for 44 weeks.

In the second stage, the soil was mixed with yard waste and formed into a 2-m high pile to begin the aerobic treatment. The pile was located on top of a synthetic liner system with leachate control.

Remark : Soil bioremediated in this study stems from Gyttorp facility in Sweden, which was used for explosives manufacturing from 1864 to 1995.

Result : During the anaerobic period (stage 1) the concentration of 2,4-DNT was reduced from 4 g/kg to 19 mg/kg. The total concentration of other nitrotoluene explosives was reduced from 5,3 g/kg to 22 mg/kg. Amino-substituted degradation intermediates were detected by GC/MS during the initial phase of the anerobic treatment, but not at the end of this treatment. During the aerobic stage (stage 2) leachate contained <2 µg/l of 2,4-DNT.

Reliability : (2) valid with restrictions
Basic data given

Flag : Critical study for SIDS endpoint

22.01.2004 (49)

Type : aerobic
Inoculum : other: Burkholderia cepacia wildtype and genetically modified strain
Contact time :
Degradation : (±) % after
Result : other: Burkholderia cepacia wildtype and genetically modified strain grow on 2,4-DNT as sole source of carbon (mineralization not quantified)
Deg. product :
Method : other: Batch degradation experiments
Year : 2001
GLP : no data
Test substance : other TS: 2,4-DNT

Method : Bacteria and plasmids:
Burkholderia cepacia NRRL B-14180 maintained on tryptic soy agar (TSA); plasmids were pUC8:16, pUC18:NotI, pUTminiTn5(Cm) and pUT-miniTn5:vgb(Cm)

Result : Vitreoscilla hemoglobin gene (vgb)-bearing Burkholderia cepacia strain (BcJC) had a growth advantage over the wildtype strain (BcWT) at ca. 90 ppm, but not at ca. 120 ppm 2,4-DNT; no difference in DNT degradation between the two strains at low aeration.

Test condition : Shake flask cultures (either vgb-free or vgb-bearing; vbg: Vitreoscilla hemoglobin gene) to be used as inocula were grown in 25 ml of TSA for 12 h at 30 °C and 200 rpm. Approximately 0.1 ml of culture was harvested by centrifugation and inoculated into 50 or 200 ml of medium. The medium included initially 120 or 180 ppm DNT. The actual DNT concentrations measured at time of inoculation were 88-94 and 119-125 ppm. The reason for the differences is not known, but may be related to autoclaving of the medium.
Normal aeration was 200 rpm in flasks in which the medium volume was 20 % of the flask volume; low aeration was 50 rpm in flasks in which the medium volume was 80 % of the flask volume. The numbers of viable cells were determined by plating dilutions (in 0.85 % NaCl) on Luria broth. Colonies were counted after growth for 16 h at 30 °C. Experiments with B. cepacia lasted 72 h.
During growth cells were removed by centrifugation and supernatant fluids were analyzed by HPLC (Varian Star Chromatography 9012Q, solvent delivery system with 9050 UV-Vis detector)

Test substance : 2,4-DNT solved in tryptic soy agar (TSA)

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles

Flag : Critical study for SIDS endpoint

22.01.2004 (68)

Type : aerobic

Inoculum : activated sludge, domestic
Concentration : 20 mg/l related to DOC (Dissolved Organic Carbon)
related to
Contact time :
Degradation : 33 (±) % after 28 day(s)
Result :
Kinetic of testsubst. : 7 day(s) 31 %
14 day(s) 40 %
21 day(s) 65 %
28 day(s) 33 %
%
Deg. product :
Method : OECD Guide-line 301 E "Ready biodegradability: Modified OECD Screening Test"
Year : 1982
GLP : no data
Test substance : other TS: 2,4-DNT, purity not given

Remark : Large but unexplained scattering of results
Result : A precipitation was observed during the test. The authors concluded, that this was due to crystallization.
Test condition : - Test control performed with aniline
- 2 replicates
- DOC-analysis
- Toxicity control was also performed (see chapter 4.4)
Reliability : (3) invalid
Basic data given

22.01.2004 (69)

Type : aerobic
Inoculum : other: bacterial strains from contaminated munition site
Contact time :
Degradation : (±) % after
Result : other: degradation of 2,4-DNT and 2,6-DNT to aromatic amines
Control substance : other: ammonium sulfate
Kinetic : %
%
Deg. product : yes
Method : other
Year : 1989
GLP : no data
Test substance : other TS: mixture of nitroaromatics including 2,4-DNT and 2,6-DNT
Deg. products : aromatic amines

Method : Isolation of microorganisms from contaminated soil and activated carbon filter, culture of bacteria in standard and TNT or DNT containing growing medium, analysis of nitroaromatics by GC, GC-MS and HPLC
Result : 2,4-DNT and 2,6-DNT (each with 100 mg/l) were degraded to aromatic amines by bacteria isolated from TNT contaminated soil. Within 14 days concentration decreased to 1 mg/l (2,4-DNT) and 24 mg/l (2,6-DNT).
Reliability : (2) valid with restrictions
Basic data given

20.11.2003 (70)

Type : aerobic
Inoculum : other: Azotobacter agilis, adapted to 2,4,6-TNT
Contact time :
Degradation : 100 (±) % after 36 hour(s)

Result	:	other: 100 % of nitro compounds and 97 % of intermittantly formed amino compounds degraded
Deg. product	:	
Method	:	other: 2 stage model by Bringmann and Kuehn (1971). Simulation of a munition industrial sewage treament plant
Year	:	1971
GLP	:	no
Test substance	:	other TS: 2,4-and 2,6-DNT separately; no purity given
Remark	:	1st stage: aeration vessel inoculated with Azotobacter (25 °C); the drain of this stage was directed to a sampling vessel. Every hour the content of the vessel was pumped to the 2nd stage: a trickling filter inoculated with activated sludge (domestic); specific analytical monitoring of 2,4-DNT and its metabolic reduction products
Result	:	146 mg/l 2,4-DNT were degraded to 91.8 % after first stage and 100 % after second stage. 128 mg/l 2,6-DNT was degraded to 83.6 % after first stage and 99.9 % after second stage. By diazotation without and with reduction it was distiguished between nitro compounds and amino compounds. Amino compounds were formed from DNT but were removed during the incubation period
Test condition	:	- The bacteria (Azetobacter agilis) were isolated from a compost soil sample suspended in nutrient solution containing 130 mg/l 2,4,6-trinitrotoluene - Incubations were done in a model 2 step wastewater treatment plant (both steps aerobic) although the inoculum does not represent the activated sludge of an ordinary wastewater treatment plant. 1st stage: aeration vessel inoculated with Azotobacter the drain of this stage was directed to a sampling vessel. Every hour the content of the vessel was pumped to the 2nd stage: a trickling filter inoculated with activated sludge (domestic. - For incubation of 2,4- and 2,6-DNT was dissolved in bidestilled water, filtered, and 1g/l K2HPO4, 5 g/l Glucose, and 5 mg/l Na2MoO4 x 2 H2O were added - Additional nutrients were supplied daily directly into the model wastewater treatment plant - Temperature 25 °C - Residence time in aeration vessel: 36 h - Spectrometric analysis after reduction and azo coupling at 490 nm - 1st stage: aeration vessel inoculated with Azotobacter; the drain of this stage was directed to a sampling vessel. Every hour the content of the vessel was pumped to the 2nd stage: a trickling filter inoculated with activated sludge (domestic); specific analytical monitoring of 2,4-DNT and 2,6-DNT and its metabolic reduction products.
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles

22.01.2004 (71)

Type	:	aerobic
Inoculum	:	other: Burkholderia cepacia JS872 (for 2,4-DNT), B. cepacia JS850 and Hydrogenophaga palleronii JS863 (for 2,6-DNT)
Concentration	:	1775 mg/l related to Test substance 1973 mg/l related to Test substance
Contact time	:	
Degradation	:	ca. 100 (±) % after 2 day(s)
Result	:	other: 2,4-DNT and 2,6-DNT were mineralized

Deg. product : yes
Method : other: Batch degradation experiment
Year : 2000
GLP : no data
Test substance : other TS: Mixture of nitroaromatics including 2,4-DNT and 2,6-DNT
Deg. products : nitrite

Result : VAAP soil: After inoculation with 2,4-DNT degrading bacteria 2,4-DNT was degraded from concentrations as high as 9750 µM (1.78 g/l) near to detection limits in 42 h
BAAP soil: After inoculation with 2,4-DNT degrading bacteria 2,4-DNT was degraded from concentrations as high as 10840 µM (1.97 g/l) near to detection limits in 46 h
Degradation of 2,6-DNT is inhibited by high concentration of 2,4-DNT. This requires a dual-stage approach to achieve complete degradation of both isomers. Operating 2 reactors in series, where 2,4-DNT was degraded in the first reactor and 2,6-DNT was degraded in the second reactor, allowed for stable draw-and-fill operation. High nitrite concentrations resulting from 2,4-DNT degradation in the first reactor had no apparent impact on subsequent 2,6-DNT degradation
Test condition : Contaminated soils from Volunteer Army Ammunition Plant (VAAP; Chattanooga, TN) and Badger Army Ammunition Plant (BAAP; Baraboo, WI). Concentrations of 2,4-DNT and 2,6-DNT were 19 and 1.38 g/kg in VAAP soil and 8.9 and 0.48 g/kg in BAAP soil. Soils were homogenized and subjected to a soil washing process; the resulting soil slurry was subsequently fed to an Eimco bioreactor (70 l) operated in a draw-and-fill mode. Determination of DNT concentrations by HPLC analysis using a Hewlett-Packard series 1050 HPLC equipped with an UV detector
Reliability : (2) valid with restrictions
Study well documented; meets generally accepted scientific principles

15.01.2004 (46)

Type : aerobic
Inoculum : Pseudomonas sp. (Bacteria)
Contact time :
Degradation : (±) % after
Result : other: Biotransformation products of 2,4-DNT and 2,6-DNT found
Deg. product :
Method : other: Batch degradation experiments
Year : 1996
GLP : no data
Test substance : other TS: 2,4-DNT and 2,6-DNT, purity not given
Deg. products : 2,2´-dinitro-4,4´-azoxytoluene
2,4´-dinitro-2´,4-azoxytoluene
6,6´-dinitro-2,2´-azoxytoluene
119-32-4 204-314-0 3-nitro-p-toluidine
603-83-8 210-059-6 3-nitro-o-toluidine
99-55-8 202-765-8 5-nitro-o-toluidine

Remark : Pseudomonas sp. clone A cultured in minimal medium with fructose was able to grow on 2,4-DNT and 2,6-DNT (0.1-2 g 2,4-DNT/l) as N-source. From cultures grown with 2,4-DNT two possible monoamino derivates, namly, 2-amino-4-nitrotoluene and 4-amino-2-nitrotoluene were isolated. From these cultures only three azzoxytoluenes derived from 2,4-DNT metabolism , namly, 4,4´-dinitro-2,2´-azoxytoluene, 2,2´-dinitro-4,4´-azoxytoluene and 2,4´-dinitro-2´,4-azoxytoluene were isolated and identified.
From the culture supernatant of bacteria grown with 2,6-DNT, 2-amino-6-nitrotoluene was isolated. 6,6´-dinitro-2,2´-azoxytoluene, the sole possible

azoxytoluene expected, was also found and isolated.

Test condition : Cells were grown for 1-7 days under aerobic conditions at 30 °C.
Reliability : (2) valid with restrictions
Study well documented

07.11.2003 (72)

Type : aerobic
Inoculum : other: 3 Burkholderia (Pseudomonas) sp. strains, 2 of them genetically engineered
Concentration : 50 mg/l related to Test substance
200 mg/l related to Test substance
Contact time :
Degradation : 26 - 88 (±) % after 72 hour(s)
Result : other: biodegradable by three Burkholderia strains
Deg. product :
Method : other: Batch degradation experiments
Year : 2001
GLP : no data
Test substance : other TS: 2,4-DNT

Method : Growth and degradation of 2,4-DNT were compared in liquid cultures in shake flasks for 3 Burkholderia sp. strains under several conditions: varied aeration rate, initial 2,4-DNT concentration, and concentration and type of cosubstrate
Remark : Burkholderia was previously identified as Pseudomonas sp.
Result : 2,4-DNT degradation increased with increasing cosubstrate concentration and was greater for strain YV1 than for strain DNT under most conditions tested. The greatest advantages of YV1 (up to 3.5-fold) occurred under limited aeration. Strain YV1m had increased 2,4-DNT degradation (up to 1.3-fold compared to YV1) at 200 ppm 2,4-DNT.
Depending on aeration and cosubstrate biodegradation varied from 6-100 % within 48 h.
Test condition : - Inoculum: Burkholderia sp. strain DNT (strain DNT), Burkholderia sp. strain DNT engineered to produce Vitreoscilla (bacterial) Hb (strain YV1), and a third strain (YV1m), derived from YV1 by repeated growth on 2,4-DNT-containing medium
- Incubation in liquid cultures in shake flasks
- Parameters varied included aeration rate, initial 2,4-DNT concentration (50 and 200 ppm), and concentration and type of cosubstrate (yeast extract, succinate, casamino acids, and tryptic soy broth)
- Analysis: HPLC with UV-VIS detector set at 230 nm
- Statistics: t-test
Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles

22.08.2003 (73)

Type : aerobic
Inoculum : other: aquatic microcosms
Concentration : 50 mg/l related to Test substance
10 mg/l related to Test substance
Contact time : 36 day(s)
Degradation : 27 - 48 (±) % after 36 day(s)
Result : other: 2,4-DNT and 2,6-DNT are both primarily degradable in aquatic microcosms
Deg. product : no
Method : other: Batch degradation experiments in aquatic microcosms
Year : 1999
GLP : no
Test substance : other TS: amended wastewater sample

Result : Percentage decrease of the test substances
- Sterile: 2,4-DNT 28 %, 2,6-DNT 27 %,
- Nonsterile (microbial consortium): 2,4-DNT 40 %, 2,6-DNT 48 %,
- Nonsterile half-strength: 2,4-DNT 37 %, 2,6-DNT 43 %,
Calculated half-life in the nonsterile microcosm: 2,4-DNT 13 d, 2,6-DNT 16 d

Test condition : Microcosms were established in 500 ml Schott glas bottles. For incubation, each bottle contained 350 g sand and 350 ml wastewater, 50 ml buffer (and nutrients in non-sterile samples), 10 ml microbial consortia (or 10 ml sterile effluent for controls)
Wastewater were amended with nitroaromatics and incubated in 3 different microcosms systems during 36 days in a sterile, a nonsterile, and a half-strength system in the dark.
A combination of aerobic and anaerobic conditions was employed

Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles. Basic data given

15.01.2004 (52)

Type : aerobic
Inoculum : other bacteria: Burkholderia cepacia, Alcaligenesis sp.
Contact time :
Degradation : (±) % after
Result : other: 2,4-DNT and 2,6-DNT were mineralized by several bacterial strains
Deg. product : yes
Method : other: Batch degradation tests
Year : 2000
GLP : no data
Test substance : other TS: 2,4-DNT, 2,6-DNT, both at highest purity available
Deg. products : 2-hydroxy-5-nitro-6-oxohepta-2,4-dienoic acid
2-hydroxy-5-nitropenta-2,4-dienoic acid
nitrite
205375-84-4 3-methyl-4-nitrocatechol

Method : Initial steps of pathway of 2,6-DNT degradation were determined by simultaneous induction experiments, enzyme assays, and identification of metabolites by MS and NMR

Result : Burkholderia cepacia (R34; PR7; JS872), Alcaligenes denitrificans and Alcaligenes xylosoxidans isolated from DNT contaminated soil and surface water were able to degrade 2,4-DNT. The strains R34 and PR7 completely removed 2,4-DNT within 1-2 days. In the presence of 2,6-DNT (0.2 mM) up to 60 % degradation of 2,4-DNT (1 mM) was observed after 3 days.
2,6-DNT-degrading strains (B. cepacia strain JS850, Hydrogenophaga palleronii) isolated from soil and activated sludge were not able to grow with 2,4-DNT as the sole organic substrate.
The pathway of 2,6-DNT degradation: 2,6-DNT was dioxygenated to 3-methyl-4-nitrocatechol accompanied by nitrite release. 3-Methyl-4-nitrocatechol was the substrate for extradiol ring cleavage to yield 2-hydroxy-5-nitro-6-oxohepta-2,4-dienoic acid, which was metabolized to 2-hydroxy-5-nitropenta-2,4-dienoic acid.

Test condition : Enrichment cultures were isolated from contaminated soil, groundwater, and activated sludge. 1 ml of water or activated sludge or 1 g of soil was inoculated into 100 ml of nitrogen-free minmal medium (BLK) containing 2,4-DNT (18 mg/l) or 2,6-DNT (9 mg/l) as sole source of nitrogen and organic carbon. Cultures were shaken at 30 °C. DNT was monitored by HPLC, and when decreasing, the cultures were transfered to fresh medium. After 2-14 months (several transfers) culture samples were

spread on 1/4 strength tryptic soy agar or on DNT plates (0.5 g/l DNT in agar). Thes samples were incubated for 1-6 weeks. Freshly grown isolates were incubated in microtiter plates containing BLK with either 2,4-DNT (18 mg/l), 2,6-DNT (9 mg/l) or a mixture of 2,4-DNT (18 mg/l) and 2,6-DNT (9 mg/l) for 3-5 d at 30 °C.

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles

22.01.2004 (74)

Type : aerobic
Inoculum : other bacteria: mixed culture
Deg. product :
Method : other: Degradation in shake flask
Year : 1981
GLP : no
Test substance : other TS: 2,3-; 2,4-; 2,5-; 2,6-; 3,4-; 3,5-DNT; 98%

Result : Screening test with Waconda Bay organisms:
The mixed culture, capable of using 2,4-DNT as the sole carbon and energy source, mineralized 2,4-DNT but did not mineralize other DNT isomers.
Metabolites:
The compounds 2,4-DNT and 3,4-DNT were biotransformed by high populations (10E09 CFU/ml) of the Searsville Lake pond microorganisms in 2-amino-4-nitrotoluene (2,4-DNT) and 4-amino-3-nitrotoluene (3,4-DNT).
Biotransformation rate constant:
Degradation rates observed with yeast extract added to Waconda Bay organisms (4.6x10E09 cell/ml):
2,3-DNT: 3.1x10E10 cell-1 h-1
2,4-DNT: 0.37x10E10 cell-1 h-1
2,5-DNT: 2.3x10E10 cell-1 h-1
2,6-DNT: 0.28x10E10 cell-1 h-1
3,4-DNT: 0.89x10E10 cell-1 h-1
3,5-DNT: 0.46x10E10 cell-1 h-1
Degradation rates observed with Searsville pond water organisms of 9.6x10E08 cell-1 h-1
2,3-DNT: 5.9x10E10 cell-1 h-1
2,4-DNT: 3.4x10E10 cell-1 h-1
2,5-DNT: 12.0x10E10 cell-1 h-1
2,6-DNT: 0.083x10E10 cell-1 h-1
3,4-DNT: 6.1x10E10 cell-1 h-1
3,5-DNT: 5.1x10E10 cell-1 h-1

Test condition : Screening test:
An enriched mixed culture from Waconda Bay was inoculated into shaker flasks containing 100 ppm of 2,4-DNT and a basal salts medium (BSM). The flask was incubated at 25°C in the dark for 3 days. The microorganisms were inoculated, each containing 30 ppm of one DNT isomer in BSM. The degradation of DNT isomer was determined by broth turbidity and by UV scanning (190-400 nm) of a hexane extract of the broth.

Biotransformation rate study with high cell population:
A mixed culture of Waconda Bay water organisms plus yeast extract in BSM and a mixed culture of Searsville Lake pond water organisms were grown for 16 to 19 hours and harvested near the end of the growth phase. Then the cells were centrifuged, washed with BSM, centrifuged again and resuspended. The organisms were diluted with BSM and transferred to flasks and DNT isomeres were added to achieve the final concentration of 10 ppm. The flasks were gently shaken in the dark. Aliquots were

withdrawn periodically for microbial plate counts and chemical analyses. Bacterial counts were made by serial dilution of the water sample followed by the growing of the organisms on Difco Plate Count Agar. After 3 days of incubation, the organisms were counted.
For the metabolite study, media, exposed to high cell populations, were extracted and analysed by HPLC and GC-MS.

Reliability : (2) valid with restrictions
Study well documented

20.11.2003 (38)

Type : aerobic
Inoculum : Escherichia sp. (Bacteria)
Contact time :
Degradation : (±) % after
Result : other: Dioxygenase from E. coli oxidizes 2,4-DNT and 2,6-DNT to catechols
Deg. product : yes
Method : other: Molecular characterization
Year : 2002
GLP : no data
Test substance : other TS: several nitroaromatics including 2,4-DNT and 2,6-DNT
Deg. products : 205375-84-4 3-methyl-4-nitrocatechol
68906-21-8 4-methyl-5-nitrocatechol

Result : 2,4-DNT and 2,6-DNT are oxidized to 4-methyl-5-nitrocatechol or 3-methyl-4-nitrocatechol (CAS 68906-21-8 or 205375-84-4, respectively) by nitrobenzene dioxygenase (NBDO) or 2-nitrotoluene dioxygenase (2NTDO) from E. coli DH5alpha(pDTG927).
Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles

19.08.2003 (75)

Type : aerobic
Inoculum : other: bioslurry from 2 contaminated sites, augmented with DNT-mineralizing bacteria
Concentration : 3300 mg/l related to Test substance
262 mg/l related to Test substance
Contact time :
Degradation : 100 (±) % after 2 day(s)
Result : other: 2,4-DNT and 2,6-DNT were mineralized
Deg. product : yes
Method : other: Sequential reactor degradation experiments
Year : 2001
GLP : no
Test substance : other TS: Mixture of nitroaromatics including 2,4-DNT and 2,6-DNT
Deg. products : nitrite

Result : Both DNT isomers were nearly completely degraded when two reactors were operating in series, the first one degrading 2,4-DNT, the second one 2,6-DNT.
Nitrite was released in near stoichiometric (>80 %) amounts. Per mole of DNT, about 5.6-6.8 moles of oxygen (O2) were taken up by the inoculum.
In general, degradation was completed within 2 d.
Test condition : 75 l Eimco Biolift slurry reactor (Model B75LA, Tekno Associates, Salt Lake City, UT); maintained at 30 °C and pH 6.75-7.25; monitoring of DNT concentrations throughout feeding cycles by HPLC analysis; nitrite analysis by modified colorimetric method on an EL340 Automated Microplate Reader.

Concentration of TS:
VAAP soil

ss (g/l)	40	160	250
2,4-DNT (µM)	2990	11960	17940
2,6-DNT (µM)	240	960	1440

BAAP soil

ss (g/l)	7	13	26	39	52
2,4-DNT (µM)	2450	4900	9800	14700	19600
2,6-DNT (µM)	130	260	520	780	1040

ss: Suspended solid
VAAP: Volunteer Army Ammunition Plant (Chattanooga, TN)
BAAP: Badger Army Ammunition Plant (Baraboo, WI)

Reliability : (2) valid with restrictions
Study well documented; meets generally accepted scientific principles
20.11.2003 (76)

Type : aerobic
Inoculum : other: soil from TNT production sites
Contact time :
Degradation : 100 (±) % after 7 month
Result : other: Conditions of 2,4-DNT and 2,6-DNT mineralization elucidated
Deg. product :
Method : other: Shake flask and soil column studies
Year : 2000
GLP : no
Test substance : other TS: 2,4-DNT and 2,6-DNT

Result : Indigenous bacteria from contaminated site readily acclimated (2 weeks) to degrade 2,4-DNT and 2,6-DNT. The pH optimum was pH 6.8 - 7.5. At 20-50 mM nitrite DNT degradation became inhibited. The addition of phosphate buffer to bring up moisture in soil to field capacity stimulated the degradation considerably. The ratio of 2,4-DNT to 2,6-DNT affected the degradation rates.
Test condition : - Indigenous bacterial consortia in soil from contaminated site (Badger Army Ammunition Plant)
- 10 g DNT/kg soil
- 2,4-DNT, 2,6-DNT ca. 25 :1
Reliability : (4) not assignable
Abstract
15.01.2004 (77)

Type : aerobic
Inoculum : other: soil from TNT production sites and several isolated bacterial strains
Contact time :
Degradation : 82 - 100 (±) % after 6 month
Result : other: 2,3-DNT, 2,4-DNT and 2,6-DNT mineralized by indigenous soil bacteria, 2,4-DNT and 2,6-DNT by isolated bacterial strains
Deg. product : yes
Method : other: Shake flask and soil column studies
Year : 2001
GLP : no
Test substance : other TS: 2,4-DNT, 2,6-DNT
Deg. products : nitrite

Result : Indigenous bacteria from contaminated sites readily acclimated to degrade 2,4-DNT, but 2,6-DNT degradation was slow and did not occur until 2,4-DNT was removed. The onset of degradation was 6-8 weeks at 13 °C, the in situ soil temperature. The lag period was shortened to 1-2 weeks when

incubations were conducted at 20 °C. There was a broad pH optimum around pH 8, and degradation stopped below pH 7. The addition of 10 mM phosphate stimulated the degradation considerably. In column studies conducted with contaminated soil from Badger Army Ammunition Plant, initial concentrations of 14.03 g/kg and 0.55 g/kg of 2,4- and 2,6-DNT were reduced to 2-14 mg/kg and 4-12 mg/kg in inoculated columns at room temperature, and to 5-9 mg/kg and 11-98 mg/kg in inoculated columns at 13 °C.
2,4-DNT and 2,6-DNT were used as the sole source of bacterial carbon, nitrogen, and energy. Nitrite was released nearly stochiometrically (=> 80 %)

Test condition : Soil:
Soil from two contaminated sites (Badger Army Ammunition Plant [BAAP] Propellant Burning Ground [PBG] and Deterent Burning Ground [DBG]) was composited, dried at room temperature, sieved (20 mesh), and stored at 4 °C.
Groundwater: Clean groundwater from BAAP was stored at 4 °C in the shipping drum until use.
Column studies:
Dried soil (75 g) was placed in autoclaved glass columns (2.5 cm x 30 cm) on top of a 3 cm layer of washed, autoclaved sand, then topped with additional sand (total approximately 70 g). Groundwater (pH 7.7) was recirculated upwards through the columns at approximately 5 ml/min. Filtered air was pumped through the reservoir to provide oxygen. Samples were withdrawn for analysis of pH, DNT, nitrite (Smibert and Krieg, 1994), and nitrate (Parsons et al., 1984).
DNT-degrading bacteria:
A mixed culture inoculated with Burkholderia sp. DNT, Burkholderia cepacia JS872, B. cepacia JS922, Hydrogenophaga palleronii JS863, and B. cepacia JS850 was grown in 250 ml shake flasks containing nitrogen-free nutrient medium. 2,4-DNT (1 mM) and 2,6-DNT (0.25 mM) were provided as needed. Incubation with shaking at 30 °C.
A DNT-degrading culture (IB) enriched from shake flasks inoculated with soil from the PBG was maintained under identical conditions. Both cultures were transferred periodically and cells were harvested as needed for inoculation of shake flask and column studies. A portion of the IB culture was subcultured at room temperature. When growth on the added DNT was complete, the bacteria were harvested by centrifugation and the entire pellet was used to inoculate a new culture which was incubated at a temperature 2 degrees lower than the previous temperature. Five similar transfers were made over a 3-week period to obtain a DNT-degrading culture adapted to growth at 13 °C (LTIB).

Reliability : (2) valid with restrictions
Basic data given

15.01.2004 (47)

Type : aerobic
Inoculum : other: genetically modified E.coli strain JM109
Contact time :
Degradation : (±) % after
Result : other: 2,4-DNT was degraded by modified E.coli
Deg. product : yes
Method : other: shake flask degradation experiments
Year : 2000
GLP : no data
Test substance : other TS: 2,4-DNT
Deg. products : 4-Methyl-5-nitrocatechol

Method : Bacteria: Escherischia coli strain JM109; plasmids: pJS39 bearing the DNT dioxygenase genes (dntAa-dntAd) from Burkholdria sp. strain DNT and

pHG1 containing the Vitreoscilla hemoglobin gene (vgb). Cells were transformed with pHG1 (strain PFHG1), pJS39 (strain PFJS39), or both (strain PF6). Strains were maintained on Luria-Bertaini plates. PFHG1 and PF6 were incubated in LB medium at 150 rpm and 37 °C. PFJS39 and PF6 were grown in DNT minimal medium which contained 110 µM (20 ppm) 2,4-DNT. Normal aeration was 150 rpm, 37 °C, 200 ml medium in 1000 ml Erlenmeyer flask and restricted aeration was 50 rpm, 37 °C, 200 ml medium in 250 Erlenmeyer flask.

Result : PF6 outgrew PFJS39 in LB medium and at restricted aeration in minimal medium containing 110 µM (20 ppm) 2,4-DNT. When grown in minimal medium containing 110 µmM 2,4-DNT with normal aeration, PF6 and PFJS39 converted 2,4-DNT to methyl nitrocatechol at almost the same rate, while with restricted aeration the rate for PF6 was twice that of PFJS39.

Vmax(µmol/h/mg protein)	PFJS39	PF6
whole cells/normal aeration	0.94	4.31
lysed cells/normal aeration	1.26	3.77
whole cells/restricted aeration	1.08	3.17
lysed cells/restricted aeration	1.12	3.48

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles

20.11.2003 (78)

Type : anaerobic
Inoculum : other: Clostridium acetobutylicum
Contact time :
Degradation : 75 - 100 (±) % after 30 hour(s)
Result : other: Reductive biotransformation of both isomers within a few hours
Deg. product : yes
Method : other: 14C batch degradation experiments
Year : 1999
GLP : no data
Test substance : other TS: 96% 14C-2,4-DNT and 97% 14C-2,6-DNT
Deg. products : 2,6-dihydroxylaminotoluene
2-hydroxylamino-6-nitrotoluene
119-32-4 204-314-0 3-nitro-p-toluidine
99-55-8 202-765-8 5-nitro-o-toluidine

Result : Nearly all of the 2,4-DNT was transformed in 2 h, forming 2-amino-4-nitrotoluene and 4-amino-2-nitrotoluene at a combined yield of 74 %. A longer reaction time was required (3.5 h) for a similar extent of 2,6-DNT transformation, which resulted in 2-amino-6-nitrotoluene with a yield of 75 %. In the case of 2,4-DNT, a transformation pathway from 2,4-DNT to the final product 2,4-diaminotoluene, through various hydroxylamino intermediates (i.e.4-hydroxyamino-2-nitrotoluene and 2-hydroxylamino-4-nitrotoluene resulted in the formation of 2,4-dihydroxylaminotoluene) was observed. The transformation of 2,6-DNT was noted to proceed with the transient formation of 2-hydroxylamino-6-nitrotoluene and the subsequent formation of 2,6-dihydroxylaminotoluene. 2-hydroxylamino-6-nitrotoluene was tentatively identified as a precursor to the end-product of 2,6-diaminotoluene after up to 30 h.

Test condition : Cell cultures (50 ml) were spiked during the log-growth phase with either 5 mg TS dissolved in 0.5 ml of methanol containing 7.5 E+5 dpm of [U-ring-14C]-2,4-DNT or 5 mg TS dissolved in 0.5 ml of methanol containing 1.2 E+6 dpm of [U-ring-14C]-2,6-DNT. Subsequent experiments were carried out to isolate other metabolites of DNT transformation using longer incubatins and a higher cell extract concentration.

Reliability : (2) valid with restrictions

study well documented

15.01.2004 (79)

Type : anaerobic
Inoculum : other: soil from contaminated munition site
Contact time :
Degradation : 100 (±) % after 6 month
Result : other: in methanogenic soil environment 2,4-DNT is degraded completely
Deg. product : yes
Method : other: Anaerobic bioventing in soil columns
Year : 2000
GLP : no
Test substance : other TS: 2,4-DNT, purity 97 %
Deg. products : 74-82-8 200-812-7 methane

Method : Degradation of nitroaromatics found in munition waste streams is accelerated under anaerobic conditions followed by aerobic treatment of the degradation products. The establishment of anaerobic environment in a vadose zone was accomplished by feeding an appropriate anaerobic gas mixture ("anaerobic bioventing")
Result : Under methanogenic conditions DNT completely disappeared after six months of operation and no intermediates (e.g. 2-amino-4nitrotoluene, 4-amino-2-nitrotoluene, 2,4-diaminotoluene) could be detected.
Test condition : Pexiglas columns (2 replicates), 7 cm diameter, 15 cm long, filled with 1 kg of soil to simulate anaerobic zone operated as anaerobic up-flow continuous reactor by passing through a gas mixture containing 1% hydrogen, 1% carbon dioxide, 5 % helium, and 93 % nitrogen
- Soil: 83 % spiked sand, 5 % sea shells (pH buffer), 2% garden top soil, 10 % water
- 2,4-DNT: 77 mg/kg soil at start of incubation
- Analysis: Soxhlet extraction of soil with methanol and methylene chloride. HPLC with accubond C18 column with isocratic mobile phase 50 % water, 50 % acetonitrile (or 100 % acetonitrile for diaminotoluene)
Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles

15.01.2004 (80)

Type : anaerobic
Inoculum : other: aquatic microcosms
Contact time :
Degradation : (±) % after
Result : other: Both 2,4-DNT and 2,6-DNT were degraded
Deg. product :
Method : other: Batch degradation experiments in aquatic microcosms
Year : 1999
GLP : no data
Test substance : other TS:2,4-DNT and 2,6-DNT

Result : 2,4,6-Trinitrotoluene was the most easily degraded of several munition compounds, followed by 2,4-DNT, 3-nitrotoluene, and 2,6-DNT. Aerobic conditions and amendment with nutrients (e.g. phosphate) increased the degradation rate. A non-amended degradation rate is given for 2,4-DNT, but no dimension is reported.
Reliability : (4) not assignable
Documentation insufficient for assessment

19.08.2003 (54)

Type :
Inoculum : Alcaligenes sp. (Bacteria)

Concentration : 30 mg/l related to Test substance
related to
Contact time :
Degradation : (±) % after
Result : other: Nitrite is released from 2,4-DNT under aerobic and anaerobic conditions
Deg. product : yes
Method : other: Batch removal experiments under aerobic and anaerobic conditions
Year : 2001
GLP : no
Test substance : other TS: 2,4-DNT
Deg. products : nitrite

Method : Dependence of transformation of 2,4-DNT by Alcaligenes JS867 on electron acceptor availability was examined.
Result : Complete 2,4-DNT removal was observed under oxygen excess with near stoichiometric release (83 %) of nitrite. At decreasing oxygen availability, rates and extent of 2,4-DNT transformation and nitrite production decreased.
Depending on growth conditions, JS867 was able to use nitrite as a terminal electron acceptor. The nearly constant molar ratios of DNT removed over nitrite released under various degrees of oxygen limitation suggested that oxygenolytic denitration pathways continued. No evidence of nitroreduction obtained.
Test condition : Bacterial inoculum:
Alcaligenes Strain JS867 was obtained from Shirley Nishino at AFRL/MLQR (Tyndall AFB, FL) and was closely related to Alcaligenes xylosoxidans (via 16S rDNA ribotyping) and Alcaligenes xylosoxidans subsp. denitrificans. Cultivation was at 30 °C on solid or in liquid defined media free of inorganic N and organic C supplemented with 2,4-DNT (3 mM) as sole carbon and nitrogen source. Maintenance media contained sorptive XAD-7 beads (10 g/l; Sigma, St. Louis, MO) to maintain a sub-toxic, longterm aqueous supply of DNT.
HPLC analysis:
C6-hexyl column (Spherisorb, Alltech, Deerfield, IL) and UV-detection at 254 nm. Eluent was H2O: methanol (50 : 50) delivered at 1 ml/min. 10 µl sample volumes were injected; retention were recorded for 2,4-DNT (10.3 min), 2-amino-4-nitrotoluene (7.3 min) and 2-nitro-4-aminotoluene (6.4 min). The method's linear dynamic range was from 0.001 to 0.3 mM 2,4-DNT.
Nitrite analysis:
by a modification of the sulfanilamid standard method (Gerhardt et al. 1994).
Batch 2,4-DNT removal experiments:
Serum bottles (125 ml) containing 75 ml of BLKN with 2,4-DNT (approximately 30 mg/l) were inoculated with a known cell mass of JS867 pregrown aerobically in a medium free of inorganic N and organic C containing 3 mM 2,4-DNT. Bottles were closed with rubber septa and aluminum crimp seals and incubated on a shaker table (150 rpm) at 30 °C. To attain oxygen limited conditions, sealed bottles were purged with a stream of N2 gas before injecting a microfiltered quantity (5, 2, or 1 ml) of air directly into the liquid phase. Actual oxygen concentrations were measured in replicate bottles, via GC/TCD (HP 5890 Series II).
Aqueous samples (1 ml) for HPLC analysis were immediately mixed with MeOH and centrifuged for 5 min at 14.000 rpm (Eppendorf, 5415C).
Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles

19.08.2003 (81)

Deg. product :

Method	:	
Year	:	2001
GLP	:	
Test substance	:	
Result	:	Short review on DNT degradation reports of the participants of the "Federal Integrated Biotreatment Research Consortium (Flask to Field)"
Reliability	:	(4) not assignable Secondary literature

18.08.2003 (82)

3.6 BOD5, COD OR BOD5/COD RATIO

13.08.2003

3.7 BIOACCUMULATION

Species	:	Cyprinus carpio (Fish, fresh water)
Exposure period	:	56 day(s) at 25 °C
Concentration	:	.025 mg/l
BCF	:	3.2 - 21.2
Elimination	:	
Method	:	other: OECD TG 305C "Bioaccumulation: Test for the Degree of Bioconcentration in Fish"
Year	:	1992
GLP	:	no data
Test substance	:	other TS: 2,4-DNT/2,6-DNT (technical mixture)
Remark	:	The test was conducted in accordance with "Bioaccumulation test of chemical substance in fish and shellfish" stipulated in the Order Prescribing the Items of the Test Relating to the New Chemical Substance (1974, Order of the Prime Minister, the Minister of Health and Welfare, the Minister of International Trade and Industry No. 1). This guideline corresponds to "305C, Bioaccumulation: Degree of Bioconcentration in Fish" stipulated in the OECD Guidelines for Testing of Chemicals (1981)
Result	:	With a concentration of 0.25 mg/l, a BCF of 0.6 - 2.9 was obtained.
Test condition	:	- Fish were supplied by Sugishama fish farm - After external desinfection under static conditions with 50 mg/l Terramycin and 7 g/l sodium chloride, the fish were reared in a flow through system for about 28 d - Fish were reared in an acclimatization tank (flow through system) for another 28 d at 25 +/- 2 °C - Fish feeding with pelleted food (Japan Haigo Shiryo K.K.), about 1 % of body weight twice per day - Fish at start of incubation: ca. 30 g, ca. 10 cm, lipid content 6.8 % - Water was groundwater from the Kurume Research Laboratories - Water temperature, pH, dissolved oxygen were continuously measured - Total hardness, COD, chloride, and other parameters were measured every 6 months - Incubation of each 15-20 fish per level in glass tank containing 100 l of liquid each - 6-8 mg/l dissolved oxygen - Incubation temperature 25 +/- 2 °C - 48 h LC50 was estimated by Doudoroff method or Probit method

Reliability : (1) valid without restriction
Test procedure according to national standards, comparable with guideline
Flag : Critical study for SIDS endpoint
18.08.2003 (16)

Species : Lepomis macrochirus (Fish, fresh water)
Exposure period : 4 day(s) at °C
Concentration : mg/l
BCF : 5 - 84
Elimination : yes
Method : other
Year : 1983
GLP : no
Test substance : other TS: 14C labled 2,4-DNT

Method : - The fish were incubated in aqueous solution containing 14C labled 2,4-DNT or in synthetic wastewater containing several nitroaromatics and 14C labled 2,4-DNT
- The nominal 2,4-DNT concentration was 1 mg/l in the experiments with 2,4-DNT alone, and 1.3 mg/l in the synthetic waste water. The total nitroaromatics concentration was 3 mg/l in the synthetic wastewater.
- After 2 and 4 days of uptake and after 3 and 10 days of clearance the test organisms were collected, rinsed in clean water and radioanalyzed
Remark : Kinetics of uptake indicate that steady state might have not been reached after 4 d. However, clearance was rapid indicating that equilibrium may have been reached
Result : BCF in the viscera
- 2,4-DNT alone: uptake 2 d 53, 4 d 78; clearance 3 d 1, 10 d 0
- 2,4-DNT in synthetic wastewater: uptake 2 d 47, 4 d 84; clearance 3 d 0, 10 d 2
BCF in the muscle
- 2,4-DNT alone: uptake 2 d 4, 4 d 4; clearance 3 d 0, 10 d 2
- 2,4-DNT in synthetic wastewater: uptake 2 d 5, 4 d 5; clearance 3 d 0, 10 d 0
In the experiments with synthetic wastewater containing several nitroaromatics (e.g. 2,4,6-trinitrotoluene), the BCF might have been influenced by other compounds
Test substance : No information supplied on the purity of the 14C labled 2,4-DNT
Reliability : (2) valid with restrictions
Basic data given
Flag : Critical study for SIDS endpoint
18.08.2003 (31)

Species : Poecilia reticulata (Fish, fresh water)
Exposure period : 3 day(s) at °C
Concentration :
BCF : 204 - 274
Elimination :
Method : other: see TC
Year : 1987
GLP : no data
Test substance : other TS: 2,4-DNT and 2,6-DNT, both purity 98 %

Remark : concentration of test substance: 1/5 of LC50
Test concentration was too high: 20 % of LC50 instead of 1 % as prescribed in OECD TG 305
Result : The following results were obtained:

	log BCF	BCF
2,4-DNT	2.31 +/- 0.03	204 +/- 1

2,6-DNT 2.44 +/- 0.04 274 +/- 1

BCF on the basis of fat weight, given as value +/- standard deviation

Test condition : - Organisms: female guppies, 5-8 month of age, wet weight varied from 60-450 mg at end of experiment, mean fat content was 8 +/- 2 %, feeding with commercial fish food daily, fish reared in the laboratory of the working group and acclimatized for at least 12 days prior to experiment.
- Test water: standard water (SW) prepared according to Alabaster and Abraham (1964) which corresponds to very soft tape water (hardness: 25 mg/l as CaCO3).
- Bioconcentration factor: corresponding amount to LC50 in 1 l SW as stock solution, diluted 1:5 in SW immediately before use, yielding a concentration of 1/5 LC50 for the test solution, new solutions were prepared from stock solution every day, exposure of fish for 3 days, after having established in preliminary experiments that the uptake of the chemicals proceeded very fast.
-Concentration of TS: 2.5 mg/l for 2,4-DNT or 3.6 mg/l for 2,6-DNT, respectively, corresponding to 1/5 LC50.
- Analyses: analyses were carried out using a Tracor 550 GC equipped or a Pye Unicam 304 GC both with an electron capture detector, and a Shimadzu CRIA integrator.

Reliability : (3) invalid
Significant methodological deficiences

18.08.2003 (30)

Species : other: Daphnia magna
Exposure period : 4 day(s) at °C
Concentration : mg/l
BCF : 13 - 14
Elimination : no data
Method : other: see Method
Year : 1983
GLP : no
Test substance : other TS: 14C labled 2,4-DNT

Method : - The test organisms were feed 14C labled 2,4-DNT in aqueous solution or in synthetic wastewater in the presence of several nitroaromatics.
- The nominal 2,4-DNT concentration was 1 mg/l in the experiments with 2,4-DNT alone, and 1.3 mg/l in the synthetic waste water. The total nitroaromatics concentration was 3 mg/l in the synthetic wastewater.
- After up to 4 days the test organisms were collected, rinsed in clean water and radioanalyzed

Remark : No steady state reported
Result : BCF of
2,4-DNT alone: 13
2,4-DNT in synthetic wastewater: 14
In the experiments with synthetic wastewater containing several nitroaromatics (e.g. 2,4,6-trinitrotoluene), the BCF might have been influenced by other compounds
Test substance : No information supplied on the purity of the 14C labled 2,4-DNT
Reliability : (4) not assignable
Documentation insufficient for assessment

18.08.2003 (31)

Species : other: Lumbriculus variegatus
Exposure period : 4 day(s) at °C
Concentration : mg/l
BCF : 58 - 63
Elimination : no data
Method : other: see Method

Year : 1983
GLP : no
Test substance : other TS: 14C labled 2,4-DNT

Method : - The test organisms were feed 14C labled 2,4-DNT in aqueous solution or in synthetic wastewater in the presence of several nitroaromatics
- The nominal 2,4-DNT concentration was 1 mg/l in the experiments with 2,4-DNT alone, and 1.3 mg/l in the synthetic waste water. The total nitroaromatics concentration was 3 mg/l in the synthetic wastewater
- After up to 4 days the test organisms were collected, rinsed in clean water and radioanalyzed
Remark : No steady state reported
Result : BCF of
2,4-DNT alone: 58
2,4-DNT in synthetic wastewater: 63
In the experiments with synthetic wastewater containing several nitroaromatics (e.g. 2,4,6-trinitrotoluene), the BCF might have been influenced by other compounds
Test substance : No information supplied on the purity of the 14C labled 2,4-DNT
Reliability : (4) not assignable
Documentation insufficient for assessment

18.08.2003 (31)

Species : other: Selenastrum capricornutum
Exposure period : 4 day(s) at °C
Concentration : mg/l
BCF : 2149 - 2507
Elimination : no data
Method : other: see Method
Year : 1983
GLP : no
Test substance : other TS: 14C labled 2,4-DNT

Method : - The test organisms were feed 14C labled 2,4-DNT in aqueous solution or in synthetic wastewater in the presence of several nitroaromatics
- The nominal 2,4-DNT concentration was 1 mg/l in the experiments with 2,4-DNT alone, and 1.3 mg/l in the synthetic waste water. The total nitroaromatics concentration was 3 mg/l in the synthetic wastewater
- After up to 4 days the test organisms were collected, rinsed in clean water and radioanalyzed
Remark : No steady state reported. Information on bacterial contaminations of the algae should have been supplied since bacteria might degrade DNT to 14CO2 which is assimilated by the algae during photosynthesis
Result : BCF of
2,4-DNT alone: 2507
2,4-DNT in synthetic wastewater: 2149
The high BCF is postulated by the authors to be due to sorption to the outer cell walls (the algae have a higher specific surface than all other test species used in the study).
Within the incubation period some degradatiion of 2,4-DNT might have occured. Since BCF was determined by using radioactive 2,4-DNT it is assumed that the algae had accumulated 14C radioactivity (e.g. in the form of 14C labled CO2) and not 2,4-DNT. This explanation is consitant with the low BCF observed in non-photosynthetically active species.
In the experiments with synthetic wastewater containing several nitroaromatics (e.g. 2,4,6-trinitrotoluene), the BCF might have been influenced by other compounds
Test substance : No information supplied on the purity of the 14C labled 2,4-DNT
Reliability : (3) invalid

Significant methodological deficiences
18.08.2003 (31)

Species : other: fish (species not indicted)
Exposure period : at °C
Concentration :
BCF : 10
Elimination :
Method : other: no data
Year : 1991
GLP : no data
Test substance : other TS: 2,4-DNT and 2,6-DNT

Remark : No information on method
Result : For 2,4-DNT three results are compiled: 10.6, 11.6, and 3.8
For 2,6-DNT one result is compiled: 9.82
Reliability : (2) valid with restrictions
Data from handbook or collection of data
06.11.2003 (21)

BCF : 31.83
Elimination :
Method : other: calculated
Year : 1983
GLP :
Test substance :

Method : BCF values were calculated from the estimated log Kow (octanol water partition coefficient) values according to the method of Leo, Hansch, and Elkins (1971) [Leo A, Hansch C, and Elkins D (1971). Partition coefficients and their uses. Chem. Rev. 71, 525-616)]
Result : BCF is 31.83 for all six DNT isomers
Reliability : (2) valid with restrictions
Acceptable calculation method
Flag : Critical study for SIDS endpoint
12.08.2003 (31)

3.8 ADDITIONAL REMARKS

Memo : Adsorption on activated carbon

Result : Adsorption characteristics DNT and other nitroaromatics on granular activated C (GAC) were studied to understand the dynamic adsorption behavior for dilute aqueous solutions. A model was developed to predict adsorption dynamics and the effect of design and operating parameters on adsorption characteristics. Breakthrough characteristics obtained for GAC with different surface area (650-1500 m2/g), hydraulic loading rates (HLR) of 12-24 m3/h-m2, feed concentrations of 50-130 mg/l, and bed heights of 300-1000 mm were examined. The effect of independent parameters on breakthrough time, adsorption capacity, and min. concentration achieved in the effluent was studied. Results indicated adsorption capacity goes though a maximum when studied as a function of HLR and feed concentration. Adsorption capacity/unit surface area also exhibited a maximum of approximately 1000 m2/g.
Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles
22.08.2003 (83)

Memo : DNT in plants

Method : Hydroponic experiments: Phaseolus vulgaris was germinated and grown in commercial nutrient solution (Substral). Three weeks old plants were incubated with the roots in 300 ml nutrient solution containing 10 mg/l TNT (purity 99 %). After 15 min, 3,5, and 7 d, plants were removed from the culture medium and the roots were rinsed briefly in methanol/water (80/20 v/v). The roots were carefully wiped dry.
Outdoor experiments: 2 vegetable bed of approx. 5-10 m2 per plot were laid in 8 different plots of land in an area known to be contaminated with TNT. The 2 beds were used for for cultivation of different plant species. The soil was not homogenized prior to plant cultivation
Plant material was homogenized with a kitchen mixer, extracted with 50 ml of 1 M HCl per 2 g of plant material, hydrolysed for 1 h at 90 °C and extracted with 30 ml toluene, followed by addition of 15 ml 4 M NH3 and 40 ml methanol.

Remark : For several reasons,it cannot be decided whether TNT was degraded to 2,6-DNT in plants:
- The hydroponic experiments were not performed under sterile conditions and it was not examined to what extend bacteria and fungus present in the nutrient solution degraded 2,6-DNT.
- The TNT (10 mg/l) used in the hydroponic experiments was of 99 % purity. Neither the contents of DNT isomers nor the root mass were reported, thus no mass balance can be calculated to demonstrate the formation of DNT.
- There is no report on how the purity of TNT was checked, it is assumed that the purity might be far less and the content in 2,6-DNT far higher than 1 %.
- There is no explanation on why there are significant levels of 2,6-DNT (higher than TNT) occured in root material even after 15 min of incubation. Except from the washing with methanol and drying, no precursions were undertaken to avoid contamination with dissolved or adsorbed test substance and its contaminations.
- There is a significant scattering in the hydroponic data (µg/g fresh weight, e.g. TNT: 3 d 0.1, 5 d 1.32, 7 d 0.09; 4-Amino-2,6-DNT: 3 d 4.13, 5 d 28, 7 d 6.28) and in the outdoor data.
- No repetion of incubations
- In the outdoor experiments the soils of the plots were not homogenized and it is not clear whether the samples taken and analyzed were represantative for the corresponding plots
- Although it is stated that 16 outdoor experiments were performed, ca. 24 results were reported
- When reporting data significance rules were not observed

Result : The following concentrations of DNT (µg/g fresh weight) were recovered in the roots of bush beans after growing the plants in 10 mg/l TNT (purity 99 %) (hydroponic experiments)

Incubation	TNT	2,4-DNT	2,6-DNT
15 min	0.15	n.d.	0.12
3 d	0.1	0.13	0.77
5 d	1.32	0.09	1.42
7 d	0.09	0.17	3.86

After the outdooor experiments traces of DNT were found in plants which were grown in soil plots were no DNT was found in soil samples.

Reliability : (4) not assignable
Documentation insufficient for assessment

10.11.2003 (84)

Memo : Fate of TNT by Klebsiella sp. strain C1

Result : Before adaptation, the bacteria could completely remove within 6 h, 100 and 200 mg/l of TNT.
First major metabolite after 20 min (after the adaptation process) was Hydroxylamino-dinitrotoluene, after 6 h this was removed and monoamino derivates appeared. DNTs were also formed but in lower concentrations (< 10 mg/l, data taken from the graphic).

Test condition : - Inocolum was obtained from activated sludge from a municipal treatment plant. The culture was incubated for 6 months at room temperature, with increase of TNT (10 to 300 mg/l).Afterwards pure cultures were isolated and the bacteria with the fastest removal rate was chosen (Klebsiella sp.strain C1).
- The isolated strain (20 ml) was put in a growth medium and one day incubated.
- TNT was then added to 100 mg/l.
- The residual TNT and its metabolites were analysed by HPLC .
- The culture was incubated under aerobic conditions and at 30 °C, during 48 hours.
- All experiments were carried out duplicate or triplicate. Mean values were reported.

Test substance : Analytical-grade, purchased from Supelco Co. and AccuStandard Inc.

Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles

12.11.2003 (85)

Memo : Rate-limiting step in nitro-reduction of TNT

Result : The standard one-electron redox potentials at pH 7 for nitroarenes have been measured by pulse radiolysis. The internally consistent values were -0.397 V for 2,4-DNT, and -0.402 V for 2,6-DNT. The reduction kinetics of the nitroarenes was investigated using a bacterial nitroreductase, NAD(P)H:FMN oxidoreductase that uses NADH+H+ as a cosubstrate. A log-linear relationship was observed between the standard one-electron redox potentials and the enzymatic reduction rates for nitroarenes, suggesting that transfer of the first electron is the rate-limiting step in nitroreduction.

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles

19.11.2003 (86)

Memo : Reduction of DNT by elemental Fe

Result : Rapid reduction of DNT by elemental nanoscale Fe yielding diaminotoluenes.

Reliability : (2) valid with restrictions
Basic data given

22.08.2003 (87)

Memo : Uptake of TNT in plants

Method : Field trial:
- 3 different levels of soil contamination were tested: high contamination, low contamination, and uncontaminated soil control.
- The investigation was performed between February 1992 and March 1993, in the site of a former munitions factory in Stadtallendorf (Germany).
- The seeds of 8 different plant species were sown in soil after purging and stirring the soil to obtain a homogeneous soil.
- The soil with the high contamination level was prepared by addition of >100 mg/kg loading of nitroaromatic compounds by sieving of the existing soil.

- 3 soil samples were taken from each test system during the test period of one year and were analyzed with GC/ECD. - Plants were harvested depending on the species and were also with GC/ECD analyzed.
- Plants were poured weekly with tap water.

Remark : It cannot be decided whether TNT was degraded to 2,4- and 2,6-DNT in plants:
- There is a significant scattering in the data of nitroaromatics-content in soil and plants, thus a median value is not representative.
- Except from the washing and drying, no precautions were undertaken to avoid contamination with dissolved or adsorbed nitroaromatics.
- Highly contaminated soil was not homogenously contaminated but contained (undissolved) macroscopic TNT/nitroaromatics particles.

Result : NITROAROMATICS-CONTENT IN SOIL
- In the control soil no contaminants were detected under the detection limit of 0.003 mg/kg.
- The low contaminated soil contained a mean concentration of 3 mg/kg nitroaromatics in soil, about
  *0.03-0.2 mg/kg was 2,4-DNT
  *0.1-0.83 mg/kg was 2,6-DNT
- In the high contaminated soil a mean concentration of 499 mg/kg nitroaromatics was reported, mainly of TNT and very little Aminodinitrotoluenes (ADNT), but no dinitrotoluenes were detected.

NITROAROMATICS IN PLANTS
In plants were observed that the TNT initial concentration declined and the amount of ADNT increased.
GROWTH INHIBITION
Growth rate was also observed. However only plants in the high contaminated soil system presented some inhibition.
UPTAKE OF NITROAROMATICS IN PLANTS
Uptake depends on species, organs (roots = most uptake) and contaminant-content in soil.
In contrast to the soil, ADNT was more abundant in plants than TNT.
In the highly contaminated soil mostly ADNT and less TNT were found.
DNT was also found in roots of Phaseolus vulgaris with 1.7 % of 2,4-DNT and 9.3 % of 2,6-DNT parts of the total nitroaromatics-content. DNT in other species was not detected or found in << 1%.
In the low contaminated soil, nitroaromatics could be determined only in roots of two species: P. vulgaris and Medicago sativa, among others DNT was identified (<< 1 %).

Test condition : Tested species:
Allium schoenoprasum
Daucus carota
Fragaria vesca
Lactuca sativa
Medicago sativa
Petroselinum crispum
Phaseolus vulgaris
Raphanus sativus
Valerianella locusta

Reliability : (3) invalid
Significant methodological deficiences, see remarks

27.11.2003 (88)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	other: static or semistatic
Species	:	Oryzias latipes (Fish, fresh water)
Exposure period	:	48 hour(s)
Unit	:	mg/l
LC50	:	27
Limit test	:	
Analytical monitoring	:	no data
Method	:	other: Japanese Industrial Standard (JIS K 0102-1986-71) "Testing methods for industrial waste water"
Year	:	1992
GLP	:	no data
Test substance	:	other TS: 2,4-/2,6-Dinitrolouene (technical mixture)
Test condition	:	- Orange-red killifish (Oryzias latipes) was obtained from Nakashima fish farm, Daimyojin Nagasu-cho Tamana-gun Kumamot 869-01 Japan - After external desinfection, the fish were reared in a flow through system for 3 - 5 weeks - Fish were reared in an acclimatization tank for 28 d at 25 +/- 2 °C - Water was groundwater from the Kurume Research Laboratories - Water temperature, pH, dissolved oxygen were continuously measured - Total hardness, COD, chloride, and other parameters were measured every 6 months - Incubation of each 10 fish in round glass vessels containing 4 l of liquid each - Incubation temperature 25 +/- 2 °C - 10 fish per concentration level - 48 h LC50 was estimated by Doudoroff method or Probit method
Reliability	:	(1) valid without restriction Test procedure in accordance with national standard methods
Flag	:	Critical study for SIDS endpoint

16.08.2003 (16)

Type	:	static
Species	:	Pimephales promelas (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
Limit test	:	no
Analytical monitoring	:	no
Method	:	other: EPA-660/3-75-009, "Methods for acute toxicity with fish, macroinvertebrates and amphibians", 1975
Year	:	1979
GLP	:	no
Test substance	:	other TS: all DNT isomers, no purity reported
Method	:	US EPA (1975). Methods for acute toxicity tests with Fish, Macroinvertebrates, and Amphibians. Ecological Research Series, EPA-66013-75-009, National Environmental Research Center, Office of Research and Development, Washington, DC
Result	:	The following results 96 h LC50 (mg/l) were obtained: 2,3-DNT 1.9 2,4-DNT 32.5 2,5-DNT 1.3 2,6-DNT 19.8 3,4-DNT 1.5 3,5-DNT 22.0

Test condition : - Pimephales promelas obtained from SRI stock cultures
- Incubation temperature 20 °C,
- Test water: Dechlorinated tap water was used to prepare stock and test solutions of the test substance and to rear and maintain the test animals.
- Quality of water (municipal water, dechlorinated by passage through a series of 6 activated carbon columns: hardness (26 ppm) as CaCO3, alkalinity (45 ppm) as CaCO3, pH 7.2-8.6
- Food: During the static test no food provided
- Fish: 90 d +/- 2 d old at start of incubation
- Probit data analysis

Reliability : (2) valid with restrictions
Test procedure in accordance with national standard methods without detailed documentation

Flag : Critical study for SIDS endpoint

17.01.2004 (45)

Type : semistatic
Species : Poecilia reticulata (Fish, fresh water)
Exposure period : 14 day(s)
Unit : mg/l
LC50 : 12.6 - 17.8
Limit test : no
Analytical monitoring : no data
Method : other: according to Koenemann (1981)
Year : 1987
GLP : no data
Test substance : other TS: 2,4-DNT and 2,6-DNT, both purity 98 %

Result : The following results were obtained:

	log IC50 (µM)	IC50 (mg/l)
2,4-DNT	1.84	12.6
2,6-DNT	1.99	17.8

Test condition : - Organisms: male and female guppies, age: 2 to 3 months at start of test, feeding with commercial fish food daily, fish reared in the laboratory of the working group and acclimatized for at least 12 days prior to experiment.
- Test water: standard water (SW) prepared according to Alabaster and Abraham (1964) which corresponds to very soft tape water (hardness: 25 mg/l as CaCO3).
- LC50 determination: following the procedure outlined by Koenemann (1981), oxygen after 24 h 4.5 mg/l, pH between 6.8 and 7.2, temperature 21-23 °C, calculation of LC50 by logit transformation.
- Analyses: analyses were carried out using a Tracor 550 GC equipped with an electron capture detector, and a Shimadzu CRIA integrator.

Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles

20.08.2003 (30)

Type : static
Species : Pimephales promelas (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
Limit test : no
Analytical monitoring : no
Method : other: Method by US-EPA 1975 (EPA-660/3-75-009)
Year : 1983
GLP : no
Test substance : other TS: all DNT isomers

Method : US EPA (1975). Methods for acute toxicity tests with Fish,

Macroinvertebrates, and Amphibians. Ecological Research Series, EPA-66013-75-009, National Environmental Research Center, Office of Research and Development, Washington, DC

Result : The following 96 h LC50 values were obtained (mg/l)
2,3-DNT 1.8
2,4-DNT 32.8
2,5-DNT 1.3
2,6-DNT 18.5
3,4-DNT 1.5
3,5-DNT 22.6

Test condition : - Test water: Dechlorinated tap water was used to prepare stock and test solutions of the test substance and to rear and maintain the test animals. The water treatment system comprised several 75-µm particle filters and several 0.042-m3 activated carbon columns. The means of hardness 33.8 (19 mg/l as CaCO3), pH (7.7), alkalinity 38 (20 mg/l as CaCO3), conductivity (81 µs/cm), and residual chlorine of water samples (2.2 µg/l) were collected monthly during a significant portion of the study
- Test temperature: 20 °C
- Food: During the static test no food provided
- Stock solution: The stock solution was prepared by dissolving a measured amount of chemical in a known volume of water. No carrier was used. The mixing time was about 24 h. The stock solution was filtered through a 5-µm filter and analyzed for the chemical
- Fish: 90 d +/- 2 d old at start of incubation

Test substance : The DNT isomers were obtained from
2,3-DNT SRI International
2,4-DNT ICN
2,5-DNT SRI International
2,6-DNT Aldrich
3,4-DNT Aldrich
3,5-DNT SRI International

Reliability : (2) valid with restrictions
Test procedure according to national standards. Basic data given

22.01.2004 (89) (31)

Type : other: static and flow-through
Species : other: species tested see below
Exposure period : 96 hour(s)
Unit :
Limit test : no
Analytical monitoring : yes
Method : other: Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (EPA, 1987)
Year : 1983
GLP : no
Test substance : other TS: 2,4-DNT, >95 % purity

Result : LC50 (96 h) from duplicate static tests for 2,4-Dinitrotoluene:
Pimephales promelas (Fathead minnow) 28.5 mg/l
Lepomis macrochirus (Bluegill) 13.5 mg/l
Ictalurus punctatus (Channel catfish) 24.8 mg/l
Oncorhynchus myskis (Rainbow trout) 13.6 mg/l

LC50 (96 h) from duplicate flow-through tests for 2,4-Dinitrotoluene:
Pimephales promelas (Fathead minnow) 36.1 mg/l
Lepomis macrochirus (Bluegill) 16 mg/l
Ictalurus punctatus (Channel catfish) 32 mg/l
Oncorhynchus myskis (Rainbow trout) 13.9 mg/l

Exposure of 2,4-DNT to filtered UV light reduced the acute toxicity.

		Results given for non photolyzed samples: Fathead minnow: LC50 = 31.4 mg/l
Test condition	:	- EPA guidelines (1975) - Mortality controls were conducted. - During the study the concentrations of the test substance were determined with HPLC - To compute the LC50 were used either probit or the binomial method. - Test period: up to 96 h - Diluent water: Dechlorinated tap water - Toxicity was also investigated with photolyzed test samples. They were obtianed by exposing them to filtered ultraviolet light (simulated light) in photolytic reactors.
Reliability	:	(2) valid with restrictions Basic data given

10.08.2004 (90)

Type	:	static
Species	:	Pimephales promelas (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	31
NOEC	:	25
Limit test	:	
Analytical monitoring	:	yes
Method	:	other: EPA-660/3-75-009 (1975)
Year	:	1976
GLP	:	no data
Test substance	:	other TS: 2,4-DNT, no purity given
Method	:	other: Methods for acute toxicity tests with fish, macroinvertebrates and amphibians.
Test condition	:	- Dechlorinated tap water was used - Temperature: 20°C +/- 1°C - Dissolved oxygen range from 2.0 to 9.8 ppm - Hardness: 31 to 45 ppm (as CaCO3) - Alkalinity: 25 to 40 ppm (as CaCO3) - pH ranged from 8.0 to 9.5
Reliability	:	(2) valid with restrictions Test according to national standards

01.09.2003 (91)

Type	:	semistatic
Species	:	Gasterosteus aculeatus (Fish, estuary, marine)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	6.3
Limit test	:	no
Analytical monitoring	:	yes
Method	:	other: Short term toxicity tests according to Adema , report RIV 627905 001, National Institute of Public Health and Environmental Protection, Bilthoven, Netherlands
Year	:	1981
GLP	:	no data
Test substance	:	other TS: 2,4-DNT (purity 98%)
Test condition	:	fishes 4 to 5 weeks old; number of organisms: 10 (in duplicate)
Reliability	:	(2) valid with restrictions sufficient documentation

08.09.2003 (92)

Type	:	static
Species	:	Cyprinodon variegatus (Fish, estuary, marine)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	2.3
Limit test	:	no
Analytical monitoring	:	no
Method	:	
Year	:	1981
GLP	:	no
Test substance	:	other TS: 2,3-Dinitrotoluene
Result	:	LC50 (96 h) = 2.3 (1.4 - 3.4) mg/l
Test condition	:	- Fish length: 8-15 mm - Fish age: 14-28 days - Toxicity endpoint: LC50 (96 h)= 2.3 (1.4 - 3.4) mg/l - Concentration: nominal - Salt water, salinity: 10 - 31 ppt - Incubation temperature: 25 - 31 °C Test conditions: test type: static, concentration: nominal, Salt water, salinity: 10 - 31 ppt, temperature: 25 - 31 °C Reference: Heitmuller, P.T., T.A. Hollister, and P.R. Parrish. (1981) Acute Toxicity of 54 Industrial Chemicals to Sheepshead Minnows (Cyprinodon variegatus) Bull.Environ.Contam.Toxicol. 27(5):596-604
Reliability	:	(2) valid with restrictions Basic data given

10.08.2004 (93)

Type	:	semistatic
Species	:	Brachydanio rerio (Fish, fresh water)
Exposure period	:	4 day(s)
Unit	:	mg/l
LC50	:	> 16
Limit test	:	
Analytical monitoring	:	no
Method	:	other: according to a dutch national standard method: NEN 6504, 1980 (Wateronderzoek. Bepaling van de acute toxiciteit met Poecilia reticulata)
Year	:	1984
GLP	:	no
Test substance	:	other TS: 2,4-DNT, 98%
Remark	:	The toxicity tests have been carried out in two institutes: TNO and RIVM.
Result	:	1-2 days old test organisms: TNO: LC50 13 mg/l, conf. interval: 11-16 mg/l NOLC (no letal concentration): 5.6 mg/l NOEC: 1.8 mg/l RIVM: LC50 24 mg/l, conf. interval: 10-60 mg/l NOLC: 3.2 mg/l NOEC: 2.4 mg/l 4-5 weeks old test organisms: TNO: LC50 >10<32 mg/l NOLC: 10 mg/l NOEC: 4.2 mg/l RIVM: LC50 >16 mg/l NOLC: >/= 16 mg/l NOEC: 1.8 mg/l

Test condition	:	-1-2 days and 4-5 week old organisms tested -testorganisms per group: 10 -5 concentrations tested, two replicates -temperature: 23 +/- 2°C -renewal every 48 hours -test criteria: mortality and immobility
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment

11.09.2003 (94)

Type	:	semistatic
Species	:	other: see Remark
Exposure period	:	4 day(s)
Unit	:	mg/l
Limit test	:	
Analytical monitoring	:	no
Method	:	other: according to a dutch national standard method: NEN 6504, 1980 (Wateronderzoek. Bepaling van de acute toxiciteid met Poecilia reticulata)
Year	:	1981
GLP	:	no
Test substance	:	other TS: 2,4-DNT, 98%
Remark	:	The toxicity tests have been carried out in two institutes: TNO and RIV (now RIVM). Test organisms: Oryzias latipes, Jordanella floridae, Poecilia reticulata in different ages.
Result	:	Oryzias latipes (tested by RIV): Result for 1-2 day and 4-5 week old fish in 2 and 4 day tests: LC50 >16 mg/l, NOEC: 1.8 mg/l Jordanella floridae (tested by TNO): 1-2 day old fish: 2 day test: LC50 25 mg/l, conf. interval: 22-28 mg/l 4 day test: LC50 22 mg/l, conf. interval: 19-25 mg/l NOEC: 10 mg/l 4-5 week old fish: 2 day and 4 day test: LC50 >16 <32 mg/l NOEC: 5.6 mg/l Poecilia reticulata (tested by TNO and RIV): 3-4 week old fish TNO: 2 day test: LC50 33 mg/l, conf. interval: 31-34 mg/l 4 day test: LC50 25 mg/l, conf. interval: 23-27 mg/l NOEC: 3.2 mg/l RIV: 2 day and 4 day test: LC50 >16 mg/l, NOEC: 1.8 mg/l
Test condition	:	-1-2 days and 3-5 week old organisms tested -testorganisms per group: 10 -5 concentrations tested, two replicates -temperature: 23 +/- 2°C -renewal every 48 hours -test criteria: mortality and immobility
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment

17.09.2003 (95)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
Limit Test	:	no
Analytical monitoring	:	no
Method	:	other: EPA-660/3-75-009, "Methods for acute toxicity with fish, macroinvertebrates and amphibians", 1975
Year	:	1979
GLP	:	no
Test substance	:	other TS: all DNT isomers, no purity reported
Result	:	The following results 48 h EC50 (mg/l) were obtained: 2,3-DNT 4.7 2,4-DNT 35.0 2,5-DNT 3.4 2,6-DNT 21.7 3,4-DNT 3.1 3,5-DNT 45.1
Test condition	:	- Daphnia obtained from SRI stockcultures - Incubation temperature 20 °C, - Quality of water (municipal water, dechlorinated by passage through a series of 6 activated carbon columns: hardness (26 ppm) as CaCO3, alkalinity (45 ppm) as CaCO3, pH 7.2-8.6 - Probit data analysis
Reliability	:	(2) valid with restrictions Test procedure in accordance with national standard methods without detailed documentation
Flag	:	Critical study for SIDS endpoint

17.01.2004 (45)

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	24 hour(s)
Unit	:	mg/l
Analytical monitoring	:	no
Method	:	other: Immobilization test acc. to Bringmann & Kühn
Year	:	1977
GLP	:	no
Test substance	:	other TS: 2,3-DNT, 2,4-DNT, 2,6-DNT
Remark	:	Effect endpoint: immobilisation
Result	:	The following 24 h EC50 values were obtained (mg/l) 2,3-DNT 3. 2 2,4-DNT 22 2,6-DNT 14
Test condition	:	- temperature (20 - 22 °C) - quality of tap water: free from chlorine, saturated with oxygen, hardness 16° (degree dH) (corresponding to 286 mg CaCO3/l), pH (7.6 - 7.7) - 10 daphnids/vessel (age max. 24 h) - concentration range of 10 - 1000 mg/l
Reliability	:	(2) valid with restrictions Test procedure comparable to standard method and in

accordance with generally accepted scientific standards;
detailed documentation of test procedure and test conditions

17.09.2003 (96)

Type	:	flow through
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC50	:	30.4
Analytical monitoring	:	yes
Method	:	other: EPA-660/3-75-009, "Methods for acute toxicity with fish, macroinvertebrates and amphibians", 1975
Year	:	1983
GLP	:	no
Test substance	:	other TS: >= 95%, 2,4-DNT
Result	:	24 h LC50 = 31.2 mg/l 48 h LC50 = 30.4 mg/l 96 h LC50 = 23.9 mg/l 288 h LC50 = 4.1 mg/l
Test condition	:	- age of daphnids (< 12 h) - Water quality: temperature (20 °C), dissolved oxygen (8.1 - 8.6 mg/l), pH (7.1 - 7.8), hardness (20 mg/l) as CaCO3, alkalinity (25 mg/l) as CaCO3
Reliability	:	(2) valid with restrictions Test procedure in accordance with national standard methods

17.09.2003 (90)

Type	:	semistatic
Species	:	Daphnia magna (Crustacea)
Exposure period	:	24 hour(s)
Unit	:	mg/l
EC50	:	38
Analytical monitoring	:	no
Method	:	other: DIN-Standard 38 412 L11 (Daphnia, Short-time toxicity test)
Year	:	1988
GLP	:	no data
Test substance	:	other TS: 90 % 2,4-Dinitrotoluene, 90 % 2,6-Dinitrotoluene
Method	:	Method of the German Standards Institution, Berlin, Germany
Result	:	related to nominal concentration The following 24 h EC50 value (mg/l) is obtained: 2,6-DNT = 20
Test condition	:	details are listed in the publication of the reproduction test (chapter 4.5.2)
Reliability	:	(2) valid with restrictions Test procedure according national standard method.

17.09.2003 (97) (98)

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
Limit Test	:	no
Analytical monitoring	:	yes
Method	:	other: Method by US-EPA 1975 (EPA-660/3-75-009)
Year	:	1983
GLP	:	no
Test substance	:	other TS: all DNT isomers

Method	:	US EPA (1975). Methods for acute toxicity tests with Fish, Macroinvertebrates, and Amphibians. Ecological Research Series, EPA-66013-75-009, National Environmental Research Center, Office of Research and Development, Washington, DC
Remark	:	The invertebrate species were from stocks reared in the laboratory at SRI International where the study was performed.
Result	:	The following 48 h EC50 values were obtained (mg/l) 2,3-DNT 4.7 2,4-DNT 47.5 2,5-DNT 3.1 2,6-DNT 21.8 3,4-DNT 3.7 3,5-DNT 45.2
Test condition	:	- Test water: Dechlorinated tap water was used to prepare stock and test solutions of the test substance and to rear and maintain the test animals. The water treatment system comprised several 75-µm particle filters and several 0.042-m3 activated carbon columns. The means of hardness 33.8 (19 mg/l as CaCO3), pH (7.7), alkalinity 38 (20 mg/l as CaCO3), conductivity (81 µs/cm), and residual chlorine of water samples (2.2 µg/l) were collected monthly during a significant portion of the study - Test temperature: 20 °C - Food: During the static test no food provided - Stock solution: The stock solution was prepared by dissolving a measured amount of chemical in a known volume of water. No carrier was used. The mixing time was about 24 h. The stock solution was filtered through a 5-µm filter and analyzed for the chemical
Test substance	:	The DNT isomers were obtained from 2,3-DNT SRI International 2,4-DNT ICN 2,5-DNT SRI International 2,6-DNT Aldrich 3,4-DNT Aldrich 3,5-DNT SRI International
Reliability	:	(2) valid with restrictions Test procedure according to national standards. Basic data given

11.08.2003 (31)

Type	:	flow through
Species	:	other: Lumbriculus variegatus (aquatic worm)
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC50	:	80.9
Analytical monitoring	:	yes
Method	:	other: EPA-660/3-75-009, "Methods for acute toxicity with fish, macroinvertebrates and amphibians", 1975
Year	:	1984
GLP	:	no
Test substance	:	other TS: 2,4-DNT, purity >= 95%
Result	:	24 h LC50 = 99.5 mg/l 48 h LC50 = 80.9 mg/l 96 h LC50 = 47.2 mg/l 336 h LC50 = 30.4 mg/l
Test condition	:	temperature: 21°C; dissolved oxygen: 7.7-8.5 mg/l; pH 7.0-7.6; hardness: 45 mg/l as CaCO3; alkalinity: 55 mg/l as CaCO3
Reliability	:	(2) valid with restrictions test procedure in accordance with national standard methods

22.08.2003 (90)

Type	:	other: not specified

Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC50	:	22
Limit Test	:	no
Analytical monitoring	:	no
Method	:	other: Bringmann and Kuehn (1982)
Year	:	1982
GLP	:	no
Test substance	:	other TS: 2,3-DNT, 2,6-DNT
Result	:	The following 48 h EC50-values were obtained (mg/): 2,3-DNT 3,9 2,6-DNT 21
Reliability	:	(2) valid with restrictions Method comparable with test guideline

05.09.2003 (99) (69)

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC50	:	35
Limit Test	:	no
Analytical monitoring	:	no
Method	:	other: EPA-660/3-75-009 (1975)
Year	:	1976
GLP	:	no
Test substance	:	other TS: 2,4-DNT, no purity given
Test condition	:	- Dechlorinated tap water was used - Temperature: 20°C +/- 1°C - Dissolved oxygen range from 2.0 to 9.8 ppm - Hardness: 31 to 45 ppm (as CaCO3) - Alkalinity: 25 to 40 ppm (as CaCO3) - pH ranged from 8.0 to 9.5
Reliability	:	(2) valid with restrictions Test according to national standards

01.09.2003 (91)

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
IC50	:	5.6 - 34
Limit Test	:	no
Analytical monitoring	:	no
Method	:	other: Dutch Standard Organisation NEN 6501 (1980)
Year	:	1988
GLP	:	no data
Test substance	:	other TS: 2,3-DNT, 2,4-DNT, 2,6-DNT, 3,4-DNT
Remark	:	The data were published as a dissertation and in a journal
Result	:	The following results were obtained:

	log IC50 (µM)	IC50 (mg/l)
2,3-DNT	1.49	5.6
2,4-DNT	2.27	34
2,6-DNT	2.27	34
3,4-DNT	1.49	5.6

Test condition	:	- Organisms: Freshwater crustacean Daphnia magna cultured in the laboratory according to the Dutch standard NPR 6503 (1980). The culture medium used was Lake Ijssel water. - Chemical Composition of Lake Ijssel Water (mg/l): Na 59.2, K 6.9, Ca 72.0, Mg 10.7, Mn 0.015, Fe 0.162, Si 4.1, NH4-N 0.13, NO3-N 4.46, NO2-N 0.08, PO4-P 0.28, SO4-- 78.0, HCO3- 159, Cl- 133, Hardness (as CaCO3) 224, Chlorophyll a 0.002, TOC 4.5, pH 8.1. Daphnias were fed with cells of the unicellular alga Chlorella pyrenoidosa (strain: Wisconsin 2005 from Culture Centre of Algae and Protozoa, Cambridge) - Synthetic test medium was prepared as described in NPR 6503 (1980) with a CaCO3 hardness of 200 mg/l and a pH of 8.4 ± 0.1, saturated with air prior to use - Daphnia magna was less than 24 h old at start of incubation - During incubation newborn (0 to 24-hr) Daphnias were fed with Chlorella pyrenoidosa (10E+8 cells/l at start of experiment) according to NEN 6502 (1980) - Incubation: 10 Daphnias per concentration, one animal per jar containing 50 ml test medium; 12 h/d illumination at 20 +/- 0.5 °C - Endpoint: immobilization - The LC50 values, confidence limits, and X2 fit were determined by the method of Litchfield and Wilcoxon (1949). When necessary, corrections were made for mortality in the controls. Population growth was analyzed according to Kooyman et al. (1983)
Reliability	:	(2) valid with restrictions Test procedure in accordance with national standard methods with acceptable restrictions

10.08.2004 (100) (101)

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC50	:	26.2
Analytical monitoring	:	no data
Method	:	other: Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (EPA-660/3-75-009) 1975
Year	:	1980
GLP	:	no
Test substance	:	other TS: 2,4-DNT
Result	:	95% confidence interval EC50: 22.5 - 30.5 mg/l nominal concentration
Test condition	:	- Test organisms: 12 +/- 12 h old (first instar) - Temperature 22 °C - Diluent source: springfed pond - Measured hardness as CaCO3 over the period of use: 154.5 mg/l (average concentration)
Reliability	:	(2) valid with restrictions Study in accordance with generally accepted scientific standards and described in sufficient detail

10.08.2004 (102)

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC50	:	< 16
Analytical monitoring	:	no

Method	:	other: according to a dutch national standard method: NEN 6501, 1980 (Wateronderzoek. Bepaling van de acute toxiciteit met Daphnia magna)
Year	:	1981
GLP	:	no
Test substance	:	other TS: 2,4-DNT, 98%
Remark	:	The toxicity tests have been carried out in two institutes: TNO and RIV (now RIVM).
Result	:	Results for 24 and 48 hours of both institutes: EC50 >10 mg/l and <16 mg/l, NOEC: 10 mg/l
Test condition	:	-test organisms per group: 25 -5 concentrations tested, two replicates -temperature: 19 +/- 1°C -test criteria: immobilisation
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment

10.08.2004 (95)

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC50	:	.66
Limit Test	:	no
Analytical monitoring	:	no
Method	:	
Year	:	1980
GLP	:	no
Test substance	:	other TS: 2,3-Dinitrotoluene
Remark	:	Cited according to EU (2004). Draft Risk assessment report 2,4-Dinitrotoluene, Draft of May 2004.
Result	:	LC50 (48 h) = 0.66 (0.42 - 1.1) mg/L
Test condition	:	- Age of water fleas <24 h - Dissolved oxygen > 60 %, - Water hardness: 173 mg/L CaCO3 - pH: 8.4 ± 1 - Incubation temperature 22 °C
Reliability	:	(4) not assignable Original literature not available

10.08.2004 (103)

Type	:	
Species	:	other aquatic crustacea: Americamysis bahia (Opossum shrimp)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	.59
Analytical monitoring	:	no data
Method	:	other: no data
Year	:	1978
GLP	:	no
Test substance	:	other TS: 2,3-Dinitrotoluene
Test condition	:	Salt water, no other data available
Reliability	:	(4) not assignable Documentation insuffcient for assessment

10.08.2004 (104)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Selenastrum capricornutum (Algae)
Endpoint	:	growth rate
Exposure period	:	96 hour(s)
Unit	:	mg/l
Limit test	:	
Analytical monitoring	:	yes
Method	:	other: 96-well microplate method
Year	:	1999
GLP	:	no data
Test substance	:	other TS: highest grade of purity available, 2,4-DNT, 2,6-DNT
Method	:	According to "Biological Test Method: Growth Inhibition Test using the Freshwater Alga Selenastrum capricornutum", EPS 1/RM/25, Environment Canada (1992)
Result	:	for 2,4-DNT: EC50 = 2.6 mg/l EC20 = 1.6 mg/l for 2,6-DNT: EC50 = 16.4 mg/l EC20 = 12.2 mg/l
Test condition	:	- algae exposed during logarithmic growth phase (cultured for 6 to 8 days) - temperature 25 +/- 1°C - continuous light 4000 +/- 400 lux - pH 6 - 7.5; - solubilizing agent DMSO (0.25%)
Reliability	:	(2) valid with restrictions study well documented, meets generally accepted scientific principles
Flag	:	Critical study for SIDS endpoint

17.01.2004 (105)

Species	:	Chlorella pyrenoidosa (Algae)
Endpoint	:	growth rate
Exposure period	:	96 hour(s)
Unit	:	mg/l
EC50	:	.7 - 6.7
Limit test	:	no
Analytical monitoring	:	no
Method	:	other: according to Van Leeuwen et al. (1985)
Year	:	1988
GLP	:	no
Test substance	:	other TS: 2,3-DNT, 2,4-DNT, 2,6-DNT, 3,4-DNT
Method	:	According to van Leeuwen et al. (1985) - Organisms: Unicellular alga Chlorella pyrenoidosa (strain: Wisconsin 2005 from Culture Centre of Algae and Protozoa, Cambridge) - Algal medium according to van Leeuwen and Maas (1985) - 10E+8 cells/l (100 ml) added into 200 ml Erlenmeyer flask at start of experiment, incubated on mechanical shaker under fluorescent light (7.5 W/m2) - Cells were counted with ZBI Coulter Counter using 70 µm aperture - Population growth was analyzed according to Kooyman et al. (1983)
Remark	:	The data were published as a dissertation and in a journal
Result	:	The following results were obtained:

Growth	log EC50 (µM)	EC50 (mg/l)
2,3-DNT	0.70	0.9
2,4-DNT	0.70	0.9
2,6-DNT	1.57	6.7
3,4-DNT	0.61	0.7

Reliability	:	(2) valid with restrictions

Basic data given

Flag : Critical study for SIDS endpoint
17.01.2004 (100) (101)

Species : other algae: see Remark
Endpoint : growth rate
Exposure period : 4 day(s)
Unit : mg/l
Limit test :
Analytical monitoring : no
Method : other: according to a Dutch national standard method: NEN 6506, 1979 (Water-Bepaling van de toxiciteit met behulp van algen)
Year : 1981
GLP : no
Test substance : other TS: 2,4-DNT, purity 98 %

Remark : Test organisms: Chlorella pyrenoidosa (green algae), Scenedesmus pannonicus (green algae), Microcystis aeruginosa (blue-green algae), Selenastrum capricornutum (green algae), Euglena gracilis (flagellate), Stephanodiscus hantzschii (diatom).
The toxicity tests have been carried out in three different institutes: TNO, RIV (now RIVM) and RID.

Result : Scenedesmus pannonicus:
EC50 = 1.6 mg/l, conf.interval: 1.5-1.6 mg/l, NOEC: 0.56 mg/l
(measured with spectrophotometer by RIV)
EC50 = 2.1 mg/l, conf.interval: 2.0-2.3 mg/l, NOEC: <1.0 mg/l
(measured with spectrophotometer by TNO)
EC50 = 2.3 mg/l, conf.interval: 2.2-2.5 mg/l, NOEC: 0.32 mg/l
(measured with Coulter counter by TNO)
Chlorella pyrenoidosa:
EC50 = 3.8 mg/l, conf.interval: 3.5-4.1 mg/l, NOEC: 0.56 mg/l
(measured with spectrophotometer by RIV)
Selenastrum capricornutum:
EC50 = 1.6 mg/l, conf.interval: 1.5-1.8 mg/l, NOEC: 0.56 mg/l
(measured with Coulter counter by RID)
Microcystis aeruginosa:
EC50 = 0.08 mg/l, conf.interval: 0.07-0.10 mg/l,
NOEC: <0.056 mg/l
(measured with Coulter counter by RID)
Euglena gracilis:
EC50 = 9.6 mg/l, conf.interval: 8.8-11 mg/l, NOEC: 1.0 mg/l
(measured with Coulter counter by TNO)
Stephanodiscus hantzschii:
EC50 = 6.2 mg/l, conf.interval: 5.8-6.8 mg/l, NOEC: 1.0 mg/l
(measured with Coulter counter by TNO)

Test condition : -static
-test time: 4 days
-temperature: 23 +/- 2°C (S.hantzschii: 17 +/- 2°C)
-5 concentrations tested
-continuous light approx. 5000 lux (M.aeruginosa and S.capricornutum approx. 2500 lux)
-Initial cell concentration approx. 10E04 (M.aeruginosa and S.capricornutum approx. 5.10E04)
-test criteria: growth monitored by spectrophotometer and/or Coulter counter
-effects were compared to the control

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint

10.08.2004 (95)

Species	:	Scenedesmus subspicatus (Algae)
Endpoint	:	growth rate
Exposure period	:	48 hour(s)
Unit	:	mg/l
Limit test	:	
Analytical monitoring	:	no
Method	:	other: Cell multiplication inhibition test acc. to DIN 38412, part 9, modified
Year	:	1988
GLP	:	no
Test substance	:	other TS: 2,4-DNT, 2,6-DNT, no data on purity
Remark	:	modifications of the test procedure: wide-neck bottles, reduction of test time to 48 h
Result	:	-Growth rate (mg/l): for 2,4-DNT: EC10 = 1.9 for 2,6-DNT: EC10 = 10 EC50 = 6.3 EC50 = 22 -Biomass (mg/l): for 2,4-DNT: EbC10 = 1.3 for 2,6-DNT: EbC10 = 7.0 EbC50 = 3.0 EbC50 = 16 (nominal concentrations)
Test condition	:	Tested concentration 0.8 - 100 mg/l
Reliability	:	(2) valid with restrictions Test procedure in accordance with national standard methods with acceptable restrictions

05.09.2003 (106)

Species	:	Selenastrum capricornutum (Algae)
Endpoint	:	growth rate
Exposure period	:	4 day(s)
Unit	:	
Limit test	:	
Analytical monitoring	:	no data
Method	:	other: EPA test method "Algal Assay Procedures: Bottle Test", 1971
Year	:	1984
GLP	:	no
Test substance	:	other TS: 2,4-DNT, purity>= 95%,
Result	:	37.4% inhibition: 0.9 mg/l > 98% inhibition: >=4.7 mg/l
Test condition	:	static
Reliability	:	(2) valid with restrictions Test procedure in accordance with national standard methods, but without detailed documentation

22.08.2003 (90)

Species	:	Selenastrum capricornutum (Algae)
Endpoint	:	growth rate
Exposure period	:	14 day(s)
Unit	:	
Limit test	:	
Analytical monitoring	:	no data
Method	:	other: EPA test method "Algal Assay Procedures: Bottle Test", 1971
Year	:	1984
GLP	:	no
Test substance	:	other TS: >= 95%; 2,4-DNT
Result	:	13.5% inhibition: 0.9 mg/l

> 99% inhibition: >=9.4 mg/l
Test condition : static
Reliability : (2) valid with restrictions
test procedure in accordance with national standard methods, but without detailed documentation
12.09.2003 (90)

Species : Scenedesmus quadricauda (Algae)
Endpoint : biomass
Exposure period : 8 day(s)
Unit : mg/l
Limit test :
Analytical monitoring : no
Method : other: cell multiplication inhibition test
Year : 1977
GLP : no
Test substance : other TS: 2,3-DNT, 2,4-DNT, 2,6-DNT

Method : according to Bringmann
Remark : The same author also published the same value, but another test period (7 days) is given.
Result : TT (toxic threshold concentration) refers to nominal test substance concentration and was determined at 3 % effect compared to the control (comparable to EC 3)

for 2,3-DNT TT = 0,83 mg/l
for 2,4-DNT TT = 2,7 mg/l
for 2,6-DNT TT = 12 mg/l
Test condition : static test; temperature: 27°C; continuous artificial light; pH 7.0; measurement of turbidity
Reliability : (2) valid with restrictions
study well documented, meets generally accepted scientific principles, acceptable for assessment
01.09.2003 (107) (108) (109) (110) (111)

Species : Scenedesmus subspicatus (Algae)
Endpoint : growth rate
Exposure period : 7 day(s)
Unit : mg/l
TT : 1.4
Limit test : no
Analytical monitoring : no data
Method : other: Bringmann and Kuehn (1980)
Year : 1982
GLP : no
Test substance : other TS: 2,4-DNT, no purity given

Result : TT: Toxicity Treshold is the 3% inhibitory concentration
Test condition : - Initial 2,4-DNT concentration was checked measuring the DOC (dissolved organic carbon)
- The test was performed under static conditions
Reliability : (2) valid with restrictions
Basic data given. Method comparable with test guideline
30.07.2003 (69)

Species : other algae: Scenedesmus obliquus
Endpoint : growth rate
Exposure period : 48 hour(s)

Unit	:	mg/l
EC50	:	6.3
Limit test	:	no
Analytical monitoring	:	no data
Method	:	OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year	:	2000
GLP	:	no data
Test substance	:	other TS: 2,4-DNT, no purity given
Remark	:	2,4-DNT was also tested in the same test system with other compounds. Each compound mixed with 2,4-DNT increased the toxicity for the algae.
Result	:	The results are given as mol/l.
Test condition	:	- Five concentrations tested - Continuous light provided - Aqueous medium prepared according to Lang (1994) - Temperature 20 °C - Four replicates and one control
Reliability	:	(2) valid with restrictions Basic data given

17.09.2003 (112)

Species	:	other algae: Scenedesmus obliquus
Endpoint	:	growth rate
Exposure period	:	48 hour(s)
Unit	:	mg/l
Limit test	:	no
Analytical monitoring	:	no data
Method	:	other: OECD-Guideline 201 (algae, Growth inhibition test, 1981)
Year	:	1995
GLP	:	no data
Test substance	:	other TS: 2,4-DNT, 2,6-DNT, no purity given
Remark	:	EC50 was also calculated with a QSAR-equation to 6.17 mg/l
Result	:	In the publication the results are given as -Log EC50 (mol/l). In mg/l: for 2,4-DNT EC50 = 5.5 for 2,6-DNT EC50 = 15.9
Reliability	:	(2) valid with restrictions Test according to guideline study

05.09.2003 (113)

Species	:	other algae: Scenedesmus obliquus
Endpoint	:	growth rate
Exposure period	:	48 hour(s)
Unit	:	mg/l
Limit test	:	
Analytical monitoring	:	no
Method	:	other: OECD-Guideline 201 (algae, Growth inhibition test, 1981)
Year	:	2001
GLP	:	no data
Test substance	:	other TS: 2,4-DNT, 2,6-DNT
Remark	:	An equation was derived for the calculation of EC50 for substituted benzenes.
Result	:	Using the equation, for 2,4-DNT the EC50 = 6.2 mg/l (measured: 5.5 mg/l) for 2,6-DNT The EC50 = 7.3 mg/l (measured: 15.9 mg/l)
Test condition	:	The test was performed under the followimg conditions: - Temperature 20 °C +/- 1 °C - Continuous light provided by white Neon lamps (4,000 lux)

- Initial cell concentration was approx. 10000 cells/ml
- Growth was monitored by electron microscope (400 times)

Reliability : (2) valid with restrictions
Guideline study without detailed documentation

12.09.2003 (114)

Species : other aquatic plant: Potamogeton pectinatus, Heteranthera dubia, Phragmites australis, Phalaris arundinacea
Endpoint : growth rate
Exposure period : 21 day(s)
Unit : mg/l
LOEC : 5 measured/nominal
Limit test : no
Analytical monitoring : yes
Method : other: see Test condition
Year : 2001
GLP : no data
Test substance : other TS: 2,4-DNT, 2,6-DNT

Result : 20 mg/l 2,4-DNT stimulated growth in reed canary grass, 5 mg/l 2,6-DNT (NOEC) were ineffective against water stargrass, all other concentrations and incubations inhibitory. LOEC 5 mg/l was the lowest tested concentration

Test condition : Effects of 2,4-DNT and 2,6-DNT were investigated in walk-in growth room
- Plant material: Submersed [Potamogeton pectinatus (sago pondweed), Heteranthera dubia (water stargrass)] and emergent plant species [Phragmites australis (common reed), Phalaris arundinacea (reed canary grass)]
- Plants were planted in silty sediment, low levels of nutrients were suplied, and the plants were aerated
- 5.7 g/l plant fresh weight or 3 plants per pot
- Incubation for 3 weeks at 23 °C
- Change of medium twice per week
- Lighting regime: 14 h/d 500 - 600 µEm-2s-1 in the photosynthetically active region, augmented with 2 % UV-B
- Concentrations of DNT isomers 0, 5, 20, 40 mg/l
- Measurement of initial and final plant mass (dry weight)

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles

02.09.2003 (37)

Species : Microcystis aeruginosa (Algae, blue, cyanobacteria)
Endpoint : biomass
Exposure period : 8 day(s)
Unit : mg/l
TT(2,3-DNT) : .22
TT(2,4-DNT) : .13
TT(2,6-DNT) : .5
Limit test : no
Analytical monitoring : no data
Method : other: Inhibition of cell reproduction according to Bringmann
Year : 1978
GLP : no
Test substance : other TS: 2,3-DNT, 2,4-DNT, 2,6-DNT

Result : TT (toxic threshold concentration) refers to nominal test substance concentration and was determined at 3% effect compared to the control (comparable to EC 3)

Test condition	:	Static test; temperature: 27°C; continuous artificial light; pH 7; measurement of turbidity
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment

16.01.2004 (115) (108) (109)

Species	:	other aquatic plant: Lemna perpusilla Torr
Endpoint	:	biomass
Exposure period	:	11 day(s)
Unit	:	mg/l
Limit test	:	no
Analytical monitoring	:	no
Method	:	other: according to Worthley (1971)
Year	:	1974
GLP	:	no
Test substance	:	other TS: 2,4-DNT, no purity given

Result	:	Number of fronds was measured and compared with the control. 2 experiments were conducted at pH 6.3 and 4 at pH 8.5 pH 6.3: LOEC at 0.1 ppm ca.50% effect at 0.5 ppm pH 8.5: LOEC at 0.1 ppm 50% effect could not be determined 100% effect at 5.0 ppm
Reliability	:	(2) valid with restrictions Basic data given

02.09.2003 (116)

Species	:	Oscillatoria sp. (Algae)
Endpoint	:	growth rate
Exposure period	:	96 hour(s)
Unit	:	mg/l
EC50	:	2.3
Limit test	:	no
Analytical monitoring	:	no
Method	:	
Year	:	1985
GLP	:	
Test substance	:	other TS: 2,4-Dinitrotoluene

Remark	:	Cited according to EU (2004). Draft Risk assessment report 2,4-Dinitrotoluene, Draft of May 2004.
Result	:	EC50 (Growth) (96 h) = 2.3 (1.8 - 2.9) mg/l
Test condition	:	- Species: Oscillatoria agardhii (Cyanobacteria) - Cyanobacterial density at start of incubation: 50,000 cells/ml in log phase - Test conditions: test type: static - pH 7.8 - 7.9 - Temperature 23 °C
Reliability	:	(4) not assignable Original literature not available

10.08.2004 (117)

Species	:	other algae: Gomphonema parvulum (Diatom)
Endpoint	:	growth rate
Exposure period	:	96 hour(s)
Unit	:	mg/l
EC50	:	1.9
Limit test	:	no

Analytical monitoring	:	no
Method	:	
Year	:	1985
GLP	:	
Test substance	:	other TS: 2,4-Dinitrotoluene
Remark	:	Cited according to EU (2004). Draft Risk assessment report 2,4-Dinitrotoluene, Draft of May 2004.
Result	:	EC50 (Growth) (96 h) = 1.9 (1.8 - 2.1) mg/l
Test condition	:	- Algal density at start of incubation: 10,000 cells/ml in log phase - Test conditions: test type: static - pH 7.8 - 7.9 - Temperature 23 °C
Reliability	:	(4) not assignable Original literature not available

10.08.2004 (117)

Species	:	Skeletonema costatum (Algae)
Endpoint	:	other: Photosynthesis
Exposure period	:	96 hour(s)
Unit	:	mg/l
EC50	:	.4
Limit test	:	
Analytical monitoring	:	no data
Method	:	other: no data
Year	:	1978
GLP	:	no
Test substance	:	other TS: 2,3-Dinitrotoluene
Test condition	:	Salt water, no other data available
Reliability	:	(4) not assignable Original literature not available

10.08.2004 (104)

Species	:	other aquatic plant: Lemna minor
Endpoint	:	growth rate
Exposure period	:	
Unit	:	mg/l
EC50	:	1.6
Limit test	:	
Analytical monitoring	:	no
Method	:	other: see Test condition
Year	:	1984
GLP	:	no
Test substance	:	other TS: 2,4-DNT, 98%
Remark	:	The toxicity test have been carried out in two institutes: TNO and RIVM.
Result	:	RIVM: EC50, growth: 1.6 mg/l, conf. interval: 1.4-1.8 mg/l NOEC, number of leaves-counted: 0.32 mg/l TNO: EC50,growth: 2.2 mg/l, conf. interval: 1.7-2.8 mg/l NOEC, number and colour of leaves: 0.32 mg/l
Test condition	:	-plants exposed during logarithmic growth phase in a sterilized nutrient solution at ph 8 (cultured for 2 weeks). -temperature 25 +/- 2°C -continuous light 35 watt/m² -5 to 9 concentrations tested, two replicates -Exposure period: 7 to 10 days -Assessment of toxicity: TNO - devided and damaged leaves

RIVM - devided leaves
-effects were compared to the control

Reliability : (2) valid with restrictions
Study well documented

10.08.2004 (118)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type	:	aquatic
Species	:	Pseudomonas putida (Bacteria)
Exposure period	:	16 hour(s)
Unit	:	mg/l
Analytical monitoring	:	no
Method	:	other: Inhibition of cell reproduction acc. to Bringmann
Year	:	1977
GLP	:	no
Test substance	:	other TS: 2,3-DNT, 2,4-DNT, 2,6-DNT
Remark	:	TT (toxicity threshold) comparable to EC3; value based on nominal concentration
Result	:	for 2,3-DNT: TT = 9 mg/l for 2,4-DNT: TT = 57 mg/l for 2,6-DNT: TT = 26 mg/l
Test condition	:	static test; temperature: 25°C; pH 7.0; measurement of turbidity
Reliability	:	(2) valid with restrictions study well documented, meets generally accepted scientific principles
Flag	:	Critical study for SIDS endpoint

23.09.2003 (107) (110) (111)

Type	:	soil
Species	:	other bacteria: natural soil organisms
Exposure period	:	28 day(s)
Unit	:	
EC13	:	5
EC43	:	50
Analytical monitoring	:	no
Method	:	other: BBA Nr. VI, 1-1 (1990) "Dehydrogenase activity of soil microflora"
Year	:	1995
GLP	:	no data
Test substance	:	other TS: 2,4-DNT, purity 99 %
Method	:	Method developed by "Biologische Bundesanstalt, Braunschweig (Germany)
Result	:	Reduction of dehydrogenase activity of: a. sandy soil microorganisms: 26 - 46 % during the whole exposure period and at both concentrations tested b. loamy soil microorganisms: at the beginning of the test, no effect was observed with a concentration of 5 mg/kg, whereas a concentration of 50 mg/kg caused an inhibition of 32 % Inhibition was highest at the 14th day of exposure at both concentrations tested (60-70 %); at the end of the test, inhibition had declined to 13 and 43 % at 2,4-DNT concentrations of 5 and 50 mg/kg, respectively.
Test condition	:	- 2 soils were used: - sandy soil: 0.9 % org. C, pH 6.8 - loamy soil: 2.2 % org. C, pH 7.3-7.4

- 2 concentrations tested: 5 and 50 mg/kg soil dw
- Incubation in the dark at 22 °C
- Water content of soil: ca. 60 % of maximum field capacity, losses were compensated by addition of deionized water

Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles. Basic data given
Flag : Critical study for SIDS endpoint
17.01.2004 (23)

Type : aquatic
Species : Pseudomonas putida (Bacteria)
Exposure period :
Unit : mg/l
EC0 : 100
Analytical monitoring : no data
Method : other: Test according to Robra (O2-Consumption)
Year : 1981
GLP : no data
Test substance : other TS: 2,4-DNT, 90 %

Method : Robra KH (1976). Bewertung toxischer Wasserinhaltsstoffe aus ihrer Inhibitorwirkung auf die Substratoxydation von Pseudomonas Stamm Berlin mit Hilfe polarographischer Sauerstoff-Messungen. gwf Wasser/Abwasser 117 (2), 80-86.
Remark : Solubilizer: Emulgator W
Reliability : (4) not assignable
Original data not available yet
22.08.2003 (3)

Type : aquatic
Species : Pseudomonas putida (Bacteria)
Exposure period : 10 hour(s)
Unit : mg/l
EC10 : 38
Analytical monitoring : no data
Method : other: Bringmann and Kuehn (1980)
Year : 1982
GLP : no
Test substance : other TS: 2,4-DNT, no purity given

Test condition : - Test conducted at pH=7
- Initial TS concentration was checked measuring the DOC (dissolved organic carbon).
- The concentration of the bacteria suspension was measured via turbidimetry, screening the scattered light.

Reliability : (2) valid with restrictions
Basic data given
17.09.2003 (69)

Type : other: growth medium
Species : Uronema parduzci (Protozoa)
Exposure period : 20 hour(s)
Unit : mg/l
Analytical monitoring : no
Method : other: Inhibition of cell reproduction acc. to Bringmann & Kühn
Year : 1980
GLP : no
Test substance : other TS: 2,3-DNT, 2,4-DNT, 2,6-DNT

Remark	:	TT (toxicity threshold) comparable to EC5; value based on nominal concentration
Result	:	for 2,3-DNT TT = 1.6 mg/l for 2,4-DNT TT = 0.55 mg/l for 2,6-DNT TT = 23 mg/l
Test condition	:	Temperature: 20°C; pH 6.9
Reliability	:	(2) valid with restrictions Study meets generally accepted scientific principles, acceptable documentation

05.09.2003 (119) (120)

Type	:	aquatic
Species	:	Entosiphon sulcatum (Protozoa)
Exposure period	:	72 hour(s)
Unit	:	mg/l
Analytical monitoring	:	no
Method	:	other: Inhibition of cell reproduction acc. to Bringmann
Year	:	1978
GLP	:	no
Test substance	:	other TS: 2,3-DNT, 2,4-DNT, 2,6-DNT

Remark	:	TT (toxicity threshold) comparable to EC5; value based on nominal concentration
Result	:	for 2,3-DNT TT = 5.9 mg/l for 2,4-DNT TT = 0.98 mg/l for 2,6-DNT TT = 11 mg/l
Test condition	:	Temperature: 25°C; pH 6.9
Reliability	:	(2) valid with restrictions Study meets generally accepted scientific principles, acceptable documentation

05.09.2003 (121) (110) (111) (120)

Type	:	aquatic
Species	:	Chilomonas paramaecium (Protozoa)
Exposure period	:	48 hour(s)
Unit	:	mg/l
Analytical monitoring	:	no
Method	:	other: Inhibition of cell reproduction acc. to Bringmann et al.
Year	:	1980
GLP	:	no
Test substance	:	other TS: 2,3-DNT, 2,4-DNT, 2,6-DNT

Remark	:	TT (toxicity threshold) comparable to EC5; value based on nominal concentration
Result	:	for 2,3-DNT TT = 1.8 mg/l for 2,4-DNT TT = 13 mg/l for 2,6-DNT TT > 20 mg/l
Test condition	:	- temperature (20 °C) - pH (6.9)
Reliability	:	(2) valid with restrictions Study meets generally accepted scientific principles, acceptable documentation

05.09.2003 (120) (122)

Type	:	aquatic
Species	:	Microcystis aeruginosa (Bacteria)
Exposure period	:	8 day(s)
Unit	:	mg/l
Analytical monitoring	:	no data

Method : other: Inhibition of cell reproduction according to Bringmann
Year : 1978
GLP : no
Test substance : other TS: 2,3-DNT, 2,4-DNT, 2,6-DNT

Result : TT (toxic threshold concentration) refers to nominal test substance concentration and was determined at 3% effect compared to the control (comparable to EC 3)

The following results were obtained (mg/l):
2,3-DNT: TT = 0.22
2,4-DNT: TT = 0.13
2,6-DNT: TT = 0.50

Test condition : Static test; temperature: 27°C; continuous artificial light; pH 7; measurement of turbidity
Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

08.09.2003 (108) (109)

Type : aquatic
Species : Tetrahymena pyriformis (Protozoa)
Exposure period : 24 hour(s)
Unit : mg/l
EC50 : 100
Analytical monitoring : no
Method : other: Cell multiplication inhibition tst
Year : 1985
GLP : no data
Test substance : other TS: 2,6-DNT

Method : Cell counting by microscope and Coulter counter
Test condition : Incubation at 30°C without agitation
Reliability : (2) valid with restrictions
Test procedure in accordance with generally accepted scientific standards and discribed in suffient datail

11.09.2003 (123)

Type : aquatic
Species : Photobacterium phosphoreum (Bacteria)
Exposure period : 15 minute(s)
Unit : mg/l
EC50 : 2.9 - 51
Analytical monitoring : no
Method : other: Microtox
Year : 1988
GLP : no
Test substance : other TS: 2,3-DNT, 2,4-DNT, 2,6-DNT, 3,4-DNT

Method : According to Beckmann Instruments Manual (1982)
Remark : The data were published as a dissertation and in a journal
Result : The following results were obtained:
luminescence

	log EC50 (µM)	EC50 (mg/l)
2,3-DNT	1.52	6.2
2,4-DNT	2.45	51
2,6-DNT	1.20	2.9
3,4-DNT	1.58	6.9

Reliability : (3) invalid

Unsuitable test system, documentation insuffcient for assessment
08.09.2003 (100) (101)

Type : aquatic
Species : other bacteria: no data
Exposure period :
Unit : mg/l
EC50 : 4.5 - 35
Method : other: Microtox
Year : 2000
GLP : no
Test substance : other TS: 2,4-DNT, 2,6-DNT

Method : - Microtox/Mutatox Model 500 Toxicity Analyzer System used
- Lead effect (not specified) used as standard
Result : EC50 (incubation period not indicated)
2,4-DNT 35.22 mg/l
2.6-DNT 4.46 mg/l
(although results are given in ppm, it is assumed that mg/l is meant)
Reliability : (4) not assignable
Documentation insufficient for assessment
14.08.2003 (124)

4.5.1 CHRONIC TOXICITY TO FISH

Species : Oncorhynchus mykiss (Fish, fresh water)
Endpoint : other: fry growth (length, weight)
Exposure period : 90 day(s)
Unit : mg/l
NOEC : .27
LOEC : .56
Analytical monitoring : yes
Method : other: early life stage test
Year : 1984
GLP : no data
Test substance : other TS: 2,4-DNT, purity >= 95 %

Remark : Oncorhynchus mykiss is the new scientific name of Salmo gairdneri
Since the data on length and weight are given only as averages, it cannot be examined why a data pair (control value vs. concentration value), which differed only slightly (0.942 vs. 0.830), was reported to be significantly different, whereas other pairs with larger difference (e.g. 0.813 vs. 0.960, 25 vs. 46) was not reported to be significantly different.
Result :

Conc. (mg/l)	Test Series	No. hatched	No.** deform	No.*** alive	Length (cm)	Weight (g)
0.00	A	34	6	25	4.78	0.942
	B	41	1	34	4.50	0.815
0.05	A	46	0	32	4.54a	0.830a
	B	48	0	44	4.36	0.720
0.12	A	47*	4	36	4.50a	0.775a
	B	47*	6	32	4.66	0.880
0.27	A	32*	1	28	4.56a	0.921
	B	34*	1	30	4.60	0.960
0.56	A	41	0	30	4.37a	0.745a
	B	43	0	37	4.33	0.746
1.17	A	46	4	40	4.08a	0.665a
	B	45	3	42	4.24a	0.730
2.26	A	52	2	46	3.82a	0.492a

	B	47	2	35	3.97a	0.582a
4.02	A	52	3	26	3.14a	0.287a
	B	47	1	36	3.03a	0.302a

*Instead of 60 eggs exposed only 55 eggs exposed
**No. deformed
***No. alive at 90th day
All concentrations of 2,4-Dinitrotoluene in the range of 0.05 to 4.02 had no significant effects on egg survival, number of deformed fry, or fry survival. The fry exposed to 4.02 mg/l were unable to swim and remained on their sides at the bottom of the tanks.
In all these experiments (with the exception at 0.27 mg/l), the number of fry hatched and the number of fry alive at 90th day were higher than in controls. The number of deformed fry did not show any relation to concentration. The authors state that in one of the two series of experiments there was a significant decrease in the average length and weight of the fry at the lowest measured concentrations of 0.05, 0.12, 0.27, and 0.56 mg/l (length reduction 5, 6, 4, and 9 %, respectively; weight reduction 12, 18, 2 [not significant], and 21 %, respectively). In another series of experiments, these concentrations had no effect on length and weight.
The authors conclude, that there is a concentration affect at 1.17 mg/l and at higher concentrations. At 0.56 mg/l and lower concentrations, they conclude that length and weight are related to the concentration of fry in the incubation vessels. This conclusion is supported by fact that the controls in the first experiments had the second lowest hatching number and the lowest survival of the fry. The controls of the first experiment were significantly longer and heavier than the controls of the second experiment.
Lowest concentration tested: 0.05 mg/l

Test condition : - Dissolved oxygen 8.8-12.2 mg/l (measured daily)
- pH 6.7-7.4 (measured daily)
- Temperature 10-13 °C
- Hardness 35-60 mg/l (as CaCO3) (measured weekly)
- Alkalinity 16-58 mg/l (as CaCO3) (measured weekly)
- Photoperiod of 16 h light and 8 h dark

Reliability : (2) valid with restrictions
Basic data given

Flag : Critical study for SIDS endpoint

31.03.2004 (125)

Species : Pimephales promelas (Fish, fresh water)
Endpoint : other: reproduction
Exposure period : 179 day(s)
Unit : mg/l
NOEC : .28
Analytical monitoring : yes
Method : other: chronic study
Year : 1984
GLP : no data
Test substance : other TS: >= 95 %, 2,4-DNT

Remark : Although the authors clearly indicate, that there was no significant effect at 0.28 mg/l, they assume that there was also a reduction in reproductive capability at the lowest concentration tested.
In an additional test on the F1 generation they observe a small statistically significant reduction in survival at 0.28 mg/l but not at 1.31 and 2.69 mg/l. The authors assume these effects not to be dose-related. A missing dose-effect relationship for length and weight is attributed by the authors to fry density.

Result : At the two highest concentrations (see below, data not shown) fry survival was reduced after more than 30 d.

At concentrations as low as 1.31. mg/l the ability of the surviving spawning pairs to produce eggs was significantly reduced at concentrations as low as 1.31. mg/l. Only in pooled series, the number of eggs per spawn was significantly decreased at 0.62 mg/l.

Effect of chronic exposure of 2.4-DNT on reproductive parameters in fathead minnows

Conc* (mg/l)	Series **	Surv. pair	spawns/ pair	eggs/ spawn	eggs/ ***	eggs
0.00	A	129	17	2870	150.6	22.2
	B	121	10	1477	141.0	12.4
0.28	A	129	18	2332	127.6	18.1
	B	131	13	1312	101.1	10.0
0.62	A	91	14	1583	101.3b	15.9
	B	99	11	1156	97.8b	16.8
1.31	A	91	4	487b	103.7b	7.2b
	B	68	8	469b	45.0a	4.3b
2.69	A	66	3a	206a	38.0b	1.6a
	B	91	2	56a	9.4a	0.4a
6.71	A	--	--	--	--	--
	B	38	0a	0a	0a	0a

*Mean measured concentration
**Spawning pair survival (days)
***eggs/pair/spawns
a Statistically significant, $p < 0.05$.
b Statistically significant, $p < 0.05$, if series are pooled.

Test condition : - at minimum 80 eggs per test chamber
- dissolved oxygen 1.9 - 8.9 mg/l (measured daily)
- pH 6.8 - 8.3 (measured daily)
- temperature 24 - 26 °C
- hardness 14 - 70 mg/l (as CaCO3) (measured weekly)
- alkalinity 13 - 75 mg/l as (CaCO3) (measured weekly)
- variable photoperiod corresponding to that of Evansville, IN (EPA 1972)

Reliability : (2) valid with restrictions
Basic data given

22.08.2003 (125)

Species : Pimephales promelas (Fish, fresh water)
Endpoint : other: Hatching, fry growth, fry survival
Exposure period : 30 day(s)
Unit : mg/l
NOEC : 3.1
LOEC : 6.9
Analytical monitoring : yes
Method : other: Early life stage test, see TC
Year : 1984
GLP : no data
Test substance : other TS: 2,4-DNT, purity >= 95 %

Result : In a 30 d early life stage study, 2,4-DNT had no appreciable effect on the hatching, fry growth, and survival except at the highest test concentration (6.8 mg/l).

Test condition : - Per egg cup 30 embryos 24 h old, 2 cups per tank
- After hatching fry were counted and transfered into larval rearing chambers
- Larvae were fed brine shrimp nauplii three times daily
- Test performed in duplicate
- Dissolved oxygen 6.8 - 7.8 mg/l (measured daily)
- pH 7.3 - 8.3 (measured daily)
- temperature 24 - 26 °C

- hardness 14.5 - 15.3 mg/l (as CaCO3) (measured weekly)
- alkalinity 19.4 - 23.1 mg/l as (CaCO3) (measured weekly)
-

Reliability : (2) valid with restrictions
Basic data given

15.08.2003 (125)

Species : Gasterosteus aculeatus (Fish, estuary, marine)
Endpoint : other: growth, length, weight
Exposure period : 35 day(s)
Unit : mg/l
NOEC : .77
Analytical monitoring : yes
Method : other: Early life stage test according to Adema, report RIV 627905 001, National Institute of Public Health and Environmental Protection, Bilthoven, Netherlands
Year : 1981
GLP : no data
Test substance : other TS: 2,4-DNT, purity 98 %

Result : LC50: 2.2 mg/l
NOEC (growth): 0.77 mg/l
NOEC (mortality): 1.4 mg/l
NOEC (embryonic stage): 2.5 mg/l
NOEC (mortality + sublethal effects): 1.4 mg/l
EC50 (mortality + sublethal effects): 2.2 mg/l
all values based on measured concentrations
Test condition : eggs < 6 h old; number of organisms: 25 (singular);
semistatic; renewing frequency 3 x per week; circadic 16 h light/ 8 h dark; pH 8.2 +/- 0.2; hardness 11.7 °dH; 19 +/- 1 °C
Reliability : (2) valid with restrictions
test procedure according comparable to guideline (OECD 210); sufficient documentation

08.09.2003 (92)

Species : Poecilia reticulata (Fish, fresh water)
Endpoint : other: growth, length, weight
Exposure period : 28 day(s)
Unit : mg/l
NOEC : 1.8
Analytical monitoring : no
Method : other: according to a dutch national standard method: NEN 6504, 1980 (Wateronderzoek. Bepaling van de acute toxiciteit met Poecilia reticulata)
Year : 1981
GLP : no
Test substance : other TS: 2,4-DNT, 98%

Remark : The toxicity tests have been carried out in two institutes: TNO and RIV (now RIVM).
Result : TNO:
LC50 (1 week) = 19 mg/l, conf.interval: 17-22 mg/l
LC50 (2 weeks) = 12 mg/l, conf.interval: 11-13 mg/l
LC50 (4 weeks) = 5.8 mg/l, conf.interval: 5.2-6.4 mg/l
NOLC (no letal concentration): 1.8 mg/l (mortality after 28d exposure)
NOEC: 1.8 mg/l (mortality and quantitative growth after 28d exposure)
RIV:
LC50 (1 week) = 22 mg/l, conf.interval: 12-39 mg/l
LC50 (2 weeks) = 16 mg/l, conf.interval: 14-17 mg/l
LC50 (4 weeks) = 6.5 mg/l, conf.interval: 4.7-8.9 mg/l

NOLC: 3.2 mg/l (mortality and quantitative growth after 28d exposure)
NOEC: 3.2 mg/l

Test condition : -3-4 week old organisms tested
-testorganisms per group: 10
-5 concentrations tested
-temperature: 23 +/- 2°C
-renewal three times a week
-test criteria: mortality and growth (length, weight)

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

12.09.2003 (95)

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Species : Daphnia magna (Crustacea)
Endpoint : reproduction rate
Exposure period : 21 day(s)
Unit : mg/l
Analytical monitoring : yes
Method : other: Provisional procedure proposed by the Federal Environmental Agency for extended toxicology with Daphnia magna (01.01.1984)
Year : 1988
GLP : no data
Test substance : other TS: 2,4-DNT; 2,6-DNT (both moistened with 10% water)

Method : Determination of NOEC for reproduction rate, mortality and the time of the first appearance of offspring; 21 d

Result : for 2,4-DNT: The NOEC nominal value was 0.04 mg/l.
for 2,6-DNT: The NOEC nominal value was 0.16 mg/l.
Analytical monitoring was performed. But as the NOEC was below the detection limit for 2,4-DNT of 0.05 mg/l and for 2,6-DNT of 0.16 mg/l, the actual concentration could not be determined.
Based on the recovery rates of higher tested concentrations:
-for 2,4-DNT a minimum NOEC of 0.02 mg/l
-for 2,6-DNT a minimum NOEC of 0.06 mg/l was estimated.

Test condition : - Semistatic test, renewal of water after 48 h
- There were four parallel test vessels per concentration level and at least four vessels for the control. Each vessel was filled with 24 h-old Daphnia (1 animal/50 ml). The total number of daphnias per concentration level was 20. Test temperature 25 +/- 1 °C.
- Dilution water: synthetic fresh water, Hardness 2.5 mmol/l Ca + Mg, Na/K-Ratio: 10:1, pH = 8.0 +/- 0.2
- pH-values and oxygen-concentration were measured during the test in two tests-vessels per concentration level. The detected variation of these parameters had no negative influence on the organisms

Reliability : (1) valid without restriction
Test procedure according to a national method proposal. Reported in sufficient detail

Flag : Critical study for SIDS endpoint

07.10.2003 (97) (98)

Species : other aquatic crustacea: Daphnia magna Strauss
Endpoint : reproduction rate
Exposure period : 21 day(s)
Unit : mg/l
NOEC : < .5
EC40 : ca. .5

Analytical monitoring	:	yes
Method	:	other: OECD Guideline 202 (adopted: 04.04.84) "Daphnia sp., Acute Immobilisation Test and Reproduction Test"
Year	:	1986
GLP	:	no
Test substance	:	other TS: 2,4-DNT
Remark	:	0.5 mg/l lowest tested concentration
Result	:	Reduction of reproduction: ca. 40 % at 0.5 mg/l ca. 80 % at 1.58 mg/l
Test condition	:	- semistatic - daphnids fed daily - temperature 20 °C - constant light 400 lux - 4 different testconcentration (0.5 - 15.8 mg/l) - analysis with HPLC
Reliability	:	(2) valid with restrictions guideline study with detailed documentation

08.09.2003 (126)

Species	:	Daphnia magna (Crustacea)
Endpoint	:	other: immobilisation, mortality, reproduction
Exposure period	:	21 day(s)
Unit	:	mg/l
LC50	:	19
Analytical monitoring	:	no
Method	:	other: according to a dutch national standard method: NEN 6502, 1980 (Wateronderzoek. Bepaling van de chronische toxiciteit met Daphnia magna)
Year	:	1981
GLP	:	no
Test substance	:	other TS: 2,4-DNT, 98%
Remark	:	The toxicity tests have been carried out in two institutes: TNO and RIV (now RIVM).
Result	:	TNO: 2 weeks: LC50 20 mg/l, conf. interval: 18-21 mg/l 3 weeks: LC50 19 mg/l, conf. interval: 17-21 mg/l NOLC (no letal concentration)for 2 and 3 weeks: 3.2 mg/l NOLC for F1-generation for 2 weeks: 3.2 mg/l NOEC for 2 and 3 weeks anf the F1-generation test: 0.32 mg/l RIV: 2 weeks: LC50 > 10 mg/l 3 weeks: LC50 > 10 mg/l NOLC (no letal concentration)for 2 and 3 weeks: 3.2 mg/l NOLC for F1-generation for 2 weeks: 3.2 mg/l NOEC for 2 and 3 weeks anf the F1-generation test: 1.0 mg/l
Test condition	:	-test organisms per group: 25 -5 concentrations tested, two replicates -temperature: 19 +/- 1°C -renewal 3 times a week -Daphnias were fed with cells of the unicellular alga Chlorella. -14 day test was performed with F1-generation -test criteria: mortality, immobility and reproduction
Reliability	:	(2) valid with restrictions Study well documented

11.09.2003 (95)

Species	:	Daphnia magna (Crustacea)
Endpoint	:	other: immobilization, growth, length
Exposure period	:	21 day(s)
Unit	:	mg/l
NOEC	:	measured/nominal
LOEC	:	.3 - 10
IC50	:	.6 - 9.6 measured/nominal
Analytical monitoring	:	no
Method	:	other: Dutch Standard Organisation NEN 6502 (1980)
Year	:	1988
GLP	:	
Test substance	:	other TS: 2,3-DNT, 2,4-DNT, 2,6-DNT, 3,4-DNT
Remark	:	The data were published as a dissertation and in a journal
Result	:	The following results were obtained:

Immobilization	log IC50 (µM)	IC50 (mg/l)
2,3-DNT	0.99	1.8
2,4-DNT	0.52	0.6
2,6-DNT	1.72	9.6
3,4-DNT	0.78	1.1

Reproduction	log LRCT*(µM)	LRCT (mg/l)
2,3-DNT	1.24	3.2
2,4-DNT	0.74	1.0
2,6-DNT	1.74	10.0
3,4-DNT	0.24	0.3

Length	log LRCT**(µM)	LRCT(mg/l)
2,3-DNT	0.74	1.0
2,4-DNT	0.74	1.0
2,6-DNT	0.74	1.0
3,4-DNT	0.24	0.3

*LRCT = Lowest rejected concentration tested that significantly (P < 0.01) lowered the population growth constant (rm) after 21 d (similar to LOEC)
**LRCT = Lowest rejected concentration tested that significantly (P < 0.01) lowered the mean length of animals after 21 d of exposure (similar to LOEC)

Test condition :
- Organisms: Freshwater crustacean Daphnia magna cultured in the laboratory according to the Dutch standard NPR 6503 (1980). The culture medium used was Lake Ijssel water.
- Chemical Composition of Lake Ijssel Water (mg/l): Na 59.2, K 6.9, Ca 72.0, Mg 10.7, Mn 0.015, Fe 0.162, Si 4.1, NH4-N 0.13, NO3-N 4.46, NO2-N 0.08, PO4-P 0.28, SO4-- 78.0, HCO3- 159, Cl- 133, Hardness (as CaCO3) 224, Chlorophyll a 0.002, TOC 4.5, pH 8.1. Daphnias were fed with cells of the unicellular alga Chlorella pyrenoidosa (strain: Wisconsin 2005 from Culture Centre of Algae and Protozoa, Cambridge)
- Synthetic test medium was prepared as described in NPR 6503 (1980) with a CaCO3 hardness of 200 mg/l and a pH of 8.4 ± 0.1, saturated with air prior to use
- Daphnia magna was less than 24 h old at start of incubation
- During incubation newborn (0 to 24 h) Daphnias were fed with Chlorella pyrenoidosa (10E+8 cells/l at start of experiment) according to NEN 6502 (1980)
- Incubation: 10 Daphnias per concentration, one animal per jar containing 50 ml test medium; 12 h/d illumination at 20 +/- 0.5 °C
- The medium was renewed daily
- At the end of the experiment (21 d) the length of the daphnids was

determined from the top of the head to the base of the spine using an ocular micrometer.
- Growth was determined in an intermittent-flow system equipped with electric valves. The water was aerated before algal cells and test substance solutions were added. The algal suspension and test substance solutions were delivered separately by a peristaltic pump and mechanical injectors, respectively. The water flow through the 20-liter test vessels was 667 ml/hr. The Chlorella pyrenoidosa concentration was 3x10E+8 cells/liter. An initial dose of the test substance was added to the test vessels 30 min before the experiment started. The test vessels consisted of four compartments, each containing one population. The test was initiated with exponentially growing populations of 20 daphnids composed of cohorts of different ages. The total number of daphnids in each test compartment (biomass) was counted at regular intervals
- The LC50 values, confidence limits, and X2 fit were determined by the method of Litchfield and Wilcoxon (1949). Population growth was analyzed according to Kooyman et al. (1983)

Reliability : (2) valid with restrictions
Test procedure in accordance with national standard methods with acceptable restrictions

19.01.2004 (100) (101)

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

Species	:	Brassica rapa (Dicotyledon)
Endpoint	:	growth
Exposure period	:	14 day(s)
Unit	:	mg/kg soil dw
EC50	:	6.5
Method	:	other: Phytotoxicity Test on a dicotyledonous plant species (Brassica rapa)
Year	:	1984
GLP	:	yes
Test substance	:	other TS: DNT 80/20 (= DNT isomers mixture, CAS 25321-14-6)
Method	:	The test was conducted according to a proposed guideline of BBA (1984) "Phytotoxicity test on a dicotyledenous plant" species (Brassica rapa ssp. rapa (DC.) Metzg.) (EC50, 14 days)
Result	:	growth reduction: 20% at 1 mg/kg 58% at 10 mg/kg 97% at 100 mg/kg 100% at 1,000 mg/kg (nominal concentrations)
Test condition	:	5 seedlings/test vessel; photoperiod 16 h light/8 h dark; radiation intensity: 120 µE/m2/s (8000 lux); temperature 20-21 °C; soil moisture adjusted daily to 60 % of the maximum water capacity
Test substance	:	The CAS-No. of 2,4-DNT is incorrectly reported to be the CAS-No. of DNT 80/20 (DNT isomers mixture, CAS-No. 25321-14-6).
Reliability	:	(2) valid with restrictions Test procedure in accordance with a proposed national standard method
Flag	:	Critical study for SIDS endpoint

19.01.2004 (127)

Species	:	Avena sativa (Monocotyledon)
Endpoint	:	growth
Exposure period	:	14 day(s)
Unit	:	mg/kg soil dw
EC50	:	65
Method	:	other: Phytotoxicity Test on a monocotyledenous plant species (Avena sativa)
Year	:	1990
GLP	:	yes
Test substance	:	other TS: DNT 80/20 (= DNT isomers mixture, CAS 25321-14-6)
Method	:	The test was conducted according to a proposed guideline of BBA (1984) "Phytotoxicity test on a monocotyledenous plant" species (Avena sativa L.) (EC50, 14 days)
Result	:	A slight stimulation of growth was observed at concentrations of 1 and 10 mg/kg, whereas at concentrations of 100 and 1,000 mg/kg growth inhibition of 71 and 98 % was observed (nominal concentrations)
Test condition	:	5 seedlings/test vessel; photoperiod 16 h light/8 h dark; radiation intensity: 120 µE/m2/s (8000 lux); temperature 20-21 °C; soil moisture adjusted daily to 80 % of the maximum water capacity
Test substance	:	The CAS-No. of 2,4-DNT is incorrectly reported to be the CAS-No. of DNT 80/20 (DNT isomers mixture, CAS-No. 25321-14-6).
Reliability	:	(2) valid with restrictions Test procedure in accordance with a proposed national standard method

19.01.2004 (127)

Species	:	Avena sativa (Monocotyledon)
Endpoint	:	growth
Exposure period	:	16 day(s)
Unit	:	mg/l
EC50	:	61
Method	:	OECD Guide-line 208 "Terrestrial Plants, Growth Test"
Year	:	1995
GLP	:	
Test substance	:	other TS: 2,4-DNT, purity 99 %
Result	:	Estimated results: NOEC Growth 10 mg/l NOEC Emergence 100 mg/l NOEC Chlorosis of the leaf tips 1 mg/l
Test condition	:	- Plants: seeds were germinated for 36-48 h, primary root 1-2 mm length - 6 plants per replicate, 4 replicates - Soil: 2.29 org. C, particles < 20 µm 10.2 %, pH 5.6, cation exchange capacity 9.7 +/- 0.3 mval/100 g, total N 0.17 +/- 0.03 %, maximum water capacity 44 % - Each pot contained 300 g (dw) of humid soil (80 % water capacity saturation) amended with the TS - Growth conditions: 20 °C, minimum 7000 lux (16 h) - Daily compensation of humidity loss in soil - 2,4-DNT concentrations: 0, 1, 10, 100, 1000 mg/l - Reference compound: Trichloroacetic acid
Reliability	:	(2) valid with restrictions Study meets generally accepted scientific principles. Basic data given

27.11.2003 (23)

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

Type	:	artificial soil
Species	:	Eisenia fetida (Worm (Annelida), soil dwelling)
Endpoint	:	mortality
Exposure period	:	14 day(s)
Unit	:	mg/kg soil dw
LC50	:	536
Method	:	OECD Guide-line 207 "Earthworm, Acute Toxcity Test"
Year	:	1997
GLP	:	
Test substance	:	other TS: 2,4-DNT
Reliability	:	(2) valid with restrictions Data from handbook or collection of data

17.01.2004 (128)

Type	:	artificial soil
Species	:	other soil dwelling worm: Eisenia fetida andrei
Endpoint	:	mortality
Exposure period	:	14 day(s)
Unit	:	mg/kg soil dw
LC50	:	668
LOEC	:	562
Method	:	other: in accordance to OECD Guideline 207 "Earthworm, Acute Toxicity Test" (4. April 1984)
Year	:	1990
GLP	:	yes
Test substance	:	other TS: DNT 80/20 (= DNT isomers mixture, CAS 25321-14-6)
Result	:	NOEC = 316 mg/kg dry weight substrate (nominal concentration) The weight alterations of the test organisms were statistically evaluated by the U-Test of Wilcoxon, Mann & Whitney (L. Sachs: Angewandte Statistik, Springer Verlag 1978)
Test condition	:	- Artificial Soil: Sand 70 % , peat 10 % (pH 5.5-6), kaolin 20 % - pH 6.0 +/- 0.5 - 1.5 l test vessels - Constant light (400-800 lux) - Relative humidity 70-90 % - Temperature 20 +/- 1 °C
Test substance	:	The CAS-No. of 2,4-DNT is incorrectly reported to be the CAS-No. of DNT 80/20 (DNT isomers mixture, CAS-No. 25321-14-6).
Reliability	:	(2) valid with restrictions Test procedure in accordance with a proposed national standard method
Flag	:	Critical study for SIDS endpoint

19.01.2004 (129)

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

In Vitro/in vivo	:	In vivo
Type	:	Absorption
Species	:	human
Number of animals		
Males	:	
Females	:	
Doses		
Males	:	
Females	:	
Vehicle	:	
Method	:	
Year	:	1985
GLP	:	no data
Test substance	:	
Result	:	Absorption was measured by quantification of excreted DNT metabolites in the urine. The urine of the workers contained more metabolites than would have resulted from the dinitrotoluene present in the inhaled air, indicating dermal absorption.
Test condition	:	The urine samples of 28 workers (20 males and 8 females) of an explosives factory were analysed for the metabolite 2,4-dinitrobenzoic acid by gas chromatography-mass spectrometry. Urine samples were collected during two separate 1-week production campaigns. Routine atmospheric monitoring using personal samples have consistently shown atmospheric DNT levels to be low (0.03 to 0.1 mg/m³) or undetectable.
Test substance	:	technical grade DNT consisting of 76% 2,4-DNT and 20% 2,6-DNT
Reliability	:	(2) valid with restrictions Limited documentation: isomers not specified
Flag	:	Critical study for SIDS endpoint

26.11.2003 (130)

In Vitro/in vivo	:	In vivo
Type	:	Absorption
Species	:	human
Number of animals		
Males	:	
Females	:	
Doses		
Males	:	
Females	:	
Vehicle	:	
Method	:	
Year	:	1983
GLP	:	no data
Test substance	:	
Result	:	The urine of the workers contained more metabolites than would have resulted from the dinitrotoluene present in the inhaled air, indicating dermal absorption. Wiping of skin suspected of being contaminated (mainly hands and forehead) showed levels from "not detected" to 179.5 µg 2,4-DNT.
Test condition	:	Urine of 17 workers (14 males and 3 females) exposed to the technical-grade compound at a DNT manufacturing plant was collected over 72 hours. Urine samples were analysed by gas chromatography-mass

spectrometry. Absorption was measured by quantification of excreted DNT metabolites in the urine.
Additionally, hands and foreheads of 8 workers were wiped and the 2,4-DNT amount on skin surfaces was analysed.

Test substance : technical-grade material:
76.4% 2,4-DNT, 18.8% 2,6-DNT and 4.8% other isomers

Reliability : (2) valid with restrictions
limited documentation

Flag : Critical study for SIDS endpoint

26.11.2003 (131)

In Vitro/in vivo :
Type : Metabolism
Species :
Number of animals
Males :
Females :
Doses
Males :
Females :
Vehicle :
Method :
Year :
GLP :
Test substance : other TS: dinitrotoluenes (not further specified)

Remark : Dinitrotoluenes are metabolized in the liver to dinitrobenzylalkohol, which is then conjugated to form a glucuronide conjugate that is excreted in bile or urine. This conjugate is thought to be hydrolyzed by intestinal microflora and subsequently reduced to a toxic metabolite or the precursor of a toxic metabolite.

Reliability : (2) valid with restrictions
Data from handbook or collection of data

Flag : Critical study for SIDS endpoint

26.11.2003 (132)

In Vitro/in vivo : In vivo
Type : Excretion
Species : human
Number of animals
Males :
Females :
Doses
Males :
Females :
Vehicle :
Method :
Year : 1983
GLP : no data
Test substance :

Result : The main metabolites found in human urine were 2,4-dinitrobenzoic acid (52.5% in men and 28.8% in woman) and 2-amino-4-nitrobenzoic acid (about 37% in men and woman). Furthermore, 2,4- and 2,6-dinitrobenzyl glucuronide (9.5% in men and 33.3% in woman) and 2(N-acetyl)amino-4-nitrobenzoic acid (0.8% in men and 0.3% in woman) were found. In all, the amount by which men exceeded women with respect to the dinitrobenzoic acids is almost exactly that by which women exceeded men in regard to the dinitrobenzyl glucuronides.

Test condition	:	Urine of 17 workers (14 males and 3 females) exposed to the technical-grade compound at a DNT manufacturing plant was collected over 72 hours. Urine samples were analysed by gas chromatography-mass spectrometry. Estimates of the maximum one-day exposure incurred by a participant in this study ranged from 0.24 to 1.00 mg/kg bw of technical-grade DNT.
Test substance	:	technical-grade material: 76.4% 2,4-DNT, 18.8% 2,6-DNT and 4.8% other isomers
Reliability	:	(2) valid with restrictions limited documentation
Flag	:	Critical study for SIDS endpoint

26.11.2003 (131)

In Vitro/in vivo	:	In vivo
Type	:	Excretion
Species	:	human
Number of animals		
Males	:	
Females	:	
Doses		
Males	:	
Females	:	
Vehicle	:	
Remark	:	Exposure to TNT and DNT.
Result	:	Air analyses yielded maximum concentrations of 20 µg/m³ for 2,4-DNT. The maximum concentrations in the urine of workers regularly exposed amounted to 2.1 µg/l of 2,4-DNT, 95.0 µg/l of 2,4-dinitrobenzoic acid, and 3.6 µg/l of 2,6-DNT. In 63 persons TNT or DNT or metabolite concentrations above the analytical detection limit were found in urine. These persons reported more frequently symptoms like bitter taste, burning eyes, and discoloration of the skin and hair than persons (n= 19) without detectable exposure.
Test condition	:	82 employees from a mechanical plant (dismantling of military waste) in Germany were studied, of whom 51 were regularly expopsed to ammunition containing TNT and DNT, 19 occasionally, and 12 not at all. To quantify the internal exposure 2,6-DNT, 2,4-DNT, and its main metabolite 2,4-dinitrobenzoic acid were determined in urine specimens of all subjects using gas chromatographic/mass spectrometric procedures. (also TNT and metabolite adducts were measured)
Test substance	:	technical grade DNT (described as a mixture of approx. 71-77% 2,4-DNT and 18-20% 2,6-DNT)
Reliability	:	(2) valid with restrictions Limited documentation, exposure to TNT and DNT
Flag	:	Critical study for SIDS endpoint

26.11.2003 (133)

In Vitro/in vivo	:	In vivo
Type	:	Excretion
Species	:	human
Number of animals		
Males	:	
Females	:	
Doses		
Males	:	
Females	:	
Vehicle	:	
Method	:	
Year	:	1985
GLP	:	no data

Test substance	:	
Remark	:	In male rats given 2,4-DNT the major metabolites excreted in urine were 2,4-dinitrobenzoid acid (44%) and 2,4-dinitrobenzyl glucuronide (27%). Females excrete equal amounts of the two metabolites (39%).
Result	:	The main metabolites found in human urine were 2,4-dinitrobenzoic acid and 2-amino-4-nitrobenzoic acid, accounting for 74 to 86% of the DNT metabolites detected. Furthermore, 2,4-DNT, 2,6-DNT, 2,6-dinitrobenzoic acid, 2,4- and 2,6-dinitrobenzyl glucuronide and 2(N-acetyl)amino-4-nitrobenzoic acid were found. The dinitrobenzyl glucuronide was a major metabolite in only one subject, a female. Excretion of metabolites peaked near the end of the workshift, but declined to either very low or undetectable concentrations by the start of work the following day. The calculated half-times for elimination of total DNT-related material detected in urine ranged from 0.9 to 2.7 hours (1. men: 0.88 hours, 2. men: 2.63 hours, woman: 1. measure 2.29 hours and 2. measure 2.76 hours), and those of individual metabolites from 0.8 to 4.5 hours.
Test condition	:	Urine of 17 workers (14 males and 3 females) exposed to the technical-grade compound at a DNT manufacturing plant was collected over 72 hours. Urine samples were analysed by gas chromatography-mass spectrometry. Estimates of the maximum one-day exposure incurred by a participant in this study ranged from 0.24 to 1.00 mg/kg bw of technical-grade DNT. Urine from 3 workers (2 males and 1 female) contained significantly greater amounts of DNT-metabolites and were used for making estimations of the half-times for elimination of the metabolites.
Test substance	:	technical-grade material: 76.4% 2,4-DNT, 18.8% 2,6-DNT and 4.8% other isomers
Reliability	:	(2) valid with restrictions limited documentation
Flag	:	Critical study for SIDS endpoint

30.03.2004 (134)

In Vitro/in vivo	:	In vivo
Type	:	Excretion
Species	:	human
Number of animals		
Males	:	
Females	:	
Doses		
Males	:	
Females	:	
Vehicle	:	
Method	:	
Year	:	1985
GLP	:	no data
Test substance	:	
Result	:	Metabolite concentrations in urine were extremely low or non-detectable prior to starting work at the beginning of the working week, but post-shift urine samples contained a mean 2,4-DNBA level of 17 mg/l. 2,4-DNBA was shown to be the major known metabolite which is excreted in human urine. The weekly mean concentration for post-shift urine samples for all workers was 17 mg/l (standard deviation 9.8 mg/l).
Test condition	:	The urine samples of 28 workers (20 males and 8 females) of an explosives factory were analysed for the metabolite 2,4-dinitrobenzoic acid by gas chromatography-mass spectrometry. Urine samples were collected during two separate 1-week production campaigns. Urine samples were also analysed for other known metabolites.

Routine atmospheric monitoring using personal samples have consistently shown atmospheric DNT levels to be low (0.03 to 0.1 mg/m³) or undetectable.

Test substance : technical grade DNT consisting of 76% 2,4-DNT and 20% 2,6-DNT
Reliability : (2) valid with restrictions
limited documentation; isomers not fully specified
Flag : Critical study for SIDS endpoint
26.11.2003 (130)

In Vitro/in vivo : In vivo
Type : Toxicokinetics
Species : human
Number of animals
Males :
Females :
Doses
Males :
Females :
Vehicle :
Method :
Year : 2000
GLP : no data
Test substance : other TS: TNT and other explosives (no further information)

Result : Nitroaromatic hemoglobin adducts were detectable throughout all groups examined. No significant differences could be detected between the exposed and the reference subjects.
Test condition : The aim of this study was to evaluate if increased blood levels of hemoglobin adducts of nitroaromatic compounds are detectable in the residents of contaminated area and in cleaning-up workers. Blood samples of 18 (Hirschagen/Waldhof) and 45 (Stadtallendorf) subjects living in an area formerly used as an industrial area for the production of trinitrotoluene and other explosives (no information on DNT exposure) were analysed for the concentrations of hemoglobin adducts of related metabolites. Reference groups of 18 and 48 reference subjects, respectively, living in non-contaminated residential areas nearby, were examined.
Reliability : (2) valid with restrictions
study well documented, however, test substance not specified as technical grade DNT
26.11.2003 (135) (136)

5.1.1 ACUTE ORAL TOXICITY

Type : LD50
Value : = 660 mg/kg bw
Species : rat
Strain : Wistar
Sex : male
Number of animals : 10
Vehicle : other: lutrol
Doses : 0.1, 0.4, 0.8, 1.5, 2.0 g/kg
Method : other
Year : 1978
GLP : no
Test substance : other TS: DNT 80/20 (ca. 80% 2,4-DNT/ca. 20% 2,6-DNT) and other isomers

Result : MORTALITY:

- Number of deaths at each dose: 0, 4, 5, 7, 10 at 0.1, 0.4, 0.8, 1.5, 2.0 g/kg
- Time of death: 3-7 d at 0.4 g/kg, 3 h - 5d at 0.8 g/kg, 2-6 d at 1.5 g/kg, 2-3 d at 2.0 g/kg
CLINICAL SIGNS: diuresis, diarrhoe, weight loss, shaggy fur, loss of hair

Test condition : TEST ORGANISMS:
- Weight at study initiation: 160-180 g
ADMINISTRATION:
- Doses: 0.1, 0.4, 0.8, 1.5, 2.0 g/kg via gavage
- Post dose observation period: 14 d
EXAMINATIONS: clinical symptoms

Reliability : (2) valid with restrictions
limited documentation

Flag : Critical study for SIDS endpoint

15.01.2004 (137)

Type : LD50
Value : = 268 mg/kg bw
Species : rat
Strain : no data
Sex : no data
Number of animals :
Vehicle : other: corn oil
Doses : no data
Method : other: evaluation within 48 hours
Year : 1980
GLP : no
Test substance :

Test substance : technical grade DNT: same lot as in CIIT bioassay (CIIT, 1978)
current analysis:
75.8 % 2,4-DNT
19.5 % 2,6-DNT
2.5 % 3,4-DNT
1.5 % 2,3-DNT
0.7 % 2,5-DNT
0.04 % 3,5-DNT

Reliability : (2) valid with restrictions
limited documentation; observation period only 48 hours; strain, sex and number of treated animals not given

Flag : Critical study for SIDS endpoint

26.11.2003 (138)

Type : LD50
Value : = 1000 mg/kg bw
Species : rat
Strain : other: white rats
Sex :
Number of animals :
Vehicle : other: vegetable oil
Doses :
Method :
Year : 1977
GLP : no
Test substance : other TS: DNT (not further specified)

Remark : no infomation on test protocol, number of animals tested, observation

period, tested dose levels
Result : clinical symptoms: disturbances of central nervous system, dyspnea, cyanosis
Reliability : (4) not assignable
limited documentation; no infomation on test protocol, number of animals tested, observation period, tested dose levels; TS not specified; secondary literature
26.11.2003 (1) (139)

Type : LD50
Value : = 2010 mg/kg bw
Species : rat
Strain : Wistar
Sex : male
Number of animals : 10
Vehicle : other: lutrol
Doses : 0.5, 1.0, 1.5, 2.5, 3.1, 5.0 g/kg bw
Method : other
Year : 1978
GLP : no
Test substance :

Result : MORTALITY:
- Number of deaths at each dose: 0, 1, 2, 7, 8, 10 at 0.5, 1.0, 1.5, 2.5, 3.1, 5.0 g/kg
- Time of death: 5 d at 1.0 g/kg, 2 d at 1.5 g/kg, 2-7 d at 2.5 g/kg, 3 h - 10 d at 3.1 g/kg, 2 d at 5.0 g/kg
CLINICAL SIGNS: sedation, weight loss
Test condition : TEST ORGANISMS:
- Weight at study initiation: 160-180 g

ADMINISTRATION:
- Doses: 0.5, 1.0, 1.5, 2.5, 3.1, 5.0 g/kg via gavage
- Post dose observation period: 14 d
EXAMINATIONS: clinical symptoms
Test substance : DNT X consisting of:
58-73% dinitrotoluoles (isomers not specified)
18-33% dinitroxylenes
4-16% dinitroethylbenzenes
(internal information of Bayer AG)
Reliability : (4) not assignable
limited documentation; test substance different to DNT technical grade
26.11.2003 (140)

Type : LD50
Value : > 5000 mg/kg bw
Species : rat
Strain : Wistar
Sex : male
Number of animals : 10
Vehicle : other: lutrol
Doses : 3.1, 5.0 g/kg bw
Method : other
Year : 1978
GLP : no
Test substance :

Result : MORTALITY:
- Time of death: -

- Number of deaths at each dose: 0, 0 at 3.1, 5.0 g/kg
CLINICAL SIGNS: all animals showed sedation, weight loss, bloody eyes

Test condition : TEST ORGANISMS:
- Weight at study initiation: 160-180 g

ADMINISTRATION:
- Doses: 3.1, 5.0 g/kg via gavage
- Doses per time period: single
- Volume administered or concentration: no data
- Post dose observation period: 14 d
EXAMINATIONS: clinical symptoms

Test substance : DNT X consisting of:
58-73% dinitrotoluoles (isomers not specified)
18-33% dinitroxylenes
1-2% dinitroethylbenzenes
(internal information of Bayer AG)

Reliability : (4) not assignable
limited documentation; test substance different to DNT technical grade

26.11.2003 (141)

Type : LD50
Value : > 500 mg/kg bw
Species : mouse
Strain : DBA
Sex : male
Number of animals :
Vehicle : other: corn oil
Doses : no data
Method :
Year : 1980
GLP : no
Test substance :

Remark : data obtained in the course of a dominant lethal assay; mice were treated with 250 mg/kg bw at two consecutive days; no further experimental details on LD50 investigation
Result : no mortality at 48 hours posttreatment
Test substance : technical grade DNT: same lot as in CIIT bioassay (CIIT, 1978)
current analysis:
75.8 % 2,4-DNT
19.5 % 2,6-DNT
2.5 % 3,4-DNT
1.5 % 2,3-DNT
0.7 % 2,5-DNT
0.04 % 3,5-DNT
Reliability : (2) valid with restrictions
limited documentation; observation period not given; number of treated animals not given
Flag : Critical study for SIDS endpoint

26.11.2003 (138)

Type : LD50
Value : = 1100 mg/kg bw
Species : mouse
Strain : other: ddY
Sex : male
Number of animals : 10
Vehicle : other: olive oil

Doses	:	6 increasing dose levels, no further data
Method	:	other: calculation of LD50 according to Litchfield and Wilcoxon (1949)
Year	:	1989
GLP	:	no data
Test substance	:	other TS: DNT (CAS-No. 25321-14-6, highest purity available)
Result	:	MORTALITY: - Time of death: no data - Number of deaths at each dose: no data CLINICAL SIGNS: clonic convulsions, dyspnea POTENTIAL TARGET ORGANS: no changes in tissues at anatomical examination
Test condition	:	TEST ORGANISMS: - Age: 6 weeks ADMINISTRATION: - Doses: 6 dose levels - Doses per time period: single - Post dose observation period: 14 days EXAMINATIONS: clinical signs, anatomical examination at death or sacrifice
Reliability	:	(2) valid with restrictions limited documentation, however, study meets generally accepted scientific principles, acceptable for assessment
Flag	:	Critical study for SIDS endpoint

26.11.2003 (142)

Type	:	LD50
Value	:	= 750 mg/kg bw
Species	:	mouse
Strain	:	other: ddY
Sex	:	female
Number of animals	:	10
Vehicle	:	other: olive oil
Doses	:	6 increasing dose levels, no further data
Method	:	other: calculation of LD50 according to Litchfield and Wilcoxon (1949)
Year	:	1989
GLP	:	no data
Test substance	:	other TS: DNT (CAS-No. 25321-14-6, highest purity available)
Result	:	MORTALITY: - Time of death: no data - Number of deaths at each dose: no data CLINICAL SIGNS: clonic convulsions, dyspnea POTENTIAL TARGET ORGANS: no changes in tissues at anatomical examination
Test condition	:	TEST ORGANISMS: - Age: 6 weeks ADMINISTRATION: - Doses: 6 dose levels - Doses per time period: single - Post dose observation period: 14 days EXAMINATIONS: clinical signs, anatomical examination at death or sacrifice
Reliability	:	(2) valid with restrictions limited documentation, however, study meets generally accepted scientific principles, acceptable for assessment
Flag	:	Critical study for SIDS endpoint

26.11.2003 (142)

Type	:	LD50
Value	:	= 1250 mg/kg bw
Species	:	mouse
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	other: vegetable oil
Doses	:	
Method	:	
Year	:	1977
GLP	:	no
Test substance	:	other TS: DNT (not further specified)
Remark	:	no infomation on test protocol, number of animals tested, observation period, tested dose levels
Result	:	clinical symptoms: disturbances of central nervous system, dyspnea, cyanosis
Reliability	:	(4) not assignable limited documentation; no infomation on test protocol, number of animals tested, observation period, tested dose levels; TS not specified; secondary literature

26.11.2003 (1) (139)

Type	:	LD50
Value	:	= 1300 mg/kg bw
Species	:	guinea pig
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	other: vegetable oil
Doses	:	
Method	:	
Year	:	1977
GLP	:	no
Test substance	:	other TS: DNT (not further specified)
Remark	:	no infomation on test protocol, number of animals tested, observation period, tested dose levels
Result	:	clinical symptoms: disturbances of central nervous system, dyspnea, cyanosis
Reliability	:	(4) not assignable limited documentation; ; no infomation on test protocol, number of animals tested, observation period, tested dose levels; TS not specified; secondary literature

26.11.2003 (1) (139)

5.1.2 ACUTE INHALATION TOXICITY

Type	:	LC50
Value	:	
Species	:	rat
Strain	:	Fischer 344
Sex	:	male/female
Number of animals	:	
Vehicle	:	
Doses	:	26, 196, 473, 694 mg/m³
Exposure time	:	6 hour(s)
Method	:	

Year	:	1991
GLP	:	no data
Test substance	:	other TS: 2,6-DNT
Remark	:	Study performed with 2,6-DNT.
Result	:	clinical signs: from 196 mg/m³ onwards: respiratory disorders, ataxia, lethargy and deaths occurred within the observation period up to 14 days after exposure. No evidence of a significant methemoglobin production. LC50 rat, male (6h): 240 (80-400) mg/m³ LC50 rat, female (6h): 660 (490-839) mg/m³ LC50 rat, male/female (6h): 430 (230-630) mg/m³ Converted to 4h-exposure LC50 rat: 0.36 mg/l/4h
Test condition	:	No. of animals: 5/sex/dose exposure: head-nose exposure vehicle: negative control (air - or acetone/polyethylene glycol 200), 26 mg/m³ (vapour), 196-694 mg/m³ (aerosol in acetone/polyethylene glycol 200) particle size: aerodynamic diameter of > 5.5 µm, 85-96% respirable
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

23.03.2004 (8)

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD50
Value	:	> 2500 mg/kg bw
Species	:	rat
Strain	:	Wistar
Sex	:	male/female
Number of animals	:	5
Vehicle	:	
Doses	:	2500 mg/kg bw
Method	:	other: according to Noakes and Sanderson, Br. J. Ind. Med. 26, 59, 1969
Year	:	1980
GLP	:	no
Test substance	:	other TS: pure 2,4-DNT
Method	:	Hair was removed from the back and flanks (about 6x8 cm); chemical was applied evenly over the skin; the trunk of the rat was encircled with a plaster in a double layer; contact time was followed by decontamination with a detergent-and-water wash.
Remark	:	No studies with technical grade DNT available
Result	:	No deaths, no signs of intoxication, no changes of gross pathology.
Test condition	:	TEST ORGANISMS: - 5/sex - Weight at study initiation: 165-176 g APPLICATION: - Doses: 2.5 g/kg - contact time presumably 4 hours - Post dose observation period: 14 d EXAMINATIONS: clinical symptoms, gross pathology
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment, pure 2,4-DNT
Flag	:	Critical study for SIDS endpoint

26.11.2003 (143)

Type	:	other
Value	:	
Species	:	rabbit
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	
Year	:	1908
GLP	:	
Test substance	:	other TS: DNT (no further information)
Result	:	After single dermal application of 1, 2, or 4 g to rabbits, there were no toxic effects

26.11.2003 (144)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Type	:	LD50
Value	:	> 500 mg/kg bw
Species	:	mouse
Strain	:	DBA
Sex	:	male
Number of animals	:	
Vehicle	:	other: corn oil
Doses	:	no data
Route of admin.	:	i.p.
Exposure time	:	
Method	:	
Year	:	1980
GLP	:	no
Test substance	:	
Remark	:	data obtained in the course of a dominant lethal assay; mice were treated with 250 mg/kg bw at two consecutive days; no further experimental details on LD50 investigation
Result	:	no lethality at 48 hours posttreatment
Test substance	:	technical grade DNT: same lot as in CIIT bioassay (CIIT, 1978), current analysis: 75.8 % 2,4-DNT 19.5 % 2,6-DNT 2.5 % 3,4-DNT 1.5 % 2,3-DNT 0.7 % 2,5-DNT 0.04 % 3,5-DNT
Reliability	:	(2) valid with restrictions limited documentation; observation period and number of treated animals not given

12.11.2003 (138)

5.2.1 SKIN IRRITATION

Species	:	rabbit
Concentration	:	500 mg

Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 2
Vehicle : water
PDII :
Result : not irritating
Classification :
Method : other
Year : 1979
GLP : no
Test substance : other TS: DNT 80/20 (ca. 80% 2,4-DNT/ca. 20% 2,6-DNT) and other isomers

Result : Score of 0 for all observation timepoints
Test condition : TEST ANIMALS:
- Strain: New Zealand white
- Sex: male/female
- Weight at study initiation: 3-4 kg
ADMINISTRATION/EXPOSURE
- Preparation of test substance: pasted with water
- Area of exposure: interior side of right ear
- Occlusion: plaster
- Postexposure period: 7 d
- Removal of test substance after 24 hrs: water and soap/oil
EXAMINATIONS
- Scoring for erythema and oedema
- Examination time points: day 0, day 2, day 7
Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles, acceptable restrictions in documentation, acceptable for assessment
Flag : Critical study for SIDS endpoint
15.01.2004 (145)

Species : rabbit
Concentration : .5 other: ml
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 2
Vehicle : other: none
PDII :
Result : not irritating
Classification :
Method : other
Year : 1978
GLP : no
Test substance :

Result : Score of 0 for all observation timepoints.
Test condition : TEST ANIMALS:
- Strain: New Zealand white
- Sex: male/female
- Weight at study initiation: 3-4 kg
ADMINISTRATION/EXPOSURE
- Area of exposure: interiour side of right ear
- Occlusion: plaster
- Postexposure period: 7 d
- Removal of test substance with water and soap/oil
EXAMINATIONS

- Scoring for erythema and oedema
- Examination time points: days 0, 2, 7

Test substance : DNT X consisting of:
58-73% dinitrotoluoles (isomers not specified)
18-33% dinitroxylenes
4-16% dinitroethylbenzenes
(internal information of Bayer AG)

Reliability : (4) not assignable
limited documentation; test substance different to DNT technical grade

26.11.2003 (146)

5.2.2 EYE IRRITATION

Species : rabbit
Concentration : 50 mg
Dose :
Exposure time :
Comment :
Number of animals : 2
Vehicle : water
Result : not irritating
Classification :
Method : other
Year : 1979
GLP : no
Test substance : other TS: DNT 80/20 (ca. 80% 2,4-DNT/ca. 20% 2,6-DNT) and other isomers

Result : At 24 hours slight erythema in one animal. Score of 0 for all other observation timepoints.

Test condition : TEST ANIMALS:
- Strain: New Zealand white
- Sex: male/female
- Weight at study initiation: 3-4 kg

ADMINISTRATION/EXPOSURE
- Preparation of test substance: pasted with water
- Amount of substance instilled in left eye: 50 mg
- Postexposure period: 7 d

EXAMINATIONS
- Examination time points: days 0, 1, 2, 3, 7
- Examination of cornea, iris, erythema, oedema
- Ophtalmoscopic examination: no data
- Scoring system: no data
- Observation period: 7 d

Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles, acceptable restrictions in documentation, acceptable for assessment

Flag : Critical study for SIDS endpoint

15.01.2004 (145)

Species : rabbit
Concentration : undiluted
Dose : .1 ml
Exposure time :
Comment :

Number of animals	:	2
Vehicle	:	none
Result	:	
Classification	:	not irritating
Method	:	other
Year	:	1978
GLP	:	
Test substance	:	
Result	:	At 24 hours slight erythema in one animal. Score of 0 for all other obeservation timepoints.
Test condition	:	TEST ANIMALS: - Strain: New Zealand white - Sex: male/female - Weight at study initiation: 3-4 kg ADMINISTRATION/EXPOSURE - Amount of substance instilled in left eye: 0.1 ml - Postexposure period: 7 d EXAMINATIONS - Examination at days 0, 1, 2, 7 - Ophtalmoscopic examination: no data - Examination of cornea, iris, erythema, oedema - Observation period: 7 d
Test substance	:	DNT X consisting of: 58-73% dinitrotoluoles (isomers not specified) 18-33% dinitroxylenes 4-16% dinitroethylbenzenes (internal information of Bayer AG)
Reliability	:	(4) not assignable limited documentation; test substance different to DNT technical grade

26.11.2003 (146)

5.3 SENSITIZATION

Type	:	Guinea pig maximization test
Species	:	guinea pig
Number of animals	:	
Vehicle	:	no data
Result	:	
Classification	:	
Method	:	
Year	:	1979
GLP	:	
Test substance	:	other TS: 2,4- and 2,6-DNT, purity 98%
Method	:	Method: According to Magnusson B., Kligman, A.M., J. Invest. Derm 52, 268-276 (1969) No. of animals: 10/TS No further information.
Result	:	2,4-DNT: negative (0/10 animals responded) 2,6-DNT: mild reponse (2/10 animals responded).
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

23.03.2004 (147)

Type	:	other: patch-test and photo-patch-test
Species	:	human
Number of animals	:	

Vehicle	:	peanut oil
Result	:	
Classification	:	
Method	:	other: Standard photo-patch test procedure of the Scandinavian Photodermatitis Research Group (Contact Dermatitis, 155-158, 1982)
Year	:	1985
GLP	:	no data
Test substance	:	other TS: DNT (isomers not specified; purity not given)
Result	:	Patch-test: 10 control persons: all tests with DNT gave negative results 1 patient: negative reaction to DNT Photo-patch-test: 5 control persons: all tests with DNT gave negative results 1 patient: DNT 0.5 % (+++) and 1.0 % (+++)
Test condition	:	Patient: A 40-year-old man, who had worked as a rock-blaster for 10 years, developed dermatitis on his hands after contamination with dynamite powder during the summer only; during winter months dermatitis healed. Patch-test: Finn Chamber technique, application of ICDRG standard test substances. DNT: 0.5 and 1.0 % in peanut oil Photo-Patch-Test: Test substances, applied in duplicate, were removed after 24 hours and irradiated on one side by 5 Joules of UVA light. Readings were done 48 hours after illumination. Photo-patch tests and patch tests with the test substances were negative in control groups of 5 and 10 individuals, respectively.
Conclusion	:	DNT was negative in patch tests and photo-patch tests with 10 and 5 healthy humans, respectively. DNT without UVA irradiation did not induce contact allergy in a single patient with dermatological lesion. However, this patient developed photosensitisation reaction when DNT exposed skin was illuminated with UVA. Exposure to UVA was the prerequisite for an allergic reaction to DNT.
Reliability	:	(2) valid with restrictions Test substance: isomers not specified and purity not given.
Flag	:	Critical study for SIDS endpoint

12.11.2003 (148)

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-acute
Species	:	rat
Sex	:	male/female
Strain	:	Fischer 344
Route of admin.	:	oral feed
Exposure period	:	4 w
Frequency of treatm.	:	continuously
Post exposure period	:	no
Doses	:	about 37.5, 75, 150 mg/kg bw and day
Control group	:	yes, concurrent no treatment
LOAEL	:	= 37.5 mg/kg bw
Method	:	other:

Year : 1977
GLP : no
Test substance : other TS: DNT (technical grade), see TS

Remark : Pilot Study with limited number of evaluated endpoints
Result : TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:
- Mortality and time to death: none
- Clinical signs: no dose-dependent effects
- Body weight gain: dose dependently reduced by 22%/16% (m/f 37.5 mg/kg), 58%/36% (m/f 75 mg/kg) and body weight loss in the 150 mg/kg groups (-22.1g/-1.8g) compared to control
- Food consumption: dose dependently decreased by 8%/2% (m/f 37.5 mg/kg), 29%/12% (m/f 75 mg/kg), and 50%/44% (m/f 150 mg/kg)
- Haematology: reticulocytes (up to 6.2 or 2.8-times over control for males and females of the highest dose groups, respectively) and Heinz bodies (up to 5.25% in males and 0.14% in females of the highest dose group in comparison to 0% in controls) significantly and dose dependently elevated for all dose groups; methemoglobin: significantly elevated in high-dose males (2.3-fold) and low- and high-dose females (up to 3.5-fold), mid-dose females only slight elevation
- Organ weights: no data
- Gross pathology: males of all dose groups (but without dose-dependence) and high dose females: spleen: darkening, enlargement, thickening, rough or granular surface; liver: discoloration, mottled appearance, rough or granular surface; kidneys: discoloration; lungs: mottled discolored areas
- Histopathology: no data

Test condition : ANIMALS
- Age: about 9 w
- Weight at study initiation:
males: 159-195 g; females: 121-138 g
- Number of animals: 10/sex/group
ADMINISTRATION / EXPOSURE
- Duration of test/exposure: 4 w
- Type of exposure: oral feeding
- Post exposure period: none
- Vehicle: food, fresh diets were prepared weekly
- Concentration in vehicle: no data
- Doses: 37.5, 75, 150 mg/kg bw/d
- Control: basal diet
- compound consumption: low dose: ranged from 19-23 mg/kg bw/d at week 1 to ca. 36 mg/kg bw/d at week 4; mit dose: ranged from 36-44 mg/kg bw/d at week 1 to 74-76 mg/kg bw/d at week 4; high dose: ranged from 67-72 mg/kg bw/d at week 1 to 154-234 mg/kg bw/d at week 4
CLINICAL OBSERVATIONS AND FREQUENCY:
- Clinical signs: weekly
- Mortality: twice daily
- Body weight: weekly
- Food consumption: weekly
- Water consumption: no data
- Ophthalmoscopic examination: no data
- Haematology: blood samples on days 27 (females) and 28 (males); methemoglobin, reticulocyte count, Heinz body formation
- Biochemistry: no data
- Urinalysis: no data
- gross pathology: lungs, liver, spleen, kidney, ovaries, vagina (only organs with gross pathological findings listed)
ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):

- Macroscopic: lungs, liver, spleen, kidney, ovaries, vagina
- Microscopic: none
STATISTICAL METHODS: analysis of variance, Student's t-test, Cochran's approximation

Test substance : technical grade DNT:
2,4-DNT 76.49 %
2,6-DNT 18.83 %
2,5-DNT 0.65 %
3,5-DNT 0.04 %
2,3-DNT 1.54 %
3,4-DNT 2.43 %

Reliability : (2) valid with restrictions
Limited documentation; pilot study: no determination of organ weights, biochemistry, urinanalysis, histopathology

Flag : Critical study for SIDS endpoint

26.11.2003 (149)

Type : Chronic
Species : rat
Sex : male/female
Strain : Fischer 344
Route of admin. : oral feed
Exposure period : 104 w
Frequency of treatm. : continuously
Post exposure period : no
Doses : 3.5, 14, 35 mg/kg bw
Control group : yes, concurrent no treatment
LOAEL : = 3.5 mg/kg bw
Method : other
Year : 1978
GLP : no
Test substance : other TS: technical grade DNT (see TS)

Remark : See also chapter 5.7 for tumor findings and 5.8.1 for fertility.
In the high-dose group all surviving rats were sacrificed prematurely at week 55 because of histopathologic findings noted at the week 52 sacrifice.

Result : mortality: control: 10/130 (m), 12/130 (f); 3.5 mg: 14/130 (m), 15/130 (f); 14 mg: 48/130 (m), 9/130 (f); 35 mg (at week 55): 8/130 (m), 2/130 (f); clinical signs: hunched, thin, and/or bloated appearance (all groups); increase in the number of palpable nodules and/or tissue masses, beginning at week 66 (14, 35 mg); a dose-related decrease in mean body weight gain up to week 52 (m/f) and a dose-related decrease in mean body weight values during weeks 54 - 104 (m/f); food consumption was dose-dependently reduced in treated males predominantly during weeks 5-20 and in females during weeks 50 - 78; 35 mg (52 or 55 week exposure): mean body weight gain in week 50 was only about 50% (m) and 57% (f) of control; increase in serum GPT (m/f), blood urea nitrogen and alkaline phosphatase (m), decreased hemoblobin content (m), erythrocyte count (m), and treatment related morphological changes in erythrocytes; increased reticulocyte (m) and leucocyte (m) count, mean corpuscular volume (m, females only up to week 26), and mean corpuscular hemoglobin (m); methemoglobin was increased in males and decreased in females at week 26; absolute liver weights (m: 215%; f: 55%) and relative liver weights (m: 270%; f: 96%) increased; absolute kidney weights increased by about 12% in males; decreases in relative weights of other organs in line with body weight reduction; hepatotoxic changes (m/f) in nearly all animals, foci/areas of cell alteration, hyperbasophilia and megalocytosis of hepatocytes, vacuolation and

necrosis of individual hepatocytes; exacerbation of chronic interstitial nephritis and spontaneous cardiomyopathy, increased red cell turnover (hemosiderosis, extramedullary hematopoiesis), increased interstitial pigment in pancreas; testicular degeneration (in 15/20 animals) plus hypospermatogenesis (in 14/20)
14 mg (104 week exposure): mean body weight gain in week 102 was only about 66% (m) and 74% (f) of control; decrease in hematocrite (m), hemoglobine content (m), and erythrocyte count (m); increase in reticulocyte (m), leucocyte (m/f) count only at week 52 and 78; methemoglobin was decreased in females at week 26 and increased at week 78; significant increase in serum GPT (m/f) and blood urea nitrogen (m) and decrease in serum phosphatase (f); absolute liver weights (m: 62%; f: 65%) and relative liver weights increased; absolute kidney weights increased by about 11% in males and 16% in females; absolute weights of ovaries enhanced by 57%, however, no dose dependent effect; hepatotoxic changes (m/f in nearly all animals, foci/areas of cell alteration, hyperbasophilia and megalocytosis of hepatocytes, vacuolation and necrosis of individual hepatocytes), exacerbation of chronic interstitial nephritis and degenerative changes in the adrenal glands
3.5 mg (104 week exposure): mean body weight gain in week 104 was about 92% (m) and 86% (f) of control; absolute liver weights (m: 14%; f: 17%) and relative liver weights increased; absolute testes weight enhanced by 13%, however, no dose dependent effect; hepatotoxic changes (only m: foci/areas of cell alteration, hyperbasophilia and megalocytosis of hepatocytes, vacuolation and necrosis of individual hepatocytes); exacerbation of chronic interstitial nephritis

Test condition : ANIMALS
- Weight at study initiation:
males: 106-208 g; females: 95-157 g
- Number of animals: 130/sex/group
ADMINISTRATION / EXPOSURE
- Duration of test/exposure: 104 w
- Type of exposure: oral feeding
- Post exposure period: none
- Vehicle: food
- Concentration in vehicle:
- Doses: about 3.5, 14, 35 mg/kg bw/d
- Control: basal diet
- Compound consumption was calculated based on target dose levels (weekly from weeks 1-14, biweekly through week 26, and monthly through week 104)
Overall compound consumption in mg/kg and day: Low dose: 3,388 (m), 3,379 (f); mid dose: 13,451 (m), 13,633 (f) The majority of the analyzed feed mixtures were within +/- 10% of the target levels. Feed mixtures of mid-dose females and high-dose males were consistently slightly below the respective target levels.
CLINICAL OBSERVATIONS AND FREQUENCY:
- Clinical signs: twice daily
- Mortality: twice daily
- Body weight and food consumption: weekly (14 weeks), biweekly (for the next 12 weeks), every fourth week (for the remaining weeks)
- Water consumption: no data
- Ophthalmoscopic examination: yes
- Haematology: hematocrit, hemoglobin and methemoglobin levels, erythrocytes, reticulocytes, Heinz bodies, total leucocyte and differential leucocyte counts, mean corpuscular hemoglobin, mean corpuscular volume, and mean corpuscular hemoglobin concentration
- Biochemistry: alkaline phosphatase, urea nitrogen, serum glutamic-pyruvic transaminase
- Urinalysis: pH, specific gravity, ketones, total protein, occult blood and

microscopic examination of the sediment
- organ weights: brain, heart, liver, kidneys, lungs, testes, epididymides, ovaries
- gross pathology: yes, all relevant organs
- histopathology: yes, all relevant organs
Interim sacrifice at week 26 and 52: 10 rats/sex/dose; at week 78: 20 rats/sex/dose; high dose: sacrifice of all surviving rats at week 55;
terminal sacrifice: at week 104 all surviving rats
STATISTICAL METHODS: analysis of variance, Barlett's test for homogeneity of variance

Test substance : technical grade DNT: (data from Rickert et al., CRC Crit. Rev. Toxicol. 13, 217-234 (1984))
76.4 % 2,4-DNT
18.8 % 2.6-DNT
2.4 % 3.4-DNT
1.5 % 2.3-DNT
0.7 % 2,5-DNT
0.04 % 3,5-DNT

Reliability : (1) valid without restriction
Comparable to guideline study and well documented

Flag : Critical study for SIDS endpoint

26.11.2003 (150) (151)

Type : Sub-acute
Species : rat
Sex : male
Strain : Fischer 344
Route of admin. : gavage
Exposure period : 5 days
Frequency of treatm. : daily
Post exposure period : sacrifice 24 hours after last dose
Doses : 75 mg/kg bw and day
Control group : yes, concurrent vehicle
Method :
Year : 1982
GLP : no data
Test substance : other TS: technical grade DNT (70% 2,4-DNT, 25% 2,6-DNT, 5% other isomers)

Remark : Study performed to investigate the effects of DNT on liver enzyme activity.
Result : DNT-exposure caused:
- increase of relative liver weight (4.5% vs. 3.2% in control)
- slight (ca. 25%) decreases of activities of cytochrome P450 and ECOD
- significant decreases (ca. 50%) of BPNDM
- slight increases of DT-diaphorase (ca. 50%)
- significant increases of epoxide hydrolase (ca. 300-350% of control)

Test condition : animals: 4/group (weight 140 to 170g)
vehicle: corn oil for gavage experiments
vehicle: DMSO for i.p. experiments
EXAMINATIONS:
Animals were anesthetized and blood was drawn by carciac puncture.
Livers were removed and weighted.
Microsomal fractions were prepared and determination of activities of the following enzymes were perfomed: cytochrome P450, EH (epoxidehydrolase), ECOD (ethoxycoumarin-O- deethylase), BPNDM (benzphetamine-N-demethylase), DT-diaphorase

Reliability : (2) valid with restrictions
Limited documentation; study performed for the investigation of enzyme

activity in the liver; only one dose tested

Flag : Critical study for SIDS endpoint

26.11.2003 (152)

Type	:	Sub-chronic
Species	:	rat
Sex	:	no data
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	90 days
Frequency of treatm.	:	daily
Post exposure period	:	
Doses	:	5, 16, 50 mg/kg bw and day
Control group	:	yes, concurrent vehicle
Method	:	
Year	:	2000
GLP	:	no data
Test substance	:	other TS: DNT 80/20 (ca. 80% 2,4-DNT and ca. 20% 2,6-DNT) purity 99.6%
Remark	:	Study performed to investigate the effects of DNT on immune function of red blood cells.
Result	:	The daily dose of 50 mg/kg bw led to a disturbances of the immune function of red cells.
Test condition	:	animals: 10 per dose vehicle: vegetable oil
Reliability	:	(4) not assignable not assignable; limited documentation; article on chinese, only English abstract

15.01.2004 (153)

Type	:	Sub-acute
Species	:	rat
Sex	:	male
Strain	:	Fischer 344
Route of admin.	:	i.p.
Exposure period	:	3 days
Frequency of treatm.	:	daily
Post exposure period	:	sacrifice 48 hours after last dose
Doses	:	5 doses between 10 and 100 mg/kg/day
Control group	:	yes, concurrent vehicle
Method	:	
Year	:	1882
GLP	:	no data
Test substance	:	other TS: technical grade DNT (70% 2,4-DNT, 25% 2,6-DNT, 5% other isomers)
Remark	:	Study performed to investigate the effects of DNT on liver enzyme activity.
Result	:	DNT dose dependently enhanced EH activity. ED50 was determined with 62 mg/kg/day)
Test condition	:	animals: 4/group (weight 140 to 170g) vehicle: DMSO EXAMINATIONS: animals were sacrificed and liver microsomal fractions were prepared for the determination of EH (epoxide hydrolase) activity
Reliability	:	(2) valid with restrictions Limited documentation; study performed for the investigation of enzyme activity in the liver; only one dose tested

26.11.2003 (152)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Ames test
System of testing	:	Salmonella typhimurium TA1535, TA1537, TA1538, TA98, TA100
Test concentration	:	up to 1000 ug/plate (see TC)
Cycotoxic concentr.	:	> 1000 µg/plate
Metabolic activation	:	with and without
Result	:	positive
Method	:	other: according to Ames B.N. et al., Mutat. Res. 31, 347-364 (1975)
Year	:	1981
GLP	:	no data
Test substance	:	other TS: technical grade DNT (see TS)
Result	:	GENOTOXIC EFFECTS: No increase in mutation frequency of TA 1535, TA 1537, and TA 100. Dose dependent increase in mutation frequency of TA 1538 and 98 (data not fully shown) - TA 1538: at 1000 µg/plate - ca. 11fold increase (- S9) at 1000 µg/plate - ca. 3fold increase (+ S9) - TA 98: dose dependent increase (+ and - S9) at 1000 µg/plate - nearly 2fold increase (- S9) at 1000 ug/plate - ca. 2fold increase (+ S9) - TA 98 (suspension assay): pos. at >= 250 µg/ml (+- S9); dose dependent effect CYTOTOXIC CONCENTRATION: - With metabolic activation: > 1000 µg/plate - Without metabolic activation: > 1000 µg/plate positive control gave the expected result
Test condition	:	SYSTEM OF TESTING - Species/cell type: Salmonella typhimurium - Strains: TA 1535, 1537, 1538, 98, 100 - Metabolic activation system: S9-mix from the livers of Aroclor-treated (500 mg/kg, i.p., 5 days prior to sacrifice) male F344 rats. TEST CONDITIONS - duration of treatment: 3 hours (with and without S9) - concentrations: 1000 ug/plate was the highest non-toxic amount tested for TA 1535, 1537, 1538, 98, 100 - TA98 tested additionally in suspension: 250- 1000 ug/ml (data shown for all tested conc.) - Number of replicates: no data - solvent: 1% DMSO - Positive and negative control groups and treatment: negative control: DMSO positive control given only for suspension assay with TA 98: B(a)P (30 µg/ml) CRITERIA FOR EVALUATING RESULTS: two-fold increase of number of mutants/plate over control
Test substance	:	technical grade DNT: 76.5 % 2,4-DNT (purity > 99.89%) 18.8 % 2.6-DNT (purity > 99.89%) 2.4 % 3.4-DNT (purity > 99.89%) 1.5 % 2.3-DNT (purity not confirmed) 0.7 % 2,5-DNT (purity not confirmed) 0.04 % 3,5-DNT (purity > 99.89%)
Reliability	:	(2) valid with restrictions Sufficient documentation, however, for 4 strains data of only one test substance concentration shown. Positive control given for only one strain.

Flag : Critical study for SIDS endpoint
26.11.2003 (154) (155)

Type : Ames test
System of testing : Salmonella typhimurium TM 677
Test concentration : 500 µg/ml
Cycotoxic concentr. : > 500 µg/ml
Metabolic activation : with and without
Result : positive
Method : other: according to Skopek et al., Proc. Natl. Acad. Sci. 75, 410-414, 1978
Year : 1981
GLP : no data
Test substance : other TS: technical grade DNT (see TS)

Result : GENOTOXIC EFFECTS:
- TM677: at 500 µg/ml - ca. 5fold increase (- and + S9)
- dose dependent increase; however, data shown only for one conc.

Test condition : SYSTEM OF TESTING
- Species/cell type: Salmonella typhimurium TM677
- forward mutation to azaguanine resistance
- Metabolic activation system: S9-mix from the livers of Aroclor-treated (500 mg/kg, i.p., 5 days prior to sacrifice) male F344 rats.

TEST CONDITIONS
- duration of treatment: 3 hours in suspension (with and without S9)
- concentrations: 500 ug/plate was the highest non-toxic conc. tested
- Number of replicates: no data
- solvent: 1% DMSO
- Positive and negative control groups and treatment:
negative control: DMSO
positive control: no data

CRITERIA FOR EVALUATING RESULTS: two-fold increase of number of mutants/plate over control

Test substance : technical grade DNT:
76.5 % 2,4-DNT (purity > 99.89%)
18.8 % 2.6-DNT (purity > 99.89%)
2.4 % 3.4-DNT (purity > 99.89%)
1.5 % 2.3-DNT (purity not confirmed)
0.7 % 2,5-DNT (purity not confirmed)
0.04 % 3,5-DNT (purity > 99.89%)

Reliability : (2) valid with restrictions
Limited documentation; data of only one test substance concentration shown. Positve control not given.

Flag : Critical study for SIDS endpoint
26.11.2003 (154) (155)

Type : HGPRT assay
System of testing : CHO K1 cell line
Test concentration : 0.2 - 2 mM
Cycotoxic concentr. : 1.6 and 1.8 mM
Metabolic activation : with and without
Result : negative
Method : other: according to O'Neill J.P. et al., Mutat. Res. 45, 91 (1977)
Year : 1982
GLP : no data
Test substance : other TS: technical grade DNT (see TS)

Result : GENOTOXIC EFFECTS:
- With metabolic activation: negative
- Without metabolic activation: negative

CYTOTOXIC CONCENTRATION:
- With metabolic activation: 50 % cell survival at 1.8 mM DNT
- Without metabolic activation: 50 % cell survival at 1.6 mM DNT
positve control gave the expected results

Test condition : SYSTEM OF TESTING
- Metabolic activation system: S9-mix from the livers of Aroclor-treated (500 mg/kg, i.p., 5 days prior to sacrifice) male F344 rats.
- Positive and negative control groups and treatment:
positive control: benzo(a)pyrene, solvent control: DMSO
- duration of treatment: 5 hours (with and without S9)

Test substance : technical grade DNT:
76.5 % 2,4-DNT (purity > 99.89%)
18.8 % 2.6-DNT (purity > 99.89%)
2.4 % 3.4-DNT (purity > 99.89%)
1.5 % 2.3-DNT (purity not confirmed)
0.7 % 2,5-DNT (purity not confirmed)
0.04 % 3,5-DNT (purity > 99.89%)

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint

26.11.2003 (156)

Type : Mouse lymphoma assay
System of testing : P 388 mouse lymphoma TK +/- cells
Test concentration : no data
Cycotoxic concentr. : no data
Metabolic activation : with and without
Result : negative
Method : other
Year : 1983
GLP : no data
Test substance : other TS: technical grade DNT (consisting of an 80:20 mixture of 2,4-DNT and 2,6-DNT)

Remark : In this study 2,4,6-TNT, 2,4-DNT, 2,6-DNT and DNT 80/20 were tested. 2,4-DNT and 2,4,6-TNT were tested up to 1000 µg/ml for cytotoxicity and for mutagenicity up to the highest non-cytotoxic concentration, in 2 experiments with each 3 replicates. It can be assumed that the same procedure was done with DNT 80/20.

Result : Dose-related decrease of cell survival (test concentrations and survival data not specified), no induction of mutations (test results not specified) with and without S9.

Reliability : (2) valid with restrictions
Limited documentation; tested concentrations not given, test results only mentioned in text as negative, no numerical values, tables or figures given.

Flag : Critical study for SIDS endpoint

15.01.2004 (157)

Type : Unscheduled DNA synthesis
System of testing : rat hepatocytes
Test concentration : 0.01, 0.1 mM
Cycotoxic concentr. : > 0.1 mM
Metabolic activation : without
Result : negative
Method : other: according to Williams G.M., Cancer Res 37, 1845-1851 (1977)
treatment interval: 18 hours, fixed with 3 washes of acetic acid:ethanol=1:3
Year : 1979

GLP	:	no
Test substance	:	other TS: technical grade DNT (see TS)
Result	:	No increase of average net nuclear grains or % of cells in repair over solvent control. Positive controls showed the expected results.
Test condition	:	SYSTEM OF TESTING - primary cultures of rat hepatocytes (of male F344 rats) - Positive and negative control groups and treatment: positive controls: dimethylnitrosamine, 2-acetylaminofluorene negative control: solvent DMSO - duration of treatment: 18 hours; cells were treated in the presence of H^3-thymidine - scoring: autoradiography of triplicate slides per dose with 50 cells each - pooled data of 3 experiments are shown - conc. of 0.1 µM - 10 mM were tested for cytotoxicity (data not shown) - UDS induction data for 0.01 and 0.1 mM shown CRITERIA FOR EVALUATING RESULTS - significant increase of average net nuclear grains (> or = 5 net grains) - % cells in repair
Test substance	:	technical grade DNT: 76.49 % 2,4-DNT 18.83 % 2,6-DNT 2.43 % 3,4-DNT 1.54 % 2,3-DNT 0.65 % 2,5-DNT 0.04 % 3,5-DNT
Reliability	:	(2) valid with restrictions Test procedure in accordance with generally accepted scientific standards. However, data of single experiments and cytotoxicity data not shown.
Flag	:	Critical study for SIDS endpoint

26.11.2003 (158)

5.6 GENETIC TOXICITY 'IN VIVO'

Type	:	Micronucleus assay
Species	:	mouse
Sex	:	male
Strain	:	other: (CBA x BalbC)F1
Route of admin.	:	i.p.
Exposure period	:	once
Doses	:	200, 400 mg/kg bw
Result	:	negative
Method	:	other: mainly according to OECD Guide-line 474
Year	:	1985
GLP	:	no data
Test substance	:	
Remark	:	The vehicle controls gave results in the expected range (0.04-0.06% micronucleated polychromatic erythrocytes). The positive control cyclophosphamide led to significantly enhanced micronucleus frequencies at the sampling times 24 h (0.84% MN) and 48 h (0.58%), but not 72 h (0.06%).
Result	:	DNT gave a negative response at any dose and sampling time. DNT 200 mg/kg: 0.02% (24h), 0.12% (48h), 0.06% (48h) DNT 400 mg/kg: 0.06% (24h), 0.0% (48%), 0.08% (48h)
Test condition	:	TEST ORGANISMS:

- Age: 10-12 weeks
- No. of animals per dose: 5/dose and sampling time
ADMINISTRATION:
- Vehicle: suspension in aqueous 0.5 % Tween 80
- Duration of test: 24 to 72 hours
- Frequency of treatment: single
- determination of micronuclei in bone marrow polychromatic erythrocytes (PCE)
- Sampling times and number of samples: 24, 48, 72 hours
- Control groups and treatment:
positive control: 40 mg cyclophosphamide/kg bw
negative control: vehicle
EXAMINATIONS:
- Clinical observations: no data
- no information on bone marrow cytotoxicity
- determination of micronuclei in 1000 PCE/animal
- Criteria for a positive result: micronucleus frequency at least double than control and statistically significance ($p<0.05$). The presence of a dose-related response assists confirmation of a positive response.
- Criteria for selection of dose: highest dose is 80 % of MTD (MTD defined in an earlier study with 4 day observation following a single ip injection; data not shown)

Test substance : technical grade DNT: containing 20% 2,6-DNT
presumably:
71-76 % 2,4-DNT
about 20 % 2,6-DNT
and < 10 % other isomers

Reliability : (2) valid with restrictions
Deficiencies: testing of males only; data for determination of MTD not shown; ratio of PCE to NCE not given;

Flag : Critical study for SIDS endpoint

26.11.2003 (159)

Type : Dominant lethal assay
Species : mouse
Sex : male
Strain : other: DBA/2J
Route of admin. : gavage
Exposure period : 2 days
Doses : 250 mg/kg bw/d in corn oil
Result : negative
Method : other
Year : 1980
GLP : no
Test substance : other TS: technical grade DNT (see TS)

Result : Number of implants: not affected by DNT
Number of post-implantation deaths: not affected by DNT
Number of fertile matings: significantly increased in the second week of mating; slightly increased in weeks 3-5 of mating

Test condition : TEST ORGANISMS:
- Age: 10-12 weeks
- No. of animals per dose: 20/dose group, 5/control group
ADMINISTRATION:
- Duration of test: 7 weeks mating procedure starting 48 hours p.a.
- Frequency of treatment: twice
- Negative control: corn oil
- positive control: ethylmethanesulfonat (2x125 mg/kg bw)

MATING PROCEDURE:
- 48 hours posttreatment males were mated with 3 female CD1 mice each for one week; this mating procedure was repeated with vergine females for in total 7 weeks
- the females were killed 17 days after initial exposure to a male and their uterine contents examined for living fetuses and postimplantation deaths
EXAMINATIONS:
- Uterine contents: % fetal deaths per pregnant female, number of implantations per pregnant female,
- Fertility (% of fertile matings based on the number of total paired females)
- Criteria for evaluating results: significant increase of fetal deaths, significant decrease of implantations, impairment of fertility
- Criteria for selection of M.T.D.: LD50

Test substance : technical grade DNT: same lot as in CIIT bioassay (CIIT, 1978)
current analysis:
75.8 % 2,4-DNT
19.5 % 2,6-DNT
2.5 % 3,4-DNT
1.5 % 2,3-DNT
0.7 % 2,5-DNT
0.04 % 3,5-DNT

Conclusion : No indications for increases in dominant lethals or reduced fertility

Reliability : (2) valid with restrictions
Test procedure in accordance with generally accepted scientific standards, however, only pooled data shown.

Flag : Critical study for SIDS endpoint

26.11.2003 (138)

Type : Dominant lethal assay
Species : mouse
Sex : male
Strain : other: DBA/2J
Route of admin. : i.p.
Exposure period : 2 days
Doses : 250 mg/kg bw/d
Result : negative
Method : other
Year : 1980
GLP : no
Test substance : other TS: technical grade DNT (see TS)

Result : Number of implants: not affected by DNT
Number of post-implantation deaths: not affected by DNT
Number of fertile matings: significantly increased in week 6 of mating; slightly increased in weeks 5 and 7 of mating

Test condition : TEST ORGANISMS:
- Age: 10-12 weeks
- No. of animals per dose: 20/dose group, 5/control group
ADMINISTRATION:
- Duration of test: 7 weeks mating procedure starting 48 hours p.a.
- Frequency of treatment: twice
- Negative control: HBSS buffer
- positive control: triethylenemelamine (2x0.15 mg/kg bw)
MATING PROCEDURE:
- 48 hours posttreatment males were mated with 3 female CD1 mice each for one week; this mating procedure was repeated with vergine females for

in total 7 weeks
- the females were killed 17 days after initial exposure to a male and their uterine contents examined for living fetuses and postimplantation deaths
EXAMINATIONS:
- Uterine contents: % fetal deaths per pregnant female, number of implantations per pregnant female,
- Fertility (% of fertile matings based on the number of total paired females)
- Criteria for evaluating results: significant increase of fetal deaths, significant decrease of implantations, impairment of fertility
- Criteria for selection of M.T.D.: LD50

Test substance : technical grade DNT: same lot as in CIIT bioassay (CIIT, 1978)
current analysis:
75.8 % 2,4-DNT
19.5 % 2,6-DNT
2.5 % 3,4-DNT
1.5 % 2,3-DNT
0.7 % 2,5-DNT
0.04 % 3,5-DNT

Conclusion : No indications for increases in dominant lethals or reduced fertility

Reliability : (2) valid with restrictions
Test procedure in accordance with generally accepted scientific standards, however, only pooled data shown.

Flag : Critical study for SIDS endpoint

26.11.2003 (138)

Type : Mouse spot test
Species : mouse
Sex : female
Strain : other: T stock and C57BL/6J
Route of admin. : i.p.
Exposure period : single
Doses : 100 mg/kg bw
Result : negative
Method : other
Year : 1980
GLP : no
Test substance : other TS: technical grade DNT (see TS)

Result : No induction of recessive spots indicating that DNT did not induce single-gene mutations at at detectable level in this study.

Test condition : TEST ORGANISMS:
- matings: BL/6 X BL/6 and T X BL/6
- Age: 10-12 weeks
- No. of animals per dose: 2
ADMINISTRATION:
- Duration of test: until delivery
- Frequency of treatment: pregnant females were treated with a single dose on gestation day 10
- vehicle: HBSS buffer
- negative control: HBSS buffer
EXAMINATIONS:
- At birth the number and morphology of offspring were recorded. At 12 to 15 days old, offspring were observed for coat color spots (recessive spots (mutagenic effect) and increase of white midventral spots (toxic effect) and morphological abnormalities.
- Criteria for selection of M.T.D.: LD50

Test substance : technical grade DNT: same lot as in CIIT bioassay (CIIT,

1978)
current analysis:
75.8 % 2,4-DNT
19.5 % 2,6-DNT
2.5 % 3,4-DNT
1.5 % 2,3-DNT
0.7 % 2,5-DNT
0.04 % 3,5-DNT

Reliability : (2) valid with restrictions
Limited documentation; only one dose tested; only 2 females per group;
Flag : Critical study for SIDS endpoint
26.11.2003 (138)

Type : Sister chromatid exchange assay
Species : rat
Sex : male
Strain : Fischer 344
Route of admin. : gavage
Exposure period : single application
Doses : 100 mg/kg bw
Result : positive
Method : other: according to Kligerman A.D. et al., Environ. Mutagen.3, 531 (1981)
Year : 1982
GLP : no data
Test substance : other TS: DNT (technical grade, no further data)

Method : SCE-test in cultures of lymphocytes of rats treated in vivo.
Result : The increase in SCE frequency over the control was <50 %. There was no cell cycle inhibition or mitotic depression. No detailed description of test results.
Test condition : - 48 hours after treatment, animals were sacrificed; blood cells were isolated, washed and cultered in vitro with phytohemagglutinin or concanavalin A for in total 54 hours to induce lymphocyte proliferation. After 24 hours of culture BrdU and 3 hours prior to harvest colcemid was added.
Reliability : (2) valid with restrictions
Limited documentation; only one dose tested; number of animals no given; isomers not specified in detail
Flag : Critical study for SIDS endpoint
26.11.2003 (160)

Type : Unscheduled DNA synthesis
Species : rat
Sex : male
Strain : Fischer 344
Route of admin. : oral feed
Exposure period : 1, 2, 4 weeks
Doses : 0.1 % DNT in food (1000 mg/kg food)
Result : ambiguous
Method : other
Year : 1982
GLP : no data
Test substance : other TS: technical grade DNT (see TS)

Method : Hepatocytes were isolated by liver perfusion and cultured with [^{3}H]-thymidine. UDS was measured by quantitative autoradiography as net grains per nucleus.
Result : UDS (net grains): negative; no significant increase at all timepoints
% cells in repair: small but significant increase at all timepoints

		% cells in S-phase (48 hours p.a.): significant increase after 2 and 4 weeks Weekly weight gain: retarded in dosed animals only during weeks 1 and 2
Test condition	:	TEST ORGANISMS: - Weight at study initiation: 130 - 150 g - No. of animals per dose: 3-6 males - determination of DNA repair in hepatocytes ADMINISTRATION: - Vehicle: DNT was dissolved in acetone and mixed to uniformity with food - dose: 1g DNT/kg food (according to approximately 75 mg/kg bw and day) - Duration of test: 1, 2 or 4 week exposure - Frequency of treatment: continuously - Sampling times and number of samples: after 1, 2, 4 weeks of exposure - Control groups and treatment: food EXAMINATIONS: - Clinical observations: weekly weight gain - Criteria for positive result: mean value above 5 net grains; cells in repair: % cells with above 5 net grains
Test substance	:	technical grade DNT: same lot as in CIIT bioassay (CIIT, 1978) current analysis: 71.1 % 2,4-DNT 19.8 % 2,6-DNT 4.3 % 2,3-DNT 4.0 % 3,4-DNT <1 % other isomers
Conclusion	:	After subacute feeding of technical grade DNT in low dosage only marginal UDS, measured as net grains, was observable in rat liver. DNT was considered to be genotoxic (significant increase of cells in repair) and hepatotoxic (significant increase of DNA replication). After subacute feeding of technical grade DNT in low dosage only marginal, not significant UDS, measured as net grains, was observable in rat liver. However, DNT was considered to significantly increase the number of cells in repair, an indication of slight genotoxic activity. Additionally, DNT increased DNA replication in the liver.
Reliability	:	(2) valid with restrictions Limited documentation; no positive compound tested;
Flag	:	Critical study for SIDS endpoint
26.11.2003		(161) (162) (163)
Type	:	Unscheduled DNA synthesis
Species	:	rat
Sex	:	male
Strain	:	Fischer 344
Route of admin.	:	gavage
Exposure period	:	once
Doses	:	25, 100, 150, 200 mg/kg bw
Result	:	positive
Method	:	other: according to Ashby et al., Mutat. Res. 156, 1-18 (1985)
Year	:	1985
GLP	:	no data
Test substance	:	
Method	:	Hepatocytes were isolated by liver perfusion and cultured with [^{3}H]- thymidine. UDS was measured by quantitative autoradiography as net grains per nucleus.
Remark	:	Toxic picnosis was evident in hepatocytes of rats treated with DNT at dose

levels greater than 200 mg/kg bw.

Result : Orally administered technical grade DNT induced DNA repair in hepatocytes of F344 rats.
Mean net grains/dose:
control (2 rats) -2.31 (NG) 2.4 % cells in repair
20 mg/kg (3 rats) 0.87 (NG) 14.7 % cells in repair
100 mg/kg (4 rats) 21.3 (NG) 81.0 % cells in repair
150 mg/kg (3 rats) 21.6 (NG) 88.7 % cells in repair
200 mg/kg (3 rats) 2.98 (NG) 30.6 % cells in repair

Test condition : TEST ORGANISMS:
- No. of animals: 3-4/dose; 2/control (+6 historical controls)
- determination of DNA repair in hepatocytes
ADMINISTRATION:
- Duration of test: 12 hours
- Frequency of treatment: single
- Sampling times: 12 hours
- vehicle: corn oil
- Control groups and treatment:
negative control: vehicle
positive control: DNT served as positive control
EXAMINATIONS:
- scoring: minimum 150 cells/3 slides of 2 animals + 50 cells/2 slides of 1 animal
- Clinical observations: no data
- Criteria for positive results: reproducible effect of > or = 3 net grains/rat and evidence of dose-response relationship
- Criteria for selection of dose: no data

Test substance : technical grade DNT: containing 20% 2,6-DNT
presumably:
71-76 % 2,4-DNT
about 20 % 2,6-DNT
and < 10 % other isomers

Reliability : (2) valid with restrictions
Limited documentation; DNT served as positive control; no information on criteria for selection of dose;

Flag : Critical study for SIDS endpoint

26.11.2003 (159)

Type : Unscheduled DNA synthesis
Species : rat
Sex : male
Strain : other: Alderly Park (AP)
Route of admin. : gavage
Exposure period : once
Doses : 100, 200 mg/kg bw
Result : positive
Method : other: according to Ashby et al., Mutat. Res. 156, 1-18 (1985)
Year : 1985
GLP : no data
Test substance :

Method : Hepatocytes were isolated by liver perfusion and cultured with [^{3}H]-thymidine. UDS was measured by quantitative autoradiography as net grains per nucleus.

Remark : Toxic picnosis was evident in hepatocytes of rats treated with DNT at dose levels greater than 200 mg/kg bw.

Result : Orally administered technical grade DNT induced DNA repair in hepatocytes of F344 rats.
Mean net grains/dose: % cells in repair

	control (3 rats) -1.75	2.1
	100 mg/kg (1 rat) 10.5	88.0
	200 mg/kg (5 rats) 2.98	58.9
Test condition	:	TEST ORGANISMS: - No. of animals per dose: 1 at 100 mg/kg bw; 5 at 200 mg/kg bw; 3 for control (+40 historical controls) - determination of DNA repair in hepatocytes ADMINISTRATION: - Duration of test: 12 hours - Frequency of treatment: single - Sampling times: 12 hours - vehicle: corn oil - Control groups and treatment: negative control: vehicle positive control: DNT served as positive control EXAMINATIONS: - scoring: 150 cells per animal (on 3 slides) - Clinical observations: no data - Criteria for positive results: reproducible effect of > or = 3 net grains/rat and evidence of dose-response relationship - Criteria for selection of dose: no data
Test substance	:	technical grade DNT: containing 20% 2,6-DNT presumably: 71-76 % 2,4-DNT about 20 % 2,6-DNT and < 10 % other isomers
Reliability	:	(2) valid with restrictions Limited documentation; DNT served as positive control; no information on criteria for selection of dose;
Flag	:	Critical study for SIDS endpoint
26.11.2003		(159)

Type	:	Unscheduled DNA synthesis
Species	:	rat
Sex	:	male
Strain	:	Fischer 344
Route of admin.	:	gavage
Exposure period	:	single application
Doses	:	10, 50, 100 and 200 mg/kg bw in corn oil
Result	:	positive
Method	:	other: according to Mirsalis J.C. et al., Carcinogenesis 1, 621-625 (1980)
Year	:	1982
GLP	:	no data
Test substance	:	other TS: technical grade DNT (see TS)
Method	:	Hepatocytes were isolated by liver perfusion and cultured with [^{3}H]-thymidine. UDS was measured by quantitative autoradiography as net grains per nucleus.
Result	:	Dose dependent increase of UDS from 10-200 mg/kg at 12 hours p.a.: Mean net grains / % cells in repair (data taken from figure): control: about -5 / 1 10 mg/kg about-1 / 10 50 mg/kg about 1 / 20 120 mg/kg about 15 / 80 200 mg/kg about 25 / 95
Test condition	:	TEST ORGANISMS: - Weight at study initiation: 200-275 g - No. of animals per dose: 3

- determination of DNA repair in hepatocytes
ADMINISTRATION:
- Sampling times and number of samples: 12 hours
- vehicle: corn oil
- Control groups and treatment: negative control corn oil, positive control: dimethylnitrosamine
EXAMINATIONS:
- scoring: 150 cells per animal (on 3 slides)
- Criteria for positive result: mean value above 5 net grains; cells in repair: % cells with above 5 net grains

Test substance : technical grade DNT: same lot as in CIIT bioassay (CIIT, 1978)
current analysis:
71.1 % 2,4-DNT
19.8 % 2,6-DNT
4.3 % 2,3-DNT
4.0 % 3,4-DNT
<1 % other isomers

Reliability : (2) valid with restrictions
Test procedure in accordance with generally accepted scientific standards, however, data shown in figures only

Flag : Critical study for SIDS endpoint

26.11.2003 (162) (163)

Type : Unscheduled DNA synthesis
Species : rat
Sex : male
Strain : other: Fischer 344 (see TC)
Route of admin. : gavage
Exposure period : single application
Doses : 100 mg/kg in corn oil
Result : positive
Method : other: according to Mirsalis J.C. et al., Carcinogenesis 1, 621-625 (1980)
Year : 1981
GLP : no data
Test substance : other TS: technical grade DNT

Method : Hepatocytes were isolated by liver perfusion and cultured with [^{3}H]-thymidine. UDS was measured by quantitative autoradiography as net grains per nucleus.

Result : Orally administered technical grade DNT induced DNA repair in hepatocytes of male rats having the normal complement of gut flora, but not in rats which have no gut flora.
UDS as net nuclear grains / % cells in repair
Fischer 344 rats with CRASF:
control -4.2 / 7 %
DNT 18.7 / 87 %
Fischer 344 rats germ-free (axenic):
control -3.6 / 3
DNT -0.8 / 14 %

Test condition : TEST ORGANISMS:
- Fischer 344 rats: animals were born and reared in a bacteria-free isolator. These germ-free rats were devided in two groups:
1) CRASF associated group: animals were associated with Charles River Associated Schaedler Flora (CRASF: a mixture of 8 anaerobic bacterial strains similar to the normal gut microflora of rats) for two weeks prior to treatment with test substance.
2) non-associated group: germ-free rats
- Age: no data
- Weight at study initiation: no data

- No. of animals: 2-4 per treatment group
- determination of DNA repair in hepatocytes
ADMINISTRATION:
- Sampling times and number of samples: 12 hours
- vehicle: sterile corn oil
- test substance: sterile DNT
- negative control: corn oil
- positive control: dimethylnitrosamine (10 mg/kg bw)
EXAMINATIONS:
- scoring: 150 cells per animal (on 3 slides)
- Criteria for positive result: mean value above 5 net grains; cells in repair: % cells with above 5 net grains

Conclusion : The presence of gut flora with its metabolic capacity is a prerequisite for the induction of UDS by DNT.
Reliability : (2) valid with restrictions
Test procedure in accordance with generally accepted scientific standards, however, not according to current guidelines
Flag : Critical study for SIDS endpoint
26.11.2003 (164) (165)

Type : Unscheduled DNA synthesis
Species : rat
Sex : male
Strain : Fischer 344
Route of admin. : gavage
Exposure period : once
Doses : 100 mg/kg in corn oil
Result : positive
Method : other: according to Mitchell A.D., J.C. Mirsalis in : A.A. Ansari and F.J. de Serres (eds.)Single-Cell Mutation Monitoring Systems, Plenum, New York, 1984, p. 165-216
Year : 1987
GLP : no data
Test substance : other TS: technical grade DNT (see TS)

Method : Hepatocytes were isolated by liver perfusion and cultured with [^{3}H]-thymidine. UDS was measured by quantitative autoradiography as net grains per nucleus.
Result : The test substance showed a positive response.
Test condition : TEST ORGANISMS:
- Age: 6-12 weeks
- Weight at study initiation: 155-360 g
- No. of animals per dose: no data
- determination of DNA repair in hepatocytes
ADMINISTRATION:
- Sampling time: 16 hours
- vehicle: corn oil
- Negative control: corn oil
- Positive control: 2-AAF (10 mg/kg) and Benzidine (35mg/kg)
EXAMINATIONS:
- scoring: 150 cells per animal (on 3 slides)
- Criteria for positive result: mean value above 5 net grains; cells in repair: % cells with above 5 net grains

Test substance : technical grade DNT:
71.1 % 2,4-DNT
19.8 % 2,6-DNT
9.1 % other isomers
Reliability : (2) valid with restrictions
Limited documentation; only one dose tested;
Flag : Critical study for SIDS endpoint

26.11.2003 (166)

Type	:	Unscheduled DNA synthesis
Species	:	rat
Sex	:	male
Strain	:	Fischer 344
Route of admin.	:	gavage
Exposure period	:	single application
Doses	:	35, 125, 250 mg/kg bw
Result	:	positive
Method	:	other: mainly according to OECD Guide-line 486
Year	:	1889
GLP	:	no data
Test substance	:	other TS: DNT (technical grade)
Method	:	Hepatocytes were isolated by liver perfusion and cultured with [^{3}H]-thymidine. UDS was measured by quantitative autoradiography as net grains per nucleus.
Result	:	Orally administered technical grade DNT induced DNA repair in rat hepatocytes.

Mean net grains/dose:		% cells in repair:
negative control (2h, 2 rats)	-6.4	1 %
negative control (12h, 52 rats)	-5.6	2 %
35 mg/kg bw (2h, 3 rats)	-11.2	4 %
35 mg/kg bw (12h, 3 rats)	-5.5	3 %
125 mg/kg bw (12h, 3 rats)	17.4	77 %
250 mg/kg bw (12h, 2 rats)	31.0	89 %

Test condition	:	TEST ORGANISMS: - No. of animals per dose: 2-3 - Weight at study initiation: 180 to 300 g - determination of DNA repair in hepatocytes ADMINISTRATION: - Sampling times and number of samples: 2 and/or 12 hours - vehicle: corn oil - Control groups: negative control: corn oil positive control: Dimethylnitrosamine (2h) 10 mg/kg positive control: 2-Acetylaminofluorene (12h) 50 mg/kg EXAMINATIONS: - Criteria for dose selection: doses were generally selected as 80%, 40% and 10% of the LD50 - scoring: 150 cells per animal (on 3 slides) - Criteria for positive result: average net nuclear grains above 0 - % cells in repair
Test substance	:	technical grade DNT (same lot that was used for the NCI rodent bioassays; CIIT 1978): 76.5% 2,4-DNT 18.8% 2,6-DNT 4.7% other isomers
Reliability	:	(2) valid with restrictions Test procedure in accordance with generally accepted scientific standards, however, only pooled data shown; composition of test substance not described in detail
Flag	:	Critical study for SIDS endpoint

26.11.2003 (167)

Type	:	Unscheduled DNA synthesis
Species	:	rat
Sex	:	male/female
Strain	:	Fischer 344

Route of admin.	:	gavage
Exposure period	:	single application
Doses	:	100 mg/kg bw in corn oil
Result	:	positive
Method	:	other: mainly according to OECD Guide-line 486
Year	:	1982
GLP	:	no data
Test substance	:	other TS: technical grade DNT (see TS)
Method	:	Hepatocytes were isolated by liver perfusion and cultured with [^{3}H]-thymidine. UDS was measured by quantitative autoradiography as net grains per nucleus.
Result	:	Orally administered technical grade DNT induced DNA repair in rat hepatocytes. Females exhibited a lower DNA repair activity and a lower replicative DNA synthesis rate than male rats after treatment with DNT. Peak activity of UDS at 12 hours p.a., peak activity of replicative DNA synthesis at 48 hours p.a.. Replicative DNA synthesis returned to control levels at 4 days p.a. Mean net grains (12h)/ % cells in repair (12h)/ % cells in S-phase (48h) Control: males -4.2 / 2 % / 0.08 % females -3.7 / 2 % / 0.16 % DNT: males 15.1 / 80 % / 4,57 % females 4.6 / 49 % / 0.60 % In the presence of 10 or 20 mM hydroxyurea, the increase in S-phase cells could be suppressed by >95%. At the same levels of hydroxyurea, however, there was no effect on the level of UDS at 12 h post-treatment.
Test condition	:	TEST ORGANISMS: - Weight at study initiation: 200 - 275 g - No. of animals per dose: 4 males and 3 females - determination of DNA repair in hepatocytes ADMINISTRATION: - Sampling times and number of samples: 1, 12, 24, 48 hours, and 4 days p.a. - vehicle: corn oil - Control groups and treatment: negative control: corn oil positive control: dimethylnitrosamine (10 mg/kg bw; 2 h) EXAMINATIONS: - scoring: 150 cells per animal (on 3 slides) - Criteria for positive result: mean value above 5 net grains; cells in repair: % cells with above 5 net grains
Test substance	:	technical grade DNT: same lot as in CIIT bioassay (CIIT, 1978) current analysis: 71.1 % 2,4-DNT 19.8 % 2,6-DNT 4.3 % 2,3-DNT 4.0 % 3,4-DNT <1 % other isomers
Conclusion	:	Technical grade DNT in male rats strongly induces UDS and DNA replication, whereas female rats show a distinctly weaker response for both endpoints. Consequently in female rats DNT is less genotoxic and less hepatotoxic.
Reliability	:	(2) valid with restrictions Test procedure in accordance with generally accepted scientific standards, however, only pooled data shown
Flag	:	Critical study for SIDS endpoint

26.11.2003 (161) (162) (163)

5.7 CARCINOGENICITY

Species	:	rat
Sex	:	male/female
Strain	:	Fischer 344
Route of admin.	:	oral feed
Exposure period	:	104 w
Frequency of treatm.	:	continuous
Post exposure period	:	no
Doses	:	3.5, 14, 35 mg/kg bw
Result	:	positive
Control group	:	yes, concurrent no treatment
Method	:	other:
Year	:	1978
GLP	:	no
Test substance	:	other TS: technical grade DNT (see TS)
Remark	:	see also chapter 5.4
Result	:	Mortality: 3.5 mg: 14/130 (m), 15/130 (f); 14 mg: 48/130 (m), 9/130 (f); 35 mg: 8/130 (m), 2/130 (f); Tumour findings: control: Liver: hepatocellular carcinomas (m, 1/120; f, 0/120), neoplastic nodules (m, 8/120; f, 5/120); mammary glands: fibroadenomas (m, 6/91; f, 16/90); at 52 weeks all 10 sacrificed control animals showed interstitial cell tumors of the testes, at 104 weeks interstitial cell tumors of the testes (82/101) 3.5 mg (104 week exposure): Liver: hepatocellular carcinomas (m, 10/130; f, 1/129), neoplastic nodules (m, 14/130; f, 15/129); testes: interstitial cell tumors (1/1) 14 mg (104 week exposure): Liver: hepatocellular carcinomas (m, 98/128; f, 45/130), neoplastic nodules (m, 64/128; f, 74/130), cholangiocarcinomas (m, 15/128; f, 0/130), skin: subcutaneous fibromas (m, 44/52; f, 11/15) and fibrosarcomas (m, 10/52; f, 4/15); mammary gland: fibroadenomas (m, 22/79; f, 29/91); testes: interstitial cell tumors (104/108) 35 mg (52 or 55 week sacrifice): Liver: hepatocellular carcinomas (m, 32/40; f, 15/40), neoplastic nodules (m, 8/40; f, 9/49), hepatocellular cholangiocarcinomas (m, 3/40; f, 15/40); testes: interstitial cell tumors (13/40); frequent occurrence of multiple tumors
Test condition	:	ANIMALS - Weight at study initiation: males: 106-208 g; females: 95-157 g - Number of animals: 130/sex/group ADMINISTRATION / EXPOSURE - Duration of test/exposure: 104 w - Type of exposure: oral feeding - Post exposure period: none - Vehicle: food - Concentration in vehicle: - Doses: about 3.5, 14, 35 mg/kg bw/d - Control: basal diet - Compound consumption was calculated based on target dose levels (weekly from weeks 1-14, biweekly through week 26, and monthly through week 104) Overall compound consumption in mg/kg and day: Low dose: 3,388 (m), 3,379 (f); mid dose: 13,451 (m), 13,633 (f) The majority of the analyzed feed mixtures were within +/- 10% of the target levels. Feed mixtures of

mid-dose females and high-dose males were consistently slightly below the respective target levels.
CLINICAL OBSERVATIONS AND FREQUENCY:
- Clinical signs: twice daily
- Mortality: twice daily
- Body weight and food consumption: weekly (14 weeks), biweekly (for the next 12 weeks), every fourth week (for the remaining weeks)
- Water consumption: no data
- Ophthalmoscopic examination: yes
- Haematology: hematocrit, hemoglobin and methemoglobin levels, erythrocytes, reticulocytes, Heinz bodies, total leucocyte and differential leucocyte counts, mean corpuscular hemoglobin, mean corpuscular volume, and mean corpuscular hemoglobin concentration
- Biochemistry: alkaline phosphatase, urea nitrogen, serum glutamic-pyruvic transaminase
- Urinalysis: pH, specific gravity, ketones, total protein, occult blood and microscopic examination of the sediment
- organ weights: brain, heart, liver, kidneys, lungs, testes, epididymides, ovaries
- gross pathology: yes, all relevant organs
- histopathology: yes, all relevant organs
Interim sacrifice at week 26 and 52: 10 rats/sex/dose; at week 78: 20 rats/sex/dose; high dose: sacrifice of all surviving rats at week 55;
terminal sacrifice: at week 104 all surviving rats
STATISTICAL METHODS: analysis of variance, Barlett's test for homogeneity of variance

Test substance : technical grade DNT: (data from Rickert et al., CRC Crit. Rev. Toxicol. 13, 217-234 (1984))
76.4 % 2,4-DNT
18.8 % 2.6-DNT
2.4 % 3.4-DNT
1.5 % 2.3-DNT
0.7 % 2,5-DNT
0.04 % 3,5-DNT

Reliability : (1) valid without restriction
Comparable to guideline study and well documented

Flag : Critical study for SIDS endpoint

26.11.2003 (151)

Species : rat
Sex : male
Strain : Fischer 344
Route of admin. : oral feed
Exposure period : 52 w
Frequency of treatm. : continuously
Post exposure period : no
Doses : 35 mg/kg bw/d
Result : positive
Control group : yes, concurrent no treatment
Method : other: bioassay for hepatocarcinogenicity
Year : 1987
GLP : no data
Test substance : other TS: see TS

Result : MORTALITY AND TIME TO DEATH: no data
CLINICAL SIGNS: no data
BODY WEIGHT GAIN: body weight gain significantly decreased from 26-52 weeks; body weight was nearly constant from 10-20 weeks till study end; body weight at 52 w: control 434 g,

DNT 321 g
CLINICAL CHEMISTRY: not significant decrease of serum ALT and not significant increase of serum GGT at 26 and 52 w
ORGAN WEIGHTS: at 26 w: sign. increase of relative liver weight; at 52 w: sign. increase of relative (control 2.38%, DNT 6.08%) and absolute liver weight
HISTOPATHOLOGY:

neoplastic findings:	control	DNT
neoplastic liver nodules	0/20	10/19
hepatocellular carcinoma	0/20	9/19
cholangiosarcoma	0/20	2/19

non-neoplastic findings:
hepatocytic degeneration and vacuolisation, acidophilic and basophilic cell foci (control 0%, DNT > 90%), bile duct hyperplasia, cholangiofibrosis
STATISTICAL RESULTS: no data for tumor incidences

Test condition : TEST ORGANISMS
- Weight at study initiation: 130-150 g
- Number of animals: 28/group
ADMINISTRATION / EXPOSURE
- Duration of test/exposure:
terminal sacrifice: 20 rats/group after 52 weeks;
interim sacrifice: 4 rats/group after 6 and 26 weeks,
CLINICAL OBSERVATIONS AND FREQUENCY
- Body weight: every 2 weeks
- Food consumption: weekly
- Clinical chemistry: serum ALT and GGT after 26 and 52 weeks
ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
- Macroscopic: only liver and lung
- Microscopic: only liver and lung
OTHER EXAMINATIONS:
- organ weights: liver and lung weights after 26 and 52 weeks
STATISTICAL METHODS: analysis of variance; Dunnett's test

Test substance : representative technical grade DNP prepared by mixing of purified DNP isomers with final composition:
76.5% 2,4-DNT
18.8% 2,6-DNT
2.43% 3,4-DNT
1.54% 2,3-DNT
0.69% 2,5-DNT
0.04% 3,5-DNT

Reliability : (2) valid with restrictions
Limited documentation; only one dose tested; exposure limited to 52 weeks; examination of liver and lung only; insufficient number of animals

Flag : Critical study for SIDS endpoint

26.11.2003 (168) (169)

Species : mouse
Sex : male
Strain : Strain A
Route of admin. : i.p.
Exposure period : 8 w
Frequency of treatm. : 3 times/w
Post exposure period : 16 w
Doses : total dose: 480, 1200, 2400 mg/kg in corn oil
Result : negative
Control group : other: yes, concurrent vehicle, concurrent no treatment

Method	:	other: lung tumour assay according to Shimkin M.B. and Stoner G:D., Adv. Cancer Res. 21, 1-58 (1975)
Year	:	1985
GLP	:	no data
Test substance	:	
Result	:	No indication of treatment related effect on body weights; survivors: (2400 mg-gr) 24/30, (1200 mg-gr) 29/30, (480 mg-gr) 26/30, (vehicle control) 41/50, (untreated control) 24/30, urethan (1000 mg) 19/20 survivors with tumors: (2400 mg-gr) 33 %, (1200 mg-gr) 21 %, (480 mg-gr) 15 %, vehicle control: 27 %, untreated control: 33 %, urethan (1000 mg): 100%
Test condition	:	30 animals/group dose selection: MTD, 1/2 MTD, 1/5 MTD single doses of 10, 25, and 50 mg/kg bw were injected positive calibration control: urethan 1g/kg bw body weight recorded every 2 weeks incidences of tumorigenicity were lung tumors (number of tumor bearing mice per group) and tumor multiplicity (the average number of tumors per lung)
Test substance	:	2,4-/2,6-DNT mixture (2:1) in acetone 2,4-DNT: purity 92-95% (impurities: 2,6-DNT) purity 98% (impurities: 2,6-DNT, 2-nitrotoluene) 2,6-DNT: purity 92-95% (impurities: 2-nitrotoluene, 2,4-DNT) purity 98% (impurities: none)
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment
Flag	:	Critical study for SIDS endpoint

26.11.2003 (170)

Species	:	rat
Sex	:	male
Strain	:	Fischer 344
Route of admin.	:	gavage
Exposure period	:	single
Frequency of treatm.	:	
Post exposure period	:	
Doses	:	75 mg/kg bw in corn oil
Result	:	
Control group	:	yes, concurrent vehicle
Method	:	other: initiation-promotion liver foci assay (Leonard et al., 1983)
Year	:	1983
GLP	:	no data
Test substance	:	other TS: see TS
Result	:	DNT was a weak initiator only when applied 12 hours post partial hepatectomy: significant increase of GGT+ foci
Test condition	:	TEST ORGANISMS - Weight at study initiation: 130-150 g - Number of animals: 8-10/timepoint DNT-application: 24, 12 and 6 h before partial hepatectomy, 6, 12, 15 and 18 h post partial hepatectomy EXAMINATIONS: the numbers of gamma-glutamyltranspeptidase-positive (GGT) foci were quantitated following 2-AAF/CCl4 growth selection STATISTICAL METHODS: analysis of variance
Test substance	:	technical grade DNT prepared by mixing of purified DNT

		isomers in a ratio that is representative of a standard technical grade: 76 % 2,4-DNT 20 % 2,6-DNT 4 % 2,3-, 2,5-, 3,4- and 3,5-DNT
Reliability	:	(2) valid with restrictions Study meets generally accepted scientific principles, acceptable restrictions in documentation, acceptable for assessment
Flag	:	Critical study for SIDS endpoint
26.11.2003		(171) (172) (173)
Species	:	rat
Sex	:	male
Strain	:	Fischer 344
Route of admin.	:	oral feed
Exposure period	:	3 or 6 w following initiation with a single dose of 150 mg DEN/kg i.p. and a 2-week recovery period
Frequency of treatm.	:	daily
Post exposure period	:	no
Doses	:	14, 35 mg DNT/kg (calculated from average food consumption (0.2 and 0.55% in feed)
Result	:	
Control group	:	yes
Method	:	other: initiation-promotion liver foci assay
Year	:	1982
GLP	:	no data
Test substance	:	other TS: see TS
Result	:	Initiation-promotion assay: 3 week DNT treatment: without DEN initiation: no increase of foci (0 foci/cm³) with DEN initiation: dose dependent increase in number of GGT positive foci (14 mg/kg 188-248 foci/cm³; 35 mg/kg 303-720 foci/cm³) and foci volume 6 week DNT treatment: without initiation: only small increase of foci (2.4-15 foci/cm³) with DEN initiation: dose dependent significant increase of number (14 mg/kg 114-412 foci/cm³; 35 mg/kg 839-1137 foci/cm³) and volume of GGT positive foci; time dependent increase relative to 3 weeks treatment growth selection properties: no selective selection of GGT+ foci by initiation + DNT + PH nuclear 3H-thymidine labeling: time course was not influenced by DNT treatment + PH
Test condition	:	TEST ORGANISMS - Weight at study initiation: 130-150 g - Number of animals: 8-10/dose-group and timepoint and control group ADMINISTRATION / EXPOSURE - Duration of test/exposure: initiation-promotion protocol: initiation with 150 mg diethylnitrosamine (DEN)/kg (single i.p. injection in water) + 2-week recovery period + 3 or 6 w feeding of DNT initiation-promotion protocol including partial hepatectomy (PH): initiation with 150 mg DEN/kg (in water) + 2-week recovery period + 1 w feeding of DNT (35 mg/kg bw/d) + 2/3 PH + 1 w feeding of DNT examination of hepatic regeneration: 1 w feeding of DNT (35

mg/kg bw/d) + 2/3 PH + administration of 3H-thymidine 60 min prior to sacrifice; sacrifice 6, 12, 15, 18, 24, 30, 36, 42, 48, 72 hours post PH
- Type of exposure: DEN i.p.; DNT oral feed
- Post exposure period: none
FOR ORAL STUDIES:
- Control groups:
initiator control: DEN i.p. + control diet
promoter control: initiator vehicle (water) i.p. + DNT diet
control: initiator vehicle (water) i.p. + control diet
CLINICAL OBSERVATIONS AND FREQUENCY
- Body weight: weekly
- Food consumption: weekly
- Mortality: no data
OTHER EXAMINATIONS:
- determination of gamma-glutamyl-transpeptidase-positive GGT positve foci: number/volume and mean volume
- determination of 3H-thymidine nuclear labeling index
STATISTICAL METHODS: t-test

Test substance : representative technical grade DNT prepared by mixing of purified DNP isomers with final composition:
76.5% 2,4-DNT
18.8% 2,6-DNT
2.4% 3,4-DNT
1.5% 2,3-DNT
0.7% 2,5-DNT
0.1% 3,5-DNT

Conclusion : DNT has promoting activity for the development of DEN-initiated liver foci. DNT application doesnot alter DNA replication of hepatocytes (3H-thymidine labeling) following partial hepatectomy. Other than by AAF there is no growth-selection of GGT+ foci by DNT.

Reliability : (1) valid without restriction
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

Flag : Critical study for SIDS endpoint

26.11.2003 (171) (174) (175) (176)

Species : mouse
Sex : no data
Strain : Sencar
Route of admin. : dermal
Exposure period : see TC
Frequency of treatm. : once
Post exposure period : see TC
Doses : 1, 5, 10 mg/mouse in acetone
Result : negative
Control group : other: TPA administration alone as control group
Method : other: dermal initiation-promotion assay (see TC)
Year : 1985
GLP : no data
Test substance :

Result : The 2:1 mixture of 2,4-DNT and 2,6-DNT showed no tumor initiating activity after a single dermal application.
10 mg: 15 %, 5 mg: 10 %, 1 mg: 7 % of mice with papillomas (control: 13 %); none with carcinomas (control: 2.5 %);
5 and 10 mg gave a significant level of epidermal hyperplasia and dark cells.

Test condition	:	Initiation-promotion protocol: initiation: 2.4-/2.6-DNT (2:1) in acetone, one application promotion: 4 ug TPA (7.12-dimethyl(a)benzene(DMBA)-12-O-tetradecanoylphorbol-13-aetate), dermal application, daily, 30 weeks; examination of papillomas after 30 weeks; negative control: TPA only positive control: B(a)P and 4-NQO Criteria for selection of dose: The dose levels were derived from the results of the toxicity studies, the amount of compound that could be disssolved in 0.2 ml of acetone and the availability of the compound. In the prechronic toxicity studies a dose was chosen that did not kill any of the animals during a one-week period. In addition, the ability of the various compounds to induce inflammation and hyperplasia in the skin was determined.
Test substance	:	2,4-/2,6-DNT mixture (2:1) in acetone 2,4-DNT: purity 92-95% (impurities: 2,6-DNT) purity 98% (impurities: 2,6-DNT, 2-nitrotoluene) 2,6-DNT: purity 92-95% (impurities: 2-nitrotoluene, 2,4-DNT) purity 98% (impurities: none)
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment
Flag	:	Critical study for SIDS endpoint
26.11.2003		(170)

Species	:	mouse
Sex	:	no data
Strain	:	Sencar
Route of admin.	:	i.p.
Exposure period	:	see TC
Frequency of treatm.	:	once
Post exposure period	:	see TC
Doses	:	1, 5, 10 mg/mouse in corn oil
Result	:	negative
Control group	:	yes, concurrent vehicle
Method	:	other: intraperitoneal initiation-dermal promotion assay (see TC)
Year	:	1985
GLP	:	no data
Test substance	:	other TS: 2.4-/2.6-DNT mixture (2:1) in corn oil
Result	:	The 2:1 mixture of 2,4-DNT and 2,6-DNT showed no tumor initiating activity after a single intraperitoneal application. Mice with papillomas: 10 mg: 8 %, 5 mg: 15 %, 1 mg: 15 %, vehicle control: 8 %; none with carcinomas 5 and 10 mg gave a significant level of epidermal hyperplasia and dark cells.
Test condition	:	Initiation-promotion protocol: initiation: 2.4-/2.6-DNT (2:1), one i.p. application promotion: 4 ug TPA (7.12-dimethyl(a)benzene(DMBA)-12-O-tetradecanoylphorbol-13-aetate), dermal application, daily, 30 weeks; examination of papillomas after 30 weeks negative contol: vehicle corn oil positive control: B(a)P The dose levels were derived from the results of the toxicity studies, the amount of compound that could be disssolved in 0.2 ml of acetone and the availability of the compound. In the prechronic toxicity studies a dose was chosen that did not kill any of the animals during a one-week period. In addition, the ability of the various compounds to induce inflammation and

hyperplasia in the skin was determined.

Test substance : 2,4-/2,6-DNT mixture (2:1) in acetone
2,4-DNT: purity 92-95% (impurities: 2,6-DNT)
purity 98% (impurities: 2,6-DNT, 2-nitrotoluene)
2,6-DNT: purity 92-95% (impurities: 2-nitrotoluene, 2,4-DNT)
purity 98% (impurities: none)

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint

26.11.2003 (170)

Species : mouse
Sex : no data
Strain : Sencar
Route of admin. : dermal
Exposure period : see TC
Frequency of treatm. : once
Post exposure period : see TC
Doses : 1, 5, 10 mg/mouse in acetone
Result : negative
Control group : other: TPA administration alone as control group
Method : other: dermal initiation-promotion assay (see TC)
Year : 1985
GLP : no data
Test substance : other TS: 2,4-/2,6-DNT mixture (1:1) in acetone

Result : The 1:1 mixture of 2,4-DNT and 2,6-DNT showed no tumor initiating activity.
10 mg: 8 %, 5 mg: 15 %, 1 mg: 15 % of mice with papillomas (control: 13 %); none with carcinomas (control: 2.5 %);
5 and 10 mg gave a significant level of epidermal hyperplasia and dark cells

Test condition : Initiation-promotion protocol:
initiation: 2,4-/2,6-DNT (1:1), one application
promotion: 4 ug TPA (7.12-dimethyl(a)benzene(DMBA)-12-O-tetradecanoylphorbol-13-acetate), dermal application, daily, 30 weeks;
examination of papillomas after 30 weeks
negative control: TPA only
positive control: B(a)P and 4-NQO

Reliability : (4) not assignable
Test substance different to technical grade DNT.

26.11.2003 (170)

5.8.1 TOXICITY TO FERTILITY

Type : other
Species : rat
Sex : male/female
Strain : Fischer 344
Route of admin. : oral feed
Exposure period : 104 w
Frequency of treatm. : continuously
Premating exposure period
Male :
Female :
Duration of test :

No. of generation studies	:	
Doses	:	3.5, 14, 35 mg/kg bw
Control group	:	yes, concurrent no treatment
NOAEL parental	:	>= 3.5 mg/kg bw
Method	:	other
Year	:	1978
GLP	:	no
Test substance	:	other TS: technical grade DNT (see TS)
Remark	:	For further details see Sections 5.4 and 5.7
Result	:	mortality: control: 10/130 (m), 12/130 (f); 3.5 mg: 14/130 (m), 15/130 (f); 14 mg: 48/130 (m), 9/130 (f); 35 mg (at week 55): 8/130 (m), 2/130 (f); for further data see Section 5.4 Effects on reproductive tissues: control: abnormally small testes in 11.5% (7/61) of males; testes degeneration in 8% (2/25) 35 mg (52 or 55 week sacrifice): mean body weight gain in week 50 was only about 50% (m) and 57% (f) of control; gross examination: abnormally small testes in 25% (25/101) of the animals, significantly reduced absolute testicular weights at w 52, however, relative weights not affected; histopathology: moderate to moderately severe testicular degeneration (in 15/20 animals) plus hypospermatogenesis (in 14/20 animals) 14 mg (104 week exposure): mean body weight gain in week 102 was only about 66% (m) and 74% (f) of control; gross examination: abnormally small testes in 29% (7/24) of the animals; increase in the absolute (57%) and statistically significant increase of the relative weight (100%) of the ovaries, however, no dose dependent effect 3.5 mg (104 week exposure): mean body weight gain in week 104 was only about 92% (m) and 86% (f) of control (not statistically signifcant); gross examination: statistically significant increase in the absolute (13%) and relative weight of the testes; absnormally small testes in 15.7% of males; Early onset of interstitial cell tumors of the testes at 14 and 35 mg, however, at the 78 week interim sacrifice also all 10 control males showed interstitial cell tumors. At week 104 82/101 control rats and 104/108 mid dose males had interstitial cell tumors. High dose males showed interstitial cell tumors in a frequency of 13/40 (52 week sacrifice)
Test condition	:	130 rats/sex/group, twice daily observations, clinical laboratory studies, interim sacrifice at week 26 and 55: 10 rats/sex/dose; at week 78: 20 rats/sex/dose; high dose: sacrifice of all surviving rats at week 55; terminal sacrifice: at week 104 all surviving rats gross examination and histopathological examination in control and high-dose group
Test substance	:	technical grade DNT: (data from Rickert et al., CRC Crit. Rev. Toxicol. 13, 217-234, 1984) 2,4-DNT 76.4 % 2,6-DNT 18.8 % 2,5-DNT 0.7 % 3,5-DNT 0.04 % 2,3-DNT 1.5 % 3,4-DNT 2.4 %
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag	:	Critical study for SIDS endpoint
26.11.2003		(151)
Type	:	other: Dominant Lethal Assay
Species	:	mouse
Sex	:	male
Strain	:	other: DBA/2J
Route of admin.	:	gavage
Exposure period	:	2 days
Frequency of treatm.	:	once daily
Premating exposure period		
Male	:	
Female	:	
Duration of test	:	
No. of generation studies	:	
Doses	:	250 mg/kg bw/d in corn oil
Control group	:	yes, concurrent vehicle
NOAEL parental	:	>= 250 mg/kg bw
Method	:	other
Year	:	1980
GLP	:	no
Test substance	:	other TS: technical grade DNT (see TS)
Result	:	Number of implants: not affected by DNT Number of post-implantation deaths: not affected by DNT Number of fertile matings: significantly increased in the second week of mating; slightly increased in weeks 3-5 of mating
Test condition	:	TEST ORGANISMS: - Age: 10-12 weeks - No. of animals per dose: 20/dose group, 5/control group ADMINISTRATION: - Duration of test: 7 weeks mating procedure starting 48 hours p.a. - Frequency of treatment: twice - Negative control: corn oil - positive control: ethylmethanesulfonat (2x125 mg/kg bw) MATING PROCEDURE: - 48 hours posttreatment males were mated with 3 female CD1 mice each for one week; this mating procedure was repeated with vergine females for in total 7 weeks - the females were killed 17 days after initial exposure to a male and their uterine contents examined for living fetuses and postimplantation deaths EXAMINATIONS: - Uterine contents: % fetal deaths per pregnant female, number of implantations per pregnant female, - Fertility (% of fertile matings based on the number of total paired females) - Criteria for evaluating results: significant increase of fetal deaths, significant decrease of implantations, impairment of fertility - Criteria for selection of M.T.D.: LD50
Test substance	:	technical grade DNT: same lot as in CIIT bioassay (CIIT, 1978); current analysis: 2,4-DNT 76.4 % 2,6-DNT 18.8 % 2,5-DNT 0.7 % 3,5-DNT 0.04 % 2,3-DNT 1.5 % 3,4-DNT 2.4 %
Conclusion	:	No indications for reduced fertility

Reliability	:	(2) valid with restrictions Test procedure in accordance with generally accepted scientific standards and described in sufficient detail
Flag	:	Critical study for SIDS endpoint
26.11.2003		(138)
Type	:	other: Dominant Lethal Assay
Species	:	mouse
Sex	:	male
Strain	:	other: DBA/2J
Route of admin.	:	i.p.
Exposure period	:	2 days
Frequency of treatm.	:	once daily
Premating exposure period		
Male	:	
Female	:	
Duration of test	:	
No. of generation studies	:	
Doses	:	250 mg/kg bw/d in corn oil
Control group	:	yes, concurrent vehicle
NOAEL parental	:	>= 250 mg/kg bw
Method	:	other
Year	:	1980
GLP	:	no
Test substance	:	other TS: technical grade DNT (see TS)
Result	:	Number of implants: not affected by DNT Number of post-implantation deaths: not affected by DNT Number of fertile matings: significantly increased in the second week of mating; slightly increased in weeks 3-5 of mating
Test condition	:	TEST ORGANISMS: - Age: 10-12 weeks - No. of animals per dose: 20/dose group, 5/control group ADMINISTRATION: - Duration of test: 7 weeks mating procedure starting 48 hours p.a. - Frequency of treatment: twice - Negative control: HBSS buffer - positive control: triethylenemelamine (2x0.15 mg/kg bw) MATING PROCEDURE: - 48 hours posttreatment males were mated with 3 female CD1 mice each for one week; this mating procedure was repeated with vergine females for in total 7 weeks - the females were killed 17 days after initial exposure to a male and their uterine contents examined for living fetuses and postimplantation deaths EXAMINATIONS: - Uterine contents: % fetal deaths per pregnant female, number of implantations per pregnant female, - Fertility (% of fertile matings based on the number of total paired females) - Criteria for evaluating results: significant increase of fetal deaths, significant decrease of implantations, impairment of fertility - Criteria for selection of M.T.D.: LD50
Test substance	:	technical grade DNT: same lot as in CIIT bioassay (CIIT, 1978); current analysis: 2,4-DNT 76.4 % 2,6-DNT 18.8 % 2,5-DNT 0.7 % 3,5-DNT 0.04 %

2,3-DNT 1.5 %
3,4-DNT 2.4 %

Conclusion : No indications for reduced fertility
Reliability : (2) valid with restrictions
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail
Flag : Critical study for SIDS endpoint
26.11.2003 (138)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : rat
Sex : female
Strain : Fischer 344
Route of admin. : gavage
Exposure period : 14d (gd 7-20)
Frequency of treatm. : daily
Duration of test : sacrifice on gestation day 20
Doses : 14, 35, 37.5, 75, 100, 150 mg/kg in corn oil
Control group : yes, concurrent vehicle
NOAEL maternal tox. : >= 14 mg/kg bw
NOAEL teratogen. : = 150 mg/kg bw
NOAEL Embryotoxicity : >= 150 mg/kg bw
NOAEL Fetotoxicity : >= 150 mg/kg bw
Method : other
Year : 1982
GLP : no data
Test substance : other TS: technical grade DNT (see TS)

Remark : Positive control hydroxyurea produced 30.6% malformed fetuses per litter.
Result : Maternal toxicity:
mortality: 4.5% (14 mg-gr), 7.7 % (35 mg-gr), 0.0 % (37.5 mg- gr), 0.0 % (75 mg-gr), 4.3 % (100 mg-gr), 46.2 % (150 mg-gr; death between gd 11 and 18), 0% (vehicle and positive control groups)
Clinical signs: 150 mg-gr.: rough coat, lethargy, hind-limb weakness
hematology: characteristic signs of DNT toxicity in 100 and 150 mg-gr. (only groups examined on gd 20): increase of methemoglobin, reticulocyte count and platelet count, red blood cell size and distribution width, decrease of red blood cell count and hematocrit
maternal weight gain: mean absolute weight gain (without gravid uterine weight) significantly decreased in comparison to control (24g) at 14 mg (17g, reduced by 29%), 35, 37.5 and 75 mg (no effect), 100 mg (15g, 37%), 150 mg (-14g); total weight gain (including gravid uterine weight) significant decrease at 150 mg (8g versus 62 g in control)
relative liver weights: significant decrease at 14 mg, significant increase at >= 75 mg/kg
relative spleen weights: significant increase at >= 35 mg (+35%), 75 mg (+22%).
Above 75 mg/kg relative organ weights affected by reduced body weight gain.

Fetal toxicity:
no significant effects on % resorptions, % dead or live fetuses; no significant effects on live litter size, fetal body weight, crown-rump length, sex distribution, fetal grouwth and morphological develop0ment; changes of fetal liver and spleen weights without dose dependence and also

occurring in control groups
100 mg: changes of hematological parameters (as in dams)
no statistically significant increased malformations at any dose: % malformed fetuses per litter (external, visceral, sceletal) in dose groups 14/35/37.5/75/100/150/vehicle/pos. control
4.1/3.2/0.8/0.0/5.8/0.0/3.8/30.6

Test condition : Total number of animals evaluated for maternal toxicity in dose groups 14, 35, 37.5, 75, 100, 150 mg/kg, in
vehicle control, positive control (200 mg hydroxyurea/kg):
22/13/22/13/23/13/37/36
Total number of pregnants evaluated in teratogenicity study:
13/7/13/7/13/6/22/22
Total number of live litters examined:
10/7/12/6/12/2/20/19
Total numer of live fetuses examined:
92/63/77/50/88/22/146/146
DOSING:
First breeding: 0, 75, or 150 mg/kg bw/day
Since the mortality in the high-dose group was unexpected high, mated animals from the second and third breedings were treated with 14, 37.5, or 100 mg/kg bw/day.
negative control: vehicle (corn oil)
positive control hydroxyurea (200 mg/kg bw/day, p.o.)
EXAMINATIONS:
dams: body weight, liver weight, spleen weight, number of corpora lutea, gravid uterine weight, and status of uterine implantation sites
fetuses: uterine position, body weight, crown-rump length, placental weight, sex, and gross morphological abnormalities (50% of fetuses recorded for visceral or skeletal malformations)
STATISTICS:
data were pooled across breedings for statistical analysis, Kruskal-Wallis one-way analysis of variance by ranks was used

Test substance : technical grade DNT:
76 % 2,4-DNT
19 % 2.6-DNT
2.4 % 3.4-DNT
1.5 % 2.3-DNT
<1 % 2,5-DNT
<1 % 3,5-DNT

Conclusion : Fetal toxicity at dose with high maternal toxicity only, no indications for a teratogenic potential of DNT.
Maternal toxicity:
150 mg/kg: strong mortality, mean body weight gain strongly reduced, bad appearance
100 mg/kg: hematologic changes, reduced body weight gain
75 mg/kg: relative spleen weights increased
35 mg/kg: relative spleen weights increased
14 mg/kg: weight gain reduction and reduced relative liver weight not regarded as relevant

Fetal toxicity:
100 and 150 mg/kg: hematological effects due to maternal toxicity

Reliability : (2) valid with restrictions
Test procedure mainly in accordance with generally accepted scientific standards and described in sufficient detail; however, insufficient number of DNT-treated pregnant animals per dose (6-13)

Flag : Critical study for SIDS endpoint
26.11.2003 (177) (178) (179)

Species : rat

Sex	:	female
Strain	:	Fischer 344
Route of admin.	:	gavage
Exposure period	:	14 d (gd 7-20)
Frequency of treatm.	:	daily
Duration of test	:	sacrifice: dams on postnatal day 30, offspring on postnatal day 60
Doses	:	14, 35, 37.5, 75, 100, 150 mg/kg in corn oil
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	>= 14 mg/kg bw
Method	:	other
Year	:	1982
GLP	:	no data
Test substance	:	other TS: technical grade DNT (see TS)
Result	:	Maternal toxicity: during gravidity: mortality: 4.5% (14 mg-gr), 7.7 % (35 mg-gr), 0.0 % (37.5 mg- gr), 0.0 % (75 mg-gr), 4.3 % (100 mg-gr), 46.2 % (150 mg-gr); 0% in control groups Clinical signs: 150 mg-gr.: rough coat, lethargy, hind-limb weakness hematology: characteristic signs of DNT toxicity in 100 and 150 mg-gr. (only groups examined on gd 20): increase of methemoglobin, reticulocyte count and platelet count, red blood cell size and distribution width, decrease of red blood cell count and hematocrit Postnatal toxicity: on postnatal day 15: 100 mg reduced body weight; on postnatal day 30: 75 mg reduced reticulocytes; no further effects Offspring: single changes of litter size, crown-rump length, body weight and hematological parameters without dose- or time-dependency age of appearance of physical signs: eye opening earlier at 14 mg and delayed at 35 and 75 mg; age of appearance of neurobehavioral signs: cliff avoidance delayed at 35 and 75 mg; wire grasping earlier at 14 mg and delayed at 35 mg female pups: decrease of rearing in the open field In view of the absence of a dose-relationship a connection of the observed effects with DNT exposure is unlikely NOAEL developmental toxicity: >= 150 mg/kg bw
Test condition	:	Total number of animals (evaluated for maternal toxicity, afterwards dividing for evaluation of teratogenic potential or developmental toxicity): in dose groups (14, 35, 37.5, 75, 100, 150 mg/kg), in vehicle control, positive control (200 mg hydroxyurea/kg): 22, 13, 22, 13, 23, 13, 37, 36 Postnatal developmental toxicity study: Number of evaluated dams in dose groups (14, 35, 37.5, 75, 100 mg/kg), in vehicle control, positive control (200 mg hydroxyurea/kg): 5, 5, 6, 5, 7, 14, 11 Evaluated parameters: body weight, crown-rump-length liver, spleen and testes weight hematological parameters age of appearance of physical signs: pinna detachment, pilation, incisor eruption, eye opening, testes descent,

vaginal opening
age of appearance of neurobehavioral signs: surface righting, cliff avoidance, auditory startle, wire grasping, mid-air righting
open-field behaviour on postnatal day 30

Test substance : technical grade DNT:
76 % 2,4-DNT
19 % 2.6-DNT
2.4 % 3.4-DNT
1.5 % 2.3-DNT
<1 % 2,5-DNT
<1 % 3,5-DNT

Conclusion : Signs of postnatal toxicity were transient both for dams and pups by postnatal days 30 and 60 respectively. During the postnatal period various dosages of DNT produced either statistically significant faciliation or retardation of growth or development, but no dose-relationship existed.

Reliability : (2) valid with restrictions
Deviations from guideline study: insufficient number of DNT-treated dams (5-7)

Flag : Critical study for SIDS endpoint

26.11.2003 (177)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

Endpoint : other: cell transformation assay
Study descr. in chapter :
Reference :
Type :
Species : other: Syrian Hamster Embryo (SHE) cells and inhibition of intercellular communication
Sex :
Strain :
Route of admin. :
No. Of animals :
Vehicle :
Exposure period :
Frequency of treatm. :
Doses : 0 - 10 µg/ml
Control group :
Observation period :
Result : negative
Method : other
Year : 1990
GLP : no data
Test substance :

Result : No induction of morphological transformation of SHE cells by DNT treatment (no further details).
Inhibition of intercellular communication at cytotoxic concentration of 200 µg DNT/ml.

Test substance : composition of DNT:
51.6 % 2,4-DNT
12.5 % 2,6-DNT

1.7 % 3,4-DNT
29.4 % 2,4-Dinitroethylbenzene
2.7 % 2,6-Dinitroethylbenzene
1.0 % 3,4-Dinitroethylbenzene
0.2 % mononitrotoluenes
0.2 % ethylbenzene

Reliability : (4) not assignable
Limited documentation; test substance different to technical grade DNT

19.11.2003 (180)

Endpoint : other: metabolic cooperation assay
Study descr. in chapter :
Reference :
Type :
Species : other: V79 cells
Sex :
Strain :
Route of admin. :
No. Of animals :
Vehicle :
Exposure period : 4 day(s)
Frequency of treatm. :
Doses : 0.001 - 1000 µM
Control group :
Observation period :
Result :
Method : other: adopted from Yotti et al., Science 206, 1089-1091,1979
Year : 1983
GLP : no data
Test substance :

Result : No inhibition of metabolic cooperation up to cytotoxic concentrations.
Test condition : Inhibition of metabolic cooperation between 6-thioguanine resistant (TG-r) and sensitive (TG-s) V79 cells by DNT was investigated.
7 tissue culture plates with TG-r and TG-s cells were treated for 4 days with the TS. Then the medium was changed to selective medium containing 6-TG without test chemical and the cultures were incubated for additional 3 days. Cells were then fixed, stained and colonies containing >50 TG-r cells were counted.
TPA (12-O-tetradecanoyophorbol-13-acetate) was used as positive control.
Cytotoxic DNT concentration: 500 µM
Test substance : technical grade DNT:
76.5 % 2,4-DNT
18.8 % 2,6-DNT
2.4 % 3,4-DNT
1.5 % 2,3-DNT
0.65 % 2,5-DNT
< 0.1 % 3,5-DNT
Reliability : (2) valid with restrictions
limited documentation

18.11.2003 (181)

5.10 EXPOSURE EXPERIENCE

Type of experience : Health records, other

Remark : No differences were found when 84 workers exposed both to dinitrotoluene (exposure level within OSHA threshold limit

value of 1.5 mg/m³) and toluenediamine and 119 unexposed workers were the subjects of a physicians urogenital examination, a reproductive and fertility questionnaire, an estimation of testicular volume, an assessment of serum follicle-stimulating hormone, and an analysis of semen for sperm count and morphology. Detailed exposure data are not available.

Test condition : Workers are divided in the following exposure groups:
1) none to minimal exposure (0-1 exposure time, working lifetime)
2) low to high (variable exposure time, > 6 months prior to study)
3) low to moderate (variable exposure time, within 6 months of study)
4) high (variable exposure time, within 6 months of study)
Because the normal sperm cycle is 72 days, using six months as the division between current and former exposure allowed more than two full sperm cycles for complete recuperation from any - except the most severe - spermatogenic insult.
Abstinence from ejaculation for at least three days prior to the examination was requested to standardize the estimates of sperm count. The semen analysis included the determination of the semen volume, total sperm count, sperm concentration, morphology of spermatozoa and classification of abnomal sperm by morphologic types. Additionally, a physicians urogenital
examination, a testicular volume estimate, and an assessment of serum follicle-stimulating hormone (FSH) was performed. The workers completed a reproductive and fertility questionnaire.

Test substance : DNT, not further specified

Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles, acceptable restrictions in documentation, acceptable for assessment

Flag : Critical study for SIDS endpoint

15.05.2003 (182)

Type of experience : Direct observation, poisoning incidents

Remark : Case report: engineer aged 28, burning with hot fumes containing a considerable concentration of dinitrotoluene (no other information): skin was burnt, corrosion of the eye lids and of the right cornea; only slow healing of the skin, the corroded conjunctiva; cicatrization, secondary scars, and deformities could not be removed completely even by repeated transplantations. The right corneal defect healed up leaving an adherent leukoma.

Reliability : (4) not assignable
Abstract; chemical composition not defined

19.11.2003 (183)

Type of experience : Health records, other

Remark : Two cohorts of 156 men (exposed to technical grade DNT consisting of approximately 76% 2,4-DNT, 19% 2,6-DNT, and 5% other isomers) and 301 men (exposed to purified DNT: 98 % 2,4-DNT and about 1% 2,6-DNT) who had worked a month or more during the 1940s and 1950s in ammunition plants with opportunity of substantial DNT exposure (no further details, also exposure to other materials, see TS) were followed through the end of 1980. Numbers of expected deaths and standardized mortality ratios (SMRs) were computed, using mortality rates of US white males as the standard.
The SMR of 129 for all causes of deaths was significantly higher (p =

0.001) than expeced. No evidence of a carcinogenic effect was found (SMR 87) but an unsuspected excess of mortality from ischemic heart disease was noted at both plants: SMRs in the two cohorts were 131 and 143. A relationship with intensity and duration of exposure was suggested. No detailed data on exposure.

Test substance : At one plant, workers had opportunities for exposure to mono- and dinitrotoluenes, nitric and sulfuric acids, and toluene. At the second plant, the opportunities for exposure to DNT as well as many other materials did exist.

Reliability : (2) valid with restrictions
Limited documentation; no measurements on exposure; exposure to DNT and other materials assumed

Flag : Critical study for SIDS endpoint
19.11.2003 (184) (185) (186) (187)

Type of experience : Health records, other

Remark : The estimation of the daily DNT (technical grade) absorption by workers in a DNT manufacturing plant ranged from 0.24 to 1.0 mg/kg bw. Inhalative exposure was estimated to range from 50-590 µg/m3. Wiping of skin suspected of being contaminated (mainly hands and forehead) showed levels of "not detected" to 179.5 µg 2,4-DNT. Absorption was measured by quantification of excreted DNT metabolites in the urine. The urine of the workers contained more metabolites than would have resulted from the dinitrotoluene present in the inhaled air, indicating dermal absorption. For further information on this study see chapter 5.0.

Test substance : technical grade DNT:
76.4% 2,4-DNT
18.8% 2,6-DNT
4,8% other isomers (not further specified)

Reliability : (2) valid with restrictions
Limited documentation: isomers not fully specified

Flag : Critical study for SIDS endpoint
22.05.2003 (131)

Type of experience : Health records, other

Remark : There was no significant difference between the fertility of workers exposed to DNT in 3 U.S. chemical plants between May 1973 and April 1976 and the fertility of unexposed workers. A total of 670 workers were subjected to a reproductive and fertility questionaire. Detailed exposure data are not available.

Test condition : Exposure in plant A:
from May 1973 to April 1976 exposure to DNT (not further specified); from April 1976 on possibility for exposure to both DNT and TDA (toluene diamine)
participants: 137 in 1979 and 91 in 1980 (of whom 84 were interviewed for the second time)
Exposure in plant B:
variety of chemicals, including DNT, TDA, and TDI (toluene diisocyanate)
participants: 207
Exposure in plant C:
variety of chemicals, partly including DNT
participants: 235
In all plants no information on extend/quantity of exposure.
All participants were interviewed and filled a questionnaire for observed births, which was then compared to the expected birth rate of unexposed

persons, giving the standardized fertility ratio (SFR).

Test substance : DNT (not further specified), TDA, TDI and other chemicals

Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles, acceptable restrictions in documentation, acceptable for assessment

Flag : Critical study for SIDS endpoint

20.11.2003 (188) (189)

Type of experience : Human

Remark : Dinitrotoluene and Toluene diamine cause decrease in sperm count, but showed no effect on sperm morphology (no details available).

Reliability : (4) not assignable
Secondary literature

20.05.2003 (190) (191)

Type of experience : Health records, other

Remark : A total of 4989 workers exposed to DNT and 7436 unexposed workers who had worked at least 5 months at the study facility between January 1949 and January 1980 were included in this investigation.
An excess of hepatobiliary cancer was observed among workers exposed to DNT in this study in 1982. Numbers of expeced deaths and standadized mortality rates (SMRs) were computed, using mortality rates of US white males as the standard. DNT-exposed cohort: 6 cases or hepatobiliary cancer, SMR 2.67; unexposed cohort: 4 cases, SMR 0.81
Exposure-response relationship between duration of DNT exposure and hepatobiliary cancer mortality could not be demonstrated.
Limitations of the study: small number of workers with long duration of DNT exposure, lack of quantitative information on exposure to DNT and other chemicals.

Reliability : (4) not assignable
limitations of the study: small number of workers with long duration of DNT exposure, lack of quantitative information on exposure to DNT and other chemicals.

Flag : Critical study for SIDS endpoint

25.11.2003 (192)

Type of experience : Human

Remark : 154 men exposed to DNT in screening houses and coating houses complained on unpleasant taste (62%), muscular weakness (51%), headache (49%), inappetence (47%), dizziness (44%), nausea (37%), insomnia (37%), pain in extremeties (26%), vomiting (23%), numbness and tingling (19%). The chief findings from clinical examinations were pallor (36%), cyanosis (34%), anemia (23%), leukocytosis (12%). The symptoms disappeared within 2 or 3 days after removal from the exposure.
No quantitative data on exposure.

Test substance : DNT, primarily the 2,4-isomer form (no further data)

Reliability : (2) valid with restrictions
Limited documentation

Flag : Critical study for SIDS endpoint

20.11.2003 (193)

Type of experience : Human

Remark : Case report: 20 year old, healthy men worked from June 25 to July 31 in 1941 as a DNT Screen Operator. There was no history of previous jaundice, nemia or other illness. He complained of headache, insomnia, weakness, nervousness, nausea, vomiting, unpleasant taste in his mouth. He was pale, his lips and finger nails were blue; there was a slight yellow tinge in his sclerae, liver was palpable 2 fingers breadth below the costal margin and was tender, his urine was dark; erythrocyte-count: 3,070,000, hemoglobin: 12.75 g. He recovered after permanent removal from dinitrotoluene exposure and additional treatment.
No quantitative data on exposure.

Test substance : DNT, primarily the 2,4-isomer form (no further data)

Reliability : (2) valid with restrictions
Limited documentation

20.05.2003 (193)

Type of experience : Human

Remark : case report: 22 years old, healthy man worked in a building where powder grains are coated with dinitrotoluene in a revolving drum from June 7 to July 31 in 1941. He complained of inappetence, an unpleasant taste in his mouth, nausea, dizziness, nervousness and pain in his left arm. There was a slight palor of his face, cyanosis of his lips, ear lobes and finger nails, and a perceptible yellow tinge in his sclerae; liver was palpably enlarged and tender, his urine was dark, pulse rate: 80. blood pressure: 138/80, erythrocyte count: 5,200,000, hemoglobin: 17 g. He recovered after permanent removal from exposure and additional treatment.
NO quantitative data on exposure.

Test substance : DNT, primarily the 2,4-isomer form (no further data)

Reliability : (2) valid with restrictions
Limited documentation

20.05.2003 (193)

Type of experience : Human

Remark : In 714 workers exposed to DNT between 1942 and 1945, headache (13.2%) and weakness (8.7%) were the most frequent complaints, a low-grade anemia (10.2%) and cyanosis (8.7%) the most frequent findings.
No quantitative data on exposure.

Test condition : In 1942 the provision of better ventilation and other alterations in the handling of DNT furnished a working environment with atmospheric concentrations usually much less than one milligram of dinitrotoluene per m^3.

Reliability : (2) valid with restrictions
Limited documentation

Flag : Critical study for SIDS endpoint

20.05.2003 (194) (195)

Type of experience : Health records, other

Remark : A total of 4989 workers exposed to DNT and 5636 unexposed workers who had worked at least 5 months at the study facility between January 1949 and January 1980 were included in this investigation.
A retrospective cohort mortality study in 1982 failed to detect an

association between dinitrotoluene exposure and an increased risk of appearance of ischemic heart disease (IHD) or cerebrovascular disease mortality.

Test condition : A modified life-table program was used to compute the expected numbers of deaths by multiplying mortality rates specific for cause, five-year age groups, and five-year calendar groups, by the corresponding person-year distribution of the study population.

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint
20.05.2003 (196)

Type of experience : Health records, other

Remark : The health hazard evaluation of workers exposed to DNT in an US chemical company suggests that there are problems of toxicity to the male reproductive system in group 3 represented by a significant decreased sperm count. Furthermore an excess of spontaneous absorptions for wives of exposed workers was reported, which was statistically not significant.
No further details available from review.

Test condition : 44 workers were divided in three groups:
1) control persons
2) low exposed, not for the last 2 years
3) exposed
air concentration: below 0.42 mg/m³
no further information

Reliability : (2) valid with restrictions
Data taken from review

Flag : Critical study for SIDS endpoint
20.05.2003 (197)

Type of experience : Health records, other

Remark : Among 500 underground miners formerly highly exposed to explosives containing technical DNT out of a total group of 3000 underground miners in a plant in the former German Democratic Republic 6 cases of urothelial cancer and 14 cases of renal cancer occurred.
Exposure durations ranged from 7-37 years and latency periods ranged from 21-46 years. Incidences of urothelial and renal cancer were increased by factors of 4.5 and 14.3 in comparison to the incidences anticipated from the local cancer registers.
The cancer cases and a representative group of 183 miners with DNT exposure were grouped into 4 exposure categories according to types and duration of contact to DNT (skin contact and inhalative exposure, no quantitative data). This categorization of the 14 renal cancer cases revealed no dose dependency and distribution of exposure categories was comparable among the 14 cases and 183 representative subjects. However, the 6 cases of urothelial cancer were predominantly confined to the high-exposure categories.
Genotyping for N-acetyltransferases and glutathione-S-transferases identified all urothelial cancer cases exclusively as slow acetylators.
The findings provide evidence to the possibility of a carcinogenic action of DNT for humans with the urothelium

tissue as target.

Test substance : Explosives with 30% technical DNT (about 80% 2,4-DNT and 20% 2,6-DNT); the composition of the remaining 70% of the explosives is not given

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint

20.05.2003 (198) (199) (200)

Type of experience : Health records, other

Remark : 161 representative underground miners, that had all been highly exposed to DNT, and 19 miners with renal or urothelial cancer formerly highly exposed to explosives containing technical DNT out of a total group of 3000 underground miners in a plant in the former German Democratic Republic were reinvestigated for signs of subclinical renal damage. Studied parameters were alpha1-microglobulin and GST alpha as biomarkers for damage of the proximal tubule and GST pi for damage of the distal tubule.
The miners were grouped into 4 exposure categories according to types and duration of contact to DNT: skin contact (at least 5 hours per day) and inhalative exposure (no quantitative data; semi-quantitative procedure).
Results indicated a dose-dependent nephrotoxic effect directed to the tubular system with an increased incidence in renal or urothelial cancer cases (n=19) in contrast to the group of representative miners without cancer (n=161).

Test substance : Explosives with 30% technical DNT (about 80% 2,4-DNT and 20% 2,6-DNT); the composition of the remaining 70% of the explosives is not given

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint

20.11.2003 (201) (202)

Type of experience : Health records, other

Remark : DNT not further specified

Result : Exposed workers showed changes of the following parameters in blood:
both exposure groups:
decrease of erythrocytes (4.75 in control to 4.22 in exposed workers), hemoglobin (145 to 139 g/l), and GST;
increase of Heinz bodies (3.7% to 24.4%), ALT, SDH and Fe
high exposure:
decrease of CuZn-SOD;
increase of methemoglobin (0.19% in control to 1.66% in exposed workers)

Test condition : The industrial hygiene of working environment and the health status of 81 DNT exposed workers were investigated.
22 worked in high and 59 in low concentration of DNT: time-weighted average concentrations (TWA) of DNT were 1.64 mg/m³ and 0.67 mg/m³, respectively.
30 persons were used as control.

Conclusion : DNT could inhibit anti-oxidant capacity and induce toxic hemolytic anemia and liver injury.

Reliability : (4) not assignable
study in chinese, only english abstract

Flag : Critical study for SIDS endpoint

20.11.2003 (203)

Type of experience : Human

Result : Air analyses yielded maximum concentrations of 20 µg/m³ for 2,4-DNT. The maximum concentrations in the urine of workers regularly exposed amounted to 2.1 µg/l of 2,4-DNT, 95.0 µg/l of 2,4-dinitrobenzoic acid, and 3.6 µg/l of 2,6-DNT. In 63 persons TNT or DNT or metabolite concentrations above the analytical detection limit were found in urine. These persons reported more frequently symptoms like bitter taste, burning eyes, and discoloration of the skin and hair than persons (n= 19) without detectable exposure.
The clinical laboratory examination revealed findings outside the normal range, but no relation to the exposure could be found on a group basis.

Test condition : 82 employees from a mechanical plant (dismantling of military waste) in Germany were studied, of whom 51 were regularly expopsed to ammunition containing TNT and DNT, 19 occasionally, and 12 not at all. To quantify the internal exposure 2,6-DNT, 2,4-DNT, and its main metabolite 2,4-dinitrobenzoic acid were determined in urine specimens of all subjects using gas chromatographic/mass spectrometric procedures. (also TNT and metabolite adducts were measured)
The investigation included a standardised questionnaire with closed questions on personal medical history, a general physical examination, extensive clinical laboratory tests and biological monitoring of exposure. Persons were divided by the results of biological monitoring in two groups. If TNT and/or DNT and/or their metabolites were above the analytical detection limit, the persons were defined as "exposed"; the others were defined as "not exposed".

Reliability : (2) valid with restrictions
limited documentation

Flag : Critical study for SIDS endpoint

11.11.2003 (133)

Type of experience : Health records, other

Remark : Two groups of workers (with 28 and 5 participants, respectively) were monitored over tow separate 1-week production campaigns. Atmospheric concentrations of DNT were mainly below the recommended limit for DNT in factory atmospheres for an 8-hr time weighted average exposure of 1.5 mg/m³. Concentrations determined by personal monitoring ranged from 0.02 to 2.68 mg/m³ (mean 0.4, standard deviation 0.65, n=25). DNT absorption is measured by determination of 2,4-DNBA, a DNT metabolite, in urine samples.
Since the atmospheric levels of DNT could not account for the observed excretion of 2,4-DNBA it is suggested that skin may be the major route of absorption in this study. For further information on this study see chapter 5.0.

Test substance : technical grade DNT:
76% 2,4-DNT
20% 2,6-DNT
(not further specified)

Reliability : (2) valid with restrictions
limited documentation: isomers not fully specified

Flag : Critical study for SIDS endpoint

20.11.2003 (130)

5.11 ADDITIONAL REMARKS

Type : other

Remark : When administered orally at doses corresponding to 0.1-0.2 LD50 values to rats for 1-3 months, the hematotoxicity of the toluene derivates decreased in the order: trinitrotoluene, dinitrotoluene, m-nitrotoluene, p-nitrotoluene, amd o-nitrotoluene. The toluene derivates caused anemia, accompanied by reticulocytosis, a decrease in the level of SH-groups and an increase in that of fibrinogen in the blood

Reliability : (4) not assignable
English abstract

23.05.2003 (204)

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