FOREWORD INTRODUCITON

1,2-Dichloro-4-nitrobenzene

CAS N°:**99-54-7**

SIDS Initial Assessment Report

For

SIAM 17

Arona, Italy, 11 - 14 November 2003

1.	**Chemical Name:**	1,2-Dichloro-4-nitrobenzene
2.	**CAS Number:**	99-54-7
3.	**Sponsor Country:**	Germany 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
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): 8 Mai 2003 (Ecotoxicology): databases CA, biosis; searchprofile CAS-No. and special search terms 1 March 2003 (Toxicology): databases medline, toxline; 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:**	August 13, 2003
10.	**Date of last Update:**	

11. Comments:

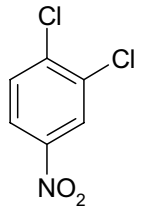
OECD/ICCA - The BUA* Peer Review Process

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.	99-54-7
Chemical Name	1,2-Dichloro-4-nitrobenzene
Structural Formula	

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

1,2-Dichloro-4-nitrobenzene is absorbed from the gastro-intestinal tract and although there are some species differences in experimental animals from the available data it can be concluded that 1,2-dichloro-4-nitrobenzene is excreted mainly via urine in the form of the mercapturic acid derivate N-acetyl-S-(2-chloro-4-nitrophenyl)-L-cysteine. Data on humans were not identified in the available literature.

There are no valid acute inhalation studies available. Based on the results of the acute dermal toxicity study with rats the LD50 is > 2000 mg/kg bw. From studies with rabbits no LD50 could be derived, the lowest Lethal Dose Level (LDLo) was 950 mg/kg bw. The acute oral toxicity in rats ranges from 625 to 950 mg/kg bw. 1,2-Dichloro-4-nitrobenzene causes the formation of methaemoglobin. Predominant signs of intoxication were lethargy, increasing weakness, collapse and coma.

1,2-Dichloro-4-nitrobenzene gave no skin irritation effects when tested for 4 hours under semiocclusive conditions according to OECD TG 404 and showed slightly irritating effects, which disappeared within 72 hours under occlusive conditions according to the method of Federal Register 38 No. 187. 1,2-Dichloro-4-nitrobenzene is slightly irritating to the eyes when tested according to OECD TG 405. 1,2-Dichloro-4-nitrobenzene was not found to induce dermal sensitization when tested according to OECD TG 406. In addition, 1,2-dichloro-4-nitrobenzene was not found to induce dermal sensitization in humans in a limited study.

The main targets identified in animal studies after repeated oral administration as well as after inhalation exposure are the haematological system and in addition the kidneys after oral application and the liver after inhalation. From a 28-day oral study performed according to OECD TG 407 a NOAEL of 4 mg/kg bw/day was derived. The NOAEL following subchronic inhalation exposure study of limited validity (limited documentation) was 0.4 mg/m³ (4 hours per day).

Changes in haematological parameters (e.g. methaemoglobinaemia, Heinz bodies) are the main target in the only available report on exposure of workers. As these findings relate to mixed exposures they cannot be clearly attributed to 1,2-dichloro-4-nitrobenzene, but would be plausible, because they were also observed in animal experiments. In the recent open literature reports of human poisoning could not be identified.

1,2-Dichloro-4-nitrobenzene exhibits mutagenic activity in *Salmonella typhimurium* but not in the HPRT test in Chinese Hamster Ovary (CHO) cells. 1,2-Dichloro-4-nitrobenzene induced chromosomal aberrations in V79 cells with metabolic activation only at the highest concentration, which was cytotoxic. In insects (*Drosophila melanogaster*) 1,2-dichloro-4-nitrobenzene revealed no mutagenic activity in the SLRL-test after application over 3 days with slight increased toxicity, but revealed mutagenic activity following a single i.p. injection of a clearly toxic dose. 1,2-Dichloro-4-nitrobenzene showed no clastogenic activity *in vivo* in a chromosomal aberrations test with rats. Overall in non-toxic doses, there was no evidence for genotoxicity *in vivo* under the conditions tested.

Studies dealing specifically with toxicity to reproduction were not identified. The subacute study with 1,2-dichloro-4-nitrobenzene yielded no damage of the reproductive organs in rats despite clear systemic toxicity up to the maximum tolerated dose of 100 mg/kg bw.

1,2-Dichloro-4-nitrobenzene commercial grade (85% 1,2-dichloro-4-nitrobenzene and 15% 1,2-dichloro-3-nitrobenzene) caused effects on development at maternally toxic doses probably due to methaemoglobinaemia in dams and foetuses. A significant dose-response trend for variations (dilated ureters) was seen in the foetuses of the >= 30 mg/kg bw/day-groups and significant reduced body weight gain of dams at dose levels of 30 mg/kg bw/day on gd 6-10 with an even stronger effect at 100 mg/kg bw/day. Thus, 10 mg/kg bw/day was determined as the NOAEL for maternal and developmental toxicity.

Environment

1,2-Dichloro-4-nitrobenzene is a yellow substance with a melting point of 43 °C, a boiling point of 255 °C, a flash point of 155 °C, and an ignition temperature of 420 °C. With a density of 1.56 g/cm³ at 15 °C and 1.487 g/cm³ at 50 °C 1.2-dichloro-4-nitrobenzene is heavier than water. The substance is slightly soluble in water with 121 mg/l at 20 °C. The vapour pressure was determined to be 2 Pa at 25 °C. A log Kow of 3.04 at 25 °C was experimentally determined.

With regard to its chemical structure 1,2-dichloro-4-nitrobenzene is not expected to hydrolyse under environmental conditions. According to the Mackay level I fugacity model, the main target compartments for 1,2-dichloro-4-nitrobenzene are air (48 %) and water (44 %). The measured Henry's law constant of 0.82 $Pa*m^3*mol^{-1}$ indicates that the compound has a low to moderate potential for volatilization from surface waters.

In the atmosphere slow photodegradation takes place by reaction with photochemically produced OH radicals. The atmospheric half-life is calculated to be 321 days with an atmospheric concentration of 0.5 x 10^6 hydroxyl radicals/cm^3 as a 24 h average. 1,2-Dichloro-4-nitrobenzene will undergo direct photolysis in air due to absorbance of environmental UV light, however, the respective half-life is not known. In water, no photolysis will occur to a significant extent.

1,2-Dichloro-4-nitrobenzene is not readily biodegradable (Manometric respirometry test: biodegradation < 10 % after 21 days based on BOD; OECD TG 301 C biodegradation 0 % within 28 days, presumably due to inhibition of inoculum). 1,2-Dichloro-4-nitrobenzene is biodegradable by adapted microorganisms under aerobic conditions and by non-adapted inocula under anaerobic conditions (primary degradation). Sewage from adapted wastewater treatment plants has significant potential to primary degrade 1,2-dichloro-4-nitrobenzene (Test method "Simulation of an industrial waste water treatment plant": after 3 days 100 %).

Bioconcentration factors determined for fish were in the range of 26 – 65. A measured Koc (Koc = 417) for sediment suggests the substance to have a medium geoaccumulation potential.

Concerning the acute toxicity of 1.2-dichloro-4-nitrobenzene towards aquatic species reliable experimental results of tests with fish, *Daphnia*, and algae are available. The acute toxicity determined for fish (*Leuciscus idus*) was of 3.1 mg/l (48 h LC_{50}) [DIN 38412 L15] and *Daphnia* (*Daphnia magna*) of 3 mg/l (24 h-EC_{50}) [DIN 38412 L11]. In the growth inhibition test with algae (*Scenedesmus obliquus)* the value 5.8 mg/l was achieved after 48 h (48 h-ErC_{50}) [OECD TG 201]. For the algae *Chlorella fusca* a value of 0.32 mg/l was found after 24 h (24 h-ErC50).
In a chronic (21 d) study with *Daphnia magna* a NOEC of 0.025 mg/l was determined for the most sensitive endpoint reproduction rate. An E_rC_{10} > 0.1 mg/l was reported for the algae *Scenedesmus subspicatus* after 48 hours
For terrestrial organisms the lowest measured 6d-EC_{50} for was 27 mg/l for the plant *Phaseolus aureus*.
Applying an assessment factor of 50 to the lowest available chronic value of 25 µg/l (21d reproduction in *D. magna*), a $PNEC_{aqua}$ of 0.5 µg/l is obtained.

Exposure

About 36,800 tonnes of 1,2-dichloro-4-nitrobenzene were produced worldwide (excluding Eastern Europe) in 2001. 1,2-Dichloro-4-nitrobenzene is a basic chemical for the synthesis of intermediates which are further processed to herbicides, bactericides, and dyestuffs. A direct use of 1,2-dichloro-4-nitrobenzene is not known in the Sponsor country. 1,2-Dichloro-4-nitrobenzene is not contained in products registered in the Danish, Finnish, Norwegian, Swedish and Swiss Product Registers.

In the Sponsor country, 1,2-dichloro-4-nitrobenzene is manufactured and processed in closed systems. From this site the effluent concentrations was below the detection limit of 2 µg/l.

In Gemany in 1999, the 90-percentile of the 1,2-dichloro-4-nitrobenzene concentrations in the River Rhine was < 0.5 µg/l and in the River Danube < 0.02 µg/l. For the River Elbe the maximum was < 0.02 µg/l.
A non-quantifiable contamination of the terrestrial compartment by 1,2-dichloro-4-nitrobenzene might result from the application of herbicides manufactured from 3,4-dichloroaniline. This assumption is based on the observation that during the biodegradation of such herbicides 3,4-dichloroaniline is formed that in trace amounts may be oxidized biotically or abiotically to 1,2-dichloro-4-nitrobenzene. However, a significant exposure of the terrestrial compartment by this source is not expected.

Exposure is well controlled in occupational settings of the main producer in the Sponsor country and the exposure of workers is well below the workplace guidance value (ARW) of 1 mg/m^3 for 1,2-dichloro-4-nitrobenzene recommended by the German Association of the Chemical Industry (VCI).
The levels of 3,4-dichloro-aniline-adducts in blood and of 3,4-dichloro-aniline in urine of manufacturing and processing plants workers were never higher than 5 % of the tolerance values (no health effect for worker in case that value is not exceeded).

Based on the very low emissions of 1,2-dichloro-4-nitrobenzene into air and water by the manufacturing and processing plants in the Sponsor country, on the very low environmental concentrations, and on the low bioaccumulation potential, a significant indirect exposure of the general public via the environment or via the food chain is not expected.

RECOMMENDATION

The chemical is currently of low priority for further work.

RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

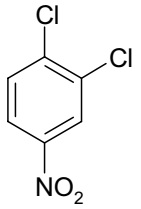
The chemical possesses properties indicating a hazard for human health (principally haematological toxicity, and developmental toxicity, probably linked to methemoglobinemia) and the environment. Based on data presented by the Sponsor country, exposure to the environment is anticipated to be low, exposure is controlled in occupational settings, and exposure of consumers is not known to occur. 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.

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number: 99-54-7
IUPAC Name: 1,2-Dichloro-4-nitrobenzene
Molecular Formula: $C_6H_3Cl_2NO_2$
Structural Formula:



Molecular Weight: 192.00 Dalton
Synonyms: 3,4-Dichloro-1-nitrobenzene

3,4-Dichloronitrobenzene

1-Nitro-3,4-dichlorobenzene

Benzene, 1,2-dichloro-4-nitro

1.2 Purity/Impurities/Additives

Purity of the commercial product (Bayer):

> 99 % w/w

Impurities: 1,2-dichloro-3-nitrobenzene (< 1 % w/w)

water (ca. 0.1 % w/w)

1.3 Physico-Chemical properties

Table 1 Summary of physico-chemical properties

Property	Value	Reference	IUCLID
Substance type	organic compound		1.1.1
Physical state	yellow crystalline substance		1.1.1
UV absorption in methanol/water at 225 nm at 276 nm at 320 nm	 log κ 3.95 log κ 3.96 log κ 3.30	Doub and Vandenbelt (1955)	1.1.2
Melting point	43 °C	Ullmann (2002)	2.1
Boiling point	255 °C	Verschueren (1977)	2.2
Density at 50 °C Density at 15 °C	1.487 g/cm^3 1.56 g/cm³	Thiem et al. (1979) Hoechst AG (1987)*	2.3
Vapour pressure at 25 °C at 114.7 °C	 0.02 hPa 6 hPa	 US EPA (2000) Thiem et al. (1979)	2.4
Partition coefficient n-octanol/water (log value)	3.04 (measured)	Niimi et al. (1989)	2.5
Henry's law constant at 25 °C	0.82 Pa m³ mol^{-1} (= 3.3 x 10^{-4})	Altschuh et al. (1999)	3.3.2
Water solubility at 20 °C	121 mg/l (measured)	Eckert (1962)	2.6.1
Solubility in organic solvents	soluble in hot ethanol, benzene, ether, and carbondisulfide	Thiem et al. (1979)	2.6.1
Flash point	155 °C	Thiem et al. (1979)	2.7
Auto flammability (ignition temperature)	420 °C (DIN 51794)	Bayer AG (2001)*	2.8
pH value in water at 23 °C	pH 5.8	Bayer AG (1991a)	2.14
Begin of thermal decomposition	370 °C	Zetkin et al. (1967)*	2.14
Conversion factor for the vapour phase	1 mg/m^3 = 0.13 ppm 1 ppm = 7.98 mg/m^3	Verschueren (1977)	2.14

*Studies with reliability not assignable

2 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

In Germany 1,2-dichloro-4-nitrobenzene is manufactured in an industrial scale only at the Bayer AG Leverkusen plant (Bayer Chemicals, 2003).

Manufacturing of 1,2-dichloro-4-nitrobenzene takes place by mono-nitration of 1,2-dichlorobenzene in a continuously working closed system. Initially a mixture of 1,2-dichloro-3-nitrobenzene (ca. 10 %) and 1,2-dichloro-4-nitrobenzene (ca. 90 %) is gained. This mixture is separated either directly by crystallization or distillation. Another way, not very commonly used, yielding directly pure 1,2-dichloro-4-nitrobenzene is based on chlorination of 4-chloronitrobenzene (Thiem et al., 1979).

Table 2 Manufacturing capacities distribution

Manufacturing Capacity	Known Capacity (%)
Western Europe	45
USA	10
South America	23
Southeast Asia	22
Eastern Europe	Unknown

In 2001 the worldwide (excluding Eastern Europe) production of 1,2-dichloro-4-nitrobenzene (worldwide distribution see Table 2) amounted to 36,800 tons by approximately 12 producers (Srour, 2001).

Bayer produces about 10,000 t/a 1,2-dichloro-4-nitrobenzene starting from 1,2-dichlorobenzene (Bayer Chemicals, 2003).

The total production volume of Bayer Chemicals is processed onsite (Bayer Chemicals, 2003).

1,2-Dichloro-4-nitrobenzene is a basic chemical, used industrially as an intermediate for processing by reduction or substitution. In Table 3 an estimation of the amount of 1,2-dichloro-4-nitrobenzene used in the first steps of different processings is given (Srour, 2001).

Table 3 Use of 1,2-dichloro-4-nitrobenzene for industrial intermediates

Intermediate	Use of 1,2-Dichloro-4-nitrobenzene (%)
3,4-dichloroaniline	82
2-chloro-4-nitroaniline	9
3-chloro-4-fluornitrobenzene	8
3-chloro-4-methoxynitrobenzene	1

These data relate to the above cited world wide production and demand in 2001. The intermediates are used mostly in the synthesis of herbicides furthermore of bactericides, and dyestuffs (Srour, 2001; Ullmann, 2002). In Germany, a direct use of 1,2-dichloro-4-nitrobenzene is not known (Bayer Chemicals, 2003).

1,2-Dichloro-4-nitrobenzene is not contained in products registered in the Danish, Finnish, Norwegian, and Swedish Product Registers (Spin Database 2003). In the Swiss Product Register (2003) four products in the category of fuels, lubricants, and heat transfer media are registered: two public products with < 1 %, one industrially used product with < 1 %, and one industrially used product with < 10 % 1,2-dichloro-4-nitrobenzene. However, further inquiries made by the Swiss authority came to the result that not the 1,2-dichloro-4-nitrobenzene, but another isomer is contained in these products. Therefore, it is confirmed that 1,2-dichloro-4-nitrobenzene is also not contained in products registered in the Swiss Product Register (Bormann, 2003).

2.2 Environmental Exposure and Fate

2.2.1 Sources of Environmental Exposure

Releases of 1,2-dichloro-4-nitrobenzene into the environment may occur during manufacturing and processing.

Information on exposure from manufacturing and processing of the chemical is available for the Bayer Chemicals plants at Leverkusen, Germany (Bayer Chemicals, 2003).

The manufacturing and processing plants contain dedicated systems in which only dichloronitro-benzenes are manufactured, separated, stored and processed. Manufacturing, processing, and filling of 1,2-dichloro-4-nitrobenzene are executed in closed systems (in general transport via pipings, as an exception in 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. 2.2 Human exposure, Bayer Chemicals, 2003).

The exhausts from manufacturing and processing of 1,2-dichloro-4-nitrobenzene are connected to air washing units and thermal exhaust purification plants. Following the Official Emission Declaration of the year 2002, virtually no 1,2-dichloro-4-nitrobenzene (< 25 kg/a) was emitted into the atmosphere from the Bayer production and processing site in Leverkusen (Bayer Chemicals, 2003).

Waste from the manufacturing and processing of 1,2-dichloro-4-nitrobenzene is incinerated in an incinerator for hazardous wastes (Bayer Chemicals, 2003).

At the Bayer Chemicals Leverkusen nitrobenzenes plants, 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 1,2-dichloro-4-nitrobenzene. The extracted wastewater is stripped and the remainder is lead to the Leverkusen industrial and municipal wastewater treatment plant, together with the wastewater with minor load (Bayer Chemicals, 2003).

The concentrated sewage sludge is incinerated in a hazardous waste incinerator especially dedicated to this sludge (Bayer Chemicals, 2003).

24 h/d, 365 d/a, the air and water emissions of the integrated production site at Leverkusen are monitored by an Environmental Surveillance Group which operates independently of any manufacturing unit. This group is equipped with mobile detectors for various potential emissions. It also operates stations with measuring and sampling devices for air and water (Bayer Chemicals, 2003).

In 2002 in the effluent of the Leverkusen wastewater treatment plant, 1,2-dichloro-4-nitrobenzene was neither detectable by the daily monitoring with a determination limit of 20 µg/l nor by 12 randomly selected fine monitoring with a determination limit of 2 µg/l (Bayer Chemicals, 2003).

The effluent of the Bayer Leverkusen plant passes into the Rhine. For the receiving river a

Predicted Environmental Concentration (PEC) of < 2.8 ng/l

is calculated taking into account the 10 percentile of the river flow (1050 m^3/s), the dilution factor (700), and the detection limit of 2 µg/l (Bayer Chemicals, 2003).

Information on environmental releases at other production and/or processing sites is not available.

The use of herbicides manufactured from 3,4-dichloroaniline may lead to an exposure of the terrestrial compartment with 3,4-dichloroaniline by degradation. As long as 3,4-dichloroaniline is not bound to soil components, 1,2-dichloro-4-nitrobenzene might be formed as a metabolite by biotic or abiotic processes. However, in soils treated with usual amount of the herbicides linuron and diuron, 1,2-dichloro-4-nitrobenzene was not detected. Therefore, a significant exposure of the terrestrial compartment by this source is not expected (BUA, 1990).

2.2.2 Photodegradation

There are no experimental data on the stability of 1,2-dichloro-4-nitrobenzene in the atmosphere. The indirect photochemical degradation in air by hydroxyl radicals is calculated via AOPWIN v. 1.90 with a half-life of 321 days using 500,000 OH radicals/cm^3 as a 24 h average (Bayer AG, 2003a).

The OH reactivity may be seen as an upper limit of stability, because direct photolysis is not taken into account. Since 1,2-dichloro-4-nitrobenzene significantly absorbs UV-B radiation [Molar absorptivity κ is 2000 M^{-1} cm^{-1} at 320 nm (Doub and Vandenbelt, 1955)], it is expected that 1,2-dichloro-4-nitrobenzene will undergo direct photolysis due to absorbance of environmental UV light, however, the respective half-life is not known. Photodegradation of 1,2-dichloro-4-nitrobenzene is summarized in Table 4.

Table 4 Photodegradation of 1,2-dichloro-4-nitrobenzene

IUCLID	Parameter	Method	Result	Source
3.1.1	Indirect photodegradation in air	Calculation 24 h average 0.5 * 10^6 OH/cm^3	t ½ = 321 d	Bayer AG (2003a)
	Direct photodegradation in air	Comparison of spectra	κ= 2000 M^{-1} cm^{-1} at 320 nm	Doub and Vandenbelt (1955)

Although 1,2-dichloro-4-nitrobenzene absorbs light at > 290 nm it does not react when irradiated in aqueous solution with light of these wavelength (BUA, 1990).

2.2.3 Stability in Water

With regard to its chemical structure 1,2-dichloro-4-nitrobenzene is not expected to hydrolyse under environmental conditions (Harris 1990).

Stability of the substance in aqueous solution is confirmed by measurements performed within the frame of a 21d Daphnia study. The substance concentrations were checked in a freshly prepared and a 2 d old test solution in closed vessels and proved to be stable (Kuehn et al., 1988).

2.2.4 Transport between Environmental Compartments

According to the Mackay Fugacity Model Level I, the main target compartments for 1,2-dichloro-4-nitrobenzene are air (48.3 %), water (44.0 %), soil (3.8 %), and sediment (3.9 %), Table 5, (Bayer AG, 2003a).

Table 5 Input parameters and results of the Mackay Fugacity Model Level I

Input Parameters	**Value**
Temperature	25 °C
Vapour pressure	2 Pa
Water solubility	121 mg/l
Molar mass	192 Dalton
log K_{ow}	3.04

Results (IUCLID 3.3.2) **Compartment**	**Calculated distribution**
Air	48.3 %
Water	44.0 %
Soil	3.8 %
Sediment	3.9 %
Susp. Sediment	< 0.1 %
Fish	< 0.1 %

The measured dimensionless Henry constant is 3.3 x 10^{-4}, which equals 0.82 Pa m³ mol^{-1} at 25 °C (Altschuh et al., 1999). It indicates a low to moderate potential for volatilization from surface waters according to the scheme of Thomas (1990).

The distribution and elimination of 1,2-dichloro-4-nitrobenzene in a sewage treatment plant with primary sedimentation was estimated according to the model Simple Treat 3.0 of Struijs (Bayer AG, 2003b). The input parameters and results are listed in Table 6.

Table 6 Input parameters and results of Simple Treat calculation

Input Parameters	Value
Sludge loading rate	0.15 kg BOD/kg dry matter/d
Sludge retention time	9.2 d
Hydraulic retention time	7.1 h
Degradation rate constant	0 h^{-1}
Henry constant	0.82 Pa m^3 mol^{-1}
Log K_{ow}	3.04

Results (Compartment)	Calculated Distribution
Air	1.8 %
Water	93.5 %
Sludge	4.7 %
Degraded	0.0 %

Effect	Removal
Removal (sum of losses to air, removal with sludge, and degradation)	6.5 %

Comparison	Removal
Removal by Bayer wastewater treatment plant	> 96 %

2.2.5 Biodegradation

Based on the available experimental data 1,2-dichloro-4-nitrobenzene is not readily biodegradable (presumably due to inhibition of the inoculum), but it is primary biodegradable by adapted organisms (see below).

The only studies available on ready biodegradability were performed at concentrations where the inoculum may have been inhibited.

Internal test results of a insufficiently documented study using the manometric respirometry test showed that the degradation of the substance (ca. 90 mg/l) was lower than 10 % within 21 days (Hoechst AG, 1982). However, the author of this study found that bacteria were inhibited by 1,2-di-chloro-4- nitrobenzene at a level of ca. 45 mg/l.

In a modified MITI I test according to OECD guideline 301 C a non adapted mixed microbial inoculum mineralised 0 % of the initial test substance concentration (100 mg/l) within 28 days (MITI, 1992). It cannot be excluded that the inoculum was inhibited by the employed test substance concentration of 100 mg/l.

There is no guideline study on inherent biodegradability. However, 1,2-dichloro-4-nitrobenzene is primary biodegradable by adapted microorganisms under aerobic conditions and by non-adapted inocula under anaerobic conditions. The key data of these experiments are listed in Table 7.

The Bayer industrial and municipal water treatment plant in Leverkusen is capable of biodegradation of 1,2-dichloro-4-nitrobenzene. In a simulation test using the sludge of this industrial and municipal wastewater treatment plant Grote et al. (1983) showed that 1,2-dichloro-4-nitrobenzene is degraded completely (100 %) within 3 days. Since the first step of the degradation is the reduction to 3,4-dichloroaniline, shock loadings of 3,4-dichloroaniline might slow down the initial reaction of the degradation of 1,2-dichloro-4-nitrobenzene (Grote et al., 1983).

The pathway of degradation of 3,4-dichloroaniline in wastewater containing also 1,2-dichloro-4-nitrobenzene was examined by Livingston and Willacy (1991). The degradation of 1,2-dichloro-4-nitrobenzene by industrial activated sludge and adapted inocula was accompanied by the release of chloride. The degradation of 1,2-dichloro-4-nitrobenzene preceeded more slowly than that of 3,4-dichloroaniline, but was completed within 15 days.

Similarily, Baumgarten et al. (1982) described that a mixture of bacteria from different sources cultivated as a biofilm and adapted to several nitro compounds is able to degrade 1,2-dichloro-4-nitrobenzene by more than 70 % within 2 days.

Chloronitrobenzenes were degraded by isolated microbial cultures and adapted mixed sludge as long as there were additional sources of carbon and nitrogen in the nutrient media (Kuhlmann, 1999).

Springer and Rast (1988) were able to genetically characterize a *Pseudomonas* strain grown on 1,2-dichlorobenzene which dehalogenated 1,2-dichloro-4-nitrobenzene by ca. 50 % within 3 days.

Non-adapted cultures of the fungus, *Mucor javanicus*, degraded about 1/3 of 1,2-dichloro-4-nitrobenzene and reduced another 1/3 to 3,4-dichloroaniline during shake flask incubation within 6 days (Hafsah et al., 1984). These authors observed a 55 % inhibition of the fungus growth at 50 mg/l.

Aquatic sediments incubated anaerobically, rapidly removed 1,2-dichloro-4-nitrobenzene under various conditions (Bunce et al., 1983; Bosma et al., 1990, 1996; Susarla et al., 1996).

Table 7 compiles the relevant data on biodegradation of 1,2-Dichloro-4-nitrobenzene.

Table 7 Biodegradation of 1,2-dichloro-4-nitrobenzene (IUCLID 3.5)

Inoculum	Procedure	Result	Source
	Aerobic tests		
Activated sludge, non-adapted	Manometric respirometry test	< 10 % degradation after 21 days (inhibition of bacteria at test concentration employed)	Hoechst AG (1982)
non adapted mixed microbial inoculum	Modified MITI I test according to OECD guideline 301 C	0 % mineralization presumably due to inhibition of the inoculum	MITI (1992)
Activated sludge, industrial	Simulation of an industrial waste water treatment plant	100 % primary degradation after 3 days	Grote et al. (1983)
Industrial activated sludge or mixed starting culture with the ability to degrade various aromatic halogenes	Sealed shaking flask	50 % primary degradation within 8 days, 100 % primary degradation within 15 days	Livingston and Willacy (1991)
Mixture of bacteria from different sources (adapted)	Shaking flask	> 70 % primary degradation within 2 days	Baumgarten et al. (1982)
Isolated microbial cultures and adapted mixed sludge	Batch	Primary Degradation	Kuhlmann (1999)
Pseudomonas strain grown on 1,2-dichlorobenzene	Shake flask	ca. 50 % dehalogenation within 3 days	Springer and Rast (1988)
Mucor javanicus (fungus, not adapted)	Shake flask	67 % removal within 6 days	Hafsah et al. (1984)
	Anaerobic tests		
Sediments of river and of river water infiltration area	Sediment columns, anaerobic	Under denitriying conditions 50 % elimination, under methanogenic conditions > 99 % elimination within 1 day	Bosma et al. (1996)
Anaerobic estuarine sediment	Anaerobic incubation	ϑ1/2 = 2.4 days (pH 5.6, 25 °C) (primary degradation)	Susarla et al. (1996)

In the Leverkusen industrial and municipal wastewater treatment plant the comparison of influent and effluent concentrations shows that the substance is removed nearly completely. In 2002, only 13 of 365 influent samples (24 h) contained 1,2-dichloro-4-nitrobenzene above the detection limit of 500 µg/l. In the effluent no 1,2-dichloro-4-nitrobenzene was detected in 365 samples with a determination limit of 20 µg/l and in 12 randomly selected fine analysis samples with a determination limit of 2 µg/l (Bayer Chemicals, 2003). It can be concluded from these data that the removal of the Leverkusen industrial and municipal wastewater treatment plant exceeds at least 96 %. This removal cannot be transferred to other wastewater treatment plants due to different wastewater composition and adaptation processes.

2.2.6 Bioaccumulation

Measured bioconcentration factors (BCF) determined for fish (*Cyprinus carpio*) according to OECD guideline 305 C, were in the range of 26 - 65 (Table 8). 1.2-Dichloro-4-nitrobenzene concentrations of 0.05 and 0.005 mg/l were tested (MITI 1992). For the isomer 1,2-dichloro-3-nitrobenzene a BCF value of 94 was calculated by Canton et al. (1985).

Table 8 Bioaccumulative properties of 1.2-dichloro-4-nitrobenzene

Parameter	Method	Result	Source	IUCLID
Bioaccumulation (Bioconcentration factor)	MITI (comparable to OECD 305 C) with Cyprinus carpio	BCF = 26 – 65 after 56 d	MITI 1992	3.7

2.2.7 Geoaccumulation

Binding to soil organic matter has been measured log Kom = 2.29 (Kom = 195, Briggs. 1981). For binding of soil organic carbon this equals a log Koc = 2.53 (Koc = 339, Hong et al.. 1996). A slightly higher experimentally determined soil sorption coefficient is reported by Wu et al. (2001) with log Koc 2.62 (Koc = 417). Hong et al. (1996) calculated a log Koc 2.55 - 2.71 (Koc = 355 - 513) from experimentally determined HPLC capacity factors. These data are compiled in Table 9. Thus it is supposed that 1,2-dichloro-4-nitrobenzene would adsorb moderately to sewage, suspended solids, and sediment. According to Litz (1990) 1,2-dichloro-4-nitrobenzene can be regarded as a substance with medium geoaccumulation properties.

Table 9 Geoaccumulative properties of 1.2-dichloro-4-nitrobenzene (IUCLID 3.3.2)

Parameter	Method	Result	Source
Soil organic matter–water	Shake flask method	log Kom = 2.29 (Kom = 195)	Briggs (1981)
Soil organic carbon–water	Calculated from Briggs data	Koc = 339	Hong et al. (1996)
	OECD Guide-line 106	Koc = 417	Wu et al. (2001)
	Calculated from measured HPLC capacity factors	Koc = 355 - 513	Hong et al. (1996)

2.2.8 Environmental Monitoring

Throughout Germany a comprehensive monitoring program on several chemicals in surface waters has been realised (Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit, 2001; update by Umweltbundesamt, 2003).

Table 10 shows the concentrations of 1,2-dichloro-4-nitrobenzene, which have been measured in German surface waters in the year 1999 (IUCLID 3.2.1).

Table 10 1,2-Dichloro-4-nitrobenzene concentrations in German surface waters in 1999

River	Measuring station	Type of Value	Result
Danube	Ulm	90 % percentile	< 0.02 µg/l
Elbe	Schnackenburg	Maximum	< 0.02 µg/l
Rhine	Kleve-Bimmen	90 % percentile	< 0.5 µg/l

Hendriks et al. (1998) reported levels of 1,2-dichloro-4-nitrobenzene in the zebra mussel (*Dreissena polymorpha*), and in the eel (*Anguilla anguilla*) from waters in the Netherlands in 1994. Zebra mussels sampled in the Rhine (sampling site Lobith), Meuse (Eijsden), and Ysselmeer, contained 1,2-dichloro-4-nitrobenzene at levels of up to 0.36 µg/kg wet weight. Eels sampled in the Rhine, Meuse, Hollands Diep location in the Rhine-Meuse delta contained 1,2-dichloro-4-nitrobenzene at levels of up to 1.2 µg/kg wet weight (Hendriks et al., 1998).

1,2-Dichloro-4-nitrobenzene was detected (6.1 ng/l) in the water of the river Elbe at Stade in 1995 (Bester et al., 1998). These authors also examined the water of the North Sea at 6 sites in the German Bight in 1990 and 1995. In 1990, 1,2-dichloro-4-nitrobenzene was found at 5 sites (0.082 - 0.27 ng/l, one site was below the limit of detection [0.05 ng/l]) in the German Bight. In 1995, the concentrations of 1,2-dichloro-4-nitrobenzene had decreased to levels below the limit of detection (Bester et al., 1998).

2.2.9 Other Information on Environmental Fate

[click here to insert text, or delete subheading as appropriate]

2.3 Human Exposure

2.3.1 Occupational Exposure

2.3.1.1 Workplaces

In principle, workers may be exposed during manufacturing and processing of 1,2-dichloro-4-nitrobenzene through the inhalational, dermal and oral routes.

All 1,2-dichloro-4-nitrobenzene manufactured by Bayer Chemicals is processed to 3,4-dichloroaniline in closed installations at the same site. For processing, 1,2-dichloro-4-nitrobenzene is transported in pipelines (Bayer Chemicals, 2003).

Any small leakage in the production and processing units would be recognized due to the aromatic odour of the 1,2-dichloro-4-nitrobenzene, its precursors (e.g. dichlorobenzene, nitrous gases), or its consecutive product 3,4-dichloroaniline (threshold odour concentration 0.047 mg/m^3, Brauer, 2002) and - in the nitration plant - due to the high visibility of nitrous gases (Bayer Chemicals, 2003).

Investigations of the workplaces have been performed also according to German Technical Guidance TRGS 402 (AGS, 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.

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, gas filter masks or a respirator with independent air supply have to be used as well as full protective clothing.

Workers in the manufacturing and processing plants of 1,2-dichloro-4-nitrobenzene are informed also by way of a material safety data sheet on the recommended safety measures (Bayer Chemicals, 2003).

2.3.1.2 Workplace Monitoring

To limit occupational exposure to chemicals, starting in 1997, the workplace guidance value (ARW) recommended by the German Association of the Chemical Industry (VCI) is 1 mg/m^3 for 1,2-dichloro-4-nitrobenzene.

At Bayer AG the exposure of workers is well below this limit. 28 workplace measurements of the 1,2-dichloro-4-nitrobenzene concentration were made between 1991 and 2002. The measurements covered representative workplaces for manufacturing and processing (nitration, hydrogenation) of 1,2-dichloro-4-nitrobenzene. One measurement was a short time value (below the detection limit). The following results were obtained for the 27 total shift values:

11 values were between 0.002 and 0.1 mg/m³, another 15 values were below the detection limit of the GC analysis (the detection limit depends on the air sampling volume and was between 0.002 and 0.1 mg/m³).

The highest value of 0.24 mg/m^3 was measured in the 1,2-dichloro-4-nitrobenzene manufacturing plant in 1994.

Since 1991 the nitrification and hydrogenation plants have completely been modernized. Although since 1991 all measured workplace values were well below the occupational exposure limits, it is expected that this modernization will contribute to further minimize workplace exposure (Bayer Chemicals, 2003).

2.3.1.3 Biological Monitoring

In humans, 1,2-dichloro-4-nitrobenzene is reduced to 3,4-dichloro-aniline, which may form adducts with hemoglobin. These adducts can be detected in the blood up to three months after exposure. Moreover, 3,4-dichloro-aniline itself can be detected in the urine up to about three days after exposure.

The levels of 3,4-dichloro-aniline -adducts in blood and of 3,4-dichloro-aniline in urine are measured at least once a year in each worker of the 1,2-dichloro-4-nitrobenzene manufacturing and processing plants (Bayer Chemicals, 2003). The measured values were never higher than 5 % of the tolerance values (no health effect for worker in case that value is not exceeded; Bayer internal experience values).

Average levels and range (minimum – maximum values) of 3,4-dichloroaniline-adducts in blood and of 3,4-dichloro-aniline in urine are listed in Table 11.

Table 11 Concentrations of 3,4-dichloroaniline-adducts in blood and of 3,4-dichloro-aniline in urine

	Worker Nitration 2002 N = 84	Worker Hydrogenation2002 N = 117	General Population	Tolerance values
3,4-dichloroaniline-adducts in blood (ng/l)	345 (< 20 – 3120)	200 (< 20 – 1550)	< 20	100,000
3,4-dichloroaniline in urine (µg/l)	10 (< 2 – 500)	3.5 (< 2 – 150)	< 2	10,000

2.3.2 Consumer Exposure

2.3.2.1 Exposure of Users of Final Products

1,2-Dichloro-4-nitrobenzene is exclusively used as an intermediate for chemical synthesis (cf Chapter Processing and Use) in the Sponsor Country. No direct use is known (Bayer Chemicals, 2003). Residual levels of 1,2-dichloro-4-nitrobenzene in the Bayer product 3,4-dichloro-aniline are below the detection limit of 100 ppm (Bayer Chemicals, 2003).

2.3.2.2 Exposure of the General Public

The only known use of 1,2-dichloro-4-nitrobenzene is that as an industrial intermediate (Bayer Chemicals, 2003). Since residues of 1,2-dichloro-4-nitrobenzene are reduced in the production chain e.g. during hydrogenations, and phase separations, final products are thought to be virtually free of 1,2-dichloro-4-nitrobenzene.

Thus, in the light of the low environmental concentrations and the low bioaccumulation potential, it is assumed that no accumulation occurs in the food chain.

Based on the very low emissions of 1,2-dichloro-4-nitrobenzene into air and water by the manufacturing and processing plants in the Sponsor Country (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

3.1.1 Toxicokinetics, Metabolism and Distribution

There are no studies, which have been performed according to current guidelines and the available studies performed between 1957 - 1959 are only dealing with the excretion pattern of 1,2-dichloro-4-nitrobenzene.

In guinea pigs about 3 % (range 2 - 5 %) of the applied dose of å 200 mg/kg bw via gavage was excreted as mercapturic acid derivate N-acetyl-S-(2-chloro-4-nitrophenyl)-L-cysteine via urine

within 24 hrs. Other metabolites were identified as sulphate ester (å 12 %) or as 3,4-dichloroaniline (å 5 %) (Bray, Franklin and James, 1959a; 1959b).

In rabbits dosed orally with 400 mg/kg bw via gavage most of the applied dose was excreted via urine within 72 hrs as mercapturic acid derivate N-acetyl-S-(2-chloro-4-nitrophenyl)-L-cysteine (å 45 %) followed by conjugates of amino dichlorophenols (å 13 % as glucuronide and å 12 % as sulphate ester)and 3,4-dichloroaniline (å 22 %) (Bray, James and Thorpe, 1957).

In rats about 19 % (range 4 - 29 %) of the applied dose (å 350 mg/kg bw via gavage) was excreted as mercapturic acid via urine within 24 hrs. In a second trial the animals were dosed with å 250 or 350 mg/kg bw and the maximum rate for excretion of mercapturic acid via urine 2 - 6 days after dosing was given with å 2 or 4 mg/kg bw/h, resp. (Barnes, James and Wood, 1959).

The stepwise conversion of 1,2-dichloro-4-nitrobenzene into N-acetyl-S-(2-chloro-4-nitrophenyl)-L-cysteine was shown also in an in vitro study by Booth, Boyland and Sirns (1961).

Monsanto (1987a) reports significantly increased methaemoglobin in both, dams and foetuses indicating the transfer of 1,2-dichloro-4-nitrobenzene to the fetus.

Conclusion

1,2-Dichloro-4-nitrobenzene is absorbed form the gastro-intestinal tract and although there are some species differences in experimental animals from the available data it can be concluded that 1,2-dichloro-4-nitrobenzene is excreted mainly via urine in the form of the mercapturic acid derivate N-acetyl-S-(2-chloro-4-nitrophenyl)-L-cysteine. Data on humans were not identified in the available literature.

3.1.2 Acute Toxicity

There are no studies according to the current OECD Test Guidelines, but there are studies, which are adequately documented and are considered of sufficient quality to allow an evaluation of this endpoint.

Studies in Animals

Inhalation

There are no valid acute inhalation studies available.

Dermal

In a study with female Wistar rats (n = 6) the occlusive application of 2000 mg/kg bw 1,2-dichloro-4-nitrobenzene (as 40 % solution) over 24 hrs caused no mortality or local irritation. The macroscopic examination 14 d p.a. gave also no adverse effects. Therefore, the LD_{50} value is > 2000 mg/kg bw (Hoechst AG, 1975a).

In another study with male and female albino rabbits (n = 6) the LDLo (lowest Lethal Dose Level) was reported with 950 mg/kg bw after occlusive application of undiluted 1,2-dichloro-4-nitrobenzene to intact skin (exposure time not mentioned) at doses of 360 – 2900 mg/kg bw. Survival time was 24 - 48 hrs. Rabbits became lethargic and lost appetite, while survivors returned to normal activity within one week. At necropsy liver discoloration and blood changes (indicative for the formation of methaemoglobin) were noted (Monsanto Co., 1955).

Oral

In male Wistar rats (n = 10) dosed with 250, 400, 630, 1000 or 1250 mg/kg bw 1,2-dichloro-4-nitrobenzene (applied as 4 % solution in sesame oil via gavage) deaths occurred at dose levels of Ó400 mg/kg bw within 1-3 days after application. In moribund animals disorders of balance were observed, while the macroscopic examination of surviving and dead animals gave no adverse effects. From this study a LD_{50} value of 625 mg/kg bw was derived (Hoechst AG, 1975b).

In male and female Sprague-Dawley rats (n = 21) dosed with 650 – 900 mg/kg bw 1,2-dichloro-4-nitrobenzene (applied as 50% solution in corn oil) the survival time was given with 4 – 36 hrs. Soon after dosing, animals showed lethargy followed by salivation, collapse and coma. At autopsy pulmonary hyperaemia and jaundice-like liver discoloration were noted, while kidneys appeared normal. From this study a LD_{50} value of 800 mg/kg bw was derived (Monsanto Co., 1955).

In another study male and female Sprague-Dawley rats (n = 5) were dosed with 1,2-dichloro-4-nitrobenzene, technical grade, consisting of 85 % 1,2-dichloro-4-nitrobenzene and 15 % 2,3-dichloro-4-nitrobenzene (dose levels 631 – 1260 mg/kg bw via gavage). As signs of intoxication weight loss, increasing weakness, salivation, ocular discharge, and collapse were reported. Deaths occurred within 1 or 2 days. Gross autopsy revealed haemorrhagic lungs, liver discoloration, in some cases darkened spleen and also acute gastrointestinal inflammation, while in survivors (14 d p.a.) viscera appeared normal. The LD_{50} value was given with 950 mg/kg bw (Monsanto Co., 1978)

Conclusion:

There are no valid acute inhalation studies available. Based on the results of the acute dermal toxicity study with rats the LD50 is > 2000 mg/kg bw. From studies with rabbits no LD50 could be derived, the lowest Lethal Dose Level (LDLo) was 950 mg/kg bw. The acute oral toxicity in rats ranges from 625 to 950 mg/kg bw. 1,2-dichloro-4-nitrobenzene causes the formation of methaemoglobin. Predominant signs of intoxication were lethargy, increasing weakness, collapse and coma.

3.1.3 Irritation

Skin Irritation

Studies in Animals

In a study with rabbits (n = 3) performed according to OECD TG 404 1,2-dichloro-4-nitrobenzene was judged as non irritating after semiocclusive application of 500 mg (moistened with physiol. saline) for 4 hrs. The mean scores (reading 30 - 60 min and 24, 48 or 72 hrs after removal of the patch) for erythema/scabbing and oedema were given with 0.2 and 0.0. The effects had disappeared at the 24-hour reading time point (Hoechst AG, 1988b).

In another study with rabbits (n = 6) performed according to Federal Register 38 (No. 187, p. 27019, § 1500.41) 0.5 ml of a 10 % solution of 1,2-dichloro-4-nitrobenzene in sesame oil was applied under occlusive conditions over 24 hrs. The irritation index (24 and 72 hrs) was given with 0.58 (maximum possible value: 8); the effects had disappeared at the 72 hour reading time point. 1,2-Dichloro-4-nitrobenzene was judged as slightly irritating (Hoechst AG, 1975c).

Eye Irritation

Studies in Animals

1,2-dichloro-4-nitrobenzene was tested in rabbits (n = 3) in a study performed according to OECD TG 405. A dose of 100 mg test substance was used and the eyes were rinsed after 24 hrs with

examinations 1, 24, 48 or 72 hrs p.a.). The average scores for cornea / iris / conjunctivae (redness) / conjunctivae (chemosis) were given with 0 / 0.1 / 1.3 / 0.2. The effects were reversible within 72 hrs. 1,2-Dichloro-4-nitrobenzene was judged as slightly irritating (Hoechst AG, 1988a).

In two other studies with rabbits (n = 6) performed by Hoechst AG (1975c) according to Federal Register 38 (no. 187) 1,2-dichloro-4-nitrobenzene was applied as I.) 0.1 ml of a 10 % solution or II.) 100 mg. In both cases the eyes were rinsed after 24 hrs. The solution of 1,2-dichloro-4-nitrobenzene was judged as non irritating while the application of 100 mg undiluted 1,2-dichloro-4-nitrobenzene caused irritation which was reversible within the 7 day observation period (no further information available).

1,2-Dichloro-4-nitrobenzene, technical grade (85 % 1,2-dichloro-4-nitrobenzene and 1,5 % 2,3-dichloro-4-nitrobenzene), was tested in a Draize Test with rabbits (n = 6). The application of 0.1 ml of the undissolved but warmed up to 37 °C compound over 24 hrs (no further information) was slightly irritating to the eyes (average score [72 hrs]: 2.7/110) The effects were reversible within 72 hours (Monsanto Co., 1978).

Conclusion:

1,2-dichloro-4-nitrobenzene gave no skin irritation effects when tested for 4 hours under semiocclusive conditions according to OECD TG 404 and showed slightly irritating effects, which disappeared within 72 hours under occlusive conditions according to the method of Federal Register 38 No. 187. 1,2-Dichloro-4-nitrobenzene is slightly irritating to the eyes when tested according to OECD TG 405.

3.1.4 Sensitisation

Studies in Animals

Skin

1,2-Dichloro-4-nitrobenzene was tested in a Guinea Pig Maximization Test according to OECD TG 406. The induction concentrations were 5 % (intracutaneous) and 50 % (topical) and the challenge concentration was 50 % (occlusive epicutaneous). A second challenge was performed with concentrations of 25 and 12 %. In this test 1,2-dichloro-4-nitrobenzene caused no skin sensitization (Bayer AG, 1991b).

In another study with guinea pigs the animals (n = 6) were treated as follows: induction (intradermal) with 0.1 ml (2.5 µg test substance in saline) followed by injection of 0.05 ml FCA after 90 min. The challenge concentration was 1 % (epicutaneous application after 13 days to the animals flanks) and the reactions were scored 24 and 48 hrs after challenge. There was no indication of a sensitizing potential for 1,2-dichloro-4-nitrobenzene also no cross-sensitization with 2,4-dinitro-1-chlorobenzene was observed (Baer and Rosenthal, 1972).

1,2-Dichloro-4-nitrobenzene also caused no sensitization in a Mouse Ear Swelling Test with WSP mice (n = 5). 0.2 ml of a 0.05 M solution in acetone was applied onto dorsal skin on day 1 and 20 µl of a 0.016 M solution onto ears on day 5. The reactions were scored 24 - 72 hrs after application but the data were not shown (Schmidt and Chung, 1992).

Studies in Humans

Skin

In a study with 10 female subjects the single dermal application of a 10 % solution of 1,2-dichloro-4-nitrobenzene in acetone followed by a challenge with a 10 or 0.01 - 1 % solution in acetone on day 28 and 49 caused no skin sensitization (Sulzberger and Baer, 1938).

Conclusion:

1,2-Dichloro-4-nitrobenzene was not found to induce dermal sensitization when tested according to OECD TG 406. In addition, 1,2-dichloro-4-nitrobenzene was not found to induce dermal sensitization in humans in a limited study.

3.1.5 Repeated Dose Toxicity

Studies in Animals

Inhalation

There is one publication available reporting of studies in rats and mice exposed to 1,2-dichloro-4-nitrobenzene via inhalation. However, these data are difficult to interpret due to the limited documentation.

In a sub-acute study, rats and mice were exposed to 28 mg/m^3 on 4 hrs/d over 21 days. Only the results of the mice study were reported: The haematological examination gave effects on blood and biochemical parameters (reduced RBC count and haemoglobin values, increase in Heinz bodies, formation of methaemoglobin, and some changes in WBC count). In the histological examination fatty and protein dystrophy of liver and kidneys and dystrophic alterations in cardiac muscle fibres were described.

In a sub-chronic study rats and mice were exposed to 0, 0.4, 3.6 or 10 mg/m^3 on 4 hrs/d over 4 months. No effects on behaviour and body weight development were reported from mice and rats, but significant changes in haematology values and clinical chemistry values were mentioned in mice and rats, with only rat data described in more detail:

From 3.6 mg/m^3 onwards increase in methaemoglobinaemia, Heinz bodies and pronounced reticulocytosis but decreases in erythrocyte count and haemoglobin level were reported. At 10 mg/m^3 significant increase in serum transaminases, liver catalase, and liver diaminooxidase were described in addition with increased levels of bilirubin in blood and cholesterol in adrenals. From the lowest concentration no adverse effects were reported. For this study no data on histological examination were given (Belyaev and Kuznetsov, 1969).

Oral

The sub-acute toxicity of 1,2-dichloro-4-nitrobenzene was tested in a 28-day study with Wistar rats (5 m/5 f per sex and dose group) according to OECD TG 407. Animals were dosed with 0, 4, 20 or 100 mg/kg bw/day in sesame oil via gavage and sacrificed on day 29. Dose levels of Ó20 mg/kg bw caused increased salivation, a slight not significant increased water intake (m/f) and dark yellow discolouration of urine (m); and at the highest concentration (m/f) additionally irregular respiration, stilted gait and dark yellow discolouration of urine (f). Clinical chemistry investigations yielded in males significantly increased, not dose-related sodium- and chloride-values (low to high versus concurrent control: 139, 142, 142 versus 135 mmol/L; and 101, 102, 102 versus 98 mmol/l), which is in the normal range of the strain used (sodium: 132 - 149 mmol/L and chloride: 95 - 106 mmol/l). Also an increase in urea values (m: > 50 %, f: not significant > 20 %) was noted, which could be an indicator for an impaired kidney function, but this could not be verified at the histopathological examination. The haematological examination revealed significant changes only in males consisting of a dose-dependent significant decrease in number of erythrocytes (low to high dose versus

concurrent control: 6.98 to 6.34 versus 7.63 10E12/L), which is in the normal range of the strain used (6.34 - 8.95 10E12/L); significantly reduced haematocrit (mid- and the high-dosed male rats) and significantly increased MCV-values and number of reticulocytes, both in high-dosed male rats. Female rats showed significantly increased MCV-values at the mid- and the high-dosage only. At necropsy, increased relative liver weights (m: > 12 % from mid-dose onwards, f: > 22 % at high dose group) and spleen weights (m/f: 41 – 70 % at highest dose group), spleenic congestion and increased extramedullary haematopoiesis and haemosiderosis were noted. From this study a NOAEL of 4 mg/kg bw was derived (Hoechst AG, 1993).

In another study, Sprague-Dawley rats (5 m/5 f per sex and dose group) were exposed to 1,2-dichloro-4-nitrobenzene, commercial grade, containing 85 % 1,2-dichloro-4-nitrobenzene. The rats were dosed continuously via diet with 0, 625, 1250, 2500, 5000 or 10,000 ppm (å 0, 62.5, 125, 250, 500 or 1000 mg/kg bw/day) for 32 days and sacrificed after termination of treatment. At Ó62.5 mg/kg bw/day a discoloration of urine was seen. Ó125 mg/kg bw/day caused a decreased feed intake and and Ó250 mg/kg bw/day caused a reduced body weight gain (at least 15%). Mean final body weight was significantly reduced in males from 250 mg/kg bw/day onwards and in females from125 mg/kg bw/day onwards. The macroscopic examination of rats showed darkening of the spleen in 1/5 male dosed with 250 mg/kg bw/day and in 1/5 male and 1/5 female rat dosed with 500 mg/kg bw/day. Urinary bladder calculi with nephritis or hydronephrosis were noted in 2/5 males of the 250 mg-group and in 1/5 male 62,5 mg-group but not in male rats of the 125 mg-, 500 mg-, 1000 mg- or control-group. In the highest dose group, mortality was increased within 3-4 weeks (3/5 m and 5/5 f) and signs of intoxication were emaciation and piloerection. In nearly all rats of this group (5/5 f, 4/5 m) minimal body fat was noted. The authors stated that the test substance was not stable in diet over one week (diet was prepared weekly), so that the NOAEL may be lower than 62.5 mg/kg bw/day (Monsanto Co., 1984).

Studies in Humans

Dermal

The validity of the only available report which relate to mixed exposures, essentially to 1,2-dichloro-4-nitrobenzene, 3,4-dichloroaniline and 3,4-dichloropropionic acid anilide (Russkikh and Lubyanskii, 1984) is not assignable. The authors describe the results of a 2.5year study on the health of workers exposed at a 1,2-dichloro-4-nitrobenzene processing plant in the former USSR. Reported were skin effects (chloracne) and changes in haematological parameters typical for exposure to amino and nitro compounds such as methaemoglobinaemia, appearance of Heinz bodies, a tendency towards reticulolymphocytosis and thrombocytosis, bilirubinaemia and dysproteinaemia. The main exposure was suspected via dermal absorption due to the low vapour pressure and the working conditions. Measurements for uncovered skin gave exposures to 0.002 - 0.2 mg/dm^2 1,2-dichloro-4-nitrobenzene and 3,4-dichloroaniline and for covered skin (clothes) 0.0013 - 0.02 mg/dm^2. For 1,2-dichloro-4-nitrobenzene and 3,4-dichloroaniline the mean individual dermal exposure per shift was given with 2.535 mg, while after cleaning of the skin the mean individual burden was still 1.066 mg. Hence, the evaluation of these findings are difficult to relate to 1,2-dichloro-4-nitrobenzene because they are compiled from mixed exposures. Nevertheless, changes in haematological parameters (e.g. methaemoglobinaemia) would be plausible, because they were also observed in the above reported animal experiments.

In the recent open literature reports of human poisoning could not be identified.

Conclusion:

The main targets identified in animal studies after repeated oral administration as well as after inhalation exposure are the haematological system and in addition the kidneys after oral application and the liver after inhalation. From a 28-day oral study performed according to OECD TG 407 a

NOAEL of 4 mg/kg bw/day was derived. The NOAEL following subchronic inhalation exposure study of limited validity (limited documentation) was 0.4 mg/m³ (4 hours per day).

Changes in haematological parameters (e.g. methaemoglobinaemia, Heinz bodies) are the main target in the only available report on exposure of workers. As these findings relate to mixed exposures they cannot be clearly attributed to 1,2-dichloro-4-nitrobenzene, but would be plausible, because they were also observed in animal experiments. In the recent open literature, reports of human poisoning could not be identified.

3.1.6 Mutagenicity

Studies in Animals

In vitro Studies

(A) Gene Mutation

1,2-Dichloro-4-nitrobenzene was tested in several Ames tests with *Salmonella typhimurium* and the results are summarized in Table 12. In these studies using plate incorporation method as well as pour-plate method or preincubation methodology, 1,2-dichloro-4-nitrobenzene had a mutagenic activity in TA 98, TA 100, TA 1530 and TA 1538 with or without metabolic activation.

Table 12 Ames tests with *Salmonella typhimurium*

Test system	Concentration	Metabolic activation	Results	Reference
TA 98, TA 100, TA 1530, TA 1535, TA 1537, TA 1538	Ò1500 µg/plate	+ / -	TA 1530: pos. (- S9) TA 100 / TA 1538: pos. (+/- S9)	Gilbert et al. (1980)
TA 98, TA 100, TA 1535, TA 1537, TA 1538	Ò6554 µg/plate	-	TA 100 : pos.	Shimizu et al. (1983)
TA 98, TA 100, TA 1535, TA 1537	Ò250 µg/plate	+ / -	TA 100 : pos. (+ S9)	Haworth et al. (1983)
TA 98, TA 100, TA 1535, TA 1537, TA 1538	Ò3000 µg/plate*	+ / -	TA 98 : weakly pos. (+/- S9) TA 100 : pos. (+/- S9)	Monsanto Co. (1982)

***** 1,2-Dichloro-4-nitrobenzene technical grade : 85 % 1,2-dichloro-4-nitrobenzene and 15 % 1,2-dichloro-3-nitrobenzene

1,2-Dichloro-4-nitrobenzene, technical grade, showed no mutagenic activity in a mammalian cell gene mutation assay (HPRT test in *Chinese Hamster* ovary (CHO) cells). Tested concentrations were 25 – 250 µg/ml with and without S9 mix and cytotoxicity was noted at Ó333 µg/ml (Monsanto Co., 1986b; 1986c).

(B) Cytogenicity

1,2-Dichloro-4-nitrobenzene was tested in V79 cells for chromosomal aberrations both with and without metabolic activation at concentrations of 15 – 150 µg/ml (higher concentrations were cytotoxic). In the presence of metabolic activation a positive result was obtained only at 150 µg/ml, the highest dose tested, which resulted in a depression of the mitotic index to 55 % of the control value indicating cytotoxicity (Hoechst AG, 1989).

(C) Indicator test

No data available

In vivo Studies

(A) Gene Mutation

The mutagenicity of 1,2-dichloro-4-nitrobenzene was tested with *Drosophila melanogaster* in SLRL-test. Following feeding of 0.2 - 0.5 ml of a 50 ppm-solution over 3 days, which caused 11 % mortality and 1 % sterility, a negative result was obtained. The single intraperitoneal injection of 0.2 - 0.3 µl of a 200 ppm-solution which caused 22% mortality and 13 % sterility, yielded a positive response. No mortality rate and no sterility rate were reported of the respective controls (Woodruff et al., 1985).

(B) Cytogenicity

In a cytogenetic assay male and female Sprague-Dawley rats (24 m/24 f per dose) were dosed once via gavage with 1,2-dichloro-4-nitrobenzene technical grade (0, 75, 250 or 750 mg/kg bw in corn oil). In rats of both sexes, a significantly reduced weight gain was noted at Ó250 mg/kg bw. Six rats per sex from each group were sacrificed 6, 12, 24 or 48 hrs p.a. and bone marrow cells were analyzed for chromosomal aberrations. According to the authors, slides from the 48 h sacrifice were not analyzed, as there was no evidence of mitotic delay seen after analysis of mitotic indices. In this study no clastogenic activity was observed (Monsanto Co., 1983).

(C) Indicator test

No data available

Conclusion

1,2-Dichloro-4-nitrobenzene exhibits mutagenic activity in *Salmonella typhimurium* but not in the HPRT test in *Chinese Hamster* Ovary (CHO) cells. 1,2-Dichloro-4-nitrobenzene induced chromosomal aberrations in V79 cells with metabolic activation only at the highest concentration, which was cytotoxic. In insects (*Drosophila melanogaster*) 1,2-dichloro-4-nitrobenzene revealed no mutagenic activity in the SLRL-test after application over 3 days with slight increased toxicity, but revealed mutagenic activity following a single i.p. injections of a clearly toxic dose. 1,2-Dichloro-4-nitrobenzene showed no clastogenic activity in vivo in a chromosomal aberrations test with rats.. Overall, in non-toxic doses, there was no evidence for genotoxicity in vivo under the conditions tested.

3.1.7 Carcinogenicity

There are no studies on carcinogenicity available.

3.1.8 Toxicity for Reproduction

Effects on Fertility

Studies dealing specifically with toxicity to reproduction were not identified.

In the sub-acute toxicity study according to OECD TG 407 Wistar rats (5 m/5 f per sex and dose group) were dosed with 0, 4, 20 or 100 mg 1,2-dichloro-4-nitrobenzene/kg bw/day in sesame oil via gavage and sacrificed on day 29 (Hoechst AG, 1993), resulting in a NOAEL for general toxicity of 4 mg/kg bw/day. For further details on general toxicity see chapter 3.1.5.

Reproductive organs of the rats of the highest dose group and of the controls were examined histopathologically. No adverse effects were noted from these organs in these groups. Therefore it can be concluded that also the reproductive organs of the mid and low dosed rats are not impaired by treatment with 1,2-dichloro-4-nitrobenzene.

Based on these results there are no indications for specific adverse effects on the reproductive organs following 28-day treatment with up to 100 mg/kg bw/day despite the fact that already at 20 mg/kg bw/day the substance leads to clear systemically toxic effects and the maximum tolerated dose was reached in that experiment.

Developmental Toxicity

The available studies are performed with 1,2-dichloro-4-nitrobenzene, commercial grade, containing 85 % 1,2-dichloro-4-nitrobenzene and 15 % 1,2-dichloro-3-nitrobenzene.

In the rat teratology study, Sprague-Dawley rats (n = 25 females per group) were dosed orally once daily via gavage with 0, 10, 30 or 100 mg/kg bw/day in corn oil from gestation days (gd) 6 – 15 and sacrificed on gd 21. Dosage was chosen based on the results of a preliminary study on developmental toxicity (200 mg/kg bw: mortality 2/6 animals; reduced mean body weights up to 32 % vs controls on gd 6 - 10) (Monsanto Co., 1986a, 1987b).

All dams survived to scheduled sacrifice. In animals dosed with Ó10 mg/kg bw/day reduced food consumption was observed. Mean body weight change was dose-related affected on gd 6-10: control 8.4 g/dam, 10 mg-gr.: 6.2 g/dam, 30 mg-gr.: 4.0 g/dam (significant, p<=0.05), 100 mg-gr.: -4.4 g/dam (significant, p <= 0.01, corresponding with a significant body weight loss of about 5 % for gd 6 - 10). Rats dosed with 100 mg/kg bw showed also significantly reduced mean bodyweights on gd 10 (267.8 g versus 284.5 g of controls), gd 13 (284.8 g versus 302.3 g of controls) and gd 16 (307.7 g [approx. 5 %] versus 324.4 g of controls) and, urogenital staining and wet or matted fur. Hydronephrosis was seen in 1/23 control rat, 1/25 low-dosed rat and 3/25 high-dosed dams. Because of the low incidence of this finding and the occurrence in the control group these findings are considered not to be treatment related. Methaemoglobin levels were not measured, neither in dams nor in live foetuses. There were no adverse effects on pregnancy rates (Ó23/25 mated rats in all groups), live or dead foetuses/dam, late resorptions/dam, total implants/dam, or corpora lutea/dam and also on foetal body weights or sex distribution. Early resorptions were increased at 100 mg/kg bw/day (1.1/dam versus 0.5/control-dam.

The examination of the foetuses gave no statistically significant differences for the incidence of total or individual malformations. However, dilated ureters, variations that are regarded to be of low concern, were increased at Ó30 mg/kg bw/day (control, low mid, high dose: 7 foetuses in 3/23 In summary, developmental effects occur in the presence of maternal toxicity (significantly reduced body weight gain on gd 6-10 from 30 mg/kg bw/day) with a NOAEL for maternal toxicity and for developmental toxicity of 10 mg/kg bw/day, respectively (Monsanto Co., 1987b).

To analyze blood of dams and foetuses for total haemoglobin and methaemoglobinemia, Sprague-Dawley female rats (n = 8 per group) were orally dosed once daily with 0 or 100 mg/kg bw in corn oil via gavage from gd 6 to gd 20 and sacrificed on gd 21 All dams survived to scheduled sacrifice. In dosed rats significantly lower body weights, body weight loss (gd 8 - 10) and reduced body weight gain were observed. Other clinical signs were alopecia, perinasal/perioral and urinary staining and wet or matted fur. There were no differences in the mean number of viable foetuses of dosed dams and control dams.

In dams total haemoglobin values were significantly decreased (mean value of total haemoglobin [g/dl]: 100 mg-group: 10.6 versus 11.8 in controls) while the differences in the foetuses were only slight (mean value of total haemoglobin [g/dl]: 100 mg-group: 10.3 versus 10.6 in controls).

However, methaemoglobin levels were significantly increased in both, dams (mean value of methaemoglobin [% of total haemoglobin]: 100 mg-group: 6.08 versus 1.24 in controls) and foetuses (mean value of methaemoglobin [% of total haemoglobin]: 100 mg-group: 2.01 versus 0.53 in controls. about 4 - 5 times compared with controls) (Monsanto Co., 1987a).

Conclusion

Studies dealing specifically with toxicity to reproduction were not identified. The subacute study with 1,2-dichloro-4-nitrobenzene yielded no damage of the reproductive organs in rats despite clear systemic toxicity up to the maximum tolerated dose of 100 mg/kg bw/day.

1,2-Dichloro-4-nitrobenzene commercial grade (85 % 1,2-dichloro-4-nitrobenzene and 15 % 1,2-dichloro-3-nitrobenzene) caused effects on development at maternally toxic doses probably due to methaemoglobinaemia in dams and foetuses. A significant dose-response trend for variations (dilated ureters) was seen in the foetuses of the >= 30 mg/kg bw/day-groups and significantly reduced body weight gain of dams at dose levels of 30 mg/kg bw/day on gd 6 - 10 with an even stronger effect at 100 mg/kg bw/day. Thus, 10 mg/kg bw/day was determined as NOAEL for maternal and developmental toxicity.

3.2 Initial Assessment for Human Health

1,2-Dichloro-4-nitrobenzene is absorbed form the gastro-intestinal tract and although there are some species differences in experimental animals from the available data it can be concluded that 1,2-dichloro-4-nitrobenzene is excreted mainly via urine in the form of the mercapturic acid derivate N-acetyl-S-(2-chloro-4-nitrophenyl)-L-cysteine. Data on humans were not identified in the available literature.

There are no valid acute inhalation studies available. Based on the results of the acute dermal toxicity study with rats the LD_{50} is > 2000 mg/kg bw. From studies with rabbits no LD_{50} could be derived, the lowest Lethal Dose Level (LDLo) was 950 mg/kg bw. The acute oral toxicity in rats ranges from 625 to 950 mg/kg bw. 1,2-dichloro-4-nitrobenzene causes the formation of methaemoglobin. Predominant signs of intoxication were lethargy, increasing weakness, collapse and coma.

1,2-Dichloro-4-nitrobenzene gave no skin irritation effects when tested for 4 hours under semiocclusive conditions according to OECD TG 404 and showed slightly irritating effects, which disappeared within 72 hours under occlusive conditions according to the method of Federal Register 38 No. 187. 1,2-Dichloro-4-nitrobenzene is slightly irritating to the eyes when tested according to OECD TG 405.

1,2-Dichloro-4-nitrobenzene was not found to induce dermal sensitization when tested according to OECD TG 406. In addition, 1,2-dichloro-4-nitrobenzene was not found to induce dermal sensitization in humans in a limited study.

The main targets identified in animal studies after repeated oral administration as well as after inhalation exposure are the haematological system and in addition the kidneys after oral application and the liver after inhalation. From a 28-day oral study performed according to OECD TG 407 a NOAEL of 4 mg/kg bw/day was derived. The NOAEL following subchronic inhalation exposure study of limited validity (limited documentation) was 0.4 mg/m³ (4 hours per day).

Changes in haematological parameters (e.g. methaemoglobinaemia, Heinz bodies) are the main target in the only available report on exposure of workers. As these findings relate to mixed exposures they cannot be clearly attributed to 1,2-dichloro-4-nitrobenzene, but would be plausible, because they were also observed in animal experiments. In the recent open literature reports of human poisoning could not be identified

1,2-Dichloro-4-nitrobenzene exhibits mutagenic activity in *Salmonella typhimurium* but not in the HPRT test in *Chinese Hamster* Ovary (CHO) cells. 1,2-Dichloro-4-nitrobenzene induced chromosomal aberrations in V79 cells with metabolic activation only at the highest concentration, which was cytotoxic. In insects (*Drosophila melanogaster*) 1,2-dichloro-4-nitrobenzene revealed no mutagenic activity in the SLRL-test after application over 3 days with slight increased toxicity, but revealed mutagenic activity following a single i.p. injections of a clearly toxic dose. 1,2-Dichloro-4-nitrobenzene showed no clastogenic activity in vivo in a chromosomal aberrations test with rats. Overall, in non-toxic doses, there was no evidence for genotoxicity in vivo under the conditions tested. Studies dealing specifically with toxicity to reproduction were not identified. The subacute study with 1,2-dichloro-4-nitrobenzene yielded no damage of the reproductive organs in rats despite clear systemic toxicity up to the maximum tolerated dose of 100 mg/kg bw/day.

1,2-Dichloro-4-nitrobenzene commercial grade (85 % 1,2-dichloro-4-nitrobenzene and 15 % 1,2-dichloro-3-nitrobenzene) caused effects on development at maternally toxic doses probably due to methaemoglobinaemia in dams and foetuses. A significant dose-response trend for variations (dilated ureters) was seen in the foetuses of the >= 30 mg/kg bw/day-groups and significantly reduced body weight gain of dams at dose levels of 30 mg/kg bw/day on gd 6 - 10 with an even stronger effect at 100 mg/kg bw/day. Thus, 10 mg/kg bw/day was determined as NOAEL for maternal and developmental toxicity.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

In this chapter in general only the lowest valid test concentrations of acute and chronic testing are presented.

Acute Toxicity Test Results

Acute toxicity to fish (*Leuciscus idus*) has been tested in accordance to the German standard method for water, wastewater and sludges DIN 38412 Part 15. A 48 h LC_{50} of 3.1 mg/l was measured (Knie et al., 1983). With *Leuciscus idus melanotus* in a static system Hoechst AG (1980) observed an acute toxicity (96 h LC_{50}) of 5.2 mg/l. Acute toxicity to fish (*Oryzias latipes*) has also been tested in a static system according to Japanese Industrial Standard (JIS) K 0102-1986-71. The result (48 h LC_{50}) was 7 mg/l (MITI, 1992).

With *Daphnia* acute tests were performed according to standard procedures. In a study according to the German standard method for water, wastewater and sludges DIN 38412 Part 11 the toxicity to *Daphnia magna* was tested during 24 h resulting in an EC_{50} of 3 mg/l (Knie et al., 1983). Using a method analog to OECD Guideline 202 Zhao et al. (1997) reported an EC_{50} (48 h) of 8.2 mg/l.

In a one generation, non-guideline study with the green alga *Chlorella fusca* (= *Scenedesmus vacuolatus*) a 24 h E_rC_{50} = 0.32 mg/l was obtained in a growth inhibition test (Schmitt et al., 2000). With *Scenedesmus obliquus* the 48h-E_rC_{50} of 5.8 mg/l was found (Liu and Lang, 1995).

All effect values are related to nominal concentrations. As 1,2-dichloro-4-nitrobenzene is low to moderately volatile (Henry's law constant 0.82 Pa*m³/mol; Thomas, 1990) it cannot be excluded that a decrease in test substance concentration has occurred during the studies that have been performed in open systems. In a stability experiment performed with 1,2-dichloro-3-nitrobenzene in an open system a continuous decrease in substance concentration was observed. After 1, 2, 4 and 8 days the concentration of 1,2-dichloro-3-nitrobenzene has decreased by 6 %, 17 %, 22 % and 36 %. The authors attributed this decline in concentration to evaporation (Canton et al., 1985). Although this stability experiment cannot be directly transferred to the 1,2-dichloro-4-nitrobenzene, it can

give an indication of the degree of test substance loss during the above mentioned ecotoxicity studies. Although neither an exact validated vapor pressure is available for 1,2-dichloro-3-nitrobenzene nor a measured Henry's law constant, it can be estimated that the volatility of 1,2-dichloro-3-nitrobenzene is in the same order than the volatility of 1,2-dichloro-4-nitrobenzene. Therefore, it can be concluded from the above mentioned stability experiment that within a period of 4 days the decrease in 1,2-dichloro-4-nitrobenzene concentration will be </= 22 % and therefore the nominal concentrations are acceptable. This is also confirmed by volatilisation studies performed for other substances with Henry's law constants in a similar range (e.g. 3-methylbut-2-en-1-ol: Henry´s law constant 0.73 - 1.4 Pa*m^3/mole, 93 % recovery rate after 4 days).

Chronic Toxicity Test Results

In a reproduction (21 d) study with *Daphnia magna* performed in a semistatic system in closed vessels Kuehn et al. (1988) found a NOEC of 0.025 mg/l for the most sensitive endpoint reproduction rate. The stability of the test substance concentration was confirmed by analytical monitoring. In the same publication also an E_rC_{10} > 0.1 mg/l was determined for the algae *Scenedesmus subspicatus* after 48 hours

Thus the lowest chronic value is the NOEC of 25 µg/l *for Daphnia magna*. is. Since there are chronic studies from 2 trophic levels, an assessment factor of 50 (following the EU Technical Guidance Document) is applied and a **$PNEC_{aqua}$ of 0.5 µg/l** is obtained.

Toxicity to Microorganisms

Regarding the toxicity to microorganisms, a O_2-consumption test in accordance to Robra with *Pseudomonas putida* during 30 minutes was performed and an EC_{10} of 44 mg/l was determined (Knie et al., 1983).

For the protozoan species *Tetrahymena pyriformis* a 40 h EC_{50} of 13 mg/l was found in a population growth inhibition test (Schultz 1999).

In an 88 h assay with the fungus *Rhizoctonia solani* Eckert (1962) observed an EC_{50} of 21 mg/l. Hafsah, Tahara, and Mizutani (1984) reported that the fungus *Mucor javanicus* showed 55 % growth inhibition at 50 mg/l of 1,2-dichloro-4-nitrobenzene.

4.2 Terrestrial Effects

Acute Toxicity Test Results

No test result with plants according to OECD-Guideline 208 (Terrestrial plant growth test) is known. In humid sand, with the endpoint growth of seedlings the 6d-EC_{50} of 1,2-dichloro-4-nitrobenzene was 27 mg/l for *Phaseolus aureus* and 56 mg/l for *Cucumis sativus* (Eckert, 1962).

For the endpoint growth of seedlings (biomass), the EC_{50} of *Lactuca sativa* was measured for various chloro(nitro)benzenes and other compounds including e.g. the isomer 1,2-dichloro-3-nitrobenzene, but not 1,2-dichloro-4-nitrobenzene. For 1,2-dichloro-3-nitrobenzene the EC_{50} was > 0.32 and < 1 mg/l after 16 to 21 days (Hulzebos et al. 1993). An equation for the calculation of the EC was derived (log EC_{50} = -0.46 log K_{ow} + 2.38 [µmol/l], Hulzebos et al., 1993), which was used to calculate the EC_{50} of 1,2-dichloro-4-nitrobenzene (log K_{ow} = 3.04) to be about 1.8 mg/l.

4.3 Other Environmental Effects

No data available.

4.4 Initial Assessment for the Environment

With regard to its chemical structure 1,2-dichloro-4-nitrobenzene is not expected to hydrolyse under environmental conditions. The favourite target compartments of 1,2-dichloro-4-nitrobenzene are air with 48 % and water with 44 % according to a Mackay calculation level I. The measured Henry's law constant of 0.82 $Pa*m^3*mol^{-1}$ indicates that the compound has a low to moderate potential for volatilization from surface waters. In the atmosphere slow photodegradation takes place by reaction with photochemically produced OH radicals. The half-life is calculated to be 321 days. 1,2-Dichloro-4-nitrobenzene will undergo direct photolysis in air due to absorbance of environmental UV light, however, the respective half-life is not known. 1,2-Dichloro-4-nitrobenzene is not readily biodegradable (Manometric respirometry test: biodegradation < 10 % after 21 days based on BOD; OECD TG 301 C biodegradation 0 % within 28 days, presumably due to inhibition of inoculum). 1,2-Dichloro-4-nitrobenzene is biodegradable by adapted microorganisms under aerobic conditions and by non-adapted inocula under anaerobic conditions (primary degradation). Sewage from adapted wastewater treatment plants has significant potential to primary degrade 1,2-dichloro-4-nitrobenzene (Test method "Simulation of an industrial waste water treatment plant": after 3 days 100 %).

Measured bioconcentration factors in fish are in the range of 26 - 65 at a 1,2-dichloro-4-nitrobenzene concentrations of 0.005 to 0.05 mg/l in the medium (*Cyprinus carpio*). A measured K_{oc} of 417 for the media water-sediment suggests the substance to have a medium geoaccumulation potential.

The lowest valid acute test results of aquatic testing determined are summarized in Table 13.

Applying an assessment factor of 50 to the lowest available chronic value of 25 µg/l (21d reproduction in *D. magna*), a

$PNEC_{aqua}$ of 0.5 µg/l

is obtained.

The lowest measured 6d-EC_{50} for terrestrial plants was 26.9 mg/l for the plant *Phaseolus aureus*.

Table 13 Acute and chronic toxicities of 1,2-dichloro-4-nitrobenzene

Trophic level	*Species*	Test	Result	Source	IUCLID
Fish	*Leuciscus idus*	48 h LC_{50}	3.1 mg/l	Knie et al. (1983)*	4.1
Fish	*Leuciscus idus melanotus*	96 h LC_{50}	5.2 mg/l	Hoechst AG (1980)	4.1
Fish	*Oryzias latipes*	48 h LC_{50}	7 mg/l	MITI (1992)	4.1
Daphnia	*Daphnia magna*	24 h EC_{50}	3 mg/l	Knie et al. (1983)*	4.2
Daphnia	*Daphnia carinata*	48 h EC_{50}	8.2 mg/l	Zhao et al. (1997)	4.2
Algae	*Chlorella fusca* (= *Scenedesmus vacuolatus*)	24 h E_rC_{50}	0.32 mg/l	Schmitt et al. (2000)	4.3
Algae	*Scenedesmus obliquus*	48h E_rC_{50}	5.8 mg/l	Liu and Lang (1995)*	4.3
Algae	*Scenedesmus subspicatus*	48h E_rC_{10}	>0.1 mg/l	Kuehn et al. (1988)*	4.3
Bacteria	*Pseudomonas putida*	30 min EC_{10}	44 mg/l	Knie et al. (1983)	4.4
Protozoa	*Tetrahymena pyriformis*	40 h EC_{50}	13 mg/l	Schultz (1999)	4.4
Fungus	*Rhizoctonia solani*	88 h EC_{50}	21 mg/l	Eckert (1962)	4.4
Fungus	*Mucor javanicus*	6 d E_rC_{55}	50 mg/l	Hafsah, Tahara, and Mizutani (1984)	4.4
Daphnia	*Daphnia magna*	21 d NOEC	0.025 mg/l	Kuehn et al. (1988)*	4.5.2
Terrestrial plant	*Phaseolus aureus*	6d EC_{50}	27 mg/l	Eckert (1962)	4.6.2

*Studies used for assessment

5 RECOMMENDATIONS

Environment:

The chemical possesses properties indicating a hazard for the environment. Based on data presented by the sponsor country, exposure to the environment is anticipated to be low, and 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.

Human Health:

The chemical possesses properties indicating a hazard for human health (principally haematological toxicity, and developmental toxicity, probably linked to methemoglobinemia). Based on data presented by the sponsor country, exposure is controlled in occupational settings, and exposure of

consumers is not known to occur. 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.

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I U C L I D

Data Set

Existing Chemical	:	ID: 99-54-7
CAS No.	:	99-54-7
EINECS Name	:	1,2-dichloro-4-nitrobenzene
EC No.	:	202-764-2
TSCA Name	:	Benzene, 1,2-dichloro-4-nitro-
Molecular Formula	:	C6H3Cl2NO2

Producer related part

Company	:	Bayer AG
Creation date	:	20.04.2000

Substance related part

Company	:	Bayer AG
Creation date	:	20.04.2000
Status	:	
Memo	:	AKTUELL / ICCA (Datensatz von Hoechst mit Update-Daten von Clariant, Teil 1 Bayer AG
Printing date	:	22.10.2004
Revision date	:	
Date of last update	:	21.09.2004
Number of pages	:	129
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

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type : other: typical for industrial intermediate
Substance type : organic
Physical status : solid
Purity : >= 99 % w/w
Colour : yellow
Odour : slightly aromatic

Remark : Information on purity from Bayer Chemicals and Thiem et al. (1979)
Information on odour from Hoechst AG
Flag : Critical study for SIDS endpoint
05.07.2003 (1) (2) (3) (4)

1.1.2 SPECTRA

Type of spectra : UV

Result : UV absorption in methanol/water:
log e 3.95 (225 nm)
log e 3.96 (276 nm)
log e 3.30 (320 nm)
Flag : Critical study for SIDS endpoint
26.03.2003 (5)

1.2 SYNONYMS AND TRADENAMES

1,2-Dichloro-4-nitrobenzene

Remark : IUPAC name
Flag : Critical study for SIDS endpoint
08.04.1994

1-Nitro-3,4-dichlorobenzene

Flag : Critical study for SIDS endpoint
08.04.1994

3,4-Dichloro-1-nitrobenzene

Flag : Critical study for SIDS endpoint
08.04.1994

Benzene, 1,2-dichloro-4-nitro-

Remark : CA-Index-Name
Flag : Critical study for SIDS endpoint
26.03.2003

1.3 IMPURITIES

Purity : other: typical for industrial intermediate
CAS-No : 7732-18-5
EC-No : 231-791-2
EINECS-Name : water
Molecular formula : H2O
Value : < .1 % w/w

05.07.2003 (6)

Purity : measured for specific batch
CAS-No : 3209-22-1
EC-No : 221-717-7
EINECS-Name : 1,2-dichloro-3-nitrobenzene
Molecular formula : C6H3Cl2NO2
Value :

Remark : 1,2-Dichloro-3-nitrobenzene is formed as a byproduct of 1,2-dichloro-4-nitrobenzene synthesis via nitration of 1,2-dichlorobenzene
05.07.2003 (7)

Purity : typical for marketed substance
CAS-No : 3209-22-1
EC-No : 221-717-7
EINECS-Name : 1,2-dichloro-3-nitrobenzene
Molecular formula : C6H3Cl2NO2
Value : < 1 % w/w

Test substance : 1,2-Dichloro-3-nitrobenzene is formed as a byproduct of 1,2-dichloro-4-nitrobenzene synthesis via nitration of 1,2-dichlorobenzene. Initial product mixture contains 7 - 13 % w/w 1,2-dichloro-3-nitrobenzene. Several methods to separate isomers are described e.g. crystallization from the molten mixture, fractionated crystallization in sulfuric acid, or selective adsorption on zeoliths
05.07.2003 (8)

Purity : other: measured before a specific experiment
CAS-No : 7732-18-5
EC-No :
EINECS-Name : water
Molecular formula : H2O
Value : = .3 % w/w

Remark : Although the purity of the substance is given as 99.67 mole %, the upper contents of 2 impurities are reported far higher (e.g. <= 0.5 % w/w) than can be deduced from the reported purity. Even the reported content of the third impurity (water, 0.3 % w/w) is in contradiction to the reported purity.
Test substance : Deduced from the reported impurity 1-chloro-4-nitrobenzene, 1,2-dichloro-4-nitrobenzene produced for this experiment was obtained by chlorination of 1-chloro-4-nitrobenzene
Reliability : (3) invalid
Reported content of impurities in contradiction to reported purity
10.07.2003 (9)

Purity : other: measured before a specific experiment
CAS-No : 100-00-5
EC-No : 202-809-6
EINECS-Name : 1-chloro-4-nitrobenzene
Molecular formula : C6H4ClNO2
Value : <= .5 % w/w

Remark : Although the purity of the substance is given as 99.67 mole %, the upper contents of 2 impurities are reported far higher (e.g. <= 0.5 % w/w) than can be deduced from the reported purity. Even the reported content of the third impurity (water, 0.3 % w/w) is in contradiction to the reported purity.
Test substance : Deduced from the reported impurity 1-chloro-4-nitrobenzene, 1,2-dichloro-4-nitrobenzene produced for this experiment was obtained by chlorination of 1-chloro-4-nitrobenzene
Reliability : (3) invalid
Reported content of impurities in contradiction to reported purity
10.07.2003 (9)

Purity : other: measured before a specific experiment
CAS-No :
EC-No :
EINECS-Name : trinitrobenzene
Molecular formula : C6H3N3O6
Value : <= .5 % w/w

Remark : Although the purity of the substance is given as 99.67 mole %, the upper contents of 2 impurities are reported far higher (e.g. <= 0.5 % w/w) than can be deduced from the reported purity. Even the reported content of the third impurity (water, 0.3 % w/w) is in contradiction to the reported purity.
Test substance : Deduced from the reported impurity 1-chloro-4-nitrobenzene, 1,2-dichloro-4-nitrobenzene produced for this experiment was obtained by chlorination of 1-chloro-4-nitrobenzene
Reliability : (3) invalid
Not reported which trinitrobenzene was present. Reported content of impurities in contradiction to reported purity. Formation of significant levels of trinitrotoluene during synthesis or purification is very unlikely
10.07.2003 (9)

1.4 ADDITIVES

1.5 TOTAL QUANTITY

Quantity : ca. 36800 - tonnes produced in 2001

Remark : Worldwide (excluding Eastern Europe) production of 1,2-dichloro-4-nitrobenzene amounted to about 36,800 tons by approximately 12 producers

Manufacturing capacities distribution

	Known Capacity (%)
Western Europe	45
USA	10
South America	23
Southeast Asia	22
Eastern Europe	unknown

05.07.2003 (10)

1.6.1 LABELLING

Labelling : provisionally by manufacturer/importer
Specific limits :
Symbols : Xn, N, ,
Nota : , ,
R-Phrases : (20/21/22) Harmful by inhalation, in contact with skin and if swallowed
(51/53) Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
S-Phrases : (28) After contact with skin, wash immediately with plenty of with plenty of water and soap, if possible with polyethylenglycol 400 too
(36/37) Wear suitable protective clothing and gloves
(61) Avoid release to the environment. Refer to special instructions/Safety data sets

05.07.2003 (1)

1.6.2 CLASSIFICATION

Classified : provisionally by manufacturer/importer
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 :

05.07.2003 (1)

Classified : provisionally by manufacturer/importer
Class of danger : harmful
R-Phrases : (20/21/22) Harmful by inhalation, in contact with skin and if swallowed
Specific limits :

05.07.2003 (1)

1.6.3 PACKAGING

1.7 USE PATTERN

Type of use : type
Category : Use in closed system

07.04.1994

Type of use : industrial
Category : Chemical industry: used in synthesis

07.04.1994

Type of use : use
Category : Intermediates

07.04.1994

1.7.1 DETAILED USE PATTERN

1.7.2 METHODS OF MANUFACTURE

Origin of substance : Synthesis
Type : Production

Remark : Manufactured by nitration of 1,2-dichlorobenzene with mixed acid (sulfuric acid and nitric acid) at 35 - 60 °C results in a mixture of 1,2-dichloro-3-nitrobenzene (about 10 %) and 1,2-dichloro-4-nitrobenzene (about 90 %), which are separated by crystallization. Another process is based on chlorination of molten 4-chloronitrobenzene (90 - 100 °C). Chlorination has the advantage that it gives a pure product during synthesis but it cannot be used when the isomeric 1,2-dichloro-3-nitrobenzene is also required.
Flag : Critical study for SIDS endpoint
08.04.2003 (3) (4)

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

Type of limit : other: maximum workplace concentration (Arbeitsplatzrichtwert, ARW)
Limit value : 1 mg/m3

Remark : maximum workplace concentration (Arbeitsplatzrichtwert, ARW) of the Bayer AG
05.07.2003 (1)

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

Classified by : KBwS (DE)
Labelled by : KBwS (DE)

Class of danger : 3 (strongly water polluting)

Remark : WGK-Kennnummer 845
10.07.2003

1.8.4 MAJOR ACCIDENT HAZARDS

1.8.5 AIR POLLUTION

Classified by : other: Hoechst AG
Labelled by :
Number : 3.1.7 (organic substances)
Class of danger : I

07.04.1994

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 : 3, 4
Date of search : 20.06.2002

Flag : Critical study for SIDS endpoint
28.03.2003

Type of search : Internal and External
Chapters covered : 5
Date of search : 01.03.2003

Flag : Critical study for SIDS endpoint
28.03.2003

1.13 REVIEWS

Memo : BUA report 52 1,2-Dichloronitrobenzenes

Flag : Critical study for SIDS endpoint
28.03.2003 (8)

2.1 MELTING POINT

Value : 43 °C
Sublimation :
Method : other: no data
Year : 1977
GLP : no
Test substance : no data

Remark : Information from Ullmann: The unstable beta-form is a liquid that reverts to the stable alpha-form at 15 °C, which has a melting point of 43 °C
Reliability : (2) valid with restrictions
Data from handbook or collection of data
Flag : Critical study for SIDS endpoint
05.07.2003 (3) (4) (11) (12)

Value : 40 - 40.5 °C
Sublimation :
Method : other: no data
Year : 1976
GLP : no
Test substance : other TS: reagent grade and recrystallised

Test condition : 1,2-Dichloro-4-nitrobenzene was recrystallised from methanol before determination of the melting point
Reliability : (2) valid with restrictions
Study according to scientific principles
05.07.2003 (13)

Value : = 39.9 °C
Sublimation :
Method : other: no data
Year : 1987
GLP :
Test substance :

Remark : Point of solidification
Reliability : (4) not assignable
Not assignable/manufacturer data without proof
15.07.2003 (2)

Value : = 41.2 °C
Sublimation :
Method :
Year : 1995
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Data obtained by an extensive literature review, critically evaluated
05.07.2003 (14)

Value : 40 - 41 °C
Sublimation :
Method : other: data reported from Japanese industry
Year : 1992
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Reliable source
15.07.2003 (15)

Value : = 42.5 °C
Sublimation :
Method : other: no data
Year : 1933
GLP : no
Test substance : other TS: synthesized

Result : The nitration products of 1,2-dichlorobenzene were analyzed. A solid fraction (1,2-dichloro-4-nitrobenzene) and a liquid fraction (1,2-Dichloro-3-nitrobenzene) were obtained. 1,2-dichloro-4-nitrobenzene was purified by repeated partial freezing followed by filtration. The melting point was 35 - 40 °C. After crystallization from alcohol the melting point was 42.5 °C
Reliability : (2) valid with restrictions
Study according to scientific principles
05.07.2003 (7)

Value : 41.9 - 42.9 °C
Sublimation :
Method : other: no data
Year : 1982
GLP : no
Test substance : other TS:Purity reported to be 99.67 mole %

Remark : Although the purity of the substance is given as 99.67 mole %, the upper contents of 2 impurities are reported far higher (e.g. <= 0.5 % w/w) than can be deduced from the reported purity. Even the reported content of the third impurity (water, 0.3 % w/w) is in contradiction to the reported purity.
Reliability : (3) invalid
Reported content of impurities in contradiction to reported purity
05.07.2003 (9)

2.2 BOILING POINT

Value : 255 °C at
Decomposition :
Method : other: no data
Year : 1977
GLP : no
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data
Flag : Critical study for SIDS endpoint
05.07.2003 (11)

Value : 255 - 256 °C at
Decomposition :
Method : other: no data
Year : 1989
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data
05.07.2003 (12)

Value : 254.9 °C at
Decomposition :
Method : other: no data
Year : 1982
GLP : no
Test substance : other TS: purity 99.67 mole %

Remark : Although the purity of the substance is given as 99.67 mole %, the upper contents of 2 impurities are reported far higher (e.g. <= 0.5 % w/w) than can be deduced from the reported purity. Even the reported content of the third impurity (water, 0.3 % w/w) is in contradiction to the reported purity.
Reliability : (3) invalid
Reported content of impurities in contradiction to reported purity
05.07.2003 (9)

Value : 263 °C at 1013 hPa
Decomposition :
Method : other: no data
Year : 2001
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data
05.07.2003 (3) (4)

Value : 105 - 107 °C at 4 hPa
Decomposition :
Method : other: Reported from Japanese industry
Year : 1992
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Reliable source
05.07.2003 (15)

Value : 188.5 - 189 °C at 133 hPa
Decomposition :
Method : other: no data
Year : 1933
GLP : no
Test substance : other TS: synthesized

Remark : The nitration products of 1,2-dichlorobenzene were analyzed. A solid fraction (1,2-dichloro-4-nitrobenzene) and a liquid fraction (1,2-Dichloro-3-nitrobenzene) were obtained. 1,2-dichloro-4-nitrobenzene was purified by repeated partial freezing followed by filtration
Reliability : (2) valid with restrictions
Study according to scientific principles
05.07.2003 (7)

Value : = 255 °C at 1013 hPa
Decomposition : yes
Method : other: no data
Year : 1987
GLP : no
Test substance : no data

Result : Decomposition temperature is 370 °C
Test condition : Decomposition temperature was determined with DTA (Increase of temperature: 10 K/min)
Reliability : (4) not assignable
Not assignable/manufacturer data without proof
16.07.2003 (2)

2.3 DENSITY

Type : density
Value : = 1.56 g/cm³ at 15 °C
Method : other: no data
Year : 1987
GLP : no
Test substance : no data

Reliability : (4) not assignable
Not assignable/manufacturer data without proof
16.07.2003 (2)

Type : density
Value : 1.487 g/cm³ at 50 °C
Method :
Year : 1979
GLP : no
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data
Flag : Critical study for SIDS endpoint
05.07.2003 (3)

Type : density
Value : 1.464 g/cm³ at 70 °C
Method :
Year : 1979
GLP : no
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data
05.07.2003 (3)

Type : density
Value : 1.4588 g/cm³ at 75 °C
Method : other: no data
Year : 1989
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data
05.07.2003 (4) (12)

Type : density
Value : 1.441 g/cm³ at 90 °C
Method :
Year : 1979
GLP : no

Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data
05.07.2003 (3)

Type : density
Value : 1.43 at 100 °C
Method :
Year : 1979
GLP : no
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data
05.07.2003 (3)

Type : density
Value : 1.456 g/cm³ at 75 °C
Method : other: no data
Year : 1982
GLP : no data
Test substance : other TS: Purity 99.67 mole %

Remark : Although the purity of the substance is given as 99.67 mole %, the upper contents of 2 impurities are reported far higher (e.g. <= 0.5 % w/w) than can be deduced from the reported purity. Even the reported content of the third impurity (water, 0.3 % w/w) is in contradiction to the reported purity.
Reliability : (3) invalid
Reported content of impurities in contradiction to reported purity
05.07.2003 (9)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value : .02 hPa at 25 °C
Decomposition :
Method : other (calculated): EPIWIN v3.10
Year : 2000
GLP :
Test substance :

Remark : EPIWIN also reports the Daubert and Danner 1991 value of 0.0103 mm Hg which equals 1.37 Pa at 25 °C
Result : Using experimental data compiled in the EPIWIN program:
Boiling point: 255.50 °C
Melting point: 43.00 °C
the vapor pressure at 25 °C is:
VP: 0.0141 mm Hg (Antoine Method)
VP: 0.0138 mm Hg (Modified Grain Method)
VP: 0.0237 mm Hg (Mackay Method)
Selected VP: 0.0138 mm Hg (Modified Grain Method) = 1.82 Pa

Using experimental data compiled in the Table 1 of the SIAR:
Boiling point: 255.00 °C
Melting point: 43.00 °C

Vapor Pressure Estimations (25 deg C):
VP: 0.0145 mm Hg (Antoine Method)
VP: 0.0141 mm Hg (Modified Grain Method)
VP: 0.0243 mm Hg (Mackay Method)
Selected VP: 0.0141 mm Hg (Modified Grain Method) = 1.86 Pa

Reliability : (2) valid with restrictions
Accepted calculation method
Flag : Critical study for SIDS endpoint
23.10.2003 (16)

Value : = 6 hPa at 114.7 °C
Decomposition :
Method :
Year : 1979
GLP : no
Test substance : other TS: purity > 99 %

Reliability : (2) valid with restrictions
Data from handbook or collection of data
Flag : Critical study for SIDS endpoint
05.07.2003 (3)

Value : = 20 hPa at 138.6 °C
Decomposition :
Method :
Year : 1979
GLP : no
Test substance : other TS: purity > 99 %

Reliability : (2) valid with restrictions
Data from handbook or collection of data
23.10.2003 (3)

Value : = 80 hPa at 173.3 °C
Decomposition :
Method :
Year : 1979
GLP : no
Test substance : other TS: purity > 99 %

Reliability : (2) valid with restrictions
Data from handbook or collection of data
23.10.2003 (3)

Value : = 100 hPa at 225.5 °C
Decomposition :
Method :
Year : 1979
GLP : no
Test substance : other TS: purity > 99 %

Reliability : (2) valid with restrictions
Data from handbook or collection of data
23.10.2003 (3)

Value : .02 hPa at 25 °C
Decomposition :
Method :
Year : 2001
GLP : no data
Test substance : other TS: Purity > 99 %

Reliability : (4) not assignable
Not assignable/manufacturer data without proof
23.10.2003 (1)

2.5 PARTITION COEFFICIENT

Partition coefficient : octanol-water
Log pow : 3.04 at °C
pH value :
Method : other (measured): Flask-shaking Method
Year : 1989
GLP : no data
Test substance : no data

Method : According to Karickhoff SW, Brown DS (1979) Determination of octanol/water distribution coefficients, water solubilities, and sediment/water partition coefficients for hydrophobic pollutants. EPA-600/4-79-032- USEPA, Environmental Research Laboratory, Athens, GA
(6 replicates performed)
Reliability : (2) valid with restrictions
Study according to scientific principles. National standard method used
Flag : Critical study for SIDS endpoint
05.07.2003 (17)

Partition coefficient : octanol-water
Log pow : 3.27 at °C
pH value :
Method : other (calculated)
Year : 1989
GLP :
Test substance :

Method : Calculated from the Fragment method of Hansch C, Leo A (1979) Substituent Constants for Correlation Analysis in Chemistry and Biology. John Wiley and Sons, New York
Reliability : (2) valid with restrictions
Accepted calculation method
05.07.2003 (17)

Partition coefficient : octanol-water
Log pow : 2.99 at 20 °C
pH value :
Method : other (measured): Flask-shaking Method
Year : 1981
GLP : no data
Test substance : other TS: purity not reported but checked by TLC and NMR

Method : According to Fujita T, Iwasa J, Hansch C (1964) A New Substituent Constant Derived from Partition Coefficients. J Am Chem Soc 86: 5175 - 5180
Reliability : (2) valid with restrictions
Well documented study
05.07.2003 (18)

Partition coefficient : octanol-water
Log pow : 2.95 at °C
pH value :

Method	:	other (measured): measured at the Japanese Chemicals Inspection and Testing Institute
Year	:	1992
GLP	:	no data
Test substance	:	no data
Reliability	:	(2) valid with restrictions Reliable source

05.07.2003 (15)

Partition coefficient	:	octanol-water
Log pow	:	= 3.12 at °C
pH value	:	
Method	:	other (measured)
Year	:	1983
GLP	:	no
Test substance	:	no data
Method	:	Determination of the octanol/water partition coefficient according to Yalkowsky and Valvani [Yalkowsky SH, Valvani SC (1980) Solubility and Partitioning I: Solubility of Nonelectrolytes in Water. J Pharmaceutical Sciences 69 (8): 912 - 922] in water saturated octanol and in octanol saturated water by UV and GC methods. No description of the method, the calculation, the statistics or on quality parameters given
Reliability	:	(3) invalid Insufficient documentation

10.07.2003 (19)

Partition coefficient	:	octanol-water
Log pow	:	3.12 at °C
pH value	:	
Remark	:	All studies cite the data of Kaiser (1983 [Kaiser KLE (1983) A Non-linear Function for the Approximation of Octanol/Water Partition Coefficients of Aromatic Compounds with Multiple Chlorine Substitution. Chemosphere 12: 1159 - 1167] - directly: Kaiser and Ribo (1985); Hansch and Leo (1995) - indirectly: Schmitt et al. (2000) cite Hansch and Leo (1995); Klamer and Beekman (1995) cite the Pomona MedChem data compilation which is based on the data compilations of Hansch and Leo
Reliability	:	(4) not assignable Secondary literature based on sparsely documented study

05.07.2003 (20) (21) (22) (23)

Partition coefficient	:	octanol-water
Log pow	:	3.12 at °C
pH value	:	
Method	:	
Year	:	1995
GLP	:	no data
Test substance	:	
Remark	:	Cronin et al. (1998) cite the value 3.12 as either a measured or computer-estimated value from the CLOGP for Windows software from Biobyte Corp., Claremont, CA. Sabljic et al. (1995) cite MedChemMaster file or a calculation by ClogP software.
Reliability	:	(3) invalid It is not clear from which source data have been derived

05.07.2003 (24) (25)

Partition coefficient	:	octanol-water

Log pow	:	3.12 at °C
pH value	:	
Method	:	
Year	:	1999
GLP	:	no data
Test substance	:	
Method	:	It is stated that the octanol/water coefficients "were measured or estimated with the CLOGP version 3.55 software." Temperature not reported
Reliability	:	(3) invalid It is not clear whether data have been measured or calculated

05.07.2003 (26)

Partition coefficient	:	octanol-water
Log pow	:	3.29 at 25 °C
pH value	:	
Method	:	OECD Guide-line 107 "Partition Coefficient (n-octanol/water), Flask-shaking Method"
Year	:	2001
GLP	:	no data
Test substance	:	other TS: Purity was more than 99 %
Method	:	Flask-shaking method according to the OECD-Guidelines for testing of chemicals (OECD 1987)
Remark	:	Wu et al. (2001) report some details of the experimental determination method. However, the data of 7 substances (1,2-dichloro-4-nitrobenzene, 3,4-dichlorobenzonitrile, 3,4-dichloroaniline, pentachlorophenol and 2,4-dichlorophenol, 4-chlorobenzaldehyd, 4-chlorobenzonitrile) are exactly the same (3 digits) as data published by the senior author (L.-S. Wang) in 1993 [Zhao Y, Wang L, Gao H, Zhang Z (1993) Quantitative Structure - Activity Relationships - Relationship between Toxicity of Organic Chemicals to Fish and to Photobacterium phosphoreum. Chemosphere 26 (11): 1971 - 1979]. Thus, it is assumed that these data were not measured by Wu et al. (2001) but that published data have been used . Furthermore, it is assumed that these data have not been measured by this group, since in the paper of Zhao et al. (1993), these data are cited as "Data from Yalkowsky et al [1, 17] and calculated according to Hansch and Leo [14]" (The first reference of this citation does not refer to a Yalkowsky paper). In the paper of Zhao et al. (1997) [Zhao Y-H, Yuan X, Ji G-D, Sheng L-X, Wang L-S (1997) Quantitative Structure-Activity Relationships of Nitroaromatic Compounds to Four Aquatic Organisms. Chemosphere 34 (8): 1837 - 1844] with the same senior author, log Kow is cited as being from Yalkowsky et al. without refering to a publication of Yalkowsky.
Reliability	:	(3) invalid Although Wu et al. (2001) describe the experimental procedure in some detail, the same data are cited in foregoing papers as being derived from other publications

05.07.2003 (27)

Partition coefficient	:	octanol-water
Log pow	:	3.29 at °C
pH value	:	
Method	:	
Year	:	1993
GLP	:	no data
Test substance	:	
Remark	:	The authors make contradictory statements on the source of the data, e.g. in the first publication: "Data from Yalkowsky et al [1, 17] and calculated according to Hansch and Leo [14]" (The first reference of this citation does not refer to a Yalkowsky paper).

Reliability : (3) invalid
It is not clear whether data were measured or calculated
05.07.2003 (28) (29) (30)

Partition coefficient : octanol-water
Log pow : 3.29 at °C
pH value :
Method :
Year : 1997
GLP :
Test substance :

Remark : The authors state that they obtained octanol/water partition coefficients from Yalkowsky et al. [1,5], however the literature cited is not from Yalkowski et al.
Reliability : (3) invalid
Secondary literature. Inconsistent information given on the source of data.
05.07.2003 (31)

Partition coefficient : octanol-water
Log pow : 3.04 at °C
pH value :
Method :
Year : 1996
GLP :
Test substance :

Remark : Devillers et al. (1996) cite the work of Niimi et al. (1989)
Reliability : (4) not assignable
Secondary literature
05.07.2003 (32)

Partition coefficient : octanol-water
Log pow : 2.94 at °C
pH value :
Method :
Year : 1985
GLP : no data
Test substance :

Remark : No information on source of data
Reliability : (3) invalid
Insufficient documentation
10.07.2003 (33)

Partition coefficient : octanol-water
Log pow : 2.99 at 20 °C
pH value :
Method :
Year : 1996
GLP :
Test substance :

Reliability : (4) not assignable
Secondary literature
05.07.2003 (34)

Partition coefficient : octanol-water
Log pow : 3.09 at °C
pH value :
Method :

Year	:	2000
GLP	:	
Test substance	:	
Reliability	:	(4) not assignable Original reference in Chinese
15.04.2003		(35)
Partition coefficient	:	octanol-water
Log pow	:	3.29 at °C
pH value	:	
Method	:	other (calculated): using SRC-WSKOW software
Year	:	2002
GLP	:	
Test substance	:	
Reliability	:	(2) valid with restrictions Accepted calculation method
05.07.2003		(36)
Partition coefficient	:	octanol-water
Log pow	:	= 3.3 at 25 °C
pH value	:	
Method	:	other (calculated): Medchem Software CLOGP3, Release 3.42, Pomona College, Cleremont CA.
Year	:	1986
GLP	:	no data
Test substance	:	no data
Source	:	not available
Reliability	:	(2) valid with restrictions Accepted calculation method
10.07.2003		(37)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	121 mg/l at 20 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	other: see TC
Year	:	1962
GLP	:	no
Test substance	:	other TS: Recrystallized from 95% ethanol but no purity given
Test condition	:	Preparation of saturated solution at 23 °C, than equilibrated for 1 day at 20 °C. Spectrometric measurement of the test substance after extraction with 2,2,4-trimethylpentan
Reliability	:	(2) valid with restrictions Study according to scientific principles
Flag	:	Critical study for SIDS endpoint
05.07.2003		(38)

Solubility in	:	Water
Value	:	121 mg/l at °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	other: Validated literature review/calculations
Year	:	1995
GLP	:	no data
Test substance	:	no data
Remark	:	Kuehne et al. (1995) report that the result is taken from extensive literature review including validation
Result	:	Water solubility Sw is reported to be - measured: log Sw = -3.20 [mol/l] = 121 mg/l - calculated according to Group method at 25 °C: log Sw = -3.41 [mol/l] = 75 mg/l
Reliability	:	(2) valid with restrictions Data obtained by an extensive literature review, critically evaluated

16.07.2003 (14)

Solubility in	:	Water
Value	:	= 151 mg/l at 20 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	yes
Deg. product	:	
Method	:	other: no data
Year	:	1987
GLP	:	no
Test substance	:	no data
Reliability	:	(4) not assignable Not assignable/manufacturer data without proof

16.07.2003 (2)

Solubility in	:	Water
Value	:	140 mg/l at °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	other: measured
Year	:	1992
GLP	:	no data
Test substance	:	no data
Remark	:	Measured at the Japanese Chemicals Inspection and Testing Institute
Reliability	:	(2) valid with restrictions

Reliable source
05.07.2003 (15)

Solubility in : Organic Solvents
Value : at °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :

Result : Hardly soluble in cold ethanol, soluble in hot ethanol, benzene, ether, and carbondisulfide
Reliability : (2) valid with restrictions
Data from handbook or collection of data
Flag : Critical study for SIDS endpoint
05.07.2003 (3)

Solubility in : Water
Value : 121 mg/l at °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method :
Year : 1999
GLP : no data
Test substance : no data

Result : Water solubility Sw is reported to be
- measured (cited from Kühne et al. (1995) [Kuehne R, Ebert R-U, Keint D, Schmidt G, Schüürmann G (1995) Group Contribution Methods to Estimate Water Solubility of Organic Compounds. Chemosphere 30 (11): 2061 - 2077]: log Sw = -3.20 [mol/l] = 121 mg/l
- calculated according to the equation of Abraham and Le (1999) taking into account the hydrogen bond acidity and basicity: log Sw = -3.436 [mol/l] = 70 mg/l

Reliability : (4) not assignable
Secondary literature in regard to measured value
05.07.2003 (39)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

Value : = 155 °C
Type :
Method : other: no data
Year : 1979
GLP : no
Test substance : other TS: purity > 99 %

Reliability : (2) valid with restrictions
Data from handbook or collection of data
Flag : Critical study for SIDS endpoint
15.07.2003 (3)

Value : ca. 123 °C
Type : closed cup
Method : other: DIN 51758
Year : 2001
GLP : no data
Test substance : other TS: purity > 99 %

Reliability : (4) not assignable
Not assignable/manufacturer data without proof
15.07.2003 (1)

Value : = 124 °C
Type :
Method : other: no data
Year : 1987
GLP : no
Test substance : no data

Reliability : (4) not assignable
Not assignable/manufacturer data without proof
15.07.2003 (2)

2.8 AUTO FLAMMABILITY

Value : = 420 °C at
Method : other: DIN 51794
Year : 2001
GLP : no
Test substance : no data

Remark : Ignition temperature is reported
Reliability : (4) not assignable
Not assignable/manufacturer data without proof
Flag : Critical study for SIDS endpoint
15.07.2003 (1)

Value : 420 °C at
Method :
Year : 1987
GLP : no data
Test substance : no data

Remark : Ignition temperature is reported
Reliability : (4) not assignable
Not assignable/manufacturer data without proof
15.07.2003 (2)

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

Value : 2.87 - mPa s (dynamic) at 60 °C
Result :
Method :
Year : 1979
GLP : no
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data
05.07.2003 (3)

Value : 2.37 - mPa s (dynamic) at 70 °C
Result :
Method :
Year : 1979
GLP : no
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data
05.07.2003 (3)

Value : 1.52 - mPa s (dynamic) at 100 °C
Result :
Method :
Year : 1979
GLP : no
Test substance : no data

Reliability : (2) valid with restrictions
Data from handbook or collection of data
05.07.2003 (3)

2.14 ADDITIONAL REMARKS

Memo : pH value

Result : In water at 23 °C: pH 5.8
Reliability : (2) valid with restrictions
Basic data given
Flag : Critical study for SIDS endpoint
23.10.2003 (40)

Memo : Conversion factors volume/weight concentration

Remark : Conversion factor for the vapour phase
1 mg/m3 = 0.13 ppm
1 ppm = 7.98 mg/m3
Reliability : (2) valid with restrictions

Data from handbook or collection of data

Flag : Critical study for SIDS endpoint
23.10.2003 (11)

Memo : Begin of thermal decomposition

Remark : Cited according to BUA (Advisory Committee on Existing Chemicals of the Association of German Chemists (GDCh)) (1991) BUA report No. 52 1,2-Dichloronitrobenzenes (1,2-Dichloro-3-nitrobenzene, 1,2-Dichloro-4-nitrobenzene) Weinheim, VCH Verlagsgesellschaft

Result : Begin of thermal decomposition at 370°C

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

Flag : Critical study for SIDS endpoint
23.10.2003 (41)

Memo : Begin of thermal decomposition

Method : Hoechst data determined with DTA [Differential Thermal Analysis], heating rate 10 °C/min

Result : Begin of thermal decomposition: 370°C
Dangerous decomposition products according to Hoechst AG: Hydrogen chloride and nitrous fumes

Reliability : (4) not assignable
Not assignable/manufacturer data without proof
23.10.2003 (2)

Memo : Begin of thermal decomposition

Result : Begin of thermal decomposition: 210°C
Dangerous decomposition products: Hydrogen chloride, carbon monoxide, carbon dioxide, nitrogen oxides, and other toxic gases in case of fire or thermal decomposition

Reliability : (4) not assignable
Company data without proof
23.10.2003 (1)

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	500000 molecule/cm³
Rate constant	:	.0000000000000501 cm³/(molecule*sec)
Degradation	:	50 % after 321 day(s)
Deg. product	:	
Method	:	other (calculated): with SRC-AOPWIN v. 1.90 (2000)
Year	:	2003
GLP	:	
Test substance	:	
Remark	:	The calculated half-life is based on a mean OH radical concentration of 0.5E+6 OH radicals/cm3 as 24h average.
Reliability	:	(2) valid with restrictions Accepted calculation method
Flag	:	Critical study for SIDS endpoint

02.12.2003 (42)

Type	:	air
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	500000 molecule/cm³
Rate constant	:	.000000000000051 cm³/(molecule*sec)
Degradation	:	= 50 % after 315 day(s)
Deg. product	:	
Method	:	other (calculated): Atkinson (1988): Environ. Toxicol. Chem. 7, 435 - 442
Year	:	1988
GLP	:	no
Test substance	:	
Reliability	:	(4) not assignable Secondary literature

06.07.2003 (8)

3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH4	:	at °C
t1/2 pH7	:	at °C
t1/2 pH9	:	at °C
Result	:	With regard to its chemical structure 1,2-dichloro-4-nitrobenzene is not expected to hydrolyse under environmental conditions
Reliability	:	(2) valid with restrictions Prediction in accordance to scientific principles
Flag	:	Critical study for SIDS endpoint

02.12.2003 (43)

Type	:	abiotic

t1/2 pH4	:	at °C
t1/2 pH7	:	at °C
t1/2 pH9	:	at °C
Deg. product	:	
Method	:	other
Year	:	1988
GLP	:	no data
Test substance	:	no data
Remark	:	In the frame of a 21 d-reproduction test with Daphnia magna the stability of the TS was checked by chemical analysis at appointed concentrations (0.1 - 0.012 mg/l) in freshly prepared test solution and in 2 d old test solution.
Result	:	TS proved to be stable in aqueous solution during 2 days
Reliability	:	(2) valid with restrictions Study meets generally accepted scientific prinicples

10.07.2003 (44)

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

Type of measurement	:	background concentration
Media	:	surface water
Concentration	:	< .5 µg/l
Method	:	
Result	:	Throughout Germany a comprehensive monitoring program on several chemicals in surface waters has been realised to check whether the limit values are not exceeded. The following concentrations of 1,2-dichloro-4-nitrobenzene have been measured in the year 1999 (UBA): River Measuring station (Type of Value) Danube Ulm (90 % percentile) < 0.02 µg/l Elbe Schnackenburg (maximum) < 0.02 µg/l Rhine Kleve-Bimmen (90 % percentile) < 0.5 µg/l For 1,2-dichloro-4-nitrobenzene the limit values in surface waters have been set at 20 µg/l to protect aquatic life and at 1 µg/l to protect drinking water. These values have not been exceeded in the years 1996 - 2001 (Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit 2001; update by Umweltbundesamt 2003).
Reliability	:	(2) valid with restrictions Measuring program with longstanding experience
Flag	:	Critical study for SIDS endpoint

07.07.2003 (45) (46)

Type of measurement	:	background concentration
Media	:	surface water
Concentration	:	0 - .0061 µg/l
Method	:	HPLC/GC-MS
Method	:	The water of the river Elbe was examined at Stade (Germany) in 1995. The water of the North Sea was examined at 6 sites in the German Bight in 1990 and 1995. Some of these sites were inside and some of them outside the plume of the Elbe river.
Result	:	1,2-Dichloro-4-nitrobenzene was detected (6.1 ng/l) in the water of the river Elbe at Stade in 1995. In 1990, 1,2-dichloro-4-nitrobenzene was found at 5 sites (0.082 - 0.27 ng/l, one site was below the limit of detection [0.05 ng/l]) in the German Bight. In

1995, the concentrations of 1,2-dichloro-4-nitrobenzene had decreased to levels below the limit of detection

Reliability : (2) valid with restrictions
Basic data given

Flag : Critical study for SIDS endpoint
10.07.2003 (47)

Type of measurement : other: concentration in Dreissena polymorpha and Anguilla anguilla
Media : biota
Concentration :
Method : GC

Result : Levels of 1,2-dichloro-4-nitrobenzene are reported from the zebra mussel (Dreissena polymorpha), and from the eel (Anguilla anguilla) from waters in the Netherlands in 1994. Zebra mussels sampled in the Rhine (sampling site Lobith), Meuse (Eijsden), and the Ysselmeer, contained 1,2-dichloro-4-nitrobenzene at levels of up to 0.36 µg/kg wet weight. Eels sampled in the Rhine, Meuse, and Hollands Diep location in the Rhine-Meuse delta contained 1,2-dichloro-4-nitrobenzene at levels of up to 1.2 µg/kg wet weight.

Reliability : (2) valid with restrictions
Basic data given

Flag : Critical study for SIDS endpoint
07.07.2003 (48)

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

3.3.2 DISTRIBUTION

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

Remark : Data used in the calculation:
Temperature (°C): 25
Molar Mass (g/mol): 192
Vapor pressure (Pa): 2
Water Solubility (g/m3): 121
log Pow: 3.04

Air: 6*10^9 m³
water: 7*10^6 m³
soil: 4.5*10^4 m³ 1500 kg/m³ 2 % org. C
sediment: 2.1*10^4 m³ 1300 kg/m³ 5 % org. C
suspended sediment: 35 m³ 1500 kg/m³ 16.7 % org. C.
aerosols: 0.12 m³ 1500 kg/m³
aquatic biota: 7 m³ 1000 kg{m³ 5 % fat

Result : Calculated distribution between environmental compartments:
air 48.3 %
water 44.0 %
soil 3.8 %
sediment 3.9 %
susp. sediment < 0.1 %
biota (fish) < 0.1 %

Reliability : (2) valid with restrictions
Accepted calculation method
Flag : Critical study for SIDS endpoint
07.08.2003 (42)

Media : water - air
Method : other (measurement): thermodynamic method
Year : 1999

Method : Thermodynamic column method of Brunner et al. 1990 applied [Brunner S, Hornung E, Santl H, Wolff E, Pringer OG, Altschuh J, Brueggemann R (1990) Henry´s law constants for polychlorinated biphenyls: Experimental determination and structure-property relationship. Environ Sci Technol 24, 1751 - 1754]:
- Aqueous solution of the TS produced in a generator column
- Solution is passed through gas liquid desorption column where it contacts a gas stream and the partition equilibrium is reached
- Gas and water are separated: water flows to the receiver dosing funnel, the gas is conducted into an absorption vessel where the TS is absorbed in organic solvent

Result : Measured dimensionless Henry's Law Constant (H):
log H = - 3.48 at 25 °C
H = 0.00033 (dimensionless)
H = 0.00033 x 8,314 Pa m3/mol K x 298 K = 0.818 Pa m3 mol-1 at 25°C
Bond method: calculated log H = - 3.32
H = 0.00048 (dimensionless) = 1.19 Pa m3 mol-1 at 25°C
Group method: calculated log H = - 2.88
H = 0.0013 (dimensionless) = 3.27 Pa m3 mol-1 at 25°C
The authors argue that the results of the Group method are less reliable than these of the Bond method

Test condition : - Temperature 25 °C
- Gas phase: Nitrogen
- Liquid phase: Demineralized, distilled water
- Analysis: GC/ECD

Reliability : (2) valid with restrictions
Basic data given
Flag : Critical study for SIDS endpoint
07.08.2003 (49)

Media : water - soil
Method : other (measurement): shake flask method
Year : 1981

Remark : The work of Briggs (1981) is cited by Hong et al. (1996), who converted Kom to Koc by multiplying Kom value by 1.724. Sabljic et al. (1995) report that they obtained their Koc of data compilations of Gerstl (1990) and Meylan et al. (1992). It is assumed that the data of Briggs (1981) were cited.

Result : log Kom = 2.29 (soil organic matter - water distribution, Kom = 195) equals log Koc = 2.53 (Koc = 339, see Hong et al. 1996)

Test condition : Soil
- Air dried and ground to pass 2-mm sieve
- Organic carbon was determined by the Walkley-Black method
Soil adsorption:
- 1 g of soil shaken for 2 h with 10 ml of test substance in 0.01 M CaCl2 in 40-ml glass centrifuge tubes with glass stoppers
- Temperature 20 +/- 2 °C
- 4 TS concentrations used: 20, 15, 10, and 5 ppm
- After centrifugation for 10 min, the chemical remaining in solution is measured
- Test substance determined at an appropriate wavelength by UV

absorption against a soil blank

Soil Characteristics

No.	Soil series	Texture	Soil history
1	Batcombe	silt loam	100 year arable
2	Batcombe	silt loam	100 year arable
3	Batcombe	silt loam	old grass, then arable
4	Batcombe	silt loam	old grass, then arable

No.	Organic matter (%)	pH (CaCl2)
1	1.09	7.5
2	2.51	6.7
3	3.53	6.1
4	4.25	6.2

Test substance : Purity was checked by TLC and NMR
Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles
Flag : Critical study for SIDS endpoint
10.07.2003 (18)

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

Result : Hong et al. (1996) convert the Kom of Briggs (1981) to Koc by multiplying by 1.724. The result is log Koc = 2.53, Koc = 339 (experimental, Briggs 1981). They compare this value with equations derived from experimentally determined HPLC capacity factors which yield the following results:
log Koc (calculated) 2.55 - 2.71 (Koc = 355 - 513)
Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles, acceptable calculation method
Flag : Critical study for SIDS endpoint
06.07.2003 (34)

Media : water - sediment
Method : OECD Guide-line 106
Year : 2001

Method : The sorption coefficient for the sediment was determined using the shake-flask method according to the OECD Guidelines for Testing of Chemicals (1987)
Remark : In other parts study has significant deficiencies
Result : log Koc = 2.62; Koc = 417
The koc was calculated as a function of the organic carbon content of the sediment
Test condition : The sediment of the Yangtse River was air dried, ground to pass an 80 mesh sieve and sampled. The contents of its sand, silt clay and organic carbon were determined and the pH-value was 7.44.
The experiments were conducted in triplicate, temperature 25 °C +/- 0.5 °C.
The equilibrium concentration in the aqueous phase was measured by a UV/Vis spectrophotometer against water blank
Test substance : Purity was more than 99 %
Reliability : (2) valid with restrictions
Guideline study, basic data given
Flag : Critical study for SIDS endpoint
06.07.2003 (27)

Media : water - air
Method : other (calculation): SRC-HENRYWIN v3.1, 2000

Year : 2003

Method : Calculated according to Bond Method
Result : Henry's law constant = 1.19 Pa m3 mol-1 (25 °C)
Reliability : (2) valid with restrictions
Accepted calculation method
23.10.2003 (42)

Media : water - soil
Method :
Year : 1995

Remark : Sabljic et al. (1995) report that they obtained their Koc of data compilations of Gerstl (1990) and Meylan et al. (1992)
Result : log Koc = 2.53; Koc = 339
Reliability : (4) not assignable
Secondary literature
06.07.2003 (25)

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type : aerobic
Inoculum : activated sludge, non-adapted
Concentration : 90 mg/l related to Test substance
related to
Contact time :
Degradation : < 10 (±) % after 21 day(s)
Result : under test conditions no biodegradation observed
Kinetic of testsubst. : 5 day(s) < 10 %
10 day(s) < 10 %
15 day(s) < 10 %
%
%
Deg. product : not measured
Method : other: respirometry test
Year : 1982
GLP : no
Test substance : no data

Remark : At a concentration of 45 mg/l TS the bacteria were inhibited, at 30 mg/l no inhibitory effects were observed.
Result : DOC = 35 mg/l saturated solution
COD = 75 mg O2/l saturated solution
BSB(5) = <10 mg O2/l saturated solution
TS concentration = ca. 90 mg/l
Test condition : The concentration of the test substance (ca. 90 mg/l) was obtained by direct weighing.
Reliability : (4) not assignable
Only short summary available
23.10.2003 (50)

Type : aerobic
Inoculum : activated sludge
Concentration : 100 mg/l related to Test substance
related to
Contact time :

Degradation	:	= 0 (±) % after 28 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	:	no data
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).
Test condition	:	Sludge concentration 30 mg/l, test substance concentration 100 mg/l
Reliability	:	(2) valid with restrictions Test procedure according to national standards. In general, good reliability but for 1,2-dichloro-4-nitrobenzene, it cannot be excluded that inhibitory test concentration was used
Flag	:	Critical study for SIDS endpoint
06.07.2003		(15)
Type	:	aerobic
Inoculum	:	activated sludge, industrial, adapted
Concentration	:	.5 mg/l related to Test substance related to
Contact time	:	
Degradation	:	100 (±) % after 3 day(s)
Result	:	other: biodegradable
Deg. product	:	yes
Method	:	other: Simulation of an industrial waste water treatment plant
Year	:	1983
GLP	:	no
Test substance	:	no data
Deg. products	:	95-76-1 202-448-4 3,4-dichloroaniline
Remark	:	Two tests were performed to observe biodegradation of the TS under the conditions of industrial waste water plants in a laboratory scale. The concentrations of the TS in the inffluent and effluent samples were determinated with both HPLC and GC. 1. Conditions in the first test: - Industrial wastewater from the Bayer AG, Leverkusen (Germany). Composition of the wastewater sample was not determined. Mean residence time was 12 h in the laboratory wastewater plant - Different concentrations of the TS were added to this wastewater during 8 days (d 1 - 3: 0.5 mg/l; d 4 - 5: 5 mg/l; d 6: 8 mg/l; d 9: 4 mg/l) 2. Conditions in the second test: - Synthetic wastewater, containing aniline (10 mg/l) and TS (0.5 mg/l). At day 4, the aniline concentration was increased to 100 mg/l - Compostion of the water sample was determined. The mean residence time was 3 h.
Result	:	Results of the first test - TS effluent concentration (d 1 - 3: 0.0 mg/l; d 4: 0.6 mg/l; d 5 - 7: <= 0.2 mg/l; d 8 - 9: <= 0.9 mg/l) - Intermediary degradation product: 3,4-Dichloroaniline (d 1 - 3: 0.0 mg/l; d 4: 0.1 mg/l; d 5 - 7: 0.0 mg/l; d 8: 0.4 mg/l, d 9: 0.2 mg/l) - elimination >= 90 % Results of second test - Due to the shock loading with aniline, elimination of aniline decreases shortly from > 95 % and is reestablished after about 1 day

- After the shock loading with aniline, TS concentration in the effluent increases from 0.01 mg/l to <= 0.2 mg/l
Conclusions
- Shock loadings of aniline can interfere with the degradation of the TS.
- Degradation of the TS takes place in the wastewater treatment plant of Bayer AG in Leverkusen, Germany, to the limit of detection

Reliability : (2) valid with restrictions
Study well documented and meets generally accepted scientific prinicples

Flag : Critical study for SIDS endpoint
06.07.2003 (51)

Type : aerobic
Inoculum : other: Industrial activated sludge or mixed starting culture with the ability to degrade various aromatic halogenes
Concentration : 25 mg/l related to Test substance
related to
Contact time :
Degradation : 100 (±) % after 15 day(s)
Result : other: biodegradable
Kinetic of testsubst. : 8 day(s) 50 %
15 day(s) 100 %
%
%
%
Deg. product : not measured
Method : other: sealed shake flask (see Test conditions)
Year : 1991
GLP : no data
Test substance : no data

Result : Concentrations of the organic compounds in the sealed shake flask were followed from the outset. After 8 days the 1,2-dichloro-4-nitrobenzene had decreased by 50 %. After 15 days 1,2-dichloro-4-nitrobenzene had totally disappeared with a corresponding increase in chloride
Test condition : Degradation of 3,4-Dichloroaniline was investigated in a packed-bed reactor, with both synthetical and industrial wastewater. 1,2-Dichloro-4-nitrobenzene was also contained in the industrial wastewater sample. Therefore the degradation of TS was investigated, too. Shake flasks containing minimal salts medium and the TS as single source of carbon and energy were inoculated with 5 ml of overflow from the packed-bed-reactor and incubated at 30°C.
Reliability : (2) valid with restrictions
Study well documented and meets generally accepted scientific principles.
Flag : Critical study for SIDS endpoint
06.07.2003 (52)

Type : aerobic
Inoculum : other: Mixture of bacteria from different sources (adapted)
Contact time : 4 month
Degradation : > 70 (±) % after 2 day(s)
Result : other: biodegradable
Deg. product : not measured
Method : other: see below
Year : 1982
GLP : no
Test substance : no data

Test condition : - Inoculum: Microorganisms from sewage sludge of industrial wastewater treatment plants and from soil cultures and also other species like Pseudomonas, Acinetobacter and Arthrobacter.
- During a 4 months incubation time, the inocula were adapted to the

substrat (benzene derivates) as the single carbon-source. The concentration was increased from 20 mg/l up to 100 mg/l.
- The adaption of the bacteria was continued with wastewater containing ion exchange resin. Every other day the wastewater was changed but the resin was kept.
- After some months the bacteria could degrade 1,2-dichloro-4-nitrobenzene > 70 % in wastewater after 2 days.

Reliability : (2) valid with restrictions
Report well documented and meets generally accepted scientific principles.
Flag : Critical study for SIDS endpoint
10.07.2003 (53)

Type : aerobic
Inoculum : other bacteria: Pseudomonas strain grown on 1,2-dichlorobenzene
Contact time :
Degradation : ca. 50 (±) % after 3 day(s)
Result : other: biodegradable
Deg. product :
Method : other
Year : 1988
GLP : no
Test substance : no data

Remark : The authors isolated bacteria strains from industrial waste water treatment plants of the genus Pseudomonas and Acinetobacter and demonstrated, that these microorganisms were able to use various halogenated aromatics as single carbon source.

Organically bound chlorine was released as inorganic chloride by oxidative dehalogenation.

Result : The Pseudomonas strain 1,2/6 (grown on 1,2-dichlorobenzene) was able to release chloride from 3,4-dichloronitrobenzene at a rate of 0.1 mmol/l x h x g (For 3,4-dichloroaniline the rate was 0.5 mmol/l x h x g). No metabolite could be detected. The degradation was dependent on oxygen. It was catalysed by two dioxygenases which were plasmid coded.

Reliability : (2) valid with restrictions
Study meets generally acceptable scientific priniciples
Flag : Critical study for SIDS endpoint
10.07.2003 (54)

Type : aerobic
Inoculum : other fungi: Mucor javanicus
Concentration : 50 mg/l related to Test substance
related to
Contact time :
Degradation : 36 (±) % after 6 day(s)
Result : other: about 31 % of initial substance was transformed to 3,4-dichloroaniline, another 36 % was further degraded
Deg. product : yes
Method : other
Year : 1984
GLP : no
Test substance : other TS: no purity reported (purchased from Tokyo Chem. Ind. Co.)
Deg. products : 95-76-1 202-448-4 3,4-dichloroaniline

Result : After 6 days 31% of the initial test substance 3,4-dichloronitrobenzene was reduced to 3,4-dichloroaniline, 33 % remained unchanged, rest was degraded.
A 55 % inhibition of the fungus growth was observed at 50 mg/l.
Test condition : The inocolum (fungus) was precultivated at 25°C for 72h. Then 50 ppm of the test substance were added to the culture and cultivation was continued

for 6 more days.
The metabolites were identified by GLC and GC-MS.

Reliability : (2) valid with restrictions
Study meets generally acceptable scientific priniciples

Flag : Critical study for SIDS endpoint

07.07.2003 (55)

Type : aerobic
Inoculum : other: various cultures of microorganisms
Deg. product : yes
Method : other: degradation in adapted batch cultures
Year : 1999
GLP : no data
Test substance : no data
Deg. products : 95-76-1 202-448-4 3,4-dichloroaniline

Remark : Study is a compilation of the results of the doctoral thesis of the author
Result : It is stated that in a screening test no viable and degrading cultures could be isolated with chloronitrobenzenes as sole source of C and N. Under cometabolic conditions (additional C- and N-source present) cultures were able to degrade chloronitrobenzenes. The first step in degrading chloronitrobenzenes was the reduction to the corresponding chloroanilines.

Reliability : (4) not assignable
Secondary literature

07.07.2003 (56)

Type : aerobic
Inoculum : other: activated sludge, adapted and non-adapted
Contact time :
Degradation : 0 (±) % after 20 day(s)
Result : under test conditions no biodegradation observed
Deg. product : not measured
Method :
Year : 1982
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : Emulsifier was not effective in dissolving 1,2-dichloro-4-nitrobenzene at 1000 mg/l (which equals 958 mg/l COD). The solution contained only 28 % of the theoretical COD (= 270 mg/l).
The solution had a pH of 4.7; it is not indicated whether the solution was neutralized.

Test condition : - Concentrations of 1-chloro-4-nitrobenzene: 2.4, 8, 24, 80 mg/l, prepared from stock solution (1 g/l, see Remark)
- Incubation time was 0, 5, 10, 20 days

Test substance : Emulsifier W (CAS 68130-72-3), 2 g/l, was used to emulsify the 1,2-dichloro-4-nitrobenzene stock solution.

Reliability : (3) invalid
Documentation insufficient. Apparently problems with emulsifying 1,2-dichloro-4-nitrobenzene

10.07.2003 (57)

Type : aerobic
Inoculum : other: activated sludge, adapted and non-adapted
Contact time :
Degradation : 0 (±) % after 20 day(s)
Result : under test conditions no biodegradation observed
Deg. product : not measured
Method :
Year : 1984
GLP : no

Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Although it was shown in 1982 (Bayer 1982), that 2 g/l emulsifier were not effective in dissolving 1,2-dichloro-4-nitrobenzene at 1000 mg/l (which equals 958 mg/l COD), this part of the experiment was repeated with 1 g/l of emulsifier. The solution contained only 26 % of the theoretical COD (= 253 mg/l).
Test condition	:	- Concentrations of 1-chloro-4-nitrobenzene: 2.4, 8, 24, 80 mg/l, prepared from stock solution (1 g/l) - Incubation time was 0, 5, 10, 20 days - Recovery of COD was only 26 % in stock solution
Test substance	:	Emulsifier W (CAS 68130-72-3), 1 g/l, was used to emulsify the 1,2-dichloro-4-nitrobenzene stock solution
Reliability	:	(3) invalid Documentation insufficient. Apparently problems with emulsifying 1,2-dichloro-4-nitrobenzene

10.07.2003 (58)

Type	:	aerobic
Inoculum	:	other: unknown sewage
Contact time	:	20 day(s)
Degradation	:	0 (±) % after 20 day(s)
Result	:	under test conditions no biodegradation observed
Deg. product	:	
Method	:	other: see Method
Year	:	1975
GLP	:	no
Test substance	:	no data
Method	:	- Standard Methods for the Examination of Water and Wastewater, 13th ed, 1971, American Public Health Assn NY 10019 (method used not indicated) - Unknown sewage: filtered 437 Secondary Effluent from the Midland plant of the Michigan Division general wastewater treatment system - Incubation periods 5, 10, 20 d - pH 6.5
Remark	:	Cover page of the study is from 1975, last update is from 1992
Reliability	:	(3) invalid Documentation insufficient

10.07.2003 (59)

Type	:	anaerobic
Inoculum	:	other: river sediment
Contact time	:	
Degradation	:	ca. 50 - 100 (±) % after 1 day(s)
Result	:	other: under denitrifying and methanogenic conditions 1,2-dichloro-4-nitrobenzene was removed from the medium
Deg. product	:	not measured
Method	:	other: Laboratory column experiment with river sediment
Year	:	1990
GLP	:	no
Test substance	:	no data
Method	:	according to - Van Der Meer JR, Roelofsen W, Schraa G, Zehnder AJB (1987) Degradation of low concentraion of dichlorobenzene and 1,2,4-trichlorobenzene by Pseudomonas sp. Strain P51 in non-sterile soil columns. FEMS Microbiol Ecol 45: 333 - 341 - Bosma TNP, Van Der Meer JR, Schraa G, Tros ME, Zehnder AJB (1988) Reductive dechlorination of all trichloro- and dichlorobenzenes isomers.

FEMS Microbiol Ecol 53: 223 -229

Remark : Even the authors assume a partial removal under denitrifying conditions they failed to investigate the nature of this transformation.
Under different redox conditions microbial transformation of chlorinated organics has been investigated in columns, packed with sediment from the Rhine river near Wageningen (The Netherlands) and from the dune infiltration site (for drinking water production from Rhine water) of the Municipal Water Works of Amsterdam near Zandvoort. Chlorinated organics were partially removed under denitrifying conditions. The nature of the transformation was not examined.

Result : 1,2-Dichloro-4-nitrobenzene was completely removed under anaerobic conditions with a low redox-potential (sulfate or carbondioxide present as electron acceptor) and partially degraded (50 %) in the columns with nitrate present as electron acceptor.

Test condition : Small columns (25 cm length, 5.5 cm i.d.) constructed of hard PVC were wet packed with sediment from the river Rhine near Wageningen or from the dune infiltration site of the municipal water works of Amsterdam near Zandervoort. The columns with the sediment from the river Rhine were percolated continuously at a flow rate of approx. 1 cm/h in an upflow mode and a temperature at 20 °C with a mineral medium prepared with highly purified milli-Q water (Millipore, USA) closely resembling the mineral composition of river Rhine water. The columns with sediment from the dune infiltration site were percolated with the same water that is also used for dune infiltration. Columns operated continuously for 1 - > 2 years.

Initially, three environments were created in dune and river Rhine sediment. To individual columns molecular oxygen, nitrate or sulfate were added to create aerobic, denitrifying or sulfate-reducing conditions, with a final concentration of 100 mg/l for Na2S and sulfate, and 35 mg/l for nitrate.

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

Flag : Critical study for SIDS endpoint

10.07.2003 (60)

Type : anaerobic
Inoculum : other: river sediment
Contact time :
Degradation : 50 - 99 (±) % after 1 day(s)
Result : other: under denitrifying and methanogenic conditions 1,2-dichloro-4-nitrobenzene was rapidly removed from the medium (50 % / > 99 %)
Deg. product : not measured
Method : other: Laboratory column experiment with river sediment
Year : 1996
GLP : no data
Test substance : other TS:analytical grade (purchased from E. Merck, Darmstadt, Germany)

Method : Biotransformation of organics in soil columns was examined under laboratory conditions in sediment from the Rhine river near Wageningen (The Netherlands) and the dune infiltration site (for drinking water production from Rhine water) of the Municipal Water Works of Amsterdam near Zandvoort.
The column experiments were conducted in two different laboratories having there own setup; Small columns were used at the Department of Microbiology in Wageningen while large columns were used at the Municipal Water Works of Amsterdam in Heemstede.

Remark : The authors pretend that 1,2-dichloro-4-nitrobenzene was completely removed without a lag-phase in all methanogenic columns, even if there is no evidence for a missing lag phase. They come to the conclusion that the reaction might be purely chemical. On the other side the authors made no attempt to prove the nature of the reaction.

The dispersion in the column experiments with sediments obtained from the Rhine and Dune infiltration area was small.

Result : 1,2-Dichloro-4-nitrobenzene was completely removed (> 99 %) by methanogenic columns without a lag-phase in the methanogenic columns packed with Rhine and Dune Sediment. It was also partially degraded (50 %) in the denitrifying column packed with Rhine Sediment.

Test condition :
- Temperature 20 °C
- Small columns of hard PVC (25 cm length, 5.5 cm i.d.), wet packed with sediment from the Rhine river or the dune infiltration site of the municipal water works of Amsterdam
- Continuously percolating at a flow rate of 1 cm/h in an upflow mode, with a mineral medium prepared with highly purified milli-Q water (Millipore, USA) closely resembling the mineral composition of Rhine water
- Synthetic medium continuously aerated in the presence of an excess of granulated marble which served as carbonate buffer in combination with CO2 in the air
- Depletion of the oxygen in the originally aerated medium by its continuous replacement with nitrogen gas amended with 0.5 % CO2 in a gas chamber
- Reducing conditions maintained by the addition of Na2S (10 mg/l final concentration)
- Methane in the column effluent verified by measurements on a Packard GC417 gas chromatograph
- Final concentration of test substance in influent 10 - 20 µg/l
- Final concentration of sodium nitrate 47 mg/l

- Large columns constructed of glass (60 cm length, 11 cm i.d.), wet-packed with sediment from the dune infiltration site of the municipal water works of Amsterdam, kept at a constant temperature with water mantle coupled to water bath
- Continuously percolating at a flow rate of 2.5 cm/h
- Water for the experiments, sampled from the water that was infiltrated in the dune area (pH-value of 7.7) without any further treatment
- Influent medium was kept under nitrogen pressure and amended with ethanol (48 mg/l)
- Final concentration of test substance in influent 0.5 - 20 µg/l
- Final concentration of nitrate 48 mg/l

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

Flag : Critical study for SIDS endpoint
06.07.2003 (61)

Type : anaerobic
Inoculum : Escherichia coli (Bacteria)
Concentration : 50 mg/l related to Test substance
related to
Contact time :
Degradation : > 99.9 (±) % after 5.3 day(s)
Result : other: biodegradable
Deg. product : yes
Method : other: see below
Year : 1983
GLP : no
Test substance : other TS: 100% Purity
Deg. products : 1,3-bis(dichlorophenyl)triazene
2,3,3´,4´-tetrachlorobiphenyl
3,3´,4,4´-tetrachloroazobenzene
3,3´,4,4´-tetrachlorobiphenyl
21232-47-3 3,3´,4,4´-tetrachloroazoxybenzene
86374-33-6 1-dichlorophenylazo-2-naphthol

Remark	:	E. coli was chosen as the test organism because E. coli is unusual in regard to its ability to reduce nitrate to nitrite and its inefficiency to reduce nitrite. Thus, E. coli should yield high amounts of azo byproducts
Result	:	Recovery of 1,2-dichloro-4-nitrobenzene after ca. 5.25 days was <0.1% (of the initial 1,2-dichloro-4-nitrobenzene concentration) in all three experiments. The following metabolites were detected in traces: - Incubation with 1,2-dichloro-4-nitrobenzene alone: 3,3',4,4'-Tetrachloroazobenzene (0.06 %) and 3,3',4,4'-tetrachloroazoxybenzene (1 %). - In presence of 100 ppm NaNO3: Tetrachlorobiphenyls (0.05 %), 1,3-bis(dichlorophenyl)triazene (1.2 %), 3,3',4,4'-tetrachloroazobenzene (0.04 %), and 3,3',4,4'-tetrachloroazoxybenzene (2.2 %). - In the presence of NaNO3 and 100 ppm 2-naphthol: 1,3-bis(dichlorophenyl)triazene (0.6 %), 1-dichlorophenylazo-2-naphthol (5.2 %), 3,3',4,4'-tetrachloroazobenzene (0.02 %), and 3,3',4,4'-tetrachloroazoxybenzene (0.7 %).
Test condition	:	All experiments were perfomed in duplicate. Incubation time: 5.25 d (6 h shaking, 5 d undisturbed) Test vessel: Erlenmeyer flask Temperature: 37 °C
Test substance	:	The test was conducted with three different preparations of the test substance. 1,2-Dichloro-4-nitrobenzene (3,4-DCNB) was always present (ca. 50 mg/l): - 3,4-DCNB alone - 3,4-DCNB plus 100 mg/l of NaNO3 - 3,4-DCNB plus 100 mg/l of NaNO3 + 100 mg/l of 2-naphthol
Reliability	:	(2) valid with restrictions Study meets generally accepted scientific principles

10.07.2003 (62)

Type	:	anaerobic
Inoculum	:	other: black surface sediment
Contact time	:	
Degradation	:	50 (±) % after 2.4 day(s)
Result	:	other: Biotransformation and biodegradation
Deg. product	:	yes
Method	:	other: Laboratory column experiment with river sediment
Year	:	1996
GLP	:	no data
Test substance	:	other TS: from Tokyo Kasei Kogyo Co. Ltd.
Deg. products	:	108-42-9 203-581-0 3-chloroaniline 95-76-1 202-448-4 3,4-dichloroaniline
Method	:	Transformation and dehalogenation was examined in an estuarine sediment from the Tsurumi river (Japan)
Result	:	The loss of the compound in sediment was observed without any lag period. The reaction followed a first-order reaction mechanism with a rate constant 0.289 +/- 0.015 per day. Half live was 2.4 days. In autoclaved sediment (control experiment) the rate constant was 0.0012 per day. Up to half of the 1,2-dichloro-4-nitrobenzene was recovered as 3,4-dichloroaniline, which was degraded less rapidly than the TS
Test condition	:	The surface sediment sample was collected by an Ekman-Barge sediment sampler. The sediment was black in colour indicating sulfate reducing activity. Macrobenthic organisms were present in the collected sediment. The sediment was sieved through a 2 mm screen and stored at 4 °C. The sieved sediment sample was mixed with water, collected just above the sampling point, just to make a sediment slurry with solids concentration of

272 +/- 2.8 g/kg for four replicate measurements (pH 5.6).

For each replicate, 5 ml of sediment slurry was placed in a screw top test tube and sealed under N2 atmosphere. Test tubes were sealed and kept under room temperature for one week, to ensure that the system was anaerobic.

The test was performed under the following conditions:
Test tubes were placed in an anaerobic chamber (10 % H2, 10 % CO2, 80 % N2). The initial concentration was 4 µmol/l.
After spiking at time zero samples were frozen at -20 °C, while other samples were kept at 25 °C. Test tubes were hand mixed three times a week. The test tubes were sampled once a day at the beginning of the incubation then at longer time intervals as the experiment progressed. The incubation lasted for a year. The experiments were carried out in two sets. Test tubes were stored in a freezer at -20 °C for further analysis with GC/MS.

Test substance : Stock solution 2 mmol in methanol
Reliability : (2) valid with restrictions
Study according to generally accepted scientific principles. Basic data given.

30.07.2003 (63)

3.6 BOD5, COD OR BOD5/COD RATIO

COD
Method :
Year : 1984
COD : 253 mg/g substance
GLP : no

Result : ThOD was calculated: 958 mg/g
Thus the ratio between COD and ThOD is 26%
Reliability : (3) invalid
Documentation insufficient, details missing
Flag : Critical study for SIDS endpoint
06.07.2003 (58)

COD
Method :
Year : 1982
COD : 270 mg/g substance
GLP : no

Result : ThOD was calculated: 958 mg/g
Thus the ration between COD and ThOD is 28%
Reliability : (3) invalid
Documentation insufficient, details missing
06.07.2003 (57)

BOD5
Method : other: see below
Year : 1975
Concentration : related to
BOD5 : 0 mg/l
GLP : no
COD
Method : other: see below
Year : 1975

COD : 410 mg/g substance
GLP : no
RATIO BOD5 / COD
BOD5/COD : 0

Method : Standard BOD Method from the Standard Methodes for the Examination of Water and Wastewater 13th Edition (1971), American Public Health Assn., NY 10019
Result : BOD5 = 0 mg/g
BOD10 = 0 mg/g
BOD20 = 0 mg/g
ThOD = 1080 mg/g
COD = 410 mg/g
Reliability : (3) invalid
Documentation insufficient, details about the conduction of the test are not reported (e.g. initial concentration of the test substance)
10.07.2003 (59)

BOD5
Method :
Year : 1996
Concentration : related to
BOD5 : mg/l
GLP : no data

Result : Final BOD = 0.0
Reliability : (4) not assignable
Secondary literature. The biodegradation data were determined by MITI (Japan) and was supplied by the U.S.EPA
06.07.2003 (64)

3.7 BIOACCUMULATION

Species : Cyprinus carpio (Fish, fresh water)
Exposure period : 56 day(s) at 25 °C
Concentration : 50 µg/l
BCF : 26 - 59
Elimination : no data
Method : OECD Guide-line 305 C "Bioaccumulation: Test for the Degree of Bioconcentration in Fish"
Year : 1992
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Test procedure in accordance with national standard methods with acceptable restrictions
Flag : Critical study for SIDS endpoint
02.12.2003 (15)

Species : Cyprinus carpio (Fish, fresh water)
Exposure period : 56 day(s) at 25 °C
Concentration : 5 µg/l
BCF : 37 - 65
Elimination : no data
Method : OECD Guide-line 305 C "Bioaccumulation: Test for the Degree of Bioconcentration in Fish"
Year : 1992
GLP : no data

Test substance	:	no data
Reliability	:	(2) valid with restrictions Test procedure in accordance with national standard methods with acceptable restrictions
Flag	:	Critical study for SIDS endpoint
02.12.2003		(15)
Species	:	Salmo gairdneri (Fish, estuary, fresh water)
Exposure period	:	36 day(s) at 15 °C
Concentration	:	.73 µg/l
BCF	:	117
Elimination	:	
Method	:	other: see Test conditions
Year	:	1989
GLP	:	no data
Test substance	:	other TS: no purity was given
Remark	:	Accepted new scientific name for Salmo gairdneri (Rainbow trout): Oncorhynchus mykiss
Result	:	The BCF were determined for 5 incubation periods: 104 +/- 19 for 5 days, 111 +/- 10 for 12 days, 113 +/- 14 for 20 days, 128 +/- 25 for 28 days, and 130 +/- 37 for 36 days. The mean BCF was 117 +/- 24 without significant differences (p </= 0.05) among the sample intervalls. Since the higher chlorinated nitrobenzenes are possibly dechlorinated by metabolism in fish, a BCF for 1,2-dichloro-4-nitrobenzene cannot be derived from this test design.
Test condition	:	30 fish exposed to 730 +/- 130 ng TS/l plus several other chloronitrobenzenes in a flow-through system; acetone used as solvent; samples of 6 fish each analysed at 5, 12, 20, 28 and 36 days of exposure; duplicate water samples taken every 3 or 4 days; GC analysis
Reliability	:	(3) invalid Unsuitable test system (more than one substance tested in the same vessel)
10.04.2003		(17)
Species	:	Salmo gairdneri (Fish, estuary, fresh water)
Exposure period	:	36 day(s) at 15 °C
Concentration	:	.73 µg/l
BCF	:	117
Elimination	:	
Method	:	
Year	:	1989
GLP	:	no data
Test substance	:	
Remark	:	Accepted new scientific name for Salmo gairdneri (Rainbow trout): Oncorhynchus mykiss The authors cite the work of Niimi et al. (1989)
Reliability	:	(4) not assignable Secondary literature
10.04.2003		(32) (22) (65)

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	other: not specified
Species	:	Leuciscus idus (Fish, fresh water)
Exposure period	:	48 hour(s)
Unit	:	mg/l
LC0	:	2.9
LC50	:	3.1
LC100	:	3.3
Limit test	:	
Analytical monitoring	:	no data
Method	:	other: DIN-Standard 38412 L15 (Fish short-time test)
Year	:	1983
GLP	:	no data
Test substance	:	other TS: no purity given

Method	:	Method of the German Standards Institution, Berlin, Germany
Reliability	:	(2) valid with restrictions Test procedure according to national standard method
Flag	:	Critical study for SIDS endpoint

23.10.2003 (66)

Type	:	static
Species	:	Leuciscus idus melanotus (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC0	:	= 4.5
LC50	:	= 5.2
LC100	:	= 5.6
EC0	:	= 4
Limit test	:	
Analytical monitoring	:	no data
Method	:	other: DIN 38412 L 15 (Fish, short-time test)
Year	:	1980
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Test condition	:	- Fish were obtained from commercial source and kept 20 d before start of experiment in dechlorinated water in test facility - Body weight 1.3 -2.6 g (mean 1.9) - Body length 4.9 -6.2 cm - Fed with Tetra Min (Tetra-Werke, Melle, Germany) - For experiments deionized tap water amended with 192 mg/l NaHCO3, 120 mg/l CaSO4 x 2 H2O, 120 mg/l MgSO4, and 8 mg/l KCl. The total hardiness was 9.5 °d and the carbonate hardiness 6.4 °d - pH (including fish) 7.0 - 8.4 - Aquaria 40 x 25 x 30 cm3 containing 20 l test medium at 20 +/- 1 °C, aeration 100 ml/min, oxygen > 7 mg/l - 12 h light (700 lux), 12 h dark - 65 h before start of test fish were put into aquaria - Test substance suspneded with Ultra turrax and aliquot grought into aquarium to give final concentration (including suspended test substance)
Reliability	:	(2) valid with restrictions Test procedure in accordance with national standard methods
Flag	:	Critical study for SIDS endpoint

23.10.2003 (67)

Type	:	static
Species	:	Oryzias latipes (Fish, fresh water)

Exposure period	:	48 hour(s)
Unit	:	mg/l
LC50	:	= 7.01
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: no purity given
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 - 48 h LC50 was estimated by Doudoroff method or Probit method
Reliability	:	(2) valid with restrictions Test procedure in accordance with national standard methods
Flag	:	Critical study for SIDS endpoint

23.10.2003 (15)

Type	:	static
Species	:	Leuciscus idus melanotus (Fish, fresh water)
Exposure period	:	48 hour(s)
Unit	:	mg/l
LC0	:	= 6.3
LC50	:	= 8
LC100	:	= 10
EC0	:	= 4
Limit test	:	
Analytical monitoring	:	no data
Method	:	other: DIN 38412 L 15 (Fish, short-time test)
Year	:	1980
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Test condition	:	- Fish were obtained from commercial source and kept 20 d before start of experiment in dechlorinated water in test facility - Body weight 1.3 -2.6 g (mean 1.9) - Body length 4.9 -6.2 cm - Fed with Tetra Min (Tetra-Werke, Melle, Germany) - For experiments deionized tap water amended with 192 mg/l NaHCO3, 120 mg/l CaSO4 x 2 H2O, 120 mg/l MgSO4, and 8 mg/l KCl. The total hardiness was 9.5 °d and the carbonate hardiness 6.4 °d - pH (including fish) 7.0 - 8.4 - Aquaria 40 x 25 x 30 cm3 containing 20 l test medium at 20 +/- 1 °C, aeration 100 ml/min, oxygen > 7 mg/l - 12 h light (700 lux), 12 h dark - 65 h before start of test fish were put into aquaria - Test substance suspneded with Ultra turrax and aliquot grought into aquarium to give final concentration (including suspended test substance)
Reliability	:	(2) valid with restrictions Test procedure in accordance with national standard methods

23.10.2003 (68)

Type	:	static
Species	:	Leuciscus idus (Fish, fresh water)
Exposure period	:	48 hour(s)
Unit	:	mg/l
LC0	:	= 2
LC100	:	= 50
Limit test	:	
Analytical monitoring	:	no data
Method	:	other: DIN 38412 L 15 (Fish, short-time test)
Year	:	1989
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4

Reliability : (2) valid with restrictions
Test according to national standards

23.10.2003 (69) (8)

Type	:	
Species	:	Cyprinus carpio (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	6.4
Method	:	
Year	:	1994
GLP	:	
Test substance	:	

Result : -log LC50 = 4.48 (mol/l), which equals 6.4 mg/l
Reliability : (4) not assignable
Original reference in Chinese. Most of the data have later been published by Lang et al. (1996) and Zhao et al. (1997)

16.04.2003 (70) (71)

Type	:	semistatic
Species	:	Cyprinus carpio (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	6.4
Limit test	:	
Analytical monitoring	:	no data
Method	:	other: see Test condition
Year	:	1996
GLP	:	no data
Test substance	:	other TS: no purity given

Remark : Since various data of Lang et al. (1996) match (3 digits) these published by Yuan et al. in 1994 and 1995 [Yuan X, Lang P, Long F, Lu G (1994) The Relationship Between Toxicities of Nitroaromatic Hydrocarbons to Photobacterium phosphoreum and Other Aquatic Organisms. Jilin Daxue Ziran Kexue Xuebao (4): 97 - 100; Yuan X, He Y, Lang P (1995) QSAR Study and the Toxicity of Nitroaromatic Compounds to Bacteria in the Songhua River. Huanjing Kexue 16 (5): 18 - 21], it is assumed that Lang et al. (1996) use data of the work of Yuan et al. (1994, 1995). However, the work of Yuan et al. (1994, 1995) is not cited by Lang et al. (1996)
Result : -log EC50 = 4.48 (mol/l), which equals 6.4 mg/l.
Calculation with the energy values of the lowest unoccupied molecular orbital yielded -log LC50 (mol/l) = 4.16, which equals LC50 = 13.3 mg/l
Test condition : The test was performed under the following conditions:
- 1 year old carps, average weight and length 23.8 +/- 6.4 g / 11.6 +/- 2.3

cm
- Sterilized and reared 2 weeks in 5 % (w/v) salt water
- Test water was dechlorinated tap water with 21.45 mg/l chlorine
- Temperature 15 - 18 °C
- pH 7.0 - 7.5
- Oxygen-content 6.35 mg/l at 12.3 °C
- Direct sunlight was avoided
- 5 concentrations were established
- Test aquaria contained 20 l test water and 10 fish
- Test water was replaced twice a day and 10 l, each time
- Acetone used as solvent (0.05 - 0.1 % v/v)

Reliability : (3) invalid
It is not clear how much chlorine was in the "dechlorinated" tap water (see Test conditions, the level reported is toxic to fish).
Although Lang et al. (1996) do not cite previous publications, there is strong evidence, that the data reported by Lang et al. (1996) have been published previously.

04.07.2003 (72)

Type : other: semistatic (water renewal after 12 hours)
Species : Cyprinus carpio (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : 6.4
Method : other: comparable to OECD-Guideline 203 (Fish: Acute Toxicity Test, 1992)
Year : 1994
GLP : no data
Test substance : other TS: no purity given

Remark : Data which are described to be measured by Zhao et al. (1997) have been published by Yuan et al. in 1994 and 1995. Neither the work of Yuan et al. (1994, 1995) nor the work of Lang et al. (1996) is cited by Zhao et al. (1997)
Result : -log LC50 = 4.48 (mol/l), which equals 6.4 mg/l
Test condition : - 60 fish used in each test (fish length 5 cm / fish weight 5 g)
- 10 fish in 16 l of test water
- Temperature 20 +/- 1 °C
- Stock solution was prepared in acetone

Reliability : (3) invalid
The study of Zhao et al. 1997 contains all (except one) carp data of a publication of Lang et al. (1996). However, these authors give a completly different description of their experiments acompared to one used by Zhao et al. (1997) e.g. source, size and age of carps. Since such a similarity in results (3 digits, 18 compounds) is extremely unlikely, it is thought that Zhao et al. (1997) have used published data.

16.04.2003 (31)

Type : other: not specified
Species : Brachydanio rerio (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
NOEC : .5
LC0 : 7
LC100 : 10
Limit test : no
Analytical monitoring : no
Method : other: UBA-Proposal "Letale Wirkung bei Brachidanio rerio" (1982.06.01)
Year : 1982
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Test substance	:	The stock solution was prepared by dissolving 1 g test substance in 1 g ethanol before it was diluted to the final concentration (1 g/l) with 2 g/l emulgator W. For this solution, the calculated COD was 958 mg/l, the COD measured 270 mg/l. The pH of this solution was 4.7.
Reliability	:	(3) invalid Test procedure according to national standards. Problems with emulsifying 1,2-dichloro-4-nitrobenzene

16.04.2003 (57)

Type	:	static
Species	:	Brachydanio rerio (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
NOEC	:	.1
LC0	:	9
LC100	:	10
Limit test	:	no
Analytical monitoring	:	no
Method	:	other: DIN 38412, Part 15 (Fish, short-time test)
Year	:	1984
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Test condition	:	- Brachydanio rerio 30 +/- 5 mm - Temperature: 23 +/-2°C - Aquarium 8.4 l containing 3.5 l tap water - Tap water was filtered through activated carbon and ion exchange resin (to remove copper). It had a Ca/Mg ratio of 4/1 and a German Hardiness of 15 °dH. pH of the filtered tap water was 7.0 +/- 0.2 - 10 animals in each aquarium, aerated - Stock solution was prepared by dissolving 1 g test substance in 1 g acetone before it was diluted to the final concentration (1 g/l) with 1 g/l emulgator W. For this solution, the calculated COD was 958 mg/l, the COD measured 253 mg/l. The pH of this solution was 6.5.
Reliability	:	(3) invalid Test procedure according to national standards. Problems with emulsifying 1,2-dichloro-4-nitrobenzene

16.04.2003 (58)

Type	:	static
Species	:	Brachydanio rerio (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC0	:	= 10
LC100	:	= 17.8
Limit test	:	
Analytical monitoring	:	no data
Method	:	other: DIN 38412 L 15 (Fish, short-time test)
Year	:	1986
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4

Test condition	:	- Brachydanio rerio 30 +/- 5 mm - Temperature: 23 +/-2°C - Aquarium containing 5 l water and 10 fish - No stock solution prepared but 1,2-dichloro-4-nitrobenzene given directly into the water
Reliability	:	(4) not assignable Only raw data available

14.04.2003 (73)

Type	:	static
Species	:	Leuciscus idus (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC0	:	7.5
LC50	:	10
LC100	:	13.3
Limit test	:	
Analytical monitoring	:	no
Method	:	other: DIN 38412 L 15 (Fish, short-time test)
Year	:	1986
GLP	:	no
Test substance	:	other TS: no purity given
Test condition	:	- Temperature: 23 +/-2°C - Aquarium containing 10 l water and 10 fish - No stock solution prepared but 1,2-dichloro-4-nitrobenzene given directly into the water
Reliability	:	(4) not assignable Only raw data available

27.06.2003 (74)

Type	:	static
Species	:	Pimephales promelas (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
NOEC	:	4
LC50	:	> 4
Limit test	:	no
Analytical monitoring	:	no data
Method	:	other: see below
Year	:	1975
GLP	:	no
Test substance	:	no data
Method	:	Standard Methods for the Examination of Water and Wastewater 13th Edition (1971), American Public Health Assn., NY 10019
Reliability	:	(4) not assignable Insufficient data

15.04.2003 (59)

Type	:	
Species	:	other: fish
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	4
Method	:	other: tested with standard EEC or OECD methods or comparable
Year	:	2001
GLP	:	no data
Test substance	:	other TS: no purity given
Remark	:	Ecotoxicological data were taken from literature. Major criteria for the selection of toxicity data were reliability and comparability of test methods. Very old data were generelly discarded.
Reliability	:	(4) not assignable Secondary literature

10.04.2003 (75)

Type	:	static
Species	:	Lepomis macrochirus (Fish, fresh water)

Exposure period : 3 hour(s)
Unit : mg/l
EC0 : 5
Method :
Year : 1957
GLP :
Test substance :

Method : Endpoint: Behaviour (Observation of stress effect)
Remark : Original literature not available. Cited according to Data USEPA Ecotox Database Aquire (http://www.epa.gov/cgi-bin/ecotox_search)
Reliability : (4) not assignable
Original literature not available
29.06.2003 (76)

Type : static
Species : Oncorhynchus mykiss (Fish, fresh water)
Exposure period : 1 hour(s)
Unit : mg/l
EC0 : 5
Method :
Year : 1957
GLP :
Test substance :

Method : Endpoint: Behaviour (Observation of stress effect)
Remark : Original literature not available. Cited according to Data USEPA Ecotox Database Aquire (http://www.epa.gov/cgi-bin/ecotox_search)
Result : The same result was obtained after 3 h of incubation
Reliability : (4) not assignable
Original literature not available
29.06.2003 (76)

Type : static
Species : Petromyzon marinus
Exposure period : 24 hour(s)
Unit : mg/l
EC0 : 5
Method :
Year : 1957
GLP :
Test substance :

Method : Endpoint: Behaviour (Observation of stress effect)
Remark : Original literature not available. Cited according to Data USEPA Ecotox Database Aquire (http://www.epa.gov/cgi-bin/ecotox_search)
Reliability : (4) not assignable
Original literature not available
29.06.2003 (76)

Type : static
Species : Oncorhynchus tschawytscha (Fish, fresh water, marine)
Exposure period : 24 hour(s)
Unit : mg/l
EC0 : 10
Method :
Year : 1969
GLP :
Test substance :

Method : Endpoint: Behaviour

Remark	:	Original literature not available. Cited according to Data USEPA Ecotox Database Aquire (http://www.epa.gov/cgi-bin/ecotox_search)
Reliability	:	(4) not assignable Original literature not available

29.06.2003 (77)

Type	:	static
Species	:	Ptychocheilus oregonensis (Fish, fresh water)
Exposure period	:	24 hour(s)
Unit	:	mg/l
EC0	:	10
Method	:	
Year	:	1969
GLP	:	
Test substance	:	

Method	:	Endpoint: Behaviour
Remark	:	Original literature not available. Cited according to Data USEPA Ecotox Database Aquire (http://www.epa.gov/cgi-bin/ecotox_search)
Reliability	:	(4) not assignable Original literature not available

29.06.2003 (77)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	other: not specified
Species	:	Daphnia magna (Crustacea)
Exposure period	:	24 hour(s)
Unit	:	mg/l
EC0	:	= 2
EC50	:	= 3
EC100	:	= 11
Analytical monitoring	:	no data
Method	:	other: DIN 38412 L 11 (Daphnia short-time test)
Year	:	1983
GLP	:	no data
Test substance	:	no data

Method	:	Method of the German Standards Institution Berlin, Germany
Reliability	:	(2) valid with restrictions Test procedure in accordance with national standard methods. Basic data given
Flag	:	Critical study for SIDS endpoint

10.04.2003 (66)

Type	:	static
Species	:	other: Daphnia carinata
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC50	:	8.2
Analytical monitoring	:	no data
Method	:	other: comparable to OECD 202 part 1 (Daphnia: Acute toxicity, 1984)
Year	:	1996
GLP	:	no data
Test substance	:	other TS: no purity given

Result	:	- measured log 1/IC50 = 4.37, corresponds to measured IC50 = 8.2 mg/l - calculated log 1/IC50 = 4.16, corresponds to calculated IC50 = 13 mg/l
Test condition	:	The test was performed under the following conditions:

- Temperature 22 +/- 1 °C, with a photoperiod of 14 hours light / 10 hours dark
- Cultured parthenogenetically, fed with a diet of green algae
- Each test used 60 Daphnia carinata (6 - 24 hours old), 10 of them in each 25 ml
- Daphnias were not fed during tests
- Stock solution prepared in acetone
- The results were considered valid if dissolved oxygen measured at the end of the test was at least equal to 60 % saturation, and if percentage of immobilization observed for the controls was zero

Reliability : (2) valid with restrictions
Comparable to guideline study, only basic data given
Flag : Critical study for SIDS endpoint
10.04.2003 (31)

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 24 hour(s)
Unit : mg/l
LC50 : ca. 6
Limit Test : no
Analytical monitoring : no data
Method : other: Static test in open system
Year : 1982
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Test condition : - The same test procedure was conducted in two parallel test systems. Every test vessel was filled with 10 ml test solution and per concentration 10 Daphnias were used.
- A control test was also run in addition to the treatment series.
- The concentrations tested are presented in form of the dilution factor and they range from 1:1 up to 1:100 of the test solution which contained about 117 mg/l

Reliability : (2) valid with restrictions
Basic data given
10.04.2003 (50)

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 24 hour(s)
Unit : mg/l
EC0 : > .1
Method : other: DIN-Standard 38 412 L11 (Daphnia, Short-time toxicity test)
Year : 1988
GLP : no data
Test substance : other TS: no purity given

Method : Method of the German Standards Institution, Berlin, Germany
Remark : Although the water solubility of 1,2-dichloro-4-nitrobenzene is about 120 mg/l, and results > 0.1 mg/l are reported, the authors report that due to their method of solvatation the highest concentration checked was 0.1 mg/l (in Ethanol)
It was clarified by one author, that due to an Electronic Data Processing error the authors report some experiments with 28 and 56 mg/l, however, the correct data were 0.025 and 0.05 mg/l, respectively
Result : Reported concentration range in data compilation: 0.0008 - 0.10 mg/l.
Test condition : The test was performed under the following conditions:
- Test organism: Daphnia magna Strauss, strain = IRCHA
- The test consists of 4 parallel test beakers per concentration level and at least 4 for the control

- Test system: Each beaker was filled with 24 h-old Daphnia (1 organism/50 ml), the total number per concentration level was 20 organisms
- Test temperature 25 +/- 1 °C
- Dilution water: Source = 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 test beakers per concentration level. The detected variation of these parameters had no negative influence on the organisms

Reliability : (2) valid with restrictions
Test procedure according national standard method. Reported in sufficient detail. Some minor contradictions in the report have been clarified by one of the authors

23.10.2003 (44)

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 24 hour(s)
Unit : mg/l
EC50 : 11.3
Analytical monitoring : no data
Method : other: comparable to OECD 202 part 1 (Daphnia: Acute toxicity)
Year : 1994
GLP : no data
Test substance : other TS: i.e. > 95 % purity

Result : - measured log 1/IC50 = 4.23 (both publications), corresponds to measured IC50 = 11 mg/l
- calculated log 1/IC50 = 4.41 [Zhao Y-H, He Y-B, Wang L-S (1995) Predicting Toxicities of Substituted Aromatic Hydrocarbons to Fish by Toxicities to Daphnia magna or Photobacterium phosphoreum. Toxicol Environ Chem 51: 191 -195], corresponds to calculated IC50 = 7.5 mg/l
- calculated log 1/IC50 = 4.63 [Zhao Y-H, Wang L-S (1995) Quantitative Structure-Activity Relationships of Hydrophobic Organic Chemicals. Toxicol Environ Chem 50: 167 - 172], corresponds to calculated IC50 = 4.5 mg/l

Test condition : The test was performed under the following conditions:
- Temperature 22 +/- 1 °C, with a photoperiod of 14 hours light / 10 hours dark
- Cultured parthenogenetically, fed with a diet of green algae
- Each test used 60 organisms (6 - 24 hours old), 10 of them in each 25 ml
- Daphnia magna were not fed during tests
- The results were considered valid if dissolved oxygen measured at the end of the test was at least equal to 60 % saturation, and if percentage of immobilization observed for the controls was zero

Reliability : (2) valid with restrictions
Comparable to guideline study, only basic data given. Experimental result is exactly the same in both studies, thus it is assumed that one experimental result was published twice

23.10.2003 (29) (30)

Type : other: not indicated
Species : other: Daphnia
Exposure period : 48 hour(s)
Unit : mg/l
EC50 : 3
Analytical monitoring : no data
Method : other: tested with standard EEC or OECD methods or comparable procedures
Year : 2001
GLP : no data
Test substance : other TS: no purity given

Remark	:	Ecotoxicological data were taken from literature. Major criteria for the selection of toxicity data were reliability and comparability of test methods.
Reliability	:	(4) not assignable Secondary literature, origin of data not reported

27.06.2003 (75)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	other algae: Scenedesmus obliquus
Endpoint	:	growth rate
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC50	:	= 5.8
Limit test	:	
Analytical monitoring	:	no data
Method	:	other: OECD Guideline 201 (Algae, Growth inhibition test, 1981)
Year	:	1995
GLP	:	no data
Test substance	:	other TS: no purity given
Result	:	Result reported: - log EC50 (mol/l) = 4.52 which equals 5.8 mg/l
Test condition	:	The test was performed under the following conditions: - Temperature 20 °C +/- 1 °C - pH 7.2 +/- 0.2 - Continuous light provided by white Neon lamps (3,600 lux), - Stock solution prepared in aceton (1 ml/l) - Initial cell concentration was approx. 10,000 cells/ml
Reliability	:	(2) valid with restrictions Basic data given
Flag	:	Critical study for SIDS endpoint

23.04.2003 (78)

Species	:	Scenedesmus subspicatus (Algae)
Endpoint	:	growth rate
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC10	:	> .1
EC50	:	> .1
Method	:	other: DIN 38 412, Part 9 (Cell multiplication inhibition test)
Year	:	1988
GLP	:	no data
Test substance	:	other TS: no purity given
Method	:	Method of the German Standards Institution, Berlin, Germany
Result	:	Effect levels determined the endpoint biomass and the results were the following: EC10 = > 0.10 mg/l EC50 = > 0.10 mg/l
Test condition	:	- The concentration range tested was 0.0008 - 0.10 mg/l - The cell material was used after 72 h of preculture to inoculate the dilution preparation after the cell concentration had been fixed at 1.0E5/ml - Test preparations: - Wide-neck bottles of 250 ml with ground-glass stoppers were used as the test vessels. - The test and control preparations were incubated under constant lighting and shaken daily. - Before beginning the test pH was adjusted to 8 Although some experiments were reported to be done in the range of 10 -

100 mg/l, it was clarifed by one of the authors that the concentration range tested was 0.0008 - 0.10 mg/l

Reliability : (2) valid with restrictions
Test procedure in accordance to national standard methods

Flag : Critical study for SIDS endpoint

07.08.2003 (44)

Species : Chlorella fusca (Algae)
Endpoint : growth rate
Exposure period : 24 hour(s)
Unit : mg/l
EC50 : = .32
Limit test :
Analytical monitoring : no data
Method : other: cf. Test conditions
Year : 2000
GLP : no data
Test substance : other TS: no purity given

Remark : New accepted scientific name for Chlorella fusca is Scenedesmus vacuolatus

Test condition : Test measures inhibition of one generation reproduction cycle within 24 h according to Altenburger et al. 1990 [Altenburger R, Boedecker W, Faust M, Grimme LH (1990) Evaluation of the Isobologram Method for the Assessment of Mixtures of Chemiclas. Combination of Effect Studies with Pesticides in Algal Biotests. Ecotox Environ Safety 20: 98 - 114].
The test was performed under the following conditions:
- Temperature 28 °C +/- 0.5 °C, pH 6.7
- Incubation in gastight vessels
- Initial cell concentration was approx. 1E5 cells/ml
- one day = 1 generation under the experimental conditions, cell number increases by a factor of 12 during incubation
- 14 h light, 10 h dark cycle

Reliability : (2) valid with restrictions
Basic data given

07.08.2003 (23)

Species : other algae
Endpoint : other
Exposure period : 96 hour(s)
Unit : mg/l
EC50 : = 1.055
Limit test :
Analytical monitoring : no data
Method : other: tested with standard EEC or OECD methods or comparable procedures
Year : 2001
GLP : no data
Test substance : other TS: no purity given

Remark : Ecotoxicological data were taken from literature. Major criteria for the selection of toxicity data were reliability and comparability of test methods. Very old data were generelly discarded.

Result : Result reported as Log 1/C [mmol/l] = 2.26 which equals 1.055 mg/l

Reliability : (4) not assignable
Secondary literature

15.04.2003 (75)

Species : other algae: Selenastrum obliquus
Endpoint :
Exposure period : 96 hour(s)

Unit	:	mg/l
EC50	:	6 - 15
Method	:	
Year	:	1995
GLP	:	
Test substance	:	
Remark	:	From log kow (log kow = 3.29) cited from Zhao et al. (1993) [Zhao Y, Wang L, Gao H, Zhang Z (1993) Quantitative Structure - Activity Relationships - Relationship between Toxicity of Organic Chemicals to Fish and to Photobacterium phosphoreum. Chemosphere 26 (11): 1971 - 1979] Chinese letters of 3,4-Dichloronitrobenzene (1,2-dichloro-4-nitrobenzene) were identified
Result	:	Reported results measured and calculated: log EC50 = 4.5 - 4.12 (mol/l) which equals 6 - 15 mg/l
Test condition	:	- Culturing ot the algae: 24 +/- 1 °C; 12 h light, 12 h dark; 4000 lux +/- 10 %, pH 7.5 +/- 0.2 - 10000 cells/ml - Spectrometric determination at 650 nm
Reliability	:	(4) not assignable Original reference in Chinese

15.04.2003 (79)

Species	:	Selenastrum capricornutum (Algae)
Endpoint	:	
Exposure period	:	96 hour(s)
Unit	:	mg/l
EC50	:	1
Limit test	:	
Analytical monitoring	:	no data
Method	:	
Year	:	2000
GLP	:	no data
Test substance	:	
Remark	:	From log kow Chinese letters of 3,4-Dichloronitrobenzene (1,2-dichloro-4-nitrobenzene) were identified in the publication of Zhang et al. (1995) [Zhang Y, Yu H, Han S, Zhao Y, Wang L (1995) The Toxicity of Substituted Aromatic Compounds to Algae and Quantitative Structure-Activity Relationship Studies. Huanjing Huaxue 14 (2): 140 - 144]
Result	:	measured log (1/EC50) = 2.283 mmol/l, which equals 1 mg/l; calculated log (1/EC50) = 2.042 mmol/l, which equals 1,7 mg/l
Test condition	:	- Spectrometric determination at 686 nm
Reliability	:	(4) not assignable Original reference in Chinese

15.04.2003 (35)

Species	:	other algae: Scenedesmus obliquus
Endpoint	:	growth rate
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC50	:	= 5.8
Limit test	:	
Analytical monitoring	:	no data
Method	:	other: OECD Guideline 201 (Algae, Growth inhibition test, 1981)
Year	:	1995
GLP	:	no data
Test substance	:	other TS: no purity given
Test condition	:	The test was performed under the folowing conditions: Temperature 20 °C +/- 1 °C, continuous light provided by white Neon

lamps (4.000 lux),
- Initial cell concentration was approx. 1 E4 cells/ml
- Growth was monitored by electron microscope (400 times)

Reliability : (4) not assignable
Secondary literature. Although about 60 % of the "measured" EC50 data are also reported in the paper of Liu and Lang (1995) [Liu J, Lang P (1995) Toxicities of Nitroaromatic Compounds to Scenedesmus obliquus and Toxic Symptoms. Huanjing Kexue 16: 7 - 10], there is no reference that these data have been published elsewhere.

23.10.2003 (80)

Species	:	other algae: Scenedesmus obliquus
Endpoint	:	growth rate
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC50	:	= 5.8
Limit test	:	
Analytical monitoring	:	no data
Method	:	other: OECD Guideline 201 (Algae, Growth inhibition test, 1984)
Year	:	1995
GLP	:	no data
Test substance	:	other TS: no purity given

Test condition : The test was performed under the following conditions:
Temperature 24 °C +/- 1 °C, in a schedule of 12 hours light /12 hours dark
- Stock solution prepared in aceton
- Initial cell concentration approx. 1 E4 cells/ml
- The cell density was measured after 0, 24, 48, 72 and 96 hours
- The optical density was determined at 650 nm

Reliability : (4) not assignable
Secondary literature. The authors use the data published by Liu and Lang (1995) [Liu J, Lang P (1995) Toxicities of Nitroaromatic Compounds to Scenedesmus obliquus and Toxic Symptoms. Huanjing Kexue 16: 7 - 10], although there is no reference the Liu and Lang (1995).

23.10.2003 (31)

Species	:	Haematococcus pluvialis (Algae)
Endpoint	:	other: O2 production of algae
Exposure period	:	4 hour(s)
Unit	:	mg/l
EC50	:	= 2
Limit test	:	
Analytical monitoring	:	no data
Method	:	other: Manometric determination, cf. Test conditions
Year	:	1983
GLP	:	no data
Test substance	:	no data

Remark : Test criteria: inhibitory effect on oxygen production

Test condition : Oxygen production measured (manometric determination) according to Tuempling (1972) in Warburg vessels [Tuempling, vW (1972) Ein manometrisches Verfahren zur Bestimmung der autotrophen Bioaktivität. Fortschritte Wasserchemie 14: 205 - 213]
- Cell densitiy: 80.000 cells/ml
- Incubation volume 5 ml
- Volume of a Warburg vessel ca. 40 ml
- Since the algae nutrient solution contains high buffer capacity, no neutralization assumed to be needed

Reliability : (4) not assignable
Documentation insufficient for assessment

23.10.2003 (66)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type	:	aquatic
Species	:	Pseudomonas putida (Bacteria)
Exposure period	:	30 minute(s)
Unit	:	mg/l
EC10	:	= 44
Analytical monitoring	:	no data
Method	:	other: Test according to Robra (O2-Consumption)
Year	:	1983
GLP	:	no data
Test substance	:	other TS: no purity given
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	:	Initial TS concentration was checked measuring the DOC (dissolved organic carbon)
Reliability	:	(2) valid with restrictions Study meets generally accepted scientific principles, acceptable for assessment
Flag	:	Critical study for SIDS endpoint

15.04.2003 (66)

Type	:	other: Agar medium
Species	:	other fungi: Rhizoctonia solani Kühn
Exposure period	:	88 hour(s)
Unit	:	mg/l
EC50	:	= 21
Analytical monitoring	:	no data
Method	:	other: Growth inhibition test
Year	:	1962
GLP	:	no data
Test substance	:	other TS: recrystallized
Remark	:	Although author wrote ED50 (effective dose), he apparently measured and reported EC50. Values were given in µmol/l
Result	:	ED50 = 110 µmol/l, which equals 21 mg/l
Test condition	:	- Tests were performed on an autoclaved nutrient agar at pH 6.4 - 6.6 - Test substance was dissolved in 1 % Triton X-100 in diethyl ether (1 ml) which was made up with sterile water to 50 ml of a relatively stable milky suspension - Aliquots of this suspension were added to sterile melted agar to give the desired stock agar concentration - Aliquots of this agar were diluted with fresh agar to yield 4 final test concentrations - After the agars solidified in growth tubes, each tube was inoculated with a 8 mm plug of the fungus and incubated at 24 °C - Linear growth measurements were taken after 40 and 88 hours
Reliability	:	(2) valid with restrictions Study with acceptable restrictions: up to date method by the time the study was undertaken
Flag	:	Critical study for SIDS endpoint

23.10.2003 (38)

Type	:	other: Growth medium
Species	:	other fungi: Mucor javanicus

Exposure period	:	7 day(s)
Unit	:	mg/l
EC50	:	ca. 50
Analytical monitoring	:	no data
Method	:	other: Growth rate test
Year	:	1984
GLP	:	no
Test substance	:	no data
Result	:	1,2-Dichloro-4-nitrobenzene (50 mg/l) decreases the growth of Mucor javanicus by 55 % during 7 d incubation
Test condition	:	The inocolum (fungus) was precultivated at 25°C for 72h. Then 50 ppm of the test substance were added to the culture and cultivation was continued for 6 more days. Growth was estimated from visible turbidity.
Reliability	:	(2) valid with restrictions Basic data given
Flag	:	Critical study for SIDS endpoint

23.10.2003 (55)

Type	:	aquatic
Species	:	anaerobic bact. from a domestic water treatment plant
Exposure period	:	24 hour(s)
Unit	:	mg/l
EC0	:	30
Analytical monitoring	:	no
Method	:	ETAD Fermentation tube method "Determination of damage to effluent bacteria by the Fermentation Tube Method"
Year	:	1982
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Dilution 1 : 3 of a 90 mg/l solution without inhibitory effect. Dilution 1 : 2 is inhibitory
Reliability	:	(2) valid with restrictions

16.04.2003 (50)

Type	:	aquatic
Species	:	Tetrahymena pyriformis (Protozoa)
Exposure period	:	40 hour(s)
Unit	:	mg/l
EC50	:	13.3
Analytical monitoring	:	no data
Method	:	other: Method following the protocol described by Schultz (1996)
Year	:	1998
GLP	:	no data
Test substance	:	other TS: purity > 95 %
Method	:	The method of Schultz (1996, 1997) [Schultz TW (1996) Tetrahymena in Aquatic Toxicology: QSARs and Ecological Hazard Assessment. In: Pauli W, Berger S (eds) Proceedings of the International Workshop on a Protozoan Test Protocol with Tetrahymena in Aquatic Toxicity Testing. German Federal Environmental Agency, Berlin, pp 31 - 66; Schultz TW (1997) TETRATOX: The Tetrahymena pyriformis Population Growth Impairment Endpoint - A Surrogate for Fish Lethality. Toxicol Methods /: 289 - 309] was applied
Remark	:	In the publications of Schultz (1999) and Cronin et al. (1998), the method for the determination of the toxicity to Tetrahymena pyriformis is described. However, most data seem to be taken from other publications without clearly stating this: E.g. in the publication of Schultz (1999) 4-methylphenol, 2-methylphenol, 4-ethylphenol, 4-chlorophenol, 3-nitrotoluene, 2-nitrophenol are exactly the same as in the publication of Schultz (1997) (3

digits).
In the publication of Cronin et al. (1998) e.g. the results of nitrobenzene, 1,4-dinitrobenzene, 3-chloronitrobenzene are exactly the same, whereas the data of 2-chloronitrobenzene, 3-nitrotoluene, 6-methyl-1,3-dinitrobenzene and 4,6-dichloro-1,2-dinitrobenzene are slightly different from these of Schultz (1997). Thus, in the publication of Cronin et al. (1998) there are some new data.
The publication of Schultz (1999) contains some data in common with the publication of Cronin et al. (1998). However, in the case of 3-nitrotoluene, the value of Schultz (1997) is reported. For other 7 randomly choosen substances both publications reported equal numbers with one exception. Thus, it is likely that Schultz (1999) published data of Schultz (1997) and Cronin et al. (1998) without citation.

Result : Result is given in -log IGC50 = 1.16.
IG = Impairment of (population) growth
The result equals 0.069 mmol/l or 13.3 mg/l

Test condition : The test was performed under the following conditions (Schultz 1996, Schultz 1997):
- End-point population density was quantitated spectrophotometrically at 540 nm
- Two controls were employed, one that was inoculated with T. pyriformis and a blank, which contained neither test material nor ciliates
- Each definitive test replicate consisted of six to eight different concentrations of the test material with duplicate flasks of each concentration
- Only replicates with control-absorbency values > 0.6 and < 0.75 were used in the analysis
- A minimum of 30 data points
- The IGC50 was determined by Probit analysis
- Although it is not clearly indicated in the publication, the test substance was presumably dissolved in DMSO (Dimethylsulfoxide, presumably some g/l in the test assays).

Reliability : (2) valid with restrictions
Basic data given

11.07.2003 (24) (26)

Type : other: Agar medium
Species : other fungi: Pythium ultimum Trow.
Exposure period : 88 hour(s)
Unit : mg/l
EC50 : = 23
Analytical monitoring : no data
Method : other: Growth inhibition test
Year : 1962
GLP : no data
Test substance : other TS: recrystallized

Remark : Although author wrote ED50 (effective dose), he apparently measured and reported EC50. Values were given in µmol/l

Result : ED50 = 120 µmol/l, which equals 23 mg/l

Test condition : - Tests were performed on an autoclaved nutrient agar at pH 6.4 - 6.6
- Test substance was dissolved in 1 % Triton X-100 in diethyl ether (1 ml) which was made up with sterile water to 50 ml of a relatively stable milky suspension
- Aliquots of this suspension were added to sterile melted agar to give the desired stock agar concentration
- Aliquots of this agar were diluted with fresh agar to yield 4 final test concentrations
- After the agars solidified in growth tubes, each tube was inoculated with a 8 mm plug of the fungus and incubated at 24 °C
- Linear growth measurements were taken after 40 and 88 hours

Reliability : (2) valid with restrictions
Study with acceptable restrictions: up to date method by the time the study was undertaken
23.10.2003 (38)

Type : other: Barley mash-agar
Species : Aspergillus niger (Fungi)
Exposure period : 6 day(s)
Unit : mg/l
EC0 : > 200
Analytical monitoring : no data
Method : other: Growth inhibition test
Year : 1961
GLP : no data
Test substance : no data

Method : Zsolnai T (1960) Versuche zur Entdeckung neuer Fungistatika-I Phenol-Derivate Biochem Pharmacol 5: 1 - 19
Remark : No fungistatic effect
Test condition : Solid barley mash-agar-culture medium (3 % agar), without serum protein, pH 6.0 - 6.2, temperature of incubation 30 °C
Reliability : (2) valid with restrictions
23.10.2003 (81)

Type : other: Barley mash-agar
Species : Candida albicans (Fungi)
Exposure period : 6 day(s)
Unit : mg/l
EC0 : > 200
Analytical monitoring : no data
Method : other: Growth inhibition test
Year : 1961
GLP : no data
Test substance : no data

Method : Zsolnai T (1960) Versuche zur Entdeckung neuer Fungistatika-I Phenol-Derivate. Biochem Pharmacol 5: 1 - 19
Remark : No fungistatic effect
Test condition : Solid barley mash-agar-culture medium (3 % agar), without serum protein, pH 6.0 - 6.2, temperature of incubation 30 °C
Reliability : (2) valid with restrictions
23.10.2003 (81)

Type : other: Barley mash-agar
Species : Saccharomyces cerevisiae (Fungi)
Exposure period : 6 day(s)
Unit : mg/l
EC0 : > 200
Analytical monitoring : no data
Method : other: Growth inhibition test
Year : 1961
GLP : no data
Test substance : no data

Method : Zsolnai T (1960) Versuche zur Entdeckung neuer Fungistatika-I Phenol-Derivate. Biochem Pharmacol 5: 1 - 19
Remark : No fungistatic effect
Test condition : Solid culture medium of barley mash (3 % agar), without serum, pH 6.0 - 6.2, temperature of incubation 30 °C
Reliability : (2) valid with restrictions
23.10.2003 (81)

Type : other: Barley mash-agar
Species : other fungi: Penicillium simplicissimum
Exposure period : 6 day(s)
Unit : mg/l
EC0 : > 200
Analytical monitoring : no data
Method : other: Growth inhibition test
Year : 1961
GLP : no data
Test substance : no data

Method : Zsolnai T (1960) Versuche zur Entdeckung neuer Fungistatika-I Phenol-Derivate. Biochem Pharmacol 5: 1 - 19
Remark : No fungistatic effect
Test condition : Solid culture medium of barley mash (3 % agar), without serum, pH 6.0 - 6.2, temperature of incubation 30 °C
Reliability : (2) valid with restrictions
23.10.2003 (81)

Type : other: Barley mash-agar
Species : other fungi: Trichothecium roseum
Exposure period : 6 day(s)
Unit : mg/l
EC0 : > 200
Analytical monitoring : no data
Method : other: Growth inhibition test
Year : 1961
GLP : no data
Test substance : no data

Method : Zsolnai T (1960) Versuche zur Entdeckung neuer Fungistatika-I Phenol-Derivate. Biochem Pharmacol 5: 1 - 19
Remark : No fungostatic effect
Test condition : Solid barley mash-agar-medium (3 % agar), without serum protein, pH 6.0-6.2, temperature of incubation 30 °C
Reliability : (2) valid with restrictions
23.10.2003 (81)

Type : other: Barley mash-agar-culture medium
Species : Aspergillus niger (Fungi)
Exposure period : 5 day(s)
Unit : mg/l
EC100 : > 200
Analytical monitoring : no data
Method : other: Growth inhibition test
Year : 1961
GLP : no data
Test substance : no data

Method : Zsolnai T (1960) Versuche zur Entdeckung neuer Fungistatika-I Phenol-Derivate. Biochem Pharmacol 5: 1 - 19
Remark : Partial inhibition
Test condition : Liquid barley mash-agar-medium, 10 % bovine serum protein, pH 6.0-6.2, temperature of incubation 30 °C
Reliability : (2) valid with restrictions
23.10.2003 (81)

Type : other: Barley mash-agar-culture medium
Species : Candida albicans (Fungi)
Exposure period : 5 day(s)

Unit : mg/l
EC100 : > 200
Analytical monitoring : no data
Method : other: Growth inhibition test
Year : 1961
GLP : no data
Test substance : no data

Method : Zsolnai T (1960) Versuche zur Entdeckung neuer Fungistatika-I Phenol-Derivate. Biochem Pharmacol 5: 1 - 19
Remark : no growth inhibition at highest tested concentration (200 mg/l)
Test condition : Liquid barley mash-agar-medium, 10 % bovine serum protein, pH 6.0-6.2, temperature of incubation 30 °C
Reliability : (2) valid with restrictions
23.10.2003 (81)

Type : other: Barley mash-agar-culture medium
Species : other fungi: Achorion quinckeanum
Exposure period : 10 day(s)
Unit : mg/l
EC100 : = 100
Analytical monitoring : no data
Method : other: Growth inhibition test
Year : 1961
GLP : no data
Test substance : no data

Method : Zsolnai T (1960) Versuche zur Entdeckung neuer Fungistatika-I Phenol-Derivate. Biochem Pharmacol 5: 1 - 19
Test condition : Liquid barley mash-agar-medium, 10 % bovine serum protein, pH 6.0-6.2, temperature of incubation 30 °C
Reliability : (2) valid with restrictions
23.10.2003 (81)

Type : other: Barley mash-agar-culture medium
Species : other fungi: Epidermophyton spp.
Exposure period : 10 day(s)
Unit : mg/l
EC100 : = 100
Analytical monitoring : no data
Method : other: Growth inhibition test
Year : 1961
GLP : no data
Test substance : no data

Method : Zsolnai T (1960) Versuche zur Entdeckung neuer Fungistatika-I Phenol-Derivate. Biochem Pharmacol 5: 1 - 19
Test condition : Liquid barley mash-agar-medium, 10 % bovine serum protein, pH 6.0-6.2, temperature of incubation 30 °C
Reliability : (2) valid with restrictions
23.10.2003 (81)

Type : other: Barley mash-agar-culture medium
Species : other fungi: Penicillium simplicissimum
Exposure period : 5 day(s)
Unit : mg/l
EC100 : = 200
Analytical monitoring : no data
Method : other: Growth inhibition test
Year : 1961
GLP : no data

Test substance : no data

Method : Zsolnai T (1960) Versuche zur Entdeckung neuer Fungistatika-I Phenol-Derivate. Biochem Pharmacol 5: 1 - 19
Test condition : Liquid barley mash-agar-medium, 10 % bovine serum protein, pH 6.0-6.2, temperature of incubation 30 °C
Reliability : (2) valid with restrictions
23.10.2003 (81)

Type : other: Barley mash-agar-culture medium
Species : other fungi: Trichophyton gypseum
Exposure period : 10 day(s)
Unit : mg/l
EC100 : = 100
Analytical monitoring : no data
Method : other: Growth inhibition test
Year : 1961
GLP : no data
Test substance : no data

Method : Zsolnai T (1960) Versuche zur Entdeckung neuer Fungistatika-I Phenol-Derivate. Biochem Pharmacol 5: 1 - 19
Test condition : Liquid barley mash-agar-medium, 10 % serum protein, pH 6.0-6.2, temperature of incubation 30 °C
Reliability : (2) valid with restrictions
23.10.2003 (81)

Type : other: Barley mash-agar-culture medium
Species : other fungi: Trichothecium roseum
Exposure period : 5 day(s)
Unit : mg/l
EC100 : = 200
Analytical monitoring : no data
Method : other: Growth inhibition test
Year : 1961
GLP : no data
Test substance : no data

Method : Zsolnai T (1960) Versuche zur Entdeckung neuer Fungistatika-I Phenol-Derivate. Biochem Pharmacol 5: 1 - 19
Test condition : Liquid barley mash-agar-medium, 10 % bovine serum protein, pH 6.0-6.2, temperature of incubation 30 °C
Reliability : (2) valid with restrictions
23.10.2003 (81)

Type : aquatic
Species : Pseudomonas putida (Bacteria)
Exposure period :
Unit : mg/l
Analytical monitoring : no
Method : other: Test according to Robra (O2-Consumption)
Year : 1982
GLP : no
Test substance : no data

Result : EC0 (nominal concentration) was 125 mg/l, however, it is assumed that the actual concentration was far less (about 33 mg/l)
Test substance : In the Bayer (1982) study, the stock solution was prepared by dissolving 1 g test substance in 1 g ethanol before it was diluted to the final concentration (1 g/l) with 2 g/l emulgator W. For this solution, the calculated COD was reported to be 958 mg/l, the COD measured 270 mg/l. The pH of

this solution was 4.7.
In the Bayer (1984) study, the stock solution was prepared by dissolving 1 g test substance in 1 g acetone before it was diluted to the final concentration (1 g/l) with 1 g/l emulgator W. For this solution, the calculated COD was reported to be 958 mg/l, the COD measured 253 mg/l. The pH of this solution was 6.5.
It is assumed that in both studies, only a minor part of the test substance was in the test preparation.

Reliability : (3) invalid
Problems with emulsifying 1,2-dichloro-4-nitrobenzene (COD was only about a quarter of theoretical COD)

23.10.2003 (57) (58)

Type : aquatic
Species : other bacteria: bacteria inocula taken from the Songhua river (People's republic of China)
Exposure period : 24 hour(s)
Unit : mg/l
EC50 : 68.12
Analytical monitoring : no data
Method : other: Bacterial growth inhibition test according to the protocoll from Alsop et al. (1980)
Year : 1997
GLP : no data
Test substance : other TS: no purity given

Method : Alsop GM, Waggy GT, Conway RA (1980) Bacterial Growth Inhibition Test. J Water Poll Control Fed 52: 2452
Test condition : The test was performed under the following conditions:
- Bacterial inoculum, buffering agents, nutrients, growth substrates and the test substance mixed
- Incubation for 24 hr at 22 +/- 2 °C
- Turbidities measured with a spectrophotometer at 530 nm

Reliability : (3) invalid
Insufficient documentation

23.10.2003 (82)

Type : aquatic
Species : Photobacterium phosphoreum (Bacteria)
Exposure period : 30 minute(s)
Unit : mg/l
EC50 : = 10.1
Analytical monitoring : no data
Method : other: Microtox Testsystem
Year : 1985
GLP : no data
Test substance : other TS: highest purity commercially avialable and purity checked by melting point and GC

Remark : The concentration values causing 50 % reduction of bioluminescence after 30 min of exposure were determined
Test condition : - 2 % NaCl in test solution
- Procedure according to instrument manual
Reliability : (3) invalid
Unsuitable test system

23.10.2003 (21)

Type : aquatic
Species : Photobacterium phosphoreum (Bacteria)
Exposure period : 15 minute(s)
Unit : mg/l

EC50	:	14.6
Analytical monitoring	:	no
Method	:	other: Microtox
Year	:	1993
GLP	:	no data
Test substance	:	no data
Result	:	- Results are given in log 1/EC50 (mol/l) = 4.12, which equals 14.6 mg/l - It is reported that also tests with an incubation period of 30 min had been performed, and that the results were similar to these performed with a 15 min incubation period
Test condition	:	- Microtox test applied according to manual of analyzer (Model Toxicity Analyzer DXY-2 of the Institute of Soil Science, Academia Sinica, Nanjing) - Incubation 15 min at 20 °C - Endpoint: 50 % inhibition of bioluminescence
Reliability	:	(4) not assignable Unsuitable test system, important information missing (e.g. quality criteria)

23.10.2003 (28) (29) (30)

Type	:	aquatic
Species	:	Photobacterium phosphoreum (Bacteria)
Exposure period	:	15 minute(s)
Unit	:	mg/l
EC50	:	14.9
Analytical monitoring	:	no data
Method	:	other: Microtox toxicity analyzer
Year	:	1997
GLP	:	no data
Test substance	:	other TS: no purity given
Method	:	The concentration values causing 50 % reduction of bioluminescence after 15 min of exposure were determined at 20 °C according to the procedures described in the Instrumental Manual (DXY-2, made by the Institute of Soil science, Academia Sinica, Nanjing (China)). All bioassays were carried out in duplicate or triplicate
Remark	:	This publication seems to be an excerpt of the publication of Yuan et al. (1995) [Yuan X, He Y, Lang P (1995) QSAR Study and the Toxicity of Nitroaromatic Compounds to Bacteria in the Songhua River. Huanjing Kexue 16 (5): 18 - 21]
Result	:	log 1/EC50 = 4.11 (mol/l), which equals 14.9 mg/l
Reliability	:	(3) invalid Unsuitable test system. Data origin not clear (see Remark)

23.10.2003 (82)

Type	:	aquatic
Species	:	Photobacterium phosphoreum (Bacteria)
Exposure period	:	15 minute(s)
Unit	:	mg/l
EC50	:	14.9
Method	:	other: Microtox
Year	:	1994
GLP	:	
Test substance	:	
Result	:	-log EC50 = 4.11 (mol/l), which equals 14.9 mg/l. Yuan et al. (1994) measured also the 30 min EC50 which was similar to the 15 min EC50. They derived an equation from the 15 min EC50 of 27 different substances (presumably as -log): 30 min EC50 = 0.0197 + 15 min EC50 (r = 0.994)
Reliability	:	(4) not assignable Original reference in Chinese. Unsuitable test system

23.10.2003 (70) (71)

Type	:	soil
Species	:	other fungi: Phythium ultimum
Exposure period	:	24 hour(s)
Unit	:	mg/l
EC50	:	ca. 100
EC5	:	ca. 30
EC95	:	ca. 400
Analytical monitoring	:	no data
Method	:	other: Growth inhibition test
Year	:	1968
GLP	:	no data
Test substance	:	other TS: "chemically pure"
Remark	:	Measurement of toxicity applied via the vapor phase: vapor pressure not taken into account for 1,2-dichloro-4-nitrobenzene. Concentration in results part denotes concentration in soil used for exposure
Test condition	:	Fungitoxicity of vapor released from a nonsterile compost soil amended with 1,2-dichloronitrobenzene - Dilution series by adding to soil with 1 g/kg 1,2-dichloronitrobenzene unamended soil in the ratio of 1 : 1 - At each stage of the dilution, triplicate 100 g samples were transfered to 16 oz wide mouth jars containing 10 ml of water. Screw caps were applied immediately and replaced 3 -4 h later with inverted culture plates with inoculum discs on potato-dextrose agar. - Radial growth was measured after incubation at 24 °C
Reliability	:	(3) invalid Unsuitable test system

23.10.2003 (83)

Type	:	soil
Species	:	other fungi: Rhizoctonia solani
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC50	:	ca. 125
EC5	:	ca. 50
EC95	:	ca. 400
Analytical monitoring	:	no data
Method	:	other: Growth inhibition test
Year	:	1968
GLP	:	no data
Test substance	:	other TS: "chemically pure"
Remark	:	Measurement of toxicity applied via the vapor phase: vapor pressure not taken into account for 1,2-dichloro-4-nitrobenzene. Concentration in results part denotes concentration in soil used for exposure
Test condition	:	Fungitoxicity of vapor released from a nonsterile compost soil amended with 1,2-dichloronitrobenzene - Dilution series by adding to soil with 1 g/kg 1,2-dichloronitrobenzene unamended soil in the ratio of 1 : 1 - At each stage of the dilution, triplicate 100 g samples were transfered to 16 oz wide mouth jars containing 10 ml of water. Screw caps were applied immediately and replaced 3 -4 h later with inverted culture plates with inoculum discs on potato-dextrose agar. - Radial growth was measured after incubation at 24 °C
Reliability	:	(3) invalid Unsuitable test system

23.10.2003 (83)

Type	:	soil
Species	:	other fungi: Trichoderma viride
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC50	:	ca. 250
EC5	:	ca. 70
EC95	:	ca. 1000
Analytical monitoring	:	no data
Method	:	other: Growth inhibition test
Year	:	1968
GLP	:	no data
Test substance	:	other TS: "chemically pure"
Remark	:	Measurement of toxicity applied via the vapor phase: vapor pressure not taken into account for 1,2-dichloro-4-nitrobenzene. Concentration in results part denotes concentration in soil used for exposure
Test condition	:	Fungitoxicity of vapor released from a nonsterile compost soil amended with 1,2-dichloronitrobenzene - Dilution series by adding to soil with 1 g/kg 1,2-dichloronitrobenzene unamended soil in the ratio of 1 : 1 - At each stage of the dilution, triplicate 100 g samples were transfered to 16 oz wide mouth jars containing 10 ml of water. Screw caps were applied immediately and replaced 3 -4 h later with inverted culture plates with inoculum discs on potato-dextrose agar. - Radial growth was measured after incubation at 24 °C
Reliability	:	(3) invalid Unsuitable test system

23.10.2003 (83)

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Species	:	Daphnia magna (Crustacea)
Endpoint	:	reproduction rate
Exposure period	:	21 day(s)
Unit	:	mg/l
NOEC	:	.025
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: no purity given
Method	:	Determination of NOEC for reproduction rate, mortality and the time of the first appearance of offspring. Analytical monitoring of test substance concentration by GC
Remark	:	The substance was tested far below the water solubility limit (circa 120 mg/l) with a maximum test concentration of 0.1 mg/l. It is not stated why higher concentrations have not been used.
Result	:	Tested concentration range: 0.0032 - 0.10 mg/l. The author assumes the water solubility limit with 0.1 mg/l Results of analytical monitoring Nom conc. instant analysis analysis after 2 d

		0.1 0.1 -0.12 0.09 - 0.1 0.05 0.05 - 0.06 0.04 - 0.05 0.025 0.020 - 0.030 0.020 - 0.026 0.012 0.010 - 0.02 0.010 - 0.012 Analysis after 2 d: In general, highest analytical values were obtained in controls. The lower values were measured in used Daphnia medium
Test condition	:	The test was performed under the following conditions: - Semistatic test - Test organism: Daphnia magna Strauss, strain IRCHA - 100 µg/l of testsubstance was dissolved in Ethanol - The test consists of 4 parallel test vessels (gastight) per concentration level and at least 4 for the control - Test system: Each vessel was filled with 24 h-old Dapnia (1 organism/50 ml), the total number per concentration level was 20 organisms. - The parent animals in the control and test vessels were pipetted 3 times a week into freshly prepared test and control media. - Test temperature 25 +/- 1 °C - Dilution water: Source = Synthetic fresh water, Hardness = 2.5 mmol/l Ca + Mg, Na/K ratio = 10:1, pH = 8.0 +/- 0.1 - pH-values and oxygen concentration were measured during the test in two test-vessels per concentration level. The detected variation of these parameters had no negative influence on the organisms
Reliability	:	(2) valid with restrictions Test procedure according national standard method. Reported in sufficient detail. Some minor contradictions in the report have been clarified by one of the authors
Flag	:	Critical study for SIDS endpoint

23.10.2003 (44)

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

Species	:	Phaseolus aureus (Dicotyledon)
Endpoint	:	growth
Exposure period	:	6 day(s)
Unit	:	mg/l
EC50	:	26.9
Method	:	other: germination and growth of seedlings in sand
Year	:	1962
GLP	:	no data
Test substance	:	no data
Result	:	Reduction in fresh weight after 6 days (compared to controls) was used to measure inhibition. Although the author wrote ED50 (effective dose), he apparently measured and reported EC50. Values were given in µmol/l. Result refers to concentration of aqueous solution.
Test condition	:	The test was performed under the following conditions: - Pregermination of the seeds for 24 hours - Test chemical suspended in 0.1 strength Hoagland's solution - 15 seeds were cultured in each tall form beaker (150 ml) containing 220 g (dry weight) of sand - 36 ml of test chemical was added - Incubation in the dark in a Mangelsdorf seed-germinator, temperature 25 °C, relative humidity approaching 100 %, incubation time 6 days
Reliability	:	(2) valid with restrictions Study with acceptable restrictions, up to date method by the time the study

was undertaken

Flag : Critical study for SIDS endpoint
02.12.2003 (38)

Species	:	Lactuca sativa (Dicotyledon)
Endpoint	:	growth
Exposure period	:	16 day(s)
Unit	:	mg/l
EC50	:	ca. 1.8 calculated
Method	:	OECD Guide-line 208 "Terrestrial Plants, Growth Test"
Year	:	1993
GLP	:	
Test substance	:	other TS: various nitro- and chlorocompounds but not 4-nitrotoluene

Remark : Lactuca sativa Ravel R2

Result : The 14 d EC50 of Lactuca sativa seedlings (endpoint growth) was measured for various chloro(nitro)benzenes and other compounds including e.g. the isomer 1,2-dichloro-3-nitrobenzene, but not 1,2-dichloro-4-nitrobenzene.
For 1,2-dichloro-3-nitrobenzene the EC50 was > 0.32 and < 1 mg/l after 16 to 21 days. An equation for the calculation of the EC was derived (log EC50 = -0.46 log Kow + 2.38 [µmol/l]), which was used to calculate the EC50 of 1,2-dichloro-4-nitrobenzene (Log kow = 3.04) to be about 1.8 mg/l.

Test condition : - 10 Seeds per tray. Trays covered with glass plates. Temperature 21 °C, photoperiod 16 h light / 8 h dark, light intensity 6500 lux, humidity 40 - 80 %
- After one week, 5 seedlings with roots longer than 3 cm were transferred to 1 l pots filled with nutrient solution and treated with the test substance.
- Solutions renewed 3 times a week to achieve semistatic exposure
- Duplicate pots for each concentration including controls
- Shoots harvested 16 or 21 d after sowing (experiments were performed in 2 laboratories yielding similar results)
- Determination of fresh weight
- Oxygen concentration and pH of nutrient solution monitored at each medium change
- 1,2-Dichloro-4-nitrobenzene was not tested but wide range of other chloro- and nitrocompounds including 1,2-dichloro-3-nitrobenzene
- The authors derived an equation for the QSAR for the relationship between log EC50 (µmol/l) and the log Kow:
EC50 = -0.46 log Kow + 2.38 [µmol/l]

Reliability : (2) valid with restrictions
Guideline study with acceptable restrictions

Flag : Critical study for SIDS endpoint
12.11.2003 (84)

Species	:	other terrestrial plant: Cucumis sativus var. National Pickling
Endpoint	:	growth
Exposure period	:	6 day(s)
Unit	:	mg/l
EC50	:	55.7
Method	:	other: germination and growth of seedlings in sand
Year	:	1962
GLP	:	no data
Test substance	:	no data

Result : Reduction in fresh weight after 6 days (compared to controls) was used to measure inhibition.
Although the author wrote ED50 (effective dose), he apparently measured and reported EC50 (endpoint growth). Values were given in µmol/l

Test condition : The test was performed under the following conditions:
- Pregermination of the seeds for 24 hours
- Test chemical suspended in 0.1 strength Hoagland's solution

- 15 seeds were cultured in each tall form beaker (150ml) containing 220 g (dry weight) of sand
- 36 ml of test chemical was added
- Incubation in the dark in a Mangelsdorf seed-germinator, temperature 25 °C, relative humidity approaching 100 %, incubation time 6 days

Reliability : (2) valid with restrictions
Study with acceptable restrictions, up to date method by the time the study was undertaken

22.10.2003 (38)

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

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 vitro
Type	:	Metabolism
Species	:	
Number of animals		
Males	:	
Females	:	
Doses		
Males	:	
Females	:	
Vehicle	:	
Method	:	other: see freetext TC
Year	:	1961
GLP	:	no
Test substance	:	other TS: 1,2-dichloro-4-nitrobenzene, no data on purity
Result	:	A stepwise conversion of 3,4-dichloronitrobenzene into N-acetyl-S-(2-chloro-4-nitrophenyl)-L-cysteine was shown. First reaction was the replacement of the para chlorine atom by GSH to form S-(2-chloro-4-nitrophenyl)glutathione. Kidney homogenates removed glycine and glutamic acid from S-(2-chloro-4-nitrophenyl)glutathione to form S-(2-chloro-4-nitrophenyl)-L-cysteine, which was further acetylated by liver slices to its corresponding mercapturic acid.
Test condition	:	Incubation of the following reaction mixtures at 37 degrees C for 1 h: 1. Incubation of 3,4-dichloronitrobenzene with soluble rat liver fraction, GSH, cysteine or acetylcysteine. 2. S-(2-chloro-4-nitrophenyl)glutathione and kidney homogenate 3. S-(2-chloro-4-nitrophenyl)cysteine and slices of rat liver Then reaction mixtures were studied by paper chromatography.
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific priciples, acceptable for assessment
Flag	:	Critical study for SIDS endpoint

07.01.2004 (85)

In Vitro/in vivo	:	In vivo
Type	:	Excretion
Species	:	guinea pig
Number of animals		
Males	:	
Females	:	
Doses		
Males	:	
Females	:	
Vehicle	:	water
Route of administration	:	gavage
Exposure time	:	
Product type guidance	:	
Decision on results on acute tox. tests	:	The single application of 3,4-dichloronitrobenzene caused anorexia but the animals recovered within 3 days.
Adverse effects on prolonged exposure	:	
Half-lives	:	1st:

2nd:
3rd:

Toxic behaviour :
Deg. product :
Method : other: see freetext TC
Year : 1959
GLP : no
Test substance : other TS: 1,2-dichloro-4-nitrobenzene, no data on purity

Result : About 3 % (range 2 - 5 %) of the applied dose was excreted as mercapturic acid (N-acetyl-S-(2-chloro-4-nitrophenyl)-L-cysteine) in urine via 24 hrs.
Test condition : -number/sex of animals: no data
-body weight: ca. 500 g
-number of experiments: 5
-applied dose: 1040 µmol/kg bw (ca. 200 mg/kg bw)

no further information available
Reliability : (2) valid with restrictions
Some study details are missing. However, method and results are sufficiently described.
Flag : Critical study for SIDS endpoint
07.01.2004 (86)

In Vitro/in vivo : In vivo
Type : Excretion
Species : rabbit
Number of animals
Males :
Females :
Doses
Males :
Females : 400 mg/kg bw
Vehicle : water
Route of administration : gavage
Exposure time :
Product type guidance :
Decision on results on acute tox. tests :
Adverse effects on prolonged exposure :
Half-lives : 1st:
2nd:
3rd:
Toxic behaviour :
Deg. product :
Method : other: see freetext TC
Year : 1957
GLP : no
Test substance : other TS: 1,2-dichloro-4-nitrobenzene, no data on purity

Result : Results are given as percentages (mean values) of the applied dose excreted via urine within 72 hrs:

1st trial

not absorbed 0.2 (0.1 - 0.4)
mercapturic acid 39 (16 - 70)
45 (30 - 56) [colorimetric method]
glucuronide 13 (7 - 23)
sulphate ester 12 (9 - 13)
dichloroaniline (free) 17 (8 - 23)

dichloroaniline (combined) 5 (0 - 11)
'catechols' 2 (1 - 2)
azo/oxy compounds 2
Total amount 94
(excl. 'catechols')

2nd trial

number of animals examinded	isolated compound	% of applied dose
10	3,4-dichloroaniline	5
12	N-acetyl-S-(2-chloro-4-nitrophenyl)-L-cysteine	17
6	3,4,3',4'-tetrachloroazoxy-benzene	2
12	2-aminodichlorophenol X (acetyl der.)	1

Test condition : 1st trial

-number of animals: no data
-body weight: 2000 - 3000 g
-number of experiments: 3
-applied dose given with 1000 mg/animal

2nd trial

-number of animals: 6 - 12
-body weight: 2000 - 3000 g
-applied dose given with 1000 mg/animal

no further information available

Reliability : (2) valid with restrictions
Some study details are missing. However, method and results are sufficiently described.

Flag : Critical study for SIDS endpoint

07.01.2004 (87)

In Vitro/in vivo : In vivo
Type : Excretion
Species : guinea pig
Number of animals
Males :
Females :
Doses
Males :
Females :
Vehicle : water
Route of administration : gavage
Exposure time :
Product type guidance :
Decision on results on acute tox. tests : The single application of 3,4-dichloronitrobenzene caused anorexia.
Adverse effects on prolonged exposure :
Half-lives : 1st:
2nd:

3rd:
Toxic behaviour :
Deg. product :
Method : other: see freetext TC
Year : 1959
GLP : no
Test substance : other TS: 1,2-dichloro-4-nitrobenzene, no data on purity

Result : Results are given as percentages (mean values) of the applied dose excreted via urine within 24 hrs:

mercapturic acid (N-acetyl-S-(2-chloro-4-nitrophenyl)-L-cysteine): 3 (2 - 5)
sulphate ester: 12 (7 and 17)
3,4-dichloroaniline (free/combined): 5 (4 - 5)

In two animals examined no 3,4-dichlorobenzene or 3,4-dichloroaniline was detected in the faeces during 72 hrs after dosing.

Test condition : -number/sex of animals: no data
-body weight: ca. 500 g
-number of experiments: 2-5
-applied dose: 1040 uM/kg bw (ca. 200 mg/kg bw)

no further information available

Reliability : (2) valid with restrictions
Some study details are missing. However, method and results are sufficiently described.

Flag : Critical study for SIDS endpoint

07.01.2004 (88)

In Vitro/in vivo : In vivo
Type : Excretion
Species : rat
Number of animals
Males :
Females :
Doses
Males :
Females : I. 1820 µmole/kg bw (ca. 350 mg/kg bw); II. 1300 or 1820 µmole/kg bw (ca. 250 or 350 mg/kg bw)
Vehicle : water
Route of administration : gavage
Exposure time :
Product type guidance :
Decision on results on acute tox. tests :
Adverse effects on prolonged exposure :
Half-lives : 1st:
2nd:
3rd:
Toxic behaviour :
Deg. product :
Method : other: see freetext TC
Year : 1959
GLP : no
Test substance : other TS: 1,2-dichloro-4-nitrobenzene, no data on purity

Result : I. About 19 % (range 4 - 29 %) of the applied dose was excreted as mercapturic acid in urine within 24 hrs.
II. The maximum rate for excretion of mercapturic acid via urine 2 - 6 days after dosing with 1300 or 1820 µmole/kg bw was given with 10 or 21 µmole/kg bw/h.

Test condition : -number of animals: no data

-body weight: ca. 200 g
-number of experiments: 12

no further information available

Reliability : (2) valid with restrictions
Some study details are missing. However, method and results are sufficiently described.

Flag : Critical study for SIDS endpoint

07.01.2004 (89)

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	= 625 mg/kg bw
Species	:	rat
Strain	:	Wistar
Sex	:	male
Number of animals	:	10
Vehicle	:	other: sesame oil
Doses	:	250, 400, 630, 1000 or 1250 mg/kg bw
Method	:	other: see freetext TC
Year	:	1975
GLP	:	no
Test substance	:	other TS: 1,2-dichloro-4-nitrobenzene, no data on purity

Result : Mortality:
250 mg: 0/10 rats; 400 mg: 1/10 rats; 630 mg: 5/10 rats; 1000 mg: 9/10 rats; 1250 mg: 10/10 rats
Deaths occurred within 1-3 days after application.
Signs of intoxication:
moribund animals showed disorders of balance, reduced general concition and died in prone positition.
Gross pathological examination:
The macroscopic examination of surviving and dead animals gave no adverse effects.

Test condition : test procedure:
Male animals were chosen because of their higher sensivity in preliminary examinations
rat boedy weight at the start of the test: 91 - 121 g
rats received no feed 16 hours before administration of TS and 2 hours after application of TS
TS was applied as 4 % solution
observation time: 14 days (during this time: feed and water ad libitum)
record of body weight: once weekly
rats that died during observation time were necropsied and gross examination was performed
surviving rats were killed and examined gross pathologically at termination of the observation period
LD50 was calculated according to the method of Linder and Weber

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific priciples, only male rats were used

Flag : Critical study for SIDS endpoint

07.01.2004 (90)

Type	:	LD50
Value	:	= 800 mg/kg bw
Species	:	rat

Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	21
Vehicle	:	other: corn oil
Doses	:	650,700, 800, 900 mg/kg bw
Method	:	other: see freetext TC
Year	:	1955
GLP	:	no
Test substance	:	other TS: 1,2-dichloro-4-nitrobenzene, no data on purity
Result	:	Mortality: 650 mg/group: 1 male died 700 mg-group: 1 male and 1 female died 800 mg-group: 2 male and 1 female died 900 mg-group: 2 males and 2 females died The survival time was 4 - 36 hrs. Signs of intoxication: Animals showed lethargy soon after dosing followed by salivation, collapse and coma. Gross pathological examination: At autopsy pulmonary hyperaemia and jaundice-like liver discoloration were noted, while kidneys appeared normal.
Test condition	:	10 males and 11 females: 650 mg-group: 3 males and 2 females 700 mg-Group: 2 males and 3 females 800 mg-group: 3 males and 3 females 900 mg-group: 2 males and 3 females TS given as 50 % corn oil solution record of surviving time (detailed data not given) and signs of intoxication gross pathological examination calculation of LD50 (details not given)
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific priciples, no data on purity of TS and no GLP
Flag	:	Critical study for SIDS endpoint

07.01.2004 (91)

Type	:	LD50
Value	:	= 950 mg/kg bw
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	5
Vehicle	:	other: no vehicle was used
Doses	:	631, 794, 1000, 1260 mg/kg bw
Method	:	other: see freetext TC
Year	:	1978
GLP	:	no
Test substance	:	other TS: technical grade: 85 % 1.2-dichloro-4-nitrobenzene and 15 % 2,3-dichloronitrobenzene
Result	:	-Signs of intoxication: weight loss (one to three days in survivors), increasing weakness, salivation, ocular discharge, collapse, death -Time to death: 1-2 d -mortality: 631 mg-group: 0/3 males, 0/2 females, summary: 0/5 794 mg-group: 1/2 males, 0/3 females, summary: 1/5 1000 mg-group: 2/3 males and 1/2 females, summary: 3/5 1260 mg/group: 2/2 males, 3/3 females, summary: 5/5 -Gross autopsy:

decedents: haemorrhagic lungs, liver discoloration, in some cases darkened spleen, acute gastrointestinal inflammation,
survivors (14 d): viscera appeared normal

Test condition : -Weight at study initiation: 230 - 245 g
2-3 males and 2-3 females per group
single oral dose of undiluted warmed to 40°C TS
Method for calculation of the median lethal dose is not explained

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

Flag : Critical study for SIDS endpoint

07.01.2004 (92)

Type : LD50
Value : = 643 mg/kg bw
Species : rat
Strain : no data
Sex : no data
Number of animals :
Vehicle : no data
Doses : no data
Method : other: no data
Year : 1972
GLP : no
Test substance : other TS: no data on purity

Result : LD50: 608 - 680 mg/kg bw
Reliability : (4) not assignable
Data from handbook or collection of data

08.04.2003 (93)

Type : LD50
Value : = 885 mg/kg bw
Species : rat
Strain : no data
Sex : no data
Number of animals :
Vehicle : no data
Doses : no data
Method : other: no data
Year : 1985
GLP : no data
Test substance : other TS: no data on purity

Reliability : (4) not assignable
Data from handbook or collection of data

08.04.2003 (33)

Type : LD50
Value : = 1568 mg/kg bw
Species : rat
Strain : no data
Sex : no data
Number of animals :
Vehicle : no data
Doses : no data
Method : other: no data
Year : 1982
GLP : no data
Test substance : other TS: no data on purity

Reliability : (4) not assignable
Data from handbook or collection of data
08.04.2003 (94)

Type : LD50
Value : 1600 - 3200 mg/kg bw
Species : rat
Strain : no data
Sex : no data
Number of animals : 10
Vehicle : other: application as 10 % solution in 2 % NaCS
Doses : 200 - 3200 mg/kg bw
Method : other: no data
Year : 1986
GLP : no data
Test substance : other TS: purity ca. 100 %

Result : Symtoms:
labored respiration, cyanosis, fsrk eyes, prostration
Time of death:
45 min to 2 days
Reliability : (4) not assignable
Data from handbook or collection of data: no description of the method
10.07.2003 (95)

Type : LD50
Value : = 1384 mg/kg bw
Species : mouse
Strain : no data
Sex : no data
Number of animals :
Vehicle : no data
Doses : no data
Method : other: no data
Year : 1982
GLP : no data
Test substance : other TS: no data on purity

Reliability : (4) not assignable
Data from handbook or collection of data
08.04.2003 (94)

Type : LD50
Value : 800 - 1600 mg/kg bw
Species : mouse
Strain : no data
Sex : no data
Number of animals : 10
Vehicle : other: application as 10 % solution in 2 % NaCS
Doses : 200 - 3200 mg/kg bw
Method : other: no data
Year : 1986
GLP : no data
Test substance : other TS: purity ca. 100 %

Result : Symptoms:
decrease in activity, slow respiration, dark eyes, cyanosis, slight tremor, prostration, yellow-orrage urine
time of death: 1 day
Reliability : (4) not assignable
Data from handbook or collection of data: no description of the method

25.06.2003 (95)

Type	:	other: Formation of methaemoglobin
Value	:	
Species	:	cat
Strain	:	no data
Sex	:	female
Number of animals	:	2
Vehicle	:	other: sesame oil
Doses	:	50 mg/kg bw
Method	:	other: see freetext TC
Year	:	1975
GLP	:	no data
Test substance	:	other TS: 1,2-dichloro-4-nitrobenzene, no data on purity
Result	:	Slight increased methaemoglobin formation: only in cat 1: before treatment: 0%, 24 hrs post treatment maximum: 0.4 % Heinz bodies: cat 1: from 6.5 % before treatment up to 39 % maximum at 24 hrs; cat 2: from 9 % before treatment(b.t.) up to 25 % 3 hrs post treatment [normal values were reached within 21 days p.a.]; absolute increase in leucocytes[10 exp3] (maximum value 3 hrs post treatment: cat 1: 33 versus (b.t.) 10.1, cat 2: 21.9 versus (b.t.) 14.0 strong increase in neutrophilic granulocytes: cat 1, max. value 7 hrs p.a.: 72 % versus b.t. 32 %, cat 2, max. value 3 hrs p.a.: 61 % versus 22 %, decrease in lymphocytes: cat 1, min. value 7 hrs p.a.: 22 % versus b.t. 61%, cat 2, min. value 3 hrs p.a.: 38 % versus b.t. 73 %
Test condition	:	TEST ORGANISMS: -2 female cats -Weight at study initiation: 2106 or 2465 g -controls: no Determination of haemoglobin, erythrocytes, leucocytes, haematocrit, methaemoglobin, and Heinz bodies: before administration of TS and from 1 - 48 hrs. p.a. (Heinz bodies up to 28 days)
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific priciples

07.01.2004 (96)

Type	:	LD100
Value	:	= 500 mg/kg bw
Species	:	guinea pig
Strain	:	no data
Sex	:	no data
Number of animals	:	
Vehicle	:	other: gum acacin (5 % solution)
Doses	:	200, 300, 400 and 500 mg/kg bw
Method	:	other: no data
Year	:	1957
GLP	:	no
Test substance	:	other TS: no data on purity, m.p.: 40.2°C
Result	:	Dose (mg/kg) Mortality 200 0/1 300 0/3 400 1/2 500 3/3
Reliability	:	(4) not assignable Data from handbook or collection of data

10.07.2003 (97)

5.1.2 ACUTE INHALATION TOXICITY

Type	:	other
Value	:	
Species	:	rat
Strain	:	other: albino
Sex	:	no data
Number of animals	:	40
Vehicle	:	
Doses	:	3, 6 or 10 mg/m³
Exposure time	:	4 hour(s)
Method	:	other: no data
Year	:	1969
GLP	:	no
Test substance	:	other TS: no data on purity
Result	:	<= 6 mg/m³: no adverse effects were reported 10 mg/m³: no "external" signs of intoxication; increased number of Heinz bodies and reticulocytes and decreased activity (13 % lower) of blood peroxidase 24 hrs after exposure
Reliability	:	(4) not assignable Documentation insufficient for assessment

08.04.2003 (98)

Type	:	other
Value	:	
Species	:	rat
Strain	:	no data
Sex	:	male
Number of animals	:	4
Vehicle	:	other: air
Doses	:	
Exposure time	:	6 hour(s)
Method	:	other: see freetext ME
Year	:	1955
GLP	:	no
Test substance	:	other TS: no data on purity
Method	:	4 mature male rats were placed in a small metal drum with a glass window and exposed for 6 hours to a saturated atmosphere of 3,4-Dichloronitrobenzene produced by passing a stream of compressed air through a container of the fluid located in the chamber. Pressure of the air forced through the compound by means of small openings in a glass tube was 22 pounds per square inch. average temperature inside the chamber: 79.0 °F average relative humidity inside the chamber: 66.5 % Observations were made for outward signs of toxicity and macroscopic examination of the viscera of those animals succumbing was carried out.
Remark	:	Author's opinion: Skin absorption of the dense fog may have contributed to the fatal results although marked pulmonary congestion, observed at autopsy, was probably sufficient to cause death.
Result	:	Mortality:Three of the rats died before termination of exposure (within 3-5 hours) and the fourth succumbed overnight. signs of intoxication: severe nasal and ocular irritation, excessive salivation, pawing at the face,

labored breathing soon after exposure

Reliability : (4) not assignable
Documentation insufficient for assessment

25.06.2003 (91)

Type : other: LC
Value : < 35 mg/m³
Species : rat
Strain : no data
Sex : no data
Number of animals :
Vehicle : no data
Doses : no data
Exposure time : 2 hour(s)
Method : other: no data
Year : 1982
GLP : no data
Test substance : other TS: no data on purity

Reliability : (4) not assignable
Data from handbook or collection of data

08.04.2003 (94)

Type : other: LC
Value : < 35 mg/m³
Species : rat
Strain : no data
Sex : no data
Number of animals :
Vehicle : no data
Doses : no data
Exposure time : 4 hour(s)
Method : other: no data
Year : 1982
GLP : no data
Test substance : other TS: no data on purity

Reliability : (4) not assignable
Data from handbook or collection of data

08.04.2003 (94)

Type : other
Value :
Species : mouse
Strain : no data
Sex : no data
Number of animals : 60
Vehicle :
Doses : 3, 6 or 10 mg/m³
Exposure time : 4 hour(s)
Method : other: no data
Year : 1969
GLP : no
Test substance : other TS: no data on purity

Result : <= 6 mg/m³: no adverse effects were reported

10 mg/m³: no "external" signs of intoxication; increased number of Heinz bodies (from 3.2 to 16.8 %) and reticulocytes (from 6.6 to 26.7 %) and decreased activity (30 % lower) of blood peroxidase 24 hrs after exposure

Reliability : (4) not assignable
Documentation insufficient for assessment
08.04.2003 (98)

5.1.3 ACUTE DERMAL TOXICITY

Type : LD50
Value : > 2000 mg/kg bw
Species : rat
Strain : Wistar
Sex : female
Number of animals : 6
Vehicle : other: sesame oil
Doses : 2000 mg/kg bw
Method : other: see freetext TC
Year : 1975
GLP : no
Test substance : other TS: 1,2-dichloro-4-nitrobenzene, no data on purity

Result : Mortality: 0/6
The dermal application caused no irritation and the
macroscopic examination after 14 d gave no adverse effects.
Test condition : TS (40 % solution) was applied on the clipped skin of the back under occlusive conditions
over 24 hrs, then the skin was washed,
post application observation period: 14 d
feed and water ad libitum
observation for signs of intoxication
rats were weighed daily (excluding weekend)
after termination of the observation time: rats were necropsied and gross pathologically examined
Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific priciples, only femalerats were used
Flag : Critical study for SIDS endpoint
07.01.2004 (99)

Type : LDLo
Value : = 950 mg/kg bw
Species : rabbit
Strain : other: albino
Sex : male/female
Number of animals : 6
Vehicle : no data
Doses : 360 - 2900 mg/kg bw
Method : other: no data
Year : 1955
GLP : no
Test substance : other TS: 1,2-dichloro-4-nitrobenzene, no data on purity

Result : Animals became lethargic and lost appetite.
Mortality:
Animals dosed with 950, 1450, 2900 mg/kg bw died; the survival time was 24-48 hrs.
Survivors:
returned to normal activity within one week. Animals dosed with 540 and 720 mg/kg bw showed reduced bodyweight of 5 and 7 %, respectively, 5 days after dosing.
At autopsy liver discoloration and indication of blood changes with possible

formation of methaemoglobin were noted.

Test condition	:	Occlusive application of undiluted TS to intact skin. Males: 360, 5720, 1450, 2900 mg/kg bw Females: 540, 950 mg/kg bw
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific priciples, acceptable for assessment
Flag	:	Critical study for SIDS endpoint

07.01.2004 (91)

Type	:	LD100
Value	:	= 2000 mg/kg bw
Species	:	rabbit
Strain	:	no data
Sex	:	no data
Number of animals	:	2
Vehicle	:	other: Dowanol 50B
Doses	:	2000 mg/kg bw as 25 % solution, applied volume not mentioned
Method	:	other: exposure duration: 24 hours
Year	:	1957
GLP	:	no
Test substance	:	other TS: eutectic mixture: 1,2-dichloro-4-nitrobenzene and 1,2-dichloro-3-nitrobenzene
Result	:	Mortality: 2/2; animals died from 2 to 5 days after application
Reliability	:	(4) not assignable documentation insufficient for assessment

10.07.2003 (97)

Type	:	LDLo
Value	:	794 mg/kg bw
Species	:	rabbit
Strain	:	New Zealand white
Sex	:	male/female
Number of animals	:	1
Vehicle	:	other: no vehicle was used
Doses	:	200, 316, 501, 794, 1260, 2000, 5010 mg/kg bw
Method	:	other: see freetext TC
Year	:	1978
GLP	:	no
Test substance	:	other TS: technical grade: 85 % 3,4-dichloronitrobenzene and 15 % 2,3-dichloronitrobenzene
Result	:	-Signs of intoxication: weight loss (2-3 days in survivors), increasing weakness, nasal discharge, collapse, death -Time to death: 2-3 d 200, 316, 501 mg-group: no rabbit died 794, 1260, 2000, 5010 mg-group: the exposed rabbit/dose died -Gross autopsy: decedents: haemorrhagic lungs, liver discoloration, enlarged gall bladder, darkened spleen and kidneys, gastrointestinal inflammation survivors (14 d): viscera appeared normal
Test condition	:	-Weight at study initiation: 2100 - 2600 g -1 male or 1 female per group TS was applied undiuted but warmed to 40 °C -Exposure: 24 hrs observation time: 14 days observation for signs of intoxications gross autopsy for decedents and survivors
Reliability	:	(4) not assignable

documentation insufficient for assessment, only 1 rabbit per dose group
10.07.2003 (92)

Type	:	other
Value	:	
Species	:	rabbit
Strain	:	no data
Sex	:	no data
Number of animals	:	2
Vehicle	:	other: 25 % solution in Dowanol 50B
Doses	:	2000 mg/kg bw
Method	:	other: no data
Year	:	1957
GLP	:	no
Test substance	:	other TS: no data on purity
Result	:	Exposure: 24 hrs Mortality: 2/2 within 2-5 days
Reliability	:	(4) not assignable Documentation insufficient for assessment

08.04.2003 (97)

Type	:	LD50
Value	:	= 790 mg/kg bw
Species	:	cat
Strain	:	no data
Sex	:	no data
Number of animals	:	
Vehicle	:	no data
Doses	:	no data
Method	:	other: no data
Year	:	1982
GLP	:	no data
Test substance	:	other TS: no data on purity
Reliability	:	(4) not assignable Data from handbook or collection of data

08.04.2003 (94)

Type	:	LD50
Value	:	> 1000 mg/kg bw
Species	:	guinea pig
Strain	:	no data
Sex	:	no data
Number of animals	:	3
Vehicle	:	other: water
Doses	:	250 - 1000 mg/kg bw moistioned with water
Method	:	other: no data
Year	:	1986
GLP	:	no data
Test substance	:	other TS: purity ca. 100 %
Result	:	no animal died signs of intoxication: slight to moderate edema, up to 2erythema with area narcotic and hemorrhagic, slight desquamation and scattered lt . to heavy eschar formation at 1week Lt. scattering at 2 weeks
Reliability	:	(4) not assignable Data from handbook or collection of data, no detailed description of the method used

25.06.2003 (95)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Type	:	LD50
Value	:	400 mg/kg bw
Species	:	rat
Strain	:	no data
Sex	:	no data
Number of animals	:	10
Vehicle	:	
Doses	:	200-3200 mg/kg bw as 10 % solution in 2 % NaCS
Route of admin.	:	i.p.
Exposure time	:	
Method	:	other: no further data
Year	:	1991
GLP	:	no data
Test substance	:	other TS: no data on purity
Result	:	Signs of intoxication: labored respiration, cyanosis, dark eyes, prostration time of death: 8 hrs to 1 day
Reliability	:	(4) not assignable application method is not relevant for the human situation

25.06.2003 (95)

Type	:	LD50
Value	:	400 - 800 mg/kg bw
Species	:	mouse
Strain	:	no data
Sex	:	no data
Number of animals	:	10
Vehicle	:	
Doses	:	200-3200nmg/kg bw as 10 % solution in 2 % NaCS
Route of admin.	:	i.p.
Exposure time	:	
Method	:	other: no further information
Year	:	1991
GLP	:	no data
Test substance	:	other TS: no data on purity
Result	:	signs of intoxication: decrease in activity, slow respiration, dark eyes, cyanosis, slight tremor, prostration, yellow-orange urine time to death: 4.5 hours to 1 day
Reliability	:	(4) not assignable application method is not relevant for the human situation

25.06.2003 (95)

5.2.1 SKIN IRRITATION

Species	:	rabbit
Concentration	:	10 %
Exposure	:	Occlusive
Exposure time	:	24 hour(s)
Number of animals	:	6
Vehicle	:	other: sesame oil
PDII	:	

Result	:	slightly irritating
Classification	:	
Method	:	other: Federal Register 38, No. 187, p. 27019, § 1500.41
Year	:	1973
GLP	:	no
Test substance	:	other TS: 1,2-dichloro-4-nitrobenzene, no data on purity
Result	:	scoring for erythema (maximum value possible: 4) // edema (maximum value: 4) intact/scarrified, 24 hrs-48 hrs-72 hrs - sum rabbit 1: 1/1-1/1-0/0 // 0 - 2 rabbit 2: 1/1-0-0 // 0 - 2 rabbit 3: 1/1-1/1-0 // 0 - 2 rabbit 4: 1/1-0-0 // 0 - 2 rabbit 5: 1/1-1/1-0 // 1/1 - 4 rabbit 6: 1/1-0-0 // 0 - 2 overall: average irritation index (24, 72 hrs): 0.58
Test condition	:	Performance: 6 Himalayan rabbits were housed individually and received feed and water ad libitum body weight: 1.5 - 2 kg at the right (test) and the left flank rabbits were shaved and skin of one side was scarified application of 0.5 ml TS (10 % sesame oil solution) occlussive condition treatment time: 24 hours reading 24, 48, 72 hrs post application
Reliability	:	(2) valid with restrictions no data on purity of TS and no GLP
Flag	:	Critical study for SIDS endpoint
07.01.2004		(100)

Species	:	rabbit
Concentration	:	500 mg
Exposure	:	Semiocclusive
Exposure time	:	4 hour(s)
Number of animals	:	3
Vehicle	:	physiol. saline
PDII	:	
Result	:	not irritating
Classification	:	
Method	:	other: OECD Guide-line 404, see also freetext TC
Year	:	1988
GLP	:	yes
Test substance	:	other TS: 1,2-dichloro-4-nitrobenzene, purity > 99 %
Result	:	scoring for erythema (maximum value possible: 4) // edema (maximum value possible: 4) intact: 30-60 min-24 hrs-48 hrs-72 hrs - sum rabbit 1: 2-1-0-0 - 0.3 // 1-0-0-0 -0 rabbit 2: 1-1-0-0 - 0.3 // 0-0-0-0 - 0 rabbit 3: 1-0-0-0 - 0 // 0-0-0-0 - 0 overall: mean scores for erythema/scabbing: 0.2 mean scores for edema: 0.0
Test condition	:	Performance: 3 New Zealand rabbits, body weight 2.5-3 kg, individual housing, food and tap water ad libitum

TS was moistioned with physiological saline and applied on a patch which was then fixed on the shaved areas of the back. After termination of the exposure period of 24 hrs areas were cleaned with water.
Examination time points for erythema and edema:
30-60 min and 24, 48 or 72 hrs after removal of the patch

Reliability : (1) valid without restriction
Guideline study

Flag : Critical study for SIDS endpoint

07.01.2004 (101)

Species : rabbit
Concentration : undiluted
Exposure : no data
Exposure time : no data
Number of animals : 3
Vehicle : no data
PDII :
Result : irritating
Classification :
Method : other: Draize Test, see also freetext ME
Year : 1955
GLP : no
Test substance : other TS: no data on purity

Method : the scoring method of Draize J.Pharm. Exp. Therap. 82, 1944 was used for evaluation of the skin irritation; observations were made over a period of several days following application of the undiluted compound to intact rabbit skin.

Result : Scores were given as follows:

	2 hrs	24 hrs	48 hrs	72 hrs	120 hrs
animal 1	2	3	2	0	0
animal 2	2	2	1	0	0
animal 3	3	4	3	1	0

Average maximum score: 3/8

Irritation after two hours ranged from mild to mederate redness with very little edema for an average score of 2.3 out of 8.0. redness increased over nighte to an average score of 3.0 with not much change in the degree of edema. the score dropped as the compound disappeared from the skin through absorption and evaporation. 2/3 animals were free of inflammation in 72 hours, wihin 120 hours 3/3 were free of inflammation.

Reliability : (4) not assignable
Documentation of the testprocedure descriptions results are limited, no data on the purity of TS

08.04.2003 (91)

Species : rabbit
Concentration : .5 other: ml
Exposure : no data
Exposure time : 24 hour(s)
Number of animals : 6
Vehicle : other: undiluted
PDII :
Result : highly irritating
Classification :
Method : other: Draize Test, see freetext ME
Year : 1978
GLP : no

Test substance	:	other TS: technical grade: 85 % 3,4-dichloronitrobenzene and 15 % 2,3-dichloronitrobenzene
Method	:	6 New Zwealand albine rabbits Compound applied undissolved but warmed up to 37°C: 0.5 ml
Result	:	Mean scores were given as follows: 4 hrs: 2/8; 24 hrs: 8/8; 48 hrs: 5/8; 72 hrs: 3/8; 168 hrs: 0/8 Average score (24-72 hours): 5.5/8, reversible within 168 hours. Slight defatting effect - skin flaked off in 7 to 10 days. There was no injury in depth.
Reliability	:	(4) not assignable Documentation insufficient for assement

26.06.2003 (92)

Species	:	rabbit
Concentration	:	
Exposure	:	Semiocclusive
Exposure time	:	no data
Number of animals	:	
Vehicle	:	other: see freetext ME
PDII	:	
Result	:	irritating
Classification	:	
Method	:	other: see freetext ME
Year	:	1957
GLP	:	no
Test substance	:	other TS: eutectic mixture: 1.2-Dichloro-4-nitrobenzene and 1,2-Dichloro-3-nitrobenzene
Method	:	TS held in contact with skin by means of cotton pads: (1) undiluted but moistioned with pure 95% ethanol, belly: a) intact skin, 9 applications; b) abraded skin, 3 applications; (2) as 10 % solution in Dowanol 50B, ear(intact skin only), belly; a) intact skin: ear and belly, 9 applications each, b) abraded skin: belly, 3 applications no further experimental details given
Result	:	(1) undiluted (a) intact (b) abraded skin: slight to moderate hyperemia, oedema, necrosis, exfoliation and scabbing, death after 10 days (2) 10 % solution (a) intact: ear: slight exfoliation; belly: slight hyperemia, oedema, exfoliation (b) abraded: slight hyperemia, oedema, exfoliation, scabbing, normal healing
Reliability	:	(4) not assignable Documentation insufficient for assessment

26.06.2003 (97)

5.2.2 EYE IRRITATION

Species	:	rabbit
Concentration	:	10 %
Dose	:	.1 ml
Exposure time	:	24 hour(s)
Comment	:	rinsed after (see exposure time)
Number of animals	:	6

Vehicle : other: sesame oil
Result : not irritating
Classification :
Method : other: according FDA directive: Federal Register 38, No. 187, see also freetext ME
Year : 1973
GLP : no
Test substance : other TS: 1,2-dichloro-4-nitrobenzene, no data on purity

Method : Performance:
6 Himalayan rabbits were housed individually and received feed and water ad libitum
second eye served as control
reading 1, 7, 24 hours post application.
Grading :
cornea 0-4
iris 0-2
conjunctiva (redness 0-3, chemosis 0-4)
observation time: up to 24 hours post application
Result : no findings
Reliability : (2) valid with restrictions
no data on purity and no GLP
Flag : Critical study for SIDS endpoint
07.01.2004 (100)

Species : rabbit
Concentration : undiluted
Dose : 100 other: mg
Exposure time : 24 hour(s)
Comment : rinsed after (see exposure time)
Number of animals : 3
Vehicle : none
Result : slightly irritating
Classification :
Method : other: OECD Guide-line 405, see also freetext TC
Year : 1987
GLP : yes
Test substance : other TS: 1,2-dichloro-4-nitrobenzene, purity > 99 %

Result : AVERAGE SCORE
rabbit1/2/3, 1h-24 hrs-48 hrs-72 hrs - mean score:
-Cornea: 0/0/0-0/0/0-0/0/0-0/0/0 - 0 (max. possible: 4)
-Iris: 1/1/1-0/1/0-0/0/0-0/0/0 - 0.1 (max possible: 2)
-Conjunctivae (Redness): 2/2/3-2/3/2-1/2/2-0/0/0 - 1.3 (max. possible: 3)
-Conjunctivae (Chemosis): 2/2/2-0/1/1-0/0/0-0/0/0 - 0.2 (max. possible: 4)
discharge: all rabbits at 1 hour, rabbit 2 and 3 up to 24 hours post reading
REVERSIBILITY: within 72 hrs p.a.
Test condition : Performance:
3 New Zealand rabbits, body weight 2.9-3.5 kg, individual housing, food and tap water ad libitum
24 hours after application of TS and at all reading time points when discharge from the treated eye was observed, the eyes were rinsed with physiological saline
Examination: 1, 24, 48 or 72 hrs p.a.
Reliability : (1) valid without restriction
Guideline study
Flag : Critical study for SIDS endpoint
07.01.2004 (102)

Species : rabbit
Concentration : undiluted

Dose	:	100 other: mg
Exposure time	:	24 hour(s)
Comment	:	other: rinsed after (see exposure time) with pysiological saline
Number of animals	:	6
Vehicle	:	none
Result	:	irritating
Classification	:	
Method	:	other: according FDA directive: Federal Register 38, No. 187, see also freetext ME
Year	:	1973
GLP	:	no
Test substance	:	other TS: 1,2-dichloro-4-nitrobenzene, no data on purity
Method	:	Performance: 6 Himalayan rabbits were housed individually and received feed and water ad libitum second eye served as control reading 1, 7, 24 48 and 72 hours post application and at day 7 post application. grading cornea 0-4 iris 0-2 conjunctiva (redness 0-3, chemosis 0-4)
Result	:	cornea: 1h, 7h: 3/6 (grade 1); 24h: 2/6 (grade 2) 1/6 (grade 1); 48h, 72h: 3/6 (grade 1); day 7: no findings iris: no findings conjunctiva, redness: 1h: 1/6 (grade 2), 1/6 (grade 1), 4/6 (grade 3); 7h: 6/6 (grade 3); 24h: 5/6(grade 3),1/6 (grade 2); 48h, 72h: 6/6 (grade 1) day 7: no findings conjunctiva, chemosis: 1h: 6/6 (grade 1); 7h: 4/6 (grade 2), 1/6 (grade 3) 1/6 (grade 1); 24h: 4/6 (grade 1), 1/6 (grade 3); 48h,72h, day 7: no findings
Reliability	:	(2) valid with restrictions no data on purity and no GLP
Flag	:	Critical study for SIDS endpoint

07.01.2004 (100)

Species	:	rabbit
Concentration	:	undiluted
Dose	:	.1 ml
Exposure time	:	24 hour(s)
Comment	:	
Number of animals	:	6
Vehicle	:	none
Result	:	slightly irritating
Classification	:	
Method	:	other: Draize test, see also freetext ME
Year	:	1978
GLP	:	no
Test substance	:	other TS: technical grade: 85 % 3,4-dichloronitrobenzene and 15 % 2,3-dichloronitrobenzene
Method	:	6 New Zealand Albino rabbits 0.1 ml TS applied undiluted but warmed up to 37°C Exposure time: 24 hours (no data on rinsing) Maxomal Score (Cornea, Iris, Conjunctivae) = 110 observation period: 168 hours
Result	:	Mean scores were given as follows: 1 hour: 10.3 24 hours: 7 48 hours: 1.3 72 hours: 0 Average Score: 2.7/110 Immediate: discomfort was severe with pawing, thrashing about the stocks

and eyes tightly closed
10 min: slight erythema, vera slight edema, copious discharge
1 hr: slight to moderate erythema, very slight edema, copious discharge,
24 hr: areas of barely perdeptible corneal dullness in two instances, slight erythema, slight to moderate discharge
48 hr: gradual improvement
72 hr: all scored zero

Reliability : (2) valid with restrictions
well documented, meets generally accepted scientific standard
Flag : Critical study for SIDS endpoint
07.01.2004 (92)

Species : rabbit
Concentration : undiluted
Dose : .1 ml
Exposure time : unspecified
Comment : no data
Number of animals : 3
Vehicle : no data
Result : moderately irritating
Classification :
Method : other: see freetext ME
Year : 1955
GLP : no
Test substance : other TS: no data on purity

Method : 0.1 ml of undiluted compound was placed in the conjunctival sac of the right eye of each of three albino rabbits and the degree of irritation scored according to Draize. Observation time 120 hours
Result : Scores were given as follows:
(1h / 24hrs / 72hrs / 120hrs)

animal 1: 18 / 12 / 8 / 0 / 0
animal 2: 12 / 8 / 4 / 0 / 0
animal 3: 20 / 15 / 8 / 4 / 0

Average maximum score: 16.6/110

There was moderate immediate discomfort leading to an average score after 1 hr of 16.6 out of possible 110. Lacrimation together with erythema and edema were moderate. there was slight cloudiness of the corneal area, but vision was only mildly affected.
Some improvement was noted in 24 hrs when average score was 11.6
A slight degree of inflammation remained in the eye of one rabbit after 72 hours.
All were clear in 5 days.

Reliability : (4) not assignable
Documentation insufficient for assessment
10.07.2003 (91)

Species : rabbit
Concentration : undiluted
Dose :
Exposure time : unspecified
Comment : other: rinsed & not rinsed
Number of animals :
Vehicle : none
Result : slightly irritating
Classification :
Method : other: no data
Year : 1957

GLP	:	no
Test substance	:	other TS: eutectic mixture1,2-dichloro-4-nitrobenzene and 1,2-dichloro-3-nitrobenzene
Result	:	Slight pain and conjunctiva irritation (cleared in 24 hrs)
Reliability	:	(4) not assignable Documentation insufficient for assessment

26.06.2003 (97)

Species	:	rabbit
Concentration	:	10 other: suspension
Dose	:	
Exposure time	:	
Comment	:	other: rinsed & not rinsed
Number of animals	:	
Vehicle	:	other: in propylene glycol
Result	:	slightly irritating
Classification	:	
Method	:	other: no data
Year	:	1957
GLP	:	no
Test substance	:	other TS: eutectic mixture: 1,2-dichloro-4-nitrobenzene and 1,2-dichloro-3-nitrobenzene
Result	:	Slight pain and conjunctiva irritation (cleared in 24 hrs)
Reliability	:	(4) not assignable Documentation insufficient for assessment

26.06.2003 (97)

5.3 SENSITIZATION

Type	:	Guinea pig maximization test
Species	:	guinea pig
Concentration	:	1st: other: see ME 2nd: 3rd:
Number of animals	:	20
Vehicle	:	other: PEG 400
Result	:	not sensitizing
Classification	:	
Method	:	other: OECD Guide-line 406 , see additional freetext ME
Year	:	1981
GLP	:	yes
Test substance	:	other TS: 1,2-dichloro-4-nitrobenzene, purity 99.8 %
Method	:	male guinea pig preliminary dose-finding studies for induction concentration and challenge concentration was performed: as result from these experiments the following doses and procedure were chosen: Induction (intradermal): 5% , 1 week later: Induction (topical): 50% for 48 hours, then cleaning and 3 to 4 weeks later: Challange:(occlussive epicutan) 50% for 24 hours Challenge: (occlussive epicutan) 25 and 12 % for 24 hours, then area was cleaned, and shaved reading 48 and 72 hours after beginning of challenge for: erythema and scars edema

general examination:
mortality, body weight development and clinical observations

Result : no mortality, reduced body weight gain compared to the control group
Reliability : (1) valid without restriction
Guideline study
Flag : Critical study for SIDS endpoint
07.01.2004 (103)

Type : Mouse ear swelling test
Species : mouse
Concentration : 1st: Induction .05 other: M open epicutaneous
2nd: Challenge .016 other: M open epicutaneous
3rd:
Number of animals : 5
Vehicle : other: acetone
Result : not sensitizing
Classification :
Method : other: see freetext TC
Year : 1992
GLP : no data
Test substance : other TS: 1,2-dichloro-4-nitrobenzene was recrystallized/redistilled prior to use where purity was suspect (no further information)

Test condition : -Animals: WSP mice (n = 5)
-clipping of 2 x 2 cm on the back
-applications:
induction: 0.2 ml of a 0.05 M solution in acetone on day 1 (dorsal skin)
Challenge: 20 ul of a 0.016 M solution in acetone on day 5 (ear)
as control treatment with acetone alone (no further data)
Reactions were read after further 24, 48, 72 hrs (visual assessment of redness and recording of changes in the ear thickness (via Oditest ODI 20 RK/K gauge)

details of the reading not given
Reliability : (2) valid with restrictions
limited documentation
Flag : Critical study for SIDS endpoint
07.01.2004 (104)

Type : no data
Species : guinea pig
Concentration : 1st: Induction .1 other: ml other: 2.5 ug of TS in 0.1 ml of saline were injected into each of five sites on the clipped back on day 21
2nd: .05 other: ml other: After 90 min each of the same five sites were injected with 0.05 ml FCA containing 2.5 µg Mycobacterium tuberculosis
3rd: Challenge 1 % other: application of a 1 % TS solution in olive oil after 13 days to the animals flanks
Number of animals : 6
Vehicle : other: olive oil
Result : not sensitizing
Classification :
Method : other: according to Maguire HC & Chase MW (1967) J Invest Dermatol 49, 460, see also freetext TC
Year : 1972
GLP : no
Test substance : other TS: 1,2-dichloro-4-nitrobenzene was twice recrystallized (no further information)

Result : No cross-sensitization with 2,4-dinitro-1-chlorobenzene
Test condition : strain: Hartley, albino

sex: male
bodyweight: 200-300 g
-reactions were scored 24 and 48 hrs after challenge

-the procedure was repeated with 2,4-Dinitro-1-chlorobenzene (DNCB) alone, a known sensitizer

-the procedure was repeated with
DNCB - induction and TS - challenge and
TS - induction and DNCB - challenge

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific priciples, acceptable for assessment
Flag : Critical study for SIDS endpoint
07.01.2004 (105)

Type : other: contact-type of eczematous hypersensitivity
Species : human
Concentration : 1st: Induction 10 %
2nd: Challenge 10 %
3rd: Challenge 1 %
Number of animals :
Vehicle : other: acetone
Result : not sensitizing
Classification :
Method : other: according to Wedroff & Dolgoff (1935) Arch Dermat Syph 171, 647 - 664, see freetext TC
Year : 1983
GLP : no
Test substance : other TS: 1,2-dichloro-4-nitrobenzene, no data on purity

Test condition : The authors studied the sensitizing properties of a 10 % solution of 1,2-dichloro-4-nitrobenzene in acetone in 10 female subjects. The TS was applied in acetone to the skin and 28 and 49 days later 0.03 ml of a 10 or 0.01 - 1 % solution in acetone was applied to different areas of skin in like fashion.
Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific priciples, acceptable for assessment
Flag : Critical study for SIDS endpoint
07.01.2004 (106)

Type : no data
Species : guinea pig
Number of animals : 5
Vehicle : other: 1 % in A+D+G.P.fat (not further specified)
Result :
Classification :
Method : other: "Drop on"
Year : 1986
GLP : no data
Test substance : other TS: purity ca. 100 %

Remark : No further information available.
Result : Initial score Final score
24 hrs 48 hrs 24 hrs 48 hrs

1.2 1 1.5 1.0

Results given as "sensitizer of low activity to 2/5 guinea pigs"

Reliability	:	(4) not assignable Data from handbook or collection of data
24.01.2002		(95)
Type	:	other
Species	:	guinea pig
Concentration	:	1st: Induction .1 other: ml other: repeated intradermal injection of a 1 % solution in NaCl [presumably using ethanol to produce a 0.3 % parent solution] into the back over several weeks 2nd: Challenge .1 other: ml other: intradermal injection of a 1 % solution 3rd: Challenge 1 % other: epicutaneous application of a 1 % solution in olive oil
Number of animals	:	
Vehicle	:	
Result	:	not sensitizing
Classification	:	
Method	:	other: no data
Year	:	1935
GLP	:	no
Test substance	:	other TS: no data on purity

Reliability	:	(4) not assignable Experimental details are missing (methodological details taken from other substances included in this study)
26.06.2003		(107)
Type	:	other: Microtubule disassembly
Species	:	other: in vitro study
Number of animals	:	
Vehicle	:	
Result	:	not sensitizing
Classification	:	
Method	:	other
Year	:	1990
GLP	:	no data
Test substance	:	other TS: no data on purity
Result	:	The exposure of the cells to 1,2-dichloro-4-nitrobenzene caused no disassembly of the microtubules (marker for inducing allergic contact dermatitis).
Test condition	:	-cell system: Swiss 3T3 murine fibroblasts and normal diploid human foreskin fibroblasts (strain AG1522) -incubation of the cells with 1,2-dichloro-4-nitrobenzene for 3 hrs -concentration: 100 uM for 3T3 cells or 40 uM for human AG1522 fibroblasts -monitoring of microtubule disassembly via indirect immunofluorescence
Reliability	:	(3) invalid unsuitable test system
26.06.2003		(108)

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-acute
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	oral feed

Exposure period	:	32 days
Frequency of treatm.	:	continuously in diet
Post exposure period	:	no
Doses	:	0, 625, 1250, 2500, 5000 or 10000 ppm (ca. 0, 62.5, 125, 250, 500 or 1000 mg/kg bw)
Control group	:	yes, concurrent vehicle
LOAEL	:	= 625 ppm
Method	:	other: see freetext TC
Year	:	1984
GLP	:	yes
Test substance	:	other TS: commercial grade: 85 % 3,4-dichloronitrobenzene, 15 % 2,3-dichloronitrobenzene
Remark	:	the purpose of the study was to determine the dose levels for a subchronic study.
Result	:	Mortality: 10000 ppm: increased mortality (3/5 males; 5/5 females) within 3-4 weeks; signs of intoxication: emaciation, piloerection; minimal body fat (5/5 f, 3/5 m), lung congestion (1/5 f), porphyria of the eyes (1/5 f) CLINICAL OBSERVATIONS: >= 625 ppm: discoloration of urine; the color was similiar to the test material and increased with dosage >= 1250 ppm: decreased feed intake and >= 2500 ppm decreased body weight gain (at least 15 %) PATHOLOGY: final mean body weights males: >=2500 ppm significantly reduced: 2500 ppm (n=5): 252 g, 5000 ppm (n=5): 200 g, 10000 ppm (n=2): 80 g versus control(n=5): 319 g females: >=1250 ppm significantly reduced: 1250 ppm (n=4): 168 g, 2500 ppm (n=5): 162 g, 5000 ppm (n=5): 156 g versus control (n=5): 190 g pathologic alterations: males: control: 1/5 minimal body fat 625 ppm: 1/5 bilateral hydronephrosis, 2500 ppm: 1/5 rat with marked bilateral hydronephrosis, thickened wall for the urinary bladder and urolithiasis; 1/5 with spleen one third enlarged and dark plum color, nephritis, thickened wall of the urinary bladder, urolthiasis 5000 ppm: 1/5 abnormal dark red spleen 10000 ppm: 1/5 minimal body fat females: control: 1/5 with bilateral mild hydrometra of the uterus 625 ppm: 1/5 mild hydrometra, 1/5 mild hydrometra and multiple purple-red foci in the thymus 1250 ppm: 1/5 para-ovarian cysts left side only 2500 ppm: 1/5 mild uterus hydrometra 5000 ppm: 1/5 very dark colored spleen, mild uterus hydrometra
Test condition	:	TEST ANIMALS and MAINTENANCE: -Age: 5-6 weeks -Weight at study initiation: ---males: 138-152 g; females 121-128 g -Number of animals: 5 rats/sex/group -Diet: prepared fresh weekly -Water: tap water ad libitum -Rooom temperature: 70-74°Fahrenheit -Acclimatisation: 9 days 30 male and 30 female rats were devided into 6 groups (control and 5 treatment groups and individually housed in stainless steel cages CLINICAL OBSERVATIONS AND FREQUENCY: -Clinical signs: twice daily -Mortality: twice daily -Body weight: weekly

-Food consumption: weekly
PATHOLOGY:
Unsheduled deaths: record of gross necropsy alterations
Alterationsat terminal sacrifice:
determination of final body weight
ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
-Macroscopic in-situ: abdominal cavity, posterior vena cava, cranial, thoracic and scrotal cavities, examination of hollow organs

No tissue were retained for microscopy

Statistics:
Analysis of variance,
Dunnett's test (two-tailed),
Bartlett's test
no statistical analysis on necropsy data

Conclusion : 1,2-Dichloro-4-nitrobenzene (commercial grade) was not stable in the diet for a period of one week, so that the feed consumed during the latter part of the week contained less than 625 ppm.
Therefore, the NOAEL may be lower than 625 ppm.

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific priciples, acceptable for assessment although histopathology was not performed

Flag : Critical study for SIDS endpoint

07.01.2004 (109)

Type : Sub-acute
Species : rat
Sex : male/female
Strain : Wistar
Route of admin. : gavage
Exposure period : 28 days
Frequency of treatm. : once per day
Post exposure period : sacrifice on day 29
Doses : 0, 4, 20 or 100 mg/kg bw/day
Control group : yes, concurrent vehicle
NOAEL : = 4 mg/kg bw
Method : OECD Guide-line 407 "Repeated Dose Oral Toxicity - Rodent: 28-day or 14-d Study"
Year : 1992
GLP : yes
Test substance : other TS: 1,2-dichloro-4-nitrobenzene, purity 99 %

Remark : For reproductive organ evaluation: see Chapter 5.8.3
Result : OBSERVATIONS:
No death occurred throughout the study
unspecific signs of intoxication: 100 mg-group, m/f: irregular respiration, stilted gain, >= 20 mg/kg bw/day, m/f: increased salivation;
all groups: body weight gain was not impaired, food consumption unaffected, >=20 mg/kg bw/day(m/f): slightly increased water intake (not significant, not dose dependant)
URINALYSIS:
dark yellow discolouration of urine:
m, from 20 mg/kg bw/day onwards; f, at 100 mg/kg bw/day;
pH-Value:
f, 100 mg-group, significantly decreased: pH=5.4 versus pH=6.1 (control)
HEMATOLOGY:
4 mg/kg bw/day:

male: significant decrease in erythrocyte counts (6.98 10E12/l versus 7.63 10E12/L [historical control: 6.34-8.95 10E/L])
20 mg/kg bw/day:
male: significant decrease in erythrocyte counts (6.87 10E12/L versus 7.63 10E12/L of concurrent control) and haematocrit (0.42 UNITY versus 0.46 UNITY of control);
female: significant increase in MCV (62 10E-15L versus 58 10E-15L)
100 mg/kg bw/day:
m: decrease in erythrocyte values (6.34 10E12/L versus 7.63 10E12/L of control), decrease in
haematocrit (0.42/0.39 UNITY versus 0.46/0.41 UNITY of control), decrease in haemoglobin (140/131 g/L versus 149/138 g/L of control), increase in MCV values 67/66 10E-15L versus 60/58 10E-15L of control), reticulocyte counts (0.077/0.080 UNITY versus 0.011/0.008 UNITY of control);
CLINICAL CHEMISTRY:
male: significant increase in sodium- and chlorid-ions when compared to concurrent control, which were, however, within the historicol control values of this strain:
control//4/20/100 mg/kg bw/day//historical control range:
Sodium: 135//139/142//142//132-149 mmol/L, Chlorid: 98//
101/102/102//95-106 mmol/L
20 mg/kg bw:
increase in alk. phosphatase (f: 261 U/L versus 175 U/L of control)
100 mg/kg bw/day:
increase in urea values (m/f: 8.44/8.17 mmol/L versus 5.62/6.80 mmol/L of control)m [indicative for an
impaired kidney function; however, no histopathological
correlates were found]; increase in ALAT(GPT) (m: 54 U/L versus 44 U/L), increase in alk. phosphatase (f: 267 U/L versus 175 U/L of control)
ORGAN WEIGHTS:
absolut organ weights not affected, rel. organ weights:
20 mg/kg bw
increased relative liver weight (m: 4.545 % versus 4.036 % of control),
100 mg/kg bw/day
increase in rel. liver weight (m/f: 4.571/4815 % versus 4.036/3.926 % of control), increased rel. spleen weight (m/f: 0.321/0.330 % versus 0.189/0.234 % of control);
PATHOLOGY:
100 mg/kg bw, spleen: 5/5 m and 5/5 f, dark discolouration
spleen:
m(grading)-f(grading), low, mid, high dose versus control:
congestion
low: 0/5(0) - 0/5(0), mid: 3/5(slight) - 5/5(slight), high: 5/5(moderate) - 5/5(moderate) versus contr.: 0/5 - 0/5
increased extramedullary haematopoiesis:
low: 2/5(minimal) - 2/5(minimal), mid: 5/5(minimal) - 5/5(slight), high: 5/5(slight) - 5/5(slight) versus contr.: 3/5(minimal) - 0/5
increase in haemosiderosis:
low: 0/5 - 5/5(slight), mid: 5/5(minimal) - 5/5(marked), high: 5/5(moderate) - 5/5(marked) versus contr.: 0/5 - 5/5(mild)

Test condition : TEST SPECIES AND ANIMAL HUSBANDARY.
-Age at start of the study: 6 weeks
-Number of rats: 5 m/5 f per group
-Animal maintenance: air-conditioned rooms,
-----groups of 5 rats/cage
-Acclimatisation: 5 days
-Room temperature: 22 °C
-Relative Humidity: 50 %
-Lighting time: 12 hours daily
-Food: rat diet ad libitum

-Water: tap water ad libitum
ADMINISTRATION / EXPOSURE
-Dose selection based on priliminary experiments
-Vehicle: sesame oil
-Concentration in vehicle: 0 - 2 % (w/v)
-Total volume applied: 5 ml/kg bw

CLINICAL OBSERVATIONS AND FREQUENCY
-Clinical signs: twice daily
-Body weight: at the start of the study and then twice weekly
-Food consumption: two times per week
-Water consumption: once per week
-Ophthalmoscopic examination: weekly
CLINICAL lABORATORY EXAMINATIONS
-Haematology / Clinical chemistry: at termination of the study
-Haematology: Erythrocyte count(Erys), hemoglobin (HG), ---hematocrit(HK), mean cellular volume(MCV), mean cellular ---hemoglobin (MCHC), Leucocyte ccount (Leucos(Thromocyte ---count(Thromos) Differential leucocyte count and red cell ---morphology, Reticulocyte count(reticulos), Heinz bodies ---(HB), coagulation time
-Clinical chemistry: Sodium, Potassium, Inorganic ---phophorus, Uric acid, Bilirubin total, Creatinine, ---Serum-glucose. Urea nitrogen, Calcium, Chloride, ---Aspartate aminotransferase(ASAT/GOT), Alanine ---aminotransferase (ALAT/GPT), Alkaline phosphatase (AP), ---Gamma-glutamyxltransferawe (GGT) Total protein, Albumin
-Urinalysis: a few days before termination of the study:
---Appearance, colour, pH-value, hemoglobin, protein, ---glucose, ketone bodies, bilirubin, urobilinogen, ---specific weight, sediment, volume

NECROPSY:
-Examination of skin, orifices, eyes, teeth, oral mucosa ---and internal organs
-Organ weights: heart, lung, liver, kidneys, spleen, ---ovaries, testes, epididymides, adrenals, brain, thymus
-Histopathology: heart, liver, kidneys, adrenals, spleen, ---lung, brain, thymus, trachea, stomach, jejunum, colon, ---urinary bladder, skeletal muscles, N.ischiadicus, femur ---with bone marrow, spinal cord (cervical, thoracal, ---lumbal), lymph nodes(cervical and iliacal)
--only controls and high dose groups: ovaries, uterus, ---testes, epididymides prostata, seminal vesicles
-Statistics:
---One way analyses of variance with sequentially rejective ------multiple comparison
---One way analyses of variance based on ranks with ------sequentially rejective multiple comparison
---Trend Test analyses for non-neoplastic lesions ------(ARMITAGE)

Reliability : (1) valid without restriction
Guideline study
Flag : Critical study for SIDS endpoint
07.01.2004 (110)

Type : Sub-acute
Species : other: rats or mice
Sex : no data
Strain : no data
Route of admin. : inhalation
Exposure period : 21 days
Frequency of treatm. : 4 hrs/d
Post exposure period : no data

Doses	:	28 mg/m3
Control group	:	no data specified
Method	:	other: see freetext ME
Year	:	1969
GLP	:	no
Test substance	:	other TS: 1,2-dichloro-4-nitrobenzene, no data on purity but melting point: 43°C
Method	:	Test animals: 40 rats and 60 mice (no further information) Test atmosphere: the vapor concentration in the chambers were monitored photocolorimetrically. Observations and measurements: behavior, body weight, blood count, methemoglobin concentration in blood, catalase activity in blood and liver, activity of serum transaminases , activity of liver diaminoxidase, bilirubin concentration in blood, cholesterol levels in adrenals fragility of erythrocytes, oxygen consumption histopathological examination
Result	:	Mice: reduced RBC from 9.8 to 8 million per mm3 and reduced haemoglobin from 14.1 to 13.2 g%; reticulocyte count was 118 % towards the end of the study, while Heinz bodies increased to 86 %; blood contained up to 7.5 % methaemoglobin; differential WBC revealed neutrophil leucocytosis with shift to the left, polychromatophilia, lymphopenia and eosinopenia; inhibition of blood catalase and peroxidase; increase in serum cholinesterase (35 %); increased fragility of erythrocytes: stability index: 0.68. Rats: decreased oxygen consumption (20 %). Histological examination: fatty and protein dystrophy of liver and kidneys, dystrophic alterations in cardiac muscle fibres, ischemic changes of the neurons in subcortical ganglia.
Reliability	:	(4) not assignable Documentation suffers from deficiencies mainly because the limited documentation. Nevertheless the study should be mentioned in the SIAR because these reports of repeated dose toxicity study using the inhalation route gives some additional information on the toxicological profile of the substance.
Flag	:	Critical study for SIDS endpoint

07.01.2004 (98)

Type	:	Sub-chronic
Species	:	other: rats or mice
Sex	:	no data
Strain	:	no data
Route of admin.	:	inhalation
Exposure period	:	4 months
Frequency of treatm.	:	4 hrs/d
Post exposure period	:	no data
Doses	:	0, 0.4, 3.6, 10 mg/m3
Control group	:	yes
NOAEL	:	= .4 mg/m³
Method	:	other: see freetext ME
Year	:	1969
GLP	:	no
Test substance	:	other TS: 1,2-dichloro-4-nitrobenzene, no data on purity but melting point: 43°C
Method	:	Test animals:

30 rats/group and 30 mice/group (no further information)
Test atmosphere:
the vapor concentration in the chambers were monitored photocolorimetrically.
Observations and measurements:
behavior, body weight, blood count, methemoglobin concentration in blood, catalase activity in blood and liver, activity of serum transaminases , activity of liver diaminoxidase, bilirubin concentration in blood, cholesterol levels in adrenals fragility of erythrocytes, oxygen consumption
histological examination

Result : RATS and MICE :
No effects on behaviour and body weights.
RATS and MICE:
Blood counts and biochemical tests revealed significant changes, mainly among animals treated with a concentration of 10 mg/m³
e.g. RATS:
0.4 mg/m3:
no adverse effects were reported;
3.6 mg/m3:
increase in Heinz bodies (up to 7 %) and
reticulocytes (up to 19.6 %); significantly increased activity of liver catalase (35.6 versus 26.5[catalase numbers] of control);
10 mg/m3:
significant changes in blood counts and biochemical tests: decrease in RBC of 10% towards the 18th day of treatment, decreased haemoglobin levels from 15.1g% to 12.2 g%, neutrophilic leucocytosis, pronounced reticulocytosis, increase in Heinz bodies and in methaemoglobin levels (up to 7.6 %);
significantly (sig.) increased activities of serum transaminases (Glutamic-alanine-transa.: 35.4 versus 28.9 [extinction units] of controls; Glutamatic-aspartic-transa.: 141.5 versus 121.8 [extinction units] of controls), sig. liver catalase (47.6 versus 26.5 [cataslase numbers] of controls), sig. liver diaminooxidase (56.8 versus 41.4 [ug/g tissue] of controls), sig. increased levels of bilirubin in blood (6.03 versus 2.0 [mg%] of controls), sig. increased cholesterol level in adrenals (4.7 versus 3.7 [mg/100 g tissue] of controls);
histological examination:
10 mg/m³: morphological changes of the brain (cells with hyperchromic nuclei most commonly in the subcortical ganglia).

Reliability : (2) valid with restrictions
Documentation suffers from deficiencies mainly because of limited documentation.
Nevertheless the study should be mentioned in the SIAR because these reports of repeated dose toxicity study using the inhalation route gives some additional information on the toxicological profile of the substance.

Flag : Critical study for SIDS endpoint
07.01.2004 (98)

Type : Sub-acute
Species : rabbit
Sex : no data
Strain : no data
Route of admin. : gavage
Exposure period : a.) 16-times during 21 days or
b.) 21-times during 27 days
Frequency of treatm. :
Post exposure period : no
Doses : 30 mg/kg bw
Control group : no data specified
Method : other: bodyweight gain determination, microscopic evaluation (no further data)

Year	:	1957
GLP	:	no
Test substance	:	other TS: no data on purity, m.p.: 40.2°C
Result	:	a.) Microscopic examination: liver: moderate hydropic degeneration; kidney: congestion; lung: congestion b.) Microscopic examination: liver: marked hydropic degeneration; kidney: moderate congestion; lung: congestion, stomach: normal No further information available.
Test condition	:	only one animal was used in each study
Reliability	:	(4) not assignable Data from handbook or collection of data

26.06.2003 (97)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Ames test
System of testing	:	Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538
Test concentration	:	51,2, 102.4, 204.8, 409.6, 819.2, 1638.4, 3276.8, 6553.6 ug/plate in DMSO
Cycotoxic concentr.	:	6554 ug/plate
Metabolic activation	:	without
Result	:	positive
Method	:	other: According to Ames et al., Proc. Natl. Acad. Sci (USA) 70: 782 (1973), pour-plate method, solvent: DMSO, performed in duplicate and repeated at least 3 times, solvent control, pos. Controls (ENNG, 2-NF, 9-AA)
Year	:	1983
GLP	:	no data
Test substance	:	other TS: 1,2-dichloro-4-nitrobenzene, purity 99 %
Result	:	Positive only in TA 100
Reliability	:	(2) valid with restrictions study meets the criteria of today, but is only performed without metabolic activation and information on GLP is missing
Flag	:	Critical study for SIDS endpoint

07.01.2004 (111)

Type	:	Ames test
System of testing	:	Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538
Test concentration	:	-S9-mix: <= 1000 ug/plate dissolved in DMSO +S9-mix: <= 3000 ug/plate dissolved in DMSO
Cycotoxic concentr.	:	-S9-mix: 1000 ug/plate +S9-mix: 3000 ug/plate
Metabolic activation	:	with and without
Result	:	positive
Method	:	other: according to Ames BN et al. (1975) Mutat Res 31, 347-364, see also Freetext ME
Year	:	1981
GLP	:	yes
Test substance	:	other TS: 1,2-dichloro-4-nitrobenzene, technical grade: 85 % 3,4-dichloronitrobenzene and 15 % 2,3-dichloronitrobenzene
Method	:	plate incorporation methodology: single plates were prepared for each strain/microsome/dose level

combination, 3 replicate plates for each strain/microsome/dose
S9-mix: from livers of Aroclor1254-induced male Spratue-Dawley rats and of Aroclor1254-induced male Syrian hamsters, prepared as described in Ames 1975
concurrent positive controls:
+S9-mix: TA98, TA1538: 2-acetylaminofluorene, TA100: benzopyrene, TA1535, TA1537: 2-aminoanthracene
-S9-mix: TA98, TA100, TA1538: 4-nitroquinoline-N-oxide, TA1535 $NaNO_2$, TA1537: 9-aminoanthracene
concurrent negative control: solvent
statistical analysis:
Bartlett's test,
within-levels pooled variance
one-sided t-test,
Grubb's test
regression analysis
t-test

Result : TA 98: weakly positive with/without metabolic activation
TA 100: positive with/without metabolic activation

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific priciples, acceptable for assessment, purity of used TS is technical grade

Flag : Critical study for SIDS endpoint

07.01.2004 (112)

Type : Ames test
System of testing : Salmonella typhimurium TA 98, TA 100, TA 1530, TA 1535, TA 1537, TA 1538
Test concentration : 0, 1, 10, 100, 200, 500, 800,1000, 1500 ug/plate dissolved in ethanol abs.
Cycotoxic concentr. : +S9-mix: >= 800 µg/plate: TA1537, TA1538, TA98; >=1000 µg/plate: TA1530, TA1535, TA100; -S9-mix: >=500 µg/plate: TA1537, >= 800 µg/plate: TA1535, TA1538, TA98, >=1000 ug/plate: TA100, 1500 µg/plate: TA1530
Metabolic activation : with and without
Result : positive
Method : other: plate incorporation method, incubation 48 hours, in aerobic and in anaerobic conditions, solvent: ethanol abs., solvent control, no positive controls, duplicates, evaluation as pos.: >2-fold than spontaneous revertants
Year : 1980
GLP : no data
Test substance : other TS: 1,2-dichloro-4-nitrobenzene, "purest grade availabe"

Result : At high doses slight but significant mutagenic activity towards TA 1530 in the absence of S9-mix (from 500 µg/plate) and towards TA100 and TA1538 both in the absence (at 1000 µg/plate and from 500 µg/plate) and in the presence of S9-mix (from 500 µg/plate and from 200 µg/plate), respectively.

Reliability : (2) valid with restrictions
acceptable for assessment but no positive controls, no detailed information on purity and no data on GLP

Flag : Critical study for SIDS endpoint

07.01.2004 (113)

Type : Ames test
System of testing : Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537
Test concentration : 2 - 250 ug/plate
Cycotoxic concentr. : >= 200 ug/plate
Metabolic activation : with and without
Result : positive

Method : other: preincubation methodology according to Ames BN et al. (1975) Mutat Res 31, 347-364 and Yahagi, Cancer Lett.1, 91 (1975), see also freetext ME
Year : 1983
GLP : no data
Test substance : other TS: 1,2-dichloro-4-nitrobenzene, purity 99 %

Method : S9-mix liver fractions were prepared from male Sprague Dawley rats and male Syrian Hamsters that were injected with Arcolor 1254;
positive controls: 2-aminoanthracene, 4-nitro-o-phenmylenediamine, sodium acide,9-aminoacridine
solvent: water
statistical method: analysis based on models presented by Margolin
Result : Positive in TA 100 with metabolic activation
Reliability : (2) valid with restrictions
only 4 strains of Salmonella typhimurium were used
Flag : Critical study for SIDS endpoint
07.01.2004 (114)

Type : Cytogenetic assay
System of testing : V79 cells
Test concentration : 15, 75, 150 µg/ml
Cycotoxic concentr. : >= 150 µg/ml
Metabolic activation : with and without
Result : ambiguous
Method : OECD Guide-line 473
Year : 1989
GLP : yes
Test substance : other TS: 1,2-dichloro-4-nitrobenzene, purity > 99 %

Result : preliminary experiment:
+S9-mix: >= 200 µg/ml survival rate: <= 6.8 %
-S9-mix: >= 200 µg/ml survival rate: <= 26.5 %

The mitotic index was 78.7, 71.3 and 55.1 % of control after treatment with 15, 75 and 150 µg/ml

main experiment:
7 hours after administration: negative with and without metabolic activation
18 and 28 ours after administration:
A significant increase in the number of chromosome aberrations inclusive gaps was seen 18 and 28 hrs after treatment with 150 ug/ml in the presence of metabolic activation.
A slight but non-significant increase in the number of chromosome aberrations exclusive gaps with four exchanges was seen 18 hrs after treatment with 150 ug/ml in the presence of metabolic activation.
However, there was no clear dose-response.
Negative results without metabolic activation

Positive controls were functional.
Test condition : Preliminary experiment for cytotoxicity:
0. 50-2000 µg/ml with and without S9-mix
Preparation of chromosomes after 4 h treatment with 150 ug/ml at 7, 18 and 28 h, and 18 hrs after treatment additionally with 15 and 75 ug/ml, both with and without metabolic activation.
solvent: ethanol
controls: solvent control, pos. controls: Ethylmethansulfonate(EMS, without S9-mix), cyclophophamide(CPA, with S9-mix)

Evaluation:
--The test substance is classified as mutagenic if it induced a significantly increased aberration rate as compared with negative controls with one of the concentrations tested. The significance is obvious either by an enhancement of the rate clearly exceeding the control range or it is proven by adequate biometry (Binominal statistic with Fisher's exact test).
--The test substance in classified as mutagenic if there were reproducible concentration related increase in the aberration rate.
--The test substance is classified as not mutagen when it tests negatively both with and without activation.

Conclusion : 3,4-dichloro-nitrobenzene is not mutagenic in the absence of metabolic activation in the chromosome aberration test in V79 Chinese Hamster cells. No clear dose related clastogenic effects for 3.4-Dichloro nitrobenzene is given in the presence of metabolic activation.

Reliability : (1) valid without restriction
Guideline study

Flag : Critical study for SIDS endpoint

21.09.2004 (115)

Type : HGPRT assay
System of testing : CHO cells
Test concentration : 0, 25, 50, 125, 200, 250 ug/ml
Cycotoxic concentr. : >= 333 ug/ml
Metabolic activation : with and without
Result : negative
Method : other: see freetext ME
Year : 1986
GLP : yes
Test substance : other TS: technical grade: 85 % 3,4-dichloronitrobenzene and 15 % 2,3-dichloronitrobenzene

Method : CELL LINE:
CHO-K1BH4
TOXICITY TEST PRIOR TO TESTING:
dose levels:
0.33, 1.0, 3.3, 10, 33.3, 100, 333, 1000 ug/ml in the presence of 0, 1, 2, 5, and 10 % Ariclor1254 induced rat liver S9-mix
incubation time: 5 hours
result:
1000 ug/ml: cytotoxicity at all concentrations of S9-mix
333 ug/ml: reduced relative cell survival(0-10% S9): 36%, 36% 34%, 38%, 18%
PRELIMINARY MUTAGENICITY SCREEN:
dose levels:
-S9-mix: 100, 120, 150 ug/ml
+S9-mix (1, 2, 5, 10%): 50, 150, 200 ug/ml
incubation time: 5 hours with TS and after removal of TS for 19 hours and after washing for additional 7 days
result:
survival:
-S9-mix: 99, 94, 47 %
+S9-mix: 1%: 79/45/10% survival; 2%: 86/48/25% survival; 5%: 91/73/ 42% survival; 10%: 99/80/78% survival
there were no significant increases in the mutation levels when compared to the negative controls
MAIN TEST:
performed in triplicate
S9-mix as 5 % solution
incubation time: see above
evaluation for survival and mutation frequency

positive controls:
Ethylmethansulfonate (EMS), Dimethylnitrosamine (DMN)

statistical analysis:
one-way analysis of variance method outlined by Snee and Irr (1981)
one-tailed student's t-test using pooled , intergroup variance

Result : Mean mutation frequency (with S9-mix - without S9-mix):
-negative controls:
--untreated controls: 0.6 - 1.9
--DMSO- control: 0.8 - 0.6
-positive controls:
EMS: 275 (without S9-mix)
DMN: 265 (with S9-mix)
test substance:
relative cell survival (low to high dose):
-S9-mix: 92, 70, 43, 30, 15 %
+S9-mix: 81, 71, 54, 42, 7 %
mean mutation frequency (low to high dose: with/without S9-mix):
0.0/1.4, 1.3/1.1, 0.8/0.5, 0.6/1.5, 1.0/1.4
no statistically significant difference when compared to the negative controls

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

Flag : Critical study for SIDS endpoint

07.01.2004 (116) (117)

Type : Ames test
System of testing : Salmonella typhimurium TA 98, TA 100
Test concentration : 0-500 ug/plate
Cycotoxic concentr. : no data
Metabolic activation : with and without
Result : positive
Method : other: pre-incubation method
Year : 1987
GLP : no data
Test substance : other TS: no data on purity

Result : TA 98: negative
TA 100: positive with/without metabolic activation

Reliability : (4) not assignable
2 strains only, documentation insufficient

27.06.2003 (118)

Type : other: Bacterial fluctuation test
System of testing : Salmonella typhimurium TA 100, TA 1538
Test concentration : 5 - 15 ug/ml
Cycotoxic concentr. :
Metabolic activation : with and without
Result : positive
Method : other: according to Green MHL et al. (1977) Mutat Res 48, 287-294: bacterial fluctuation test, incubation 72 hours, solvent: ethanol abs., triplicates
Year : 1980
GLP : no data
Test substance : other TS: "purest grade availabe"

Result : Weak mutagenicity in TA 100 and TA 1538 with metabolic activation

Reliability : (4) not assignable
no details given, special study

26.03.2003 (113)

Type : other: Ames test and Bacterial fluctuation test
System of testing : Salmonella typhimurium TA 98, TA 100, TA 1530, TA 1535, TA 1537, TA 1538, G 46, TA 1532, TA 1950, TA 1975, TA 1978
Test concentration : no data
Cycotoxic concentr. : no further specified
Metabolic activation : with and without
Result : positive
Method : other: according to Ames BN et al. (1975) Mutat Res 31, 347-364 and Green MHL et al. (1977) Mutat Res 48, 287-294
Year : 1978
GLP : no data
Test substance : other TS: no data on purity

Result : positive in TA 100 and TA 1538 at concentrations (+- 1 mg/plate), which were highly cytotoxic (100 %)
Reliability : (4) not assignable
Documentation insufficient for assessment

10.07.2003 (119)

Type : other: umu-test
System of testing : Salmonella typhimurium TA 1535/pSK1002
Test concentration : 100 ug/ml
Cycotoxic concentr. : no data
Metabolic activation : with and without
Result : negative
Method : other: determination of ß-galacdosidase activity after a incubation time of 4 hours
Year : 1992
GLP : no data
Test substance : other TS: no data on purity

Reliability : (4) not assignable
documentation insufficient for assessment

10.07.2003 (120)

Type : Chromosomal aberration test
System of testing : human peripheral lymphocytes
Test concentration : 0.05 .0.1, 0.5, 1 mmol/l dissolved in DMSO
Cycotoxic concentr. : no data
Metabolic activation : without
Result : negative
Method : other: see freetext TC
Year : 1995
GLP : no data
Test substance : other TS: no data on purity

Result : Dose (mmol/l) PAC (%)
0 1.8 (solvent control)
0.05 1.2
0.1 2.2
0.5 2.8
1 1.8
Test condition : Lymphocytes were obtained from a healthy male donor without any known occupational exposure to genotoxic agents.
TS was added to cultures at 48 h after culture initiation and incubated for additional 24 hours, colchicine was added 2 hours before the end of the incubation. The number of cells with chromosome aberrations among 100 well-spread metaphase cells in one culture was recorded (gaps were not recorded as aberration). The percentage of aberrant cells (PAC) was

calculated: PAC = number of aberrant cells/number of metaphase cells scored
no positive controls

Reliability : (4) not assignable
no data on purity of TS, not tested in the presence of an activation system, only one negative (solvent) control for 22 tested substances, no positive control

10.07.2003 (121) (122)

Type : Chromosomal aberration test
System of testing : no data
Test concentration : no data
Cycotoxic concentr. : no data
Metabolic activation : no data
Result : negative
Method : other: no data
Year : 2002
GLP : no data
Test substance : other TS: no data on purity

Reliability : (4) not assignable
Data from handbook or collection of data

10.07.2003 (123)

Type : Sister chromatid exchange assay
System of testing : no data
Test concentration : no data
Cycotoxic concentr. : no data
Metabolic activation : no data
Result : positive
Method : other: no data
Year : 2002
GLP : no data
Test substance : other TS: no data on purity

Reliability : (4) not assignable
Data from handbook or collection of data

10.07.2003 (123)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Drosophila SLRL test
Species : Drosophila melanogaster
Sex : no data
Strain : other: Canton S
Route of admin. : oral feed
Exposure period : 3 days
Doses : 0, 50 ppm
Result : negative
Method : other: according to Woodruff RC et al. (1984) Environ Mutagen 6, 189-202, see also freetext ME
Year : 1985
GLP : no data
Test substance : other TS: 1,2-dichloro-4-nitrobenzene, purity 99 %

Method : ---route of administration:
males were treated for 3 days in glass shell vials containing a glass fiber disc soaked with 0.2-0.5 ml of solution
---Solvent:

dissolved in ethanol and then diluted in a sterile solution of 5% sucrose in destilled water
---toxicity:
attempts were made to treat with concentrations that induced a mortality of 30%
---Palatability:
determined by feeding behavior, amount of excretion, or abdomen size; flies thst did not feed were dead by 3 days
---Age of males at beginning of treatment:
1 day
---Age of males at mating:
immediately after treatment
---Genotyp of parental females:
Basc
---Mating and brooding sheme:
indivdual males were mated with 3 new vergin females for each of 3 broods (3, 2, 2 days)
---Scoring criteria in F2:
lethal mutation was declared if no wild-type males were recovered among 20 or more Basc males or BascI+/-females
---Culturing temperature: 23-25 °C
---Cluster analysis:
by the formula for the cumulative Poisson distribution (Owen 1962): all data from a parental male producing a cluster werer excluded
---statistical methods:
Normal test (Margolin 1983)

Result : Total tests (test/controls): 6817 / 7162
Mortality (test/control): 11 % / 0
Sterility (test/control): 1 % / 0
Lethals (test - controls): Br1/Br2/Br3 = 3/0/1 - 4/2/1
Tests (test-controls): Br1/Br2/Br3 = 3886/1823/1108 - 3504/2379/1279
Total lethals(test/controls): 4/7
Percent lethals(test/controls): 0.06/0.10

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

Flag : Critical study for SIDS endpoint

07.01.2004 (124)

Type : Drosophila SLRL test
Species : Drosophila melanogaster
Sex : male
Strain : other: Canton S
Route of admin. : i.p.
Exposure period : once
Doses : 0, 200 ppm
Result : positive
Method : other: according to Woodruff RC et al. (1984) Environ Mutagen 6, 189-202, see also freetext ME
Year : 1985
GLP : no data
Test substance : other TS: 1,2-dichloro-4-nitrobenzene, purity 99 %

Method : ---route of administration:
0.2-0.3 ul of freshly prepared solution was injected. Males were allowed to recover for 24-48 hrs before mating.
---Solvent:
TS dissolved in ethanol and then deluted in sterile solution of 0.7% NaCl
---toxicity:
attempts were made to treat with concentrations that induced a mortality of 30%

---Palatability:
determined by feeding behavior, amount of excretion, or abdomen size; flies thst did not feed were dead by 3 days
---Age of males at beginning of treatment:
1-3 days
---Age of males at mating:
24-48 hour after treatment
---Genotyp of parental females:
Basc
---Mating and brooding sheme:
indivdual males were mated with 3 new vergin females for each of 3 broods (3, 2, 2 days)
---Scoring criteria in F2:
lethal mutation was declared if no wild-type males were recovered among 20 or more Basc males or Bascl+/-females
---Culturing temperature: 23-25 °C
---Cluster analysis:
by the formula for the cumulative Poisson distribution (Owen 1962): all data from a parental male producing a cluster werer excluded
---statistical methods:
Normal test (Margolin 1983)

Result : Total tests (tests/controls): 8230 / 7956
Mortality (tests/controls): 22 % / 0
(Sterility (tests/controls): 13 % / 0
Lethals (tests-controls): Br1/Br2/Br3 = 4/6/3 - 3/0/2
Tests (tests-controls): Br1/Br2/Br3 = 2657/2773/2800 - 2367/2795/2794
Total lethals (tests/controls): 13 / 5
Percent lethals (tests/controls): 0.16 / 0.06

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

Flag : Critical study for SIDS endpoint

07.01.2004 (124)

Type : other: chromosome aberration
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : single application
Doses : 0, 75, 250 or 750 mg/kg bw
Result : negative
Method : other: according to Evans HJ (1976) Cytological methods for detecting chemical mutagens. In: Hollaender A (ed.) Plenum Press, NY and Killian DJ et al. (1977) Handbook of mutagen testing. Elsevier, Amsterdam. see also freetext TC
Year : 1983
GLP : yes
Test substance : other TS: technical grade: 85 % 3,4-dichloronitrobenzene and 15 % 2,3-dichloronitrobenzene, assumed purity: 100 %

Result : Clinical observations:
no rat died
all test groups: signs of intoxication included red stains on nose and/or eyes, depression, soft feces, rough coat and urine stains. The number and severity increased with increased dose
250 or 750 mg/kg bw-group: significantly reduced body weight gain in rats of both sexes 24 and 48 hours post treatment.
cytogenetic analysis:
---modal number: the average number of chromosomes in the examined metaphases was determined for each animal and all test groups and

revealed no statistically significant difference between test and control animals.
---mitotic incices(number of cells undergoing mitosis per 500 cells counted) of the test groups compared to vehicle control revealed no statistically significant differences.
---chromosomal aberrations were not statistically significant increased when compared to the vehicle controls.
---the positive control cyclophosphamide was functional.

Test condition : -No. of animals per group: 24 m/24 f
-Age_ 50 days
-Acclimattion: 12 days
-Housing: individually in wire-mesh cages
-Food and water: ad libitum
-Light-cycle: 12 hours
-Temperature: 69-78°F
-Relative humidity: 54-76 %
-Dose selection: based on a priliminary dose range finding study: highest dose = MTD
-Vehicle: corn oil
-Positive control: 6 m/6 f (Cyclophosphamide)
-Clinical observations:
twice daily: general appearance, behavior,toxic and pharmacological effects
body weight determination
post treatment sampling times
-6 rats/sex from each group: single intraperitoneal injection of Colchicine 4,10, 22, 46 hrs after dosing
-6 rats/sex from each group were sacrificed 6, 12, 24 or 48 hrs after dosing.
-Cytogenetic analysis:
60 bone marrow cells in metaphase from 5/6 chosen rats from each sex and group were analyzed
Statistical analysis:
Kruskal-Wallis nonparametric analysis of variance
nonparametric pairwise group comparison (KW-ANOVA)
Analysis of covariance (ANOVA)

-No evidence of mitotic delay was seen after analysis of the mitotic indices. Therefore, slides from the 48 h sacrifice were not analyzed for chromosomal aberrations.

Reliability : (2) valid with restrictions
Study well documented, but TS technical grade

Flag : Critical study for SIDS endpoint

21.09.2004 (125)

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

Type : other: see chapter 5.8.3
Species :
Sex :
Strain :
Route of admin. :
Exposure period :
Frequency of treatm. :

Premating exposure period
Male :
Female :
Duration of test :
No. of generation studies :
Doses :
Control group :

10.07.2003

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rat
Sex	:	female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	gd 6 - 15
Frequency of treatm.	:	once daily
Duration of test	:	sacrifice on gd 21
Doses	:	0, 10, 30 or 100 mg/kg bw in corn oil
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	= 10 mg/kg bw
NOAEL teratogen.	:	= 10 mg/kg bw
Method	:	other: see freetext TC
Year	:	1987
GLP	:	yes
Test substance	:	other TS: commercial grade: 85 % 3,4-dichloronitrobenzene, 15 % 2,3-dichloronitrobenzene

Result :

Total mated females on study: 25/group
All dams survived to scheduled sacrifice.

CLINICAL OBSERVATIONS:
10 mg/kg bw:
significantly reduced food consumption on gd 6-10;
30 mg/kg bw:
significantly reduced food consumption for gd 6-10
100 mg/kg bw:
urogenital staining increasing number of dams with duration of pregnancy (3/25 to 16/25) and wet/matted fur (5/25 gd6-13);
significantly reduced mean bodyweights on gd 10 (267.8g versus 284.5g of controls), gd 13(284.8g versus 302.3g of controls) and gd 16 (307.7g [approx.5 %] versus 324.4g of controls); significantly reduced food consumption on gd 6-10 and gd10-13

Mean body weight change: gd 6-10, dose-related:
control 8.4 g/dam, 10 mg-gr.: 6.2 g/dam, 30 mg-g.: 4.0 g/dam (significant,p<=o.05), 100 mg-gr.: -4.4 g/dam (significant,p<=0.01), corresponding to a significant body weight loss of approximately 5 %

POST MORTEM FINDINGS:
controls: hydronephrosis of the kidneys in 1/23 rat
10 mg-group: hydronephrosis of the kidneys in 1/25 rat
100 mg-groups: hydronephrosis of the kidneys in 3/25 rats
MATERNAL REPRODUCTIVE DATA
No adverse effects on:
pregnancy rates (Total pregnant females: control, low, mid, high dose: 23/25, 25/25, 23/25, 24/25), live or dead foetuses/dam, late

resorptions/dam, total implants/dam, or corpora lutea/dam or preimplantation loss
100 mg: increased mean early resorptions (1.1/dam versus 0.5/dam in controls
FETAL DATA
control, low, mid, high dose:
Total number of litters examined (no of fetuses): 23(323), 25(353), 23(340), 24(339)
no of examined viscerally: 22(160), 25(176), 23(170), 24(169)
no examined skeletally: 23(163), 25(177), 23(170), 24(170)

No adverse effects on foetal body weights or sex distribution.
Total number with malformation: 2(4), 3(4), 4(6), 5(9)
No statistically significant differences for the incidence of total or individual malformations.
Findings observed in multiple foetuses in treated rats included anophthalmia/Microphthalmia (100 mg: 4 in 3 litters; 10 mg: 2 in 2 litters, control: 1 in 1 litter), small oral opening (100mg: 2 in 2 litters), skull misshapen (100 mg: 2 in 2 litters), nasal passages misshapen (100 mg: 2 in 2 litters and gastroschesis (100 mg: 2 in 2 litters).
None of these findings were seen in mid-dosed rats

For variations dilated ureters were elevated in mid- and high-dosed rats: control, low mid, high dose (7 fetuses in 3 litters, 8 fetuses in 4 litters, 17 fetuses in 9 litters 15 fetuses in 10 litters.)
Dilated ureters are regarded to be of low concern (ECETOC Monograph No. 31, 2003).

Test condition : TEST SPECIES AND ANIMAL HUSBANDARY
-Sprague Dawley rats
-Number of animals: 25 mated females per group
-Housing: individually in stainless steel cages
Temperature: 72°F
Relative Humidity: 40-60 %
lightening time: 12 hours artificial light per day
Food and water ad libitum
Acclimatisation: 10 days

ADMINISTRATION/EXPOSURE:
dose selection: based on preliminary experiments
-vehicle: corn oil
-Total volume applied: 10 ml/kg bw/day

-CLINICAL OBSERVATIONS AND FREQUENCY:
survival: twice daily
clinical signs: detailed examination on gd 0 and daily on gd 6-21
-Body weight: on gd 0, 6, 10, 13, 16 and 21
-Food consumption: on gd 0-6, 6-10, 10-13, 13-16 and 16-21

ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
gd 21: recording of number/relative placement of live/dead foetuses, early/late resorptions, number of corpora lutea;
dams underwent gross necropsy;
all live foetuses from surviving dams underwent external examination, were sexed and weighted; ca. 50 % of each litter were examined for visceral or skeletal malformations, resp.

STATISTICAL ANALYSIS:
in general: one-side comparison (except sex distribution of fetuses: two-side comparison):

Dunnett's test, Mann-Whitney U test, Fisher's exact test, cochran-armitrage test, Bonferroni's inequality

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

Flag : Critical study for SIDS endpoint

07.01.2004 (126)

Species	:	rat
Sex	:	female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	gd 6 - 20
Frequency of treatm.	:	once daily
Duration of test	:	sacrifice on gd 21
Doses	:	0 or 100 mg/kg bw in corn oil
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	< 100 mg/kg bw
NOAEL Fetotoxicity	:	< 100 mg/kg bw
Method	:	other: see freetext TC
Year	:	1987
GLP	:	yes
Test substance	:	other TS: commercial grade: 85 % 3,4-dichloronitrobenzene, 15 % 2,3-dichloronitrobenzene

Remark : The study was conducted to determine if treatment with the substance will induce an increase in maternal and fetal methemoglobin levels

Result : OBSERVATIONS:
All dams survived to scheduled sacrifice.
100 mg-group:
-an increase in number of rats with duration of pregnancy in alopecia (d0: 0, d6-19: 1/8, d17-21: 2/8), urinary staining (d0:0, d6-10: 2/8, d17-21: 7/8) and perinasal/perioral staining/matted fur (d0: 0, d17-21: 2/8), was noted.
-In dams significantly lower mean body weights (on gd 10: 279 g/dam versus 299 g/dam of controls, gd 13: 294 g/dam versus 315 g/dam of controls and gd 21: 377 g/dam versus 407 g/dam of controls), body weight loss (gd 8-10) and reduced body weight gain (gd 16-21 / 6-21) were observed.
PREGNANCY STATUS:
pregnant females: 8/8 of controls 8/8 in 100 mg-group
all dams were pregnant with viable foetuses:
pregnant females with live fetuses : 8/8 in controls and 8/8 in 100 mg-gr.
mean number of viable fetuses: control: 13.8/dam, 100 mg-group 14.4 /dam,
HEMATOLOGY:
---DAMS
-mean value of total hemoglobin [g/dl]: 100 mg-group: 10.6 versus 11.8 in controls (significant)
-mean value of methemoglobin [% of total hemoglobin]: 100 mg-group: 6.08 versus 1.24 in controls (significant)
---FETUSES.
-mean value of total hemoglobin [g/dl]: 100 mg-group: 10.3 versus 10.6 in controls (not significant)
-mean value of methemoglobin [% of total hemoglobin]: 100 mg-group: 2.01 versus 0.53 in controls (significant)

Dams showed significantly decreased total haemoglobin values, while the differences in the foetuses were only slightly. Methaemoglobin levels were significantly increased in both, dams and fetuses (about 4-5 times compared with controls).

Test condition : TEST ANIMAL AND HUSBANDARY:

Number of animals in study: 8 per group
Age: 12 weeks
Weight: 230-305 g
Housing: individually in stainless steel cages
Food and tap water: ad libitum
Acclimation: 10 days
Room light: 12 hours daily
Temperature: 72°F
Rel. Humidity: 40-60 %
DOSAGE:
The dose level was selected on the basis of a range finding teratology study.
Concentration: 10 mg TS/ml
Dose: 10 ml/kg bw
IN-LIFE-OBSERVATIONS:
Animals were observed twice daily for survival and a
detailed physical examination was performed on study days 0, daily on gd 6-21. Body weights were recorded on gd 0, 8, 10, 13, 16 and 21.
POSTMORTEM OBSERVATIONS AND HEMATOLOGY:
At sacrifice blood was collected and analyzed for total haemoglobin and methaemoglobin. Dams underwent gross necropsy.
STATISTICAL ANALYSIS:
One-side comparisons
Student's t-test

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

Flag : Critical study for SIDS endpoint

07.01.2004 (127)

Species	:	rat
Sex	:	female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	from gd 6 or 7 over 6 consecutive days
Frequency of treatm.	:	once daily
Duration of test	:	sacrifice one day after last dosing
Doses	:	0 or 100 mg/kg bw in corn oil
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	< 100 mg/kg bw
Method	:	other: see freetext TC
Year	:	1984
GLP	:	yes
Test substance	:	other TS: commercial grade: 85 % 3,4-dichloronitrobenzene, 15 % 2,3-dichloronitrobenzene

Remark : range finding study to determin if selected dosage was capable to induce methemoglobinemia

Result : All females survived to the day of sheduled sacrifice.
OBSERVATIONS:
100 mg-group: wet fur (study day4: 2/8), urinary staining (study day7: 1/8), nasal/oral staining (study day 4: 1/8)
Compared with controls the maternal body weight was slightly and not significantly reduced during the entire treatment period: e.g. study day7, mean body weight[g]:
295 versus 304 of controls.
PREGNANCY STATUS:
total pregnant females: 100mg: 8/8, control: 8/8
All dams were pregnant with viable foetuses
HEMATOLOGY:
-mean value of total hemoglobin [g/dl]: 100 mg-group: 12.0 versus 13.3 in

controls (not significant)
-mean value of methemoglobin [% of total hemoglobin]: 100 mg-group: 4.56 versus 0.95 in controls (significant)

A significant reduction of total haemoglobin and a five-fold increase in blood methaemoglobin levels was noted. Treated rats showed signs of maternal toxicity (weight loss and reduced weight gain).

Test condition : TEST ANIMAL AND HUSBANDARY:
Number of animals in study: 8 per group
Age: 12 weeks
Weight: 230-305 g
Housing: individually in stainless steel cages
Food and tap water: ad libitum
Acclimation: 9 days
Room light: 12 hours daily
Temperature: 72°F
Rel. Humidity: 40-60 %
DOSAGE:
The dose level was selected on the basis of a range finding teratology study.
Concentration: 10 mg TS/ml
Dose: 10 ml/kg bw
IN-LIFE-OBSERVATIONS:
Animals were observed twice daily for survival and a
detailed physical examination was performed on gd 0, and study day 1 and 4 and on day 7 prior to sacrifice. Body weights were recorded on gd 0, and study day 1,4,7.
POSTMORTEM OBSERVATIONS AND HEMATOLOGY:
At sacrifice blood was collected and analyzed for total haemoglobin and methaemoglobin. Dams underwent gross necropsy.
STATISTICAL ANALYSIS:
One-side comparisons
Student's t-test

Reliability : (2) valid with restrictions
dose-finding study

07.01.2004 (128)

Species : rat
Sex : female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : gd 6 - 15
Frequency of treatm. : once daily
Duration of test : sacrifice on gd 21
Doses : 0, 200, 300, 400, 500 or 600 mg/kg bw in corn oil
Control group : yes, concurrent vehicle
NOAEL maternal tox. : < 200 mg/kg bw
NOAEL Fetotoxicity : < 200 mg/kg bw
Method : other: see freetext TC
Year : 1986
GLP : yes
Test substance : other TS: commercial grade: 85 % 3,4-dichloronitrobenzene, 15 % 2,3-dichloronitrobenzene

Remark : Dose-finding study for a teratogenicity study.
Result : 200: mg/kg bw:
mortality 2/6;
OBSERVATIONS:
bodyweight:
reduced mean body weights (difference up to 32 % to the concurrent control) during 1st four treatment days gd 6-10 and reduced weight gain for

gd 6-10, 10-13, 13-16 and 16-21;
pregnancy rate: 100 %;
clinical findings:
respiratory problems, loss of muscle coordination, convulsions, poor general health;
pathological examination:
pale and mottled livers, brown to dark areas of the lungs, multiple haemorrhages of the pyloric region of the stomach and thymus;
PREGNANCY STATUS: 100 %
mean live fetuses per litter 13.8 versus 15.2 in controls, no dead fetuses
mean resorptions per litter: 0.8 versus 0.5 in controls
mean nidations per litter: 14.5 versus 15.7 in controls
mean fetal live weight[g]: 4.5 versus 4.8 of controls

no abnormalities during gross foetal examinations;

>= 300 mg/kg bw:
mortality: 100 %;
clinical findings prior death:
respiratory problems, loss of muscle coordination, convulsions, poor general health;
pathological examination:
pale and mottled livers, brown to dark areas of the lungs, multiple haemorrhages of the pyloric region of the stomach and thymus
Pregnancy rates (%): 300 mg-gr: 83 %, 400, 500, 600 mg-gr: 100 %

Test condition **:**

-Number of animals: 6 per group
-Total volume applied: 10 ml/kg bw

-Clinical observations / Mortality: once daily

ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
external examination, examination of the thoracic and abdominal organs, opening of the uterus, recording of foetuses (alive, dead, macerated, relative placement, number and location), live foetuses were sexed and examined grossly for external malformations and weight

TEST SPECIES AND ANIMAL HUSBANDARY
-Sprague Dawley rats
-Number of animals: 6 mated females per group
-Housing: individually in stainless steel cages
Temperature: 72°F
Relative Humidity: 40-60 %
lightening time: 12 hours artificial light per day
Food and water ad libitum
Acclimatisation: 10 days

ADMINISTRATION/EXPOSURE:
-vehicle: corn oil
-Total volume applied: 10 ml/kg bw/day

-CLINICAL OBSERVATIONS AND FREQUENCY:
survival: twice daily
clinical signs: detailed examination on gd 0 and daily on gd 6-21
-Body weight: on gd 0, 6, 10, 13, 16 and 21
-Food consumption: on gd 0-6, 6-10, 10-13, 13-16 and 16-21

ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
gd 21: recording of number/relative placement of live/dead

foetuses, early/late resorptions, number of corpora lutea;
dams underwent gross necropsy;
all live foetuses from surviving dams underwent external examination, were sexed and weighted; ca. 50 % of each litter were examined for visceral or skeletal malformations, resp.

STATISTICAL ANALYSIS:
in general: one-side comparison (except sex distribution of fetuses: two-side comparison):
Dunnett's test, Mann-Whitney U test, Fisher's exact test, cochran-armitrage test, Bonferroni's inequality

Reliability : (2) valid with restrictions
Dose-finding study well documented, meets generally accepted scientific priciples

07.01.2004 (129)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

Type	:	other: subacute study
In vitro/in vivo	:	In vivo
Species	:	rat
Sex	:	male/female
Strain	:	Wistar
Route of admin.	:	gavage
Exposure period	:	28 d
Frequency of treatm.	:	daily
Duration of test	:	28 d
Doses	:	0, 4, 20, 100 mg/kg bw/day in sesame oil
Control group	:	yes, concurrent vehicle
Result	:	see freetext RS
Method	:	other: OECD Guideline 407
Year	:	1992
GLP	:	yes
Test substance	:	other TS: 1,2-dichloro-4-nitrobenzene, purity: 99 %
Remark	:	For general toxicity see chapter 5.4
Result	:	OBSERVATIONS: No death occurred throughout the study unspecific signs of intoxication: 100 mg-group, m/f: irregular respiration, stilted gain, >= 20 mg/kg bw/day, m/f: increased salivation; all groups: body weight gain was not impaired, food consumption unaffected, >=20 mg/kg bw/day(m/f): slightly increased water intake (not significant, not dose dependant) REPRODUCTIVE ORGAN EVALUATION: no pathologic findings were reported.
Test condition	:	TEST SPECIES AND ANIMAL HUSBANDARY. -Age at start of the study: 6 weeks -Number of rats: 5 m/5 f per group -Animal maintenance: air-conditioned rooms, -----groups of 5 rats/cage -Acclimatisation: 5 days -Room temperature: 22 °C -Relative Humidity: 50 % -Lighting time: 12 hours daily -Food: rat diet ad libitum -Water: tap water ad libitum ADMINISTRATION / EXPOSURE -Dose selection based on priliminary experiments -Vehicle: sesame oil -Concentration in vehicle: 0 - 2 % (w/v)

-Total volume applied: 5 ml/kg bw

CLINICAL OBSERVATIONS AND FREQUENCY
-Clinical signs: twice daily
-Body weight: at the start of the study and then twice weekly
-Food consumption: two times per week
-Water consumption: once per week
-Ophthalmoscopic examination: weekly
CLINICAL lABORATORY EXAMINATIONS
-Haematology / Clinical chemistry: at termination of the study
-Haematology: Erythrocyte count(Erys), hemoglobin (HG), ---hematocrit(HK), mean cellular volume(MCV), mean cellular ---hemoglobin (MCHC), Leucocyte ccount (Leucos(Thromocyte ---count(Thromos) Differential leucocyte count and red cell ---morphology, Reticulocyte count(reticulos), Heinz bodies ---(HB), coagulation time
-Clinical chemistry: Sodium, Potassium, Inorganic ---phophorus, Uric acid, Bilirubin total, Creatinine, ---Serum-glucose. Urea nitrogen, Calcium, Chloride, ---Aspartate aminotransferase(ASAT/GOT), Alanine ---aminotransferase (ALAT/GPT), Alkaline phosphatase (AP), ---Gamma-glutamyxltransferawe (GGT) Total protein, Albumin
-Urinalysis: a few days before termination of the study:
---Appearance, colour, pH-value, hemoglobin, protein, ---glucose, ketone bodies, bilirubin, urobilinogen, ---specific weight, sediment, volume

NECROPSY:
-Examination of skin, orifices, eyes, teeth, oral mucosa ---and internal organs
-Organ weights: heart, lung, liver, kidneys, spleen, ---ovaries, tetes, epididymides, adrenals, brain, thymus
-Histopathology: heart, liver, kidneys, adrenals, spleen, ---lung, brain, thymus, trachea, stomach, jejunum, colon, ---urinary bladder, skeletal muscles, N.ischiadicus, femur ---with bone marrow, spinal cord (cervical, thoracal, ---lumbal), lymph nodes(cervical and iliacal)
--only controls and high dose groups: ovaries, uterus, ---testes, epididymides prostata, seminal vesicles
-Statistics:
---One way analyses of variance with sequentially rejective ------multiple comparison
---One way analyses of variance based on ranks with ------sequentially rejective multiple comparison
---Trend Test analyses for non-neoplastic lesions ------(ARMITAGE)

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
07.01.2004 (110)

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

Type of experience : Health records from industry

Result : The authors reported the results of a 2.5 year study on the health of workers exposed at a 1,2-dichloro-4-nitrobenzene processing plant in the former USSR. The workers were exposed essentially to 1,2-dichloro-4-nitrobenzene, 3,4-dichloroaniline and 3,4-dichloropropionic acid anilide.

The skin was affected (chloracne) and also alterations of peripheral blood including instable methaemoglobinaemia appearance of Heinz bodies, a tendency towards reticulolymphocytosis and thrombocytosis, bilirubinaemia and dysproteinaemia. The exposure risk was suspected via dermal absorption due to the low vapour pressure and the working conditions. Measurements for uncovered skin gave exposures to 0.002 - 0.2 mg/dm2 1,2-dichloro-4-nitrobenzene and 3,4-dichloroaniline and for covered skin (clothes) 0.0013 - 0.02 mg/dm2.
For 1,2-dichloro-4-nitrobenzene and 3,4-dichloroaniline the mean individual dermal exposure per shift was given with 2.535 mg, while after cleaning of the skin the mean individual burden was still 1.066 mg.
Due to the mixed exposure an association of the findings with exposure to 1,2-dichloro-4-nitrobenzene is uncertain.

Reliability : (4) not assignable
Secondary literature
Documentation insufficient for assessment

06.02.2002 (130)

5.11 ADDITIONAL REMARKS

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