201-14282B

Robust Summaries and Repository of Knowledge for CAS No. 108-90-7

Existing Chemical	:	ID: 108-90-7
CAS No.	:	108-90-7
EINECS Name	:	chlorobenzene
EINECS No.	:	203-628-5
TSCA Name	:	Benzene, chloro-
Molecular Formula	:	C6H5Cl

Printing date	:	29.01.2003
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1.0.1 OECD AND COMPANY INFORMATION

1.0.2 LOCATION OF PRODUCTION SITE

1.0.3 IDENTITY OF RECIPIENTS

1.1 GENERAL SUBSTANCE INFORMATION

Substance type	:	organic
Physical status	:	liquid
Purity	:	= 100 % w/w
Reliability	:	(1) valid without restriction

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1.1.0 DETAILS ON TEMPLATE

1.1.1 SPECTRA

1.2 SYNONYMS

benzene chloride
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benzene mono chloride
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benzene, chloro
29.11.2001

chlorbenzene
29.11.2001

chlorobenzol
29.11.2001

monochloro benzene
29.11.2001

phenyl chloride
29.11.2001

1.3 IMPURITIES

1.4 ADDITIVES

1.5 QUANTITY

1.6.1 LABELLING

1.6.2 CLASSIFICATION

1.7 USE PATTERN

1.7.1 TECHNOLOGY PRODUCTION/USE

1.8 OCCUPATIONAL EXPOSURE LIMIT VALUES

1.9 SOURCE OF EXPOSURE

1.10.1 RECOMMENDATIONS/PRECAUTIONARY MEASURES

1.10.2 EMERGENCY MEASURES

1.11 PACKAGING

1.12 POSSIB. OF RENDERING SUBST. HARMLESS

1.13 STATEMENTS CONCERNING WASTE

1.14.1 WATER POLLUTION

1.14.2 MAJOR ACCIDENT HAZARDS

1.14.3 AIR POLLUTION

1.15 ADDITIONAL REMARKS

1.16 LAST LITERATURE SEARCH

1.17 REVIEWS

1.18 LISTINGS E.G. CHEMICAL INVENTORIES

2.1 MELTING POINT

Value	:	= -45.2 ° C
Sublimation	:	
Method	:	OECD Guide-line 102 "Melting Point/Melting Range"
Year	:	
GLP	:	*no*
Test substance	:	as prescribed by 1.1 - 1.4
Source	:	Monsanto Bruxelles Monsanto Brussels Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Remark	:	Kirk Othmer Encyclopedia of Chemical Technology, 4th Edition, Volume 6, Page 89, cites a melting point value of -45.34° C. Ulmann's Encyclopedia of Industrial Chemistry, 5th Ed. Page 328 cites a value of –45.2° C.
Reliability	:	(2) valid with restrictions. OECD guideline study. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.

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2.2 BOILING POINT

Value	:	= 132.1 ° C at 1013 hPa
Decomposition	:	no
Method	:	OECD Guide-line 103 "Boiling Point/boiling Range"
Year	:	
GLP	:	No
Test substance	:	as prescribed by 1.1 - 1.4
Source	:	Monsanto Bruxelles Monsanto Brussels Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Remark	:	Kirk Othmer Encyclopedia of Chemical Technology, 4th Edition, Volume 6, page 89, cites a boiling point value of 131.7° C. Ulmann's Encyclopedia of Industrial Chemistry, 5th Ed., page 328, cites a value of 132.2° C.
Reliability	:	(2) valid with restrictions. OECD guideline study. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.

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2.3 DENSITY

Type	:	Density
Value	:	= 1.06 g/cm3 at 20° C
Test substance	:	as prescribed by 1.1 - 1.4
Source	:	Hoechst AG Frankfurt/Main Clariant GmbH Frankfurt am Main EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(4) valid with restrictions. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.
Flag	:	Key study for endpoint

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Type	:	relative density
Value	:	= 1.107 at 4° C
Method	:	OECD Guide-line 109 "Density of Liquids and Solids"
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Source	:	Monsanto Bruxelles Monsanto Brussels Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. OECD guideline study. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.

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2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value	:	11.7 hPa at 20° C
Test substance	:	as prescribed by 1.1 - 1.4
Source	:	Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.
Flag	:	Key study for endpoint

(1)

Value	:	= 12 hPa at 20° C
Test substance	:	as prescribed by 1.1 - 1.4
Source	:	Hoechst AG Frankfurt/Main Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000. This vapor pressure value is likely a measured value, since this chemical is a well-known, basic substance.

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2.5 PARTITION COEFFICIENT

Log pow	:	ca. 2.84 at 20 ° C
Method		Shaking
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	
Reliability	:	(2) valid with restrictions. Pre GLP and OECD guideline studies
Flag	:	Key study for endpoint

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Log pow	:	ca. 2.84 at ° C
Method		other (calculated)
Year	:	2001
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The Log Pow was estimated using the EPIWIN KOWWIN Program (v 1.66). The Log pow was calculated based on Log pow values established in the model for individual bond fragments in the molecule. These were summed to give the overall value.
Reliability	:	(2) valid with restrictions. Data were obtained by modeling.

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2.6.1 WATER SOLUBILITY

Value	:	.21 g/l at 20 ° C
Qualitative	:	
Pka	:	at 25 ° C
PH	:	at and ° C
Test substance	:	as prescribed by 1.1 - 1.4
Source	:	Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Remark	:	Ulmann's Encyclopedia of Industrial Chemistry cites a water solubility of 0.5 g/l. It is likely that this and the above value are both measured, since this substance is a basic chemical that has been well known for many decades.
Reliability	:	(2) valid with restrictions. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.
Flag	:	Key study for endpoint

(3)

Value	:	.4 g/l at 20 ° C
Qualitative	:	
Pka	:	at 25 ° C
PH	:	at and ° C
Method	:	
Year	:	1995
GLP	:	no data
Test substance	:	as prescribed by 1.1-1.4
Source	:	Petrasol B.V. Gorinchem EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.

(14)

Value	:	= .5 g/l at 30 ° C
Qualitative	:	
Pka	:	at 25 ° C
PH	:	= 7 at .5 g/l and 30 ° C
Test substance	:	as prescribed by 1.1-1.4
Remark	:	Chlorbenzol ist schwerer als Wasser. [Chlorobenzene is denser than water]
Source	:	Hoechst AG Frankfurt/Main Clariant GmbH Frankfurt am Main EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.

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2.7 FLASH POINT

Value	:	ca. 27 ° C
Type	:	closed cup
Method	:	other: DIN 51755
Year	:	
GLP	:	
Test substance	:	as prescribed by 1.1-1.4
Source	:	Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. DIN is an acceptable method. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.

(3)

Value	:	= 28 ° C
Type	:	
Test substance	:	as prescribed by 1.1-1.4
Source	:	Hoechst AG Frankfurt/Main Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.

(17)

2.8 AUTO FLAMMABILITY

Value	:	ca. 590 ° C at
Remark	:	Zündtemperatur [ignition temperature]
Test substance	:	as prescribed by 1.1-1.4
Source	:	Hoechst AG Frankfurt/Main Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.

(17)

Value	:	638 ° C at
Method	:	
Year	:	1988
GLP	:	no data
Test substance	:	as prescribed by 1.1-1.4
Source	:	Petrasol B.V. Gorinchem EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.

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2.9 FLAMMABILITY

Result	:	flammable
Test substance	:	as prescribed by 1.1-1.4
Source	:	Hoechst AG Frankfurt/Main Clariant GmbH Frankfurt am Main EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.

(19)

2.10 EXPLOSIVE PROPERTIES

Result	:	explosive under influence of a flame
Method	:	
Year	:	1984
GLP	:	no data
Test substance	:	as prescribed by 1.1-1.4
Remark	:	Upper 7.1%, lower 1.3% at 150 deg C
Source	:	Petrasol B.V. Gorinchem EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.

(20)

Result	:	Other
Remark	:	Explosive limits = 1.3 - 11 Vol.-%
Test substance	:	as prescribed by 1.1-1.4
Source	:	Hoechst AG Frankfurt/Main Clariant GmbH Frankfurt am Main EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.

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2.11 OXIDIZING PROPERTIES

Result	:	no oxidizing properties
Test substance	:	as prescribed by 1.1-1.4
Source	:	Petrasol B.V. Gorinchem EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.

2.12 ADDITIONAL REMARKS

Remark	:	Surface tension: 33 dyn/cm at 25 deg C, 1984
Test substance	:	as prescribed by 1.1-1.4
Source	:	Petrasol B.V. Gorinchem EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.

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Remark	:	Hazardous reactions with alkali and oxidizing agents

Test substance	:	as prescribed by 1.1-1.4
Source	:	Hoechst AG Frankfurt/Main Clariant GmbH Frankfurt am Main EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000. (19)
Remark	:	Gefährliche Zersetzungsprodukte: Chlorwasserstoff (HCl),[Hazardous decomposition products: hydrogen chloride (HCl)] Kohlendioxid, Kohlenmonoxid [Carbon dioxide, carbon monoxide].
Test substance	:	as prescribed by 1.1-1.4
Source	:	Hoechst AG Frankfurt/Main Clariant GmbH Frankfurt am Main EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000. (19)

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	Sun light
Light spect.	:	= 300 - 400 nm
Rel. intensity	:	= 1 based on Intensity of Sunlight
Direct photolysis	:	ca. 93.6 hour(s)
Halflife t1/2	:	ca. 8 day
Degradation	:	% after
Indirect photolysis	:	OH
Sensitizer	:	OH
Conc. of sens.	:	
Rate constant	:	cm3/(molecule*sec)
Degradation	:	% after
Deg. Product	:	
Method	:	other (measured): T. Mill, W. Mabery, et al. Laboratory Protocol for Evaluating the Fate of Organic Chemicals in Air and Water. SRI International, Menlo Park, California (1980)
Year	:	1980
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	This study tested for photodegradation with hydroxyl radical. O-chlorophenol & nitrochlorobenzene identified as immediate degradation products; no evidence for formation of chlorobiphenyls. The environmental half life (t 1/2) of mono chlorobenzene was estimated by simultaneously reacting mono chlorobenzene and a reference compound (toluene or benzene) with the photochemically generated OH. Measuring the relative reaction rates and knowing the t 1/2 for the reference compounds allowed the estimation of the t 1/2 for chlorobenzene. The reaction products were identified after repeating the experiment without a reference compound.
Source	:	Monsanto Bruxelles Monsanto Bruxelles Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Test condition	:	Dates of Exposure: 18 Aug to 11 Nov 1980; Summer/Fall. Conditions: Sunny days; temperature 10-35C Location: Roof of N Bld, Monsanto, St. Louis, Missouri
Reliability	:	(2) valid with restrictions. Assigned a reliability rating of 2 because the purity of test substance was not given. According to the material safety data sheet (30 August 2001) for Solutia Inc. (formerly a part of Monsanto) the purity of commercial chlorobenzene is 100%.
Flag	:	Key study for endpoint

01.12.2001 (30)

Type	:	air
Light source	:	Sun light
Light spect.	:	nm
Rel. intensity	:	based on Intensity of Sunlight
Direct photolysis		
Halflife t1/2	:	ca. 93.6 hour(s)
Degradation	:	% after
Quantum yield	:	

Indirect photolysis
Sensitizer : OH
Conc. of sens. : 1500000 molecule/cm3
Rate constant : ca. .00000000000077 cm3/(molecule*sec)
Degradation : % after
Test substance : as prescribed by 1.1-1.4
Remark : Photolysis was estimated using the EPIWIN AOP Program (v 1.90). The EPIWIN Program calculates an overall hydroxyl radical rate constant based on the reaction of hydroxyl radical with various bonds in the substance molecule. The contributions of these individual reactions are summed to give an overall rate constant. The half life is calculated assuming a constant OH radical concentration, using pseudo first order kinetics.
Reliability : (2) valid with restrictions. Data were obtained by modeling. Supporting study.
Flag :
01.12.2001 (11)

Type : air
Light source :
Light spect. : Nm
Rel. intensity : based on Intensity of Sunlight
Indirect photolysis
Sensitizer : OH
Conc. of sens. : 1600000 molecule/cm3
Rate constant : ca. .0000000000014 cm3/(molecule*sec)
Degradation : ca. 50 % after 7.5 day
Deg. Product :
Method : other (calculated): AOPWIN, Version 1.55
Year : 1994
GLP :
Test substance : as prescribed by 1.1-1.4
Source : Hoechst AG Frankfurt/Main
Clariant GmbH Frankfurt am Main
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (2) valid with restrictions. Data were obtained by modeling. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.
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Type : Air
Light source :
Light spect. : Nm
Rel. intensity : based on Intensity of Sunlight
Spectr. of subst. : lambda (max, >295nm) : 300 nm
epsilon (max) :
epsilon (295) :
Direct photolysis
Halflife t1/2 :
Degradation : 100 % after 1 month
Quantum yield :
Indirect photolysis
Sensitizer : OH
Conc. of sens. :
Rate constant : cm3/(molecule*sec)
Degradation : 5 - 10 % after 1 day
Deg. Product :

Method	:	
Year	:	1981
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Chlorobenzene adsorbs light in the 290-310 nm region and will photolyse in the trophosphere as well as in the surface water. Oxidation in air-NOx systems: the rate of reaction is somewhat higher when NOx is present, compared to OH. Products formed include chlorophenols, nitrochlorophenols and m-chloronitrobenzene.
Source	:	Petrasol B.V. Gorinchem EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(4) not assignable. Method not provided. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000. (20)

Type	:	air
Light source	:	Sun light
Light spect.	:	290 nm
Rel. intensity	:	= 1 based on Intensity of Sunlight
Conc. of subst.	:	.045 mg/l at 1.7 degree C
Deg. Product	:	
Method	:	other (measured): literature reference...D.G. Crosby and L.W. Moilanen, Bull. Environ. Contam. Toxicol. 2, 64 (1974)
Year	:	1974
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	MCB does not undergo direct photolysis.
Source	:	Monsanto Bruxelles Monsanto Bruxelles Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Test condition	:	Dates: 11-22 December 1978 (late fall to early winter) Conditions: Full sunlight during daylight period (@7:30 am to 4:30 pm); exception on 18-20 December when partly cloudy Temperature: Overnight low 20F, daytime high low 50's F Location: Roof of Monsanto T bld, St. Louis, Missouri Note: MCB vapor does not absorb UV radiation above 280 nm
Reliability	:	(2) valid with restrictions. Purity of the test material was not given. (29)

Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Absorbs light in 290-310 nm region. May photolyze in troposphere. Half life for MCB in surface water = 170 years in summer.
Source	:	DALTRADE LTD LONDON EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(4) not assignable. Method and study details not provided. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000. (12)

3.1.2 STABILITY IN WATER

Deg. Product	:	

Method : other (calculated)
Year : 2001
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Remark : An attempt was made to estimate hydrolysis rate using the EPIWIN HYDROWIN Program (v1.67).
Result : EPIWIN HYDROWIN Program (v1.67) cannot estimate a hydrolysis rate constant for chlorobenzene. It is generally recognized from basic organic chemistry that simple halobenzenes with no other functional groups are highly resistant to hydrolysis in water. Water hydrolysis is not an important degradation path for chlorobenzene.
Reliability : (4) not assignable
Flag : Key study for endpoint
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Type : abiotic
t1/2 pH4 : at degree C
t1/2 pH7 : at degree C
t1/2 pH9 : at degree C
t1/2 pH : 4.5 hour(s) at degree C
Deg. Product :
Method :
Year : 1983
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Remark : The primary loss will be due to evaporation. The rate of evaporation will depend on the wind speed and water movement. The half-life for evaporation is approximately 4.5 hours with moderate wind speed.
Source : Petrasol B.V. Gorinchem
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable. Method, pH, temperature not provided. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.
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3.1.3 STABILITY IN SOIL

Deg. Product :
Method :
Year : 1980
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Remark : Since chlorobenzene is fairly volatile, much of it will be lost to the atmosphere.

It is relatively mobile in sandy soil and aquifer material and biodegrades very slowly or not at all in these soils. Therefore it can be expected to leach into the groundwater. It has a moderate adsorption onto organic soil and if retained long enough it will biodegrade and even mineralize in soil. Degradation will generally be slow, but fairly rapid mineralization has been reported in one study, 20% per week.
Source : Petrasol B.V. Gorinchem
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable. Information came from IUCLID document created by

European Chemicals Bureau, creation date 10-FEB-2000.

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3.2 MONITORING DATA

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	:	fugacity model level III
Media	:	water - air
Air (level III)	:	25.5
Water (level III)	:	31.1
Soil (level I)	:	
Biota (level II / III)	:	.264
Soil (level II / III)	:	43.1
Method	:	Other: calculated. Inputs to the EPIWIN model were measured values for melting point (-45.2°C), boiling point (132.1°C), vapor pressure (9 mm Hg), water solubility (400 mg/l) and Log Kow (2.84).
Year	:	2001
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	MacKay level III fugacity modeling conducted using EPIWIN STP Fugacity Model. Model assumes a Henry's Law Constant of 0.00311 atm-m3/mole, a vapor pressure of 11.8 mm Hg, a log Kow of 2.84 and a soil Koc of 284. The EPIWIN HENRY (V3.10) program was used to calculate the Henry's Law Constant. The EPIWIN PCKOC (V1.66) program was used to estimate the Koc(soil-sediment partition constant).). Level III fugacity calculations allow non-equilibrium conditions to exist between connected media at steady state. The EPIWIN BCF (v2.14) program was used to estimate a BCF (bioconcentration factor) of 3.162 or a log BCF of 0.500.
Reliability	:	(2) valid with restrictions. Data were obtained by modeling
Flag	:	Key Study

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Type	:	fugacity model level I
Media	:	water – air
Air (level I)	:	97.9
Water (level I)	:	
Soil (level I)	:	0.7
Biota (level II / III)	:	
Soil (level II / III)	:	
Method	:	Other
Year	:	2001
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Level I EQC modeling by Mackay assumes equilibrium, steady state conditions.
Reliability	:	(2) valid with restrictions. Data were obtained by modeling
Flag	:	Key Study

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Type	:	volatility
Media	:	water - air
Air (level I)	:	
Water (level I)	:	

Soil (level I)	:	
Biota (level II / III)	:	
Soil (level II / III)	:	
Method	:	
Year	:	1979
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The evaporation rate of chlorobenzene from water gave a half-life of 10-11 hours, a figure which is based upon the Henry constant (3.56x10-3 atmos cu m/mole) for chlorobenzene.
Source	:	Petrasol B.V. Gorinchem EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(4) not assignable. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.

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Type	:	Henry's Law Constant
Media	:	Water - air
Result	:	.00393 atm m^3/mol
Method	:	Measured
Year	:	1987
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Range of measured and calculated values = .00314-.00640 atm m^3/mol
Reliability	:	(2) Valid with restrictions, some study detail lacking.

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3.5 BIODEGRADATION

Type	:	aerobic
Inoculum	:	activated sludge, industrial, non-adapted
Contact time	:	
Degradation	:	> 90 % after 15 day
Result	:	
Kinetic of test substance	:	5 day = 30 % 10 day = 70 % 15 day > 90 % % %
Deg. Product	:	
Method	:	other: Respirometrischer Test mit Sapromat [Respirometer test]
Year	:	1982
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Source	:	Hoechst AG Frankfurt/Main Bayer AG Leverkusen Hoechst AG Frankfurt/Main Clariant GmbH Frankfurt am Main EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.
Flag	:	Supportive study for endpoint

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Contact time	:	
Degradation	:	76.7 % after 2 month
Result	:	

Deg. Product	:	
Method	:	
Year	:	1987
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Concentration: 400 ppb, was added to a ground-water microcosm, incubated at 22 deg C. 76.7% was removed after 8 weeks.
Source	:	Petrasol B.V. Gorinchem EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(4) not assignable. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.
Flag	:	Supportive study for endpoint

(20)

Type	:	aerobic
Inoculum	:	predominantly domestic sewage
Contact time	:	
Degradation	:	50 - 60 % after 20 day
Result	:	
Deg. Product	:	
Method	:	OECD Guide-line 301 D "Ready Biodegradability: Closed Bottle Test"
Year	:	1973
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Source	:	Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Acceptable method. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.
Flag	:	Supportive study for endpoint

(8)

Type	:	aerobic
Inoculum	:	activated sludge
Contact time	:	
Degradation	:	15 % after 28 day
Result	:	
Deg. Product	:	
Method	:	Directive 84/449/EEC, C.7 "Biotic degradation - modified MITI test"
Year	:	1991
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4: chemically pure
Source	:	Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Acceptable method. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.
Flag	:	Supportive study for endpoint

(2)

Type	:	aerobic
Inoculum	:	other: sludge samplings from different sewage plants, rivers, bays and a lake
Concentration	:	100mg/l related to related to
Contact time	:	

Degradation	:	% after 28 day
Result	:	under test conditions no biodegradation observed
Deg. Product	:	
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	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, the Minister of Health and Welfare, the MITI No. 1). This guideline corresponds to "301C, Ready Biodegradability: Modified MITI Test I" stipulated in the OECD Guideline for Testing of Chemicals (May 12, 1981) Sludge conc. : 30 mg/l
Source	:	Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Acceptable method. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.
Flag	:	Supportive study for endpoint

(4)

Deg. Product	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	A large number of bacteria and fungi found in the environment are capable of degrading chlorobenzene and mineralizing it. 2- and 4-chlorophenol are products of this biodegradation. Degradation is generally slow in water and soil, but may be significant in some situations. Acclimation of the degrading microorganisms in an important factor.
Source	:	Petrasol B.V. Gorinchem EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(4) not assignable. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.
Flag	:	Supportive study for endpoint

(20)

Type	:	aerobic
Inoculum	:	other: activated sludge
Degradation	:	19-27 % after 63-day
Method	:	other
Year	:	1977
GLP	:	no data
Test substance	:	^{14}C-labeled monochlorobenzene
Remark	:	Cumulative $^{14}CO_2$ production after a 63-day exposure to Mississippi River water was 19 and 27% of theoretical for 0.5 and 4.3 ppm ^{14}C-labeled monochlorobenzene. Only 2% of theoretical $^{14}CO_2$ was released when ^{14}C-labeled monochlorobenzene was tested in BOD medium. Only 10% of theoretical $^{14}CO_2$ was evolved after the positive control (25 ppm linear dodecylbenzene sulfonate) was incubated in Mississippi River Water for 63 days, suggesting that the test system was not optimized.
Reliability	:	(4) invalid. The positive control did not degrade well.

(34)

3.7 BIOACCUMULATION

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	static
Species	:	Salmo gairdneri (Fish, estuary, fresh water)
Exposure period	:	24 hour(s)
Unit	:	mg/l
Analytical monitoring	:	yes
LC50	:	m = 4.1
Method	:	other
Year	:	1983
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The toxicity of 1,2 and 1,4 -dichlorobenzenes (ortho and para), and 1,2,3 and 1,2,4- trichlorobenzene also was tested in this study. The 24-hour LC50 values for these materials were 2.3, 1.18, 0.71 and 1.95 mg/l, respectively. It is assumed that the conditions under which the test material was handled were similar to the algal study reported below, since the study was performed in the same laboratory at a later date. The results are reported as 48 hour LC50 values in the study; however, the methods state that the experiment was only carried out for 24 hours. Therefore, the values are reported in this summary as 24 hour.
Result	:	The 24 hour LC50 value was 4.1 (3.87 - 4.23) mg/l. The slope of the effect concentration curve was 1.12. The concentrations used to calculate the LC50 value were nominal (not listed), as analytical concentrations were within 10% of nominal concentrations.
Test condition	:	An ISRA test (Quaderni dell'Instituto di Ricerca sulle Acgue, 11, Consiglio Nazionale delle Ricerche-Roma, 1973). was performed to establish the 24 hr LC50 value. Two closed bottles (10 l) containing 5 fish were used for each concentration (not stated). The test vessels were modified according to the method of Galassi and Vighi (Chemosphere 10: 1123, 1981) in order to maintain constant concentration and to allow for sampling of the medium without opening the vessel. Water conditions during the experiment were: hardness 320 mg CaCO3/l, pH 7.4, oxygen not less than 70% saturation at the end of the test, and a temperature of 15 degrees. Concentrations of test material in vessels were measured analytically (by gas chromatography) at the beginning and end of the tests. Dilute solutions were extracted with n-hexane before analysis. The limit of detection was 0.02 mg/l. Mortality data were analyzed according to the method of Litchfield and Wilcoxon
Test substance	:	Test material was analytical grade
Reliability	:	(2) valid with restrictions. It is unclear whether results are for 24 or 48 hours.
Flag	:	Supportive study for endpoint

29.11.2001 (9)

Type	:	static
Species	:	Brachydanio rerio (Fish, fresh water)
Exposure period	:	24 hour(s)
Unit	:	mg/l
Analytical monitoring	:	yes
LC50	:	m = 10.5
Method	:	other
Year	:	1983
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The toxicity of 1,2 and 1,4 -dichlorobenzenes (ortho and para), and 1,2,3 and 1,2,4- trichlorobenzene also were tested in this study. The 24-hour LC50 values for these materials were 6.8, 4.25, 3.1 and 6.3 mg/l, respectively. It is assumed that the conditions under which the test material was handled were similar to the algal study reported below, since the study was performed in the same laboratory at a later date. The results are reported as 48 hour LC50 values in the study; however, the methods state that the experiment was only carried out for 24 hours. Therefore, the values are reported in this summary as 24 hour.
Result	:	The 24 hour LC50 value was 10.5 (7.1 - 15.5) mg/l. The slope of the effect concentration curve was 1.87. The concentrations used to calculate the LC50 value were nominal (not listed), as analytical concentrations were within 10% of nominal concentrations.
Test condition	:	An ISRA test (Quaderni dell'Instituto di Ricerca sulle Acgue, 11, Consiglio Nazionale delle Ricerche-Roma, 1973). was performed to establish the 24 hr LC50 value. Two closed bottles (10 l) containing 5 fish were used for each concentration (not stated). The test vessels were modified according to the method of Galassi and Vighi (Chemosphere 10: 1123, 1981) in order to maintain constant concentration and to allow for sampling of the medium without opening the vessel. Water conditions during the experiment were: hardness 320 mg CaCO3/l, pH 7.4, oxygen not less than 70% saturation at the end of the test, and a temperature of 23 degrees. Concentrations of test material in vessels were measured analytically (by gas chromatography) at the beginning and end of the tests. Dilute solutions were extracted with n-hexane before analysis. The limit of detection was 0.02 mg/l. Mortality data were analyzed according to the method of Litchfield and Wilcoxon.
Test substance	:	Test material was analytical grade
Reliability	:	(2) valid with restrictions. It is unclear whether results are for 24 or 48 hours
Flag	:	Supportive study for endpoint

29.11.2001 (9)

Type	:	static
Species	:	Salmo gairdneri (Fish, estuary, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l

Analytical monitoring	:	yes
LC0	:	m = 5.8
LC50	:	m = 10.4
LC100	:	m = 18
Method	:	OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year	:	1990
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Results of analytical tests showed that under the test conditions, most of the test material was lost during the first 20 hours of the test. The fish that died either succumbed or showed abnormal behavior during the first few hours when the actual concentration was near the nominal concentration. Since it took 20 hours for all of the fish to die, the data from this test are considered to be indicative of the results of a 20-hour test. The nominal concentrations were regarded as more relevant for the evaluation of the test because all the fish that died were harmed by the high concentrations they were exposed to in the first hours of the test.
Result	:	No mortality was observed in fish treated with 5.8 mg/l (nominal concentration). Forty percent mortality was observed in the 10 mg/l group, and 100% mortality occurred at higher concentrations. All deaths occurred within 24 hours. The LC50 was calculated to be 10.4 mg/l (nominal concentration). Throughout the test, water maintained anywhere from 91-100% saturation, a pH of 7.2 to 7.8, and a temperature of 14.1 to 15.5 degrees C. Analytical measurements of test concentration in the test vessels indicated that only 0.26 to 2.2 % of test material was recovered. Results of the second test for analytical concentration revealed 10 (erroneous), 53, 3.5 and 2.4% recovery of a 5.8 mg/l nominal concentration at 0, 2, 6, and 20 hours; and 105, 60, 24 and 3.4% recovery of an 18 mg/l nominal concentration at 0, 2, 6, and 20 hours. Based on the % recovery at 20 hrs, the average analytical concentrations over a 20 hour period for 5.8 (LC0), 10.4 (LC50) and 18 mg/l (LC100) were 0.14, 0.3 and 0.61 mg/l. The value for the 10.4 mg/l concentration was calculated from an estimated average recovery of 2.9%.
Test condition	:	Healthy rainbow trout with a total length of 5 +/- 1 cm were used. They were acclimated for 3 weeks before test initiation in two 300 liter tanks containing dechlorinated water that was continuously aerated. Light was provided in a daily photoperiod of 12 hours. Fish were fed with trout food during the holding period. Fish were not fed 24 hours prior to initiation of the test. Two hours before the start of the test, clean glass tanks (350 mm x 230 mm x 260 mm) were filled with 18 liters of reconstituted water. The tanks were kept under identical conditions regarding temperature and aeration. Tanks were covered by specially fitted glass plates assuring aeration as well as free exchange of the atmosphere, but preventing the fish from jumping out. Test water was checked to verify that the dissolved oxygen concentration, temperature and pH were within specification limits (not less than 60% O2 saturation, temperature between 13 and 17 degrees C, and pH between 6.0 and 8.5). Test material was then added to separate vessels at 0.03, 1.8, 3.2, 5.8, 10, 18, 32, 58 or 100 mg/l. An additional vessel was not treated. The contents of each tank were mixed thoroughly with a mechanical stirrer to facilitate dissolving of the test material. Air was continuously bubbled into the test medium through "flow out stones" directly connected to the aerator with flexible PVC tubes. Aeration was necessary because the O2 concentration fell below 60% of saturation within 6-8 hours.

Two hours after test material was added to the tanks, 10 trout were set into each tank. Fish were inspected at 0, 24, 48 and 72 hours after addition according to a fixed test protocol. Fish were considered dead if touching of the caudal peduncle produced no reaction and if no breathing movements were observed. Dead fish were removed from the tanks and surviving fish were carefully observed for illness. Samples of test medium were taken at the beginning, after 48 hr and at the end of the test for analysis using GC-ECD.

The percentage of immobilization at each concentration was transferred into a Probit value. The probit value for 0% was assigned as 3.36 (this corresponds to 5%) and for 100% was assigned as 6.65 (this corresponds to 95%). These values were chosen because the result of 0 (or 10) dead fish can mean any percentage between 0 and 10% (or 90-100%) since there were only 10 (not 100) fish tested. With these values, a linear regression analysis of the Probit = f(log C) function was performed.

The first analytical tests were unsatisfactory because of high losses of test material during the storage time of the samples. Therefore, a second control test was performed. In this test, vessels were prepared containing only test material at concentrations equal to the LC0, LC50 and LC100 in the test. The medium was aerated the same way as it was in the test. This was performed 4 times: 0 hr, 2 hr, 6 hr and 20 hrs before sampling. Samples of medium were analyzed immediately for test material concentration by GC-ECD.

Reliability : (2) valid with restrictions. Purity of test material was not stated. Test material was lost due to volatization.

Flag : Supportive study for endpoint

27.11.2001 (22)

Type :
Species : other: freshwater fish
Exposure period : 96 hour(s)
Unit : mg/l
Analytical monitoring : no
LC50 : c = 13.545
Method : other: ECOSAR program (v 0.99g)
Year : 2002
GLP : no
Test substance : as prescribed by 1.1 - 1.4
Test condition : The LC50 value was estimated by the ECOSAR program (v0.99g), with a log Kow of 2.84 and water solubility of 400 mg/l entered by the user.
Reliability : (2) valid with restrictions. Data were obtained by modeling.
Flag : Supportive study for endpoint

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 24 hour(s)
Unit : mg/l
Analytical monitoring : yes
EC50 : m = 4.3

Method	:	other
Year	:	1983
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The toxicity of 1,2- and 1,4 -dichlorobenzenes (ortho and para), and 1,2,3- and 1,2,4- trichlorobenzenes also was tested in this study. The 24-hour EC50 values for these materials were 0.78, 1.6, 0.35, and 1.2 mg/l, respectively. It is assumed that the conditions under which the test material was handled were similar to the algal study reported below, since the study was performed in the same laboratory at a later date.
Result	:	The 24 hour EC50 value for immobilization (with confidence limits) was 4.3 (3.23 - 5.7) mg/l. The slope of the effect concentration curve was 1.32. The concentrations used to calculate the EC50 were nominal (not listed), as analytical concentrations were within 10% of nominal concentrations.
Test condition	:	An AFNOR test (Norme Experimentale NFT, 90-301, 1974) was performed to determine the IC50 (concentration for 50% of animals to be immobilized) at 24 hours. The test vessels were modified according to the method of Galassi and Vighi (Chemosphere 10: 1123, 1981) in order to maintain constant concentration and to allow for sampling of the medium without opening the vessel. Concentrations of test material in vessels were measured analytically (by gas chromatography) at the beginning and end of the tests. Dilute solutions were extracted with n-hexane before analysis. The limit of detection was 0.02 mg/l. The EC50 value was taken from curves fitted by eye on log probability paper and not elaborated, being very close to the concentrations with 0 and 100% immobilized animals.
Test substance	:	Test material was analytical grade.
Reliability	:	(2) valid with restrictions. Test conditions were not described in detail.

29.11.2001 (9)

Type	:	
Species	:	Daphnia
Exposure period	:	48 hour(s)
Unit	:	mg/l
Analytical monitoring	:	no
LC50	:	c = 15.381
Method	:	other: ECOSAR program (v 0.99g)
Year	:	2002
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Test condition	:	The LC50 value was estimated by the ECOSAR program (v0.99g), with a log Kow of 2.84 and water solubility of 400 mg/l entered by the user.
Reliability	:	(2) valid with restrictions. Data were obtained by modeling.
Flag	:	Supportive study for endpoint

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Selenastrum capricornutum (Algae)
Endpoint	:	growth rate

Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	yes
EC50	:	m = 12.5
Method	:	other: modification of AAPBT batch test
Year	:	1981
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Culture medium and test conditions were similar to the AAPBT, with the exception that the temperature was 20 +/- 1 degrees C. The toxicity of 1,2 and 1,4 -dichlorobenzenes (ortho and para), and 1,2,3- and 1,2,4- trichlorobenzene also were tested in this study. The 96-hour EC50 values for these materials were 2.2, 1.6, 0.9 and 1.4 mg/l, respectively. It was remarked that the EC50 values calculated for chlorobenzenes by this method are at least 2 fold lower than other methods that did not analytically control test material concentrations.
Result	:	Initial concentrations could not be measured due to high volatility of the test material. Within a few minutes of adding the test material to the flasks the concentration was very low compared to theoretical values. Therefore, the initial concentrations calculated from the dilution of the titrated stock solutions were assumed to be the initial concentrations. Equilibrium concentrations were calculated as the mean of the analytical concentrations in samples taken after the equilibrium period and 48 and 96 hours. For initial concentrations of 31.6, 63.2, 94.8, 126.4, 158.0, 221.2 and 284.4 mg/l, equilibrium concentrations of 6.5, 14.3, 23.3, 29.6, 37.8, 45.0 and 63.0 were determined. The mean initial concentration/ equilibrium concentration (Ci/Ceq +SD) was 4.5 +/- 0.3. Henry's constant (H) to be calculated from the equation Ci = Ceq(H x air volume in the flask/culture medium volume + 1). The value calculated from this equation (0.16) was fairly close to the reported value (0.11), confirming the validity of the method for prediction of concentrations at equilibrium. After the 24 hour equilibration period, the concentration of test material in the culture medium remained almost constant. Differences in the values obtained at equilibrium and after 48 or 96 hours were within the range of acceptable analytical variability. The 96 hour EC50 value calculated for chlorobenzene inhibition of algal fluorescence was 12.5 mg/l. The maximum tested concentration that produced no effect was < 6.8 mg/l and the minimum concentration that was 100% effective was 46.3 mg/l.
Test condition	:	A stock solution of test material was made by adding chlorobenzene at 10 times higher than the saturation solubility to distilled water in a closed vessel. The solution was stirred for 48 hours and decanted for 24 hours. The supernatant was filtered through paper filters and the concentration was measured. Final solutions were made by adding 10 ml of stock culture medium to different amounts of stock solution. Solutions were then diluted to 100 ml with distilled water and quickly transferred into the 2 liter spherical culture flasks. The medium to flask volume ratio (0.047) was low enough to avoid notable carbon dioxide deficiency. Flasks were closed by screw caps with both silicone rubber (4 mm thick) and teflon gaskets. The caps were pierced by a stainless steel needle dipped into the culture medium. Sampling for measurement of algal growth and toxicant concentrations was made through the needle by means of a syringe. The outer end of the needle was closed with Parafilm.

Capped flasks were shaken for 24 hours at 20 degrees C to let vapor and liquid phases equilibrate. The algal inoculum was then added at cell concentration of 5 x 10E6 cells/l. Culture medium and test conditions were similar to the AAPBT, with the exception that the temperature was 20 +/- 1 degrees C.

Concentrations of test material in the flasks were measured by GC after the 24 hour equilibration period and 48 and 96 hours after the inoculum was added. Aqueous solutions (4 microliters) were injected directly into the GC with a flame ionization detector.

Algal growth was measured at 24, 48, and 96 hours by in vivo fluorescence (CJ Lorenzen, Deep Sea Res. 13:223-227, 1966). Results were expressed as a percentage of the growth in the control culture and the EC50 was interpolated from the data.

Reliability : (2) valid with restrictions. Purity of test material was not stated. The control response in terms of the increase in cell concentration, and the pH, dissolved oxygen concentration and temperatures were not provided.

27.11.2001 (13)

Type	:	
Species	:	Green algae
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	no
EC50	:	c = 10.092
Method	:	other: ECOSAR program (v 0.99g)
Year	:	2002
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Test condition	:	The EC50 value was estimated by the ECOSAR program (v0.99g), with a log Kow of 2.84 and water solubility of 400 mg/l entered by the user.
Reliability	:	(2) valid with restrictions. Data were obtained by modeling.
Flag	:	Supportive study for endpoint

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6.1 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO OTHER NON-MAMM. TERRESTRIAL SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	20
Vehicle	:	
Value	:	= 1540 mg/kg bw
Method	:	other
Year	:	1976
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	There were no deaths at 1260 mg/kg, 3/5 deaths (2 males, 1 female) at 1580 mg/kg, 3/5 deaths at 2000 mg/kg (1 male, 2 females) and 5/5 deaths with 2510 mg/kg. All deaths occurred within 3 days. The LD50 value was 1540 mg/kg and the 95% confidence interval was 1380 - 1710 mg/kg.
Test condition	:	Rats (220 - 250 g) were dosed orally (2-3 males and 2-3 females/group; 5 males and females/group) with 1260, 1580, 2000, and 2510 mg/kg and were observed over 14 days. The LD50 value was calculated according to the method of deBeer (J Pharmacol Exper Ther 85:1, 1945).
Test substance	:	The material was technical grade, and therefore had a purity of > 99%.
Reliability	:	(2) valid with restrictions. Symptoms of toxicity were not described.
Flag	:	Key study for endpoint

27.11.2001 (5)

Type	:	LD50
Species	:	rat
Strain	:	Fischer 344
Sex	:	male/female
Number of animals	:	50
Vehicle	:	other: corn oil
Method	:	other
Year	:	1985
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	There were no deaths at 250 mg/kg. Deaths in the other treatment groups were as follows: 1 male at 500 mg/kg, 2 females at 1000 mg/kg, 1 male at 2000 mg/kg, and 3 males and 4 females at 4000 mg/kg. The LD50 was not calculated, but is obviously between 2000 and 4000 mg/kg. All deaths occurred within 3 days of dosing. Transient ataxia, labored breathing and prostration were observed at the two higher doses. Hyperpnea was frequently observed shortly after dosing in all rats administered test material.
Test condition	:	Rats were obtained at 4-6 weeks of age and were quarantined for 10-14 days before use. They were allowed food and water ad libitum. Animals were randomly assigned to groups and dosed when they were 6-8 weeks of age. Dose solutions were prepared in corn oil. Five rats/sex/group were gavaged with 250, 500, 1000, 2000 or 4000 mg/kg test material (total volume 0.5 ml/100 g). Animals were observed twice daily for 14 days.
Test substance	:	Test material was determined to be > 99.9% pure by gas chromatography.

Reliability : (1) valid without restriction
27.11.2001 (23) (32)

Type : LD100
Species : mouse
Strain : B6C3F1
Sex : male/female
Number of animals : 50
Vehicle : other:corn oil
Value : = 1000 mg/kg bw
Method : other
Year : 1985
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Result : Deaths in the treatment groups were as follows: 3 males and 2 females at 250 mg/kg; 1 male at 500 mg/kg, all males and 2 females at 1000 mg/kg, and all animals at 2000 and 4000 mg/kg. The dose to 100% lethality in mice was 1000 mg/kg in males and 2000 mg/kg in females. The LD50 value was not calculated. Most deaths occurred within 4 days of dosing.
Test condition : Mice were obtained at 4-6 weeks of age and were quarantined for 10-14 days before use. They were allowed food and water ad libitum. Animals were randomly assigned to groups and dosed when they were 6-8 weeks of age.

Dose solutions were prepared in corn oil. Five mice/sex/group were gavaged with 250, 500, 1000, 2000 or 4000 mg/kg test material (total volume 0.5 ml/100 g). Animals were observed twice daily for 14 days.
Test substance : Test material was determined to be > 99.9% pure by gas chromatography.
Reliability : (1) valid without restriction
27.11.2001 (23) (32)

5.1.2 ACUTE INHALATION TOXICITY

Type : LC50
Species : rat
Strain : Sprague-Dawley
Sex : male
Number of animals :
Vehicle :
Exposure time : 6 hour(s)
Value : = 2965 ppm
Method : other
Year : 1982
GLP : no
Test substance : as prescribed by 1.1 - 1.4.
Result : The LC50 value was 2965 (2787 - 3169) ppm. The regression equation was y = 10.9x + 33. The LC50 value in mg/l is 13.9.
Test condition : Rats (130 -160 g, 12 per dose group) were exposed to concentrations ranging from 2000 to 3500 ppm over 6 hours. Vapor was generated at 24 degrees C, 50 % relative humidity. Rats were observed for 14 days. A line of regression was plotted with the abscissa being the log of the concentration of test material and the ordinate being the probit of the percentage of corresponding deaths. The LC50 value was the concentration (x value) at which the y value was 50%.
Test substance : The test material was 99% pure.
Reliability : (1) valid without restriction

27.11.2001 (6)

Type	:	LC50
Species	:	mouse
Strain	:	
Sex	:	female
Number of animals	:	
Vehicle	:	
Exposure time	:	6 hour(s)
Value	:	= 1886 ppm
Method	:	other
Year	:	1979
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	The LC50 value was calculated as 1886 ppm (1781-1980) with a regression equation of y = 6.734 x + 17.06. The value in mg/l is 8.8.
Test condition	:	Female mice (21 g and 25 per dose group) were exposed in 200 l chambers to 1400 to 3000 ppm for 6 hours. Vapor was generated at 24 degrees C, 50 % relative humidity and an air flow of 40 m3/hr. There were 60 air changes/hour. Animals were observed for 14 days. A line of regression was plotted with the abscissa being the log of the concentration of test material and the ordinate being the probit of the percentage of corresponding deaths. The LC50 value was the concentration (x value) at which the y value was 50%.
Test substance	:	Purity of test material was 99%.
Reliability	:	(1) valid without restriction

27.11.2001 (7)

Type	:	LC50
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male
Number of animals	:	6
Vehicle	:	
Exposure time	:	3.75 hour(s)
Value	:	< 39.7 mg/l
Method	:	other
Year	:	1976
GLP	:	no
Test substance	:	as prescribed by 1.1 – 1.4
Result	:	All animals died within 3.75 hours. Signs of toxicity were tremors, labored breathing and collapse. Slight lung congestion was noted at autopsy.
Test condition	:	Six male rats (initial weight not stated) were exposed to 39.7 mg/l test material for 3.75 hours at ambient temperature. The chamber humidity was 80% and the volume was 35 l. The air flow rate was 4.0 l/min.
Test substance	:	The material was technical grade, which has a purity of 99%.
Reliability	:	(1) valid without restriction

27.11.2001 (5)

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD50
Species	:	rabbit
Strain	:	New Zealand white
Sex	:	male/female

Number of animals	:	3
Vehicle	:	
Value	:	> 7940 mg/kg bw
Method	:	other
Year	:	1976
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	There were no deaths.
Test condition	:	One female (1.8 kg) was dosed with 5010 mg/kg and 2 males (2.0 and 2.2 kg) were dosed with 7940 mg/kg. Animals were observed over 14 days. The LD50 value was calculated according to the method of deBeer (J Pharmacol Exper Ther 85:1, 1945).
Test substance	:	The material was technical grade, and therefore had a purity of > 99%.
Reliability	:	(4) not assignable. Only 3 animals were tested.

27.11.2001 (5)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

5.2.2 EYE IRRITATION

5.3 SENSITIZATION

5.4 REPEATED DOSE TOXICITY

Species	:	rat
Sex	:	male/female
Strain	:	Fischer 344
Route of admin.	:	gavage
Exposure period	:	13 weeks
Frequency of treatment	:	5 days/week
Post obs. period	:	no
Doses	:	60, 125, 250, 500 or 750 mg/kg/day
Control group	:	yes, concurrent vehicle
NOAEL	:	ca. 125 mg/kg bw
LOAEL	:	= 250 mg/kg bw
Method	:	other
Year	:	1985
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Results of a preliminary 14-day oral range finding study with 5 rats/sex/group dosed with 0, 125, 250, 500, 1000 or 2000 mg/kg/day cited in the same reference indicated that 1000 mg/kg/day and 2000 mg/kg/ day resulted in 100% mortality. None of the rats exposed to 500 mg/kg or lower concentrations died.
Result	:	Mortality occurred in 9/10 males and 8/10 females dosed with 750 mg/kg/day, in 4/10 males and 3/10 females dosed with 500 mg/kg/day, in

one female dosed with 60 mg/kg/day, and in one control male animal. Body weight gains appeared to be reduced in male rats at doses >= 250 mg/kg/day and in females at doses >= 500 mg/kg/day (statistical analyses were not performed on these data).

Compound-related changes in hematological parameters were not observed (except for a decreased white blood cell count in the 2 surviving female rats at 750 mg/kg and an increased reticulocyte percentage in the surviving male at this dose). Gamma glutamyl transpeptidase (GGT) and alkaline phosphatase (AP) were increased slightly in females receiving 500 or 750 mg/kg/day. The values for GGT in controls and females treated with 500 or 750 ppm were 0 +/- 0, 4.0 +/- 3.0 and 14.0 +/- 6.0 IU/l) and for AP in these groups were 83 +/- 12, 141 +/- 53, and 150 +/- 28). No other consistent effects were noted in clinical chemistries.

Urinalyses indicated a mild porphyria, increased urine volume (approximately 2 times that of control), an increased uroporphyria and coprophyria in the 1 surviving high dose male, and coprophyria in males (1649 +/- 821 vs. 343 +/- 167 ng in control) and females (1631 +/- 1048 vs. 267 +/- 195 ng in control) dosed with 500 mg/kg/day . There also was increased liver porphyrin in females dosed with 500 (81 +/- 21 vs. 53 +/- 19 ng in control) or 750 mg/kg /day (90 +/- 34 vs. 53 +/- 19 ng in control).

Relative liver weights were increased in males treated with 250, 500 or 750 mg/kg with respect to controls, and in females treated with 125, 250, 500 and 750 mg/kg/day with respect to controls. Relative kidney weights of males and females treated with 500 or 750 mg/kg/day also were higher than controls. In male rats, absolute liver and kidney weights were not increased and relative organ weight increases were observed only in animals whose body weight was depressed. In female rats, absolute kidney weight was increased only in the surviving animal at 750 mg/kg, but absolute liver weights were increased at all doses except 60 mg/kg. Absolute and relative spleen weights were decreased in all groups of treated male rats (except the one survivor at 750 mg/kg). With the exception of the high dose, the decreased spleen weights were not accompanied by histopathological changes.

Increased incidences of hepatic necrosis and degeneration (moderate in severity), renal necrosis or degeneration (mild to moderate), splenic lymphoid depletion (minimal to mild), thymic lymphoid depletion (mild to moderate) and bone marrow myeloid depletion (minimal to moderate) were noted in high dose males and females (including ones that died). Hepatic necrosis and degeneration (minimal to moderate), and renal necrosis or degeneration (mild to moderate) were noted in 2-3 males dosed with 500 mg/kg. One female at this dose exhibited hepatic necrosis. Myeloid depletion in bone marrow (minimal to moderate) was also noted in 3 males and 2 females at this dose. One female at this dose had lymphoid depletion in the thymus. Hepatic necrosis was noted in one female and 2 males treated with 250 mg/kg (minimal) and renal necrosis or degeneration was noted in one male at this dose. These lesions were not observed in controls or rats treated with 60 or 125 mg/kg.

Test condition : Rats were 4-6 weeks old upon arrival and were observed for 2 weeks prior to treatment. Animals were provided with food and water ad libitum. Ten animals/sex/group (6-8 weeks old) were treated by gavage with corn oil vehicle or 60, 125, 250, 500 or 750 mg/kg/day test material, 5 days/week for 13 weeks. Animals were observed 2 times/day for condition. Body weights and cage food consumption (5 rats/cage) were determined weekly.

Urine was collected for 24 hours one week before termination from rats housed individually in metabolism cages. Urine volume, pH, protein, glucose, ketones, bilirubin, specific gravity, creatinine and occult blood were measured. Urine was applied to an anion-exchange column for separation of porphyrins. Eluates were assayed for uroporphyrins and coproporphyrins by spectrofluometry.

Blood samples were taken one day prior to termination (via orbital bleeding) for hematologies (hemoglobin, packed cell volume, mean corpuscular volume, and total and differential white blood cell, red blood cell, platelet, and reticulocyte counts), and on day of euthanization (via cardiac puncture) for clinical chemistries (alkaline phosphatase, glutamic pyruvic transaminase, gamma glutamyl transpeptidase, bilirubin, cholesterol, glucose, triglyceride, urea nitrogen (BUN), total protein and globulins). Samples of liver were homogenized and analyzed for porphyrins.

All surviving animals were euthanized at the end of the 13-week exposure period. Body, lung, liver, heart, spleen, thymus, brain, kidney (right), and testis (right) or uterus weights were taken at necropsy. All animals were given a complete gross examination. Mandibular lymph node, salivary gland, femur, thyroid, parathyroids, small intestine, colon, liver, prostate, testis, ovary, lungs and bronchi, heart, esophagus, stomach, uterus, brain, thymus, trachea, pancreas, spleen, kidneys, adrenals, urinary bladder, pituitary, and mammary gland were removed, fixed and sectioned. All tissues were examined microscopically for the control, 500 and 750 mg/kg/day animals and from all animals that died during the study. Tissues from other dose groups were examined only if lesions were identified at the next highest dose. Frozen sections of liver were stained with Oil Red to determine the presence of lipids.

All clinical chemistry, hematology and organ weight data were analyzed with Dunnett's multiple comparison test. The level of significance was $p < 0.05$.

Test substance : Test material was determined to be > 99.9% pure by gas chromatography.
Reliability : (1) valid without restriction
Flag : Key study for endpoint
27.11.2001 (23) (32)

Species : mouse
Sex : male/female
Strain : B6C3F1
Route of admin. : gavage
Exposure period : 13 weeks
Frequency of treatment : 5 days/week
Post obs. period : none
Doses : 60, 125, 250, 500 or 750 mg/kg/day
Control group : yes, concurrent vehicle
NOAEL : = 125 mg/kg bw
LOAEL : = 250 mg/kg bw
Method : other
Year : 1985
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Remark : Results of a preliminary 14-day oral range finding study with 5 mice/sex/group dosed with 0, 30, 60, 125, 250 or 500 mg/kg/day showed

Result : no clinical signs of toxicity or increased mortality with respect to controls. General: All males that received 500 or 750 mg/kg died during the first week of the study, while all females that received 750 mg/kg were dead by week 9. Seven out of 10 females treated with 500 mg/kg died. Mortality in males and females given 250 mg/kg were 5/9 and 4/10, respectively. There were no other deaths (except for 1 control female). Final body weights appeared to be lower in males at 250 mg/kg and females at 250 mg/kg and 500 mg/kg (statistical analyses were not performed on these data).

Dose dependent, significant changes in hematological parameters and serum chemistries in surviving animals were not observed [with the exception of a small decrease in packed cell volume in males at 125 and 250 mg/kg (47 and 46 % respectively vs. 49 % in control)].

Group caging precluded a reasonable statistical analysis of individual urine outputs. However, mean 24 hour urine volume was 4.6 ml in 2 female mice treated with 500 mg/kg versus 1.7 ml in 2 controls. Urinary coproporphyrin excretion was increased at 250 and 500 mg/kg in females [(2628 (N = 1) and 1675 +/- 106 ng (N=2) vs. 119 +/- 7 ng in controls (N=2)]. No changes were observed in liver total porphyrin concentrations of either sex of treated animals.

At termination, absolute and relative liver weights were increased in male mice at 125 and 250 mg/kg and in female mice at 250 and 500 mg/kg. Absolute and relative heart weight were decreased slightly (less than 20%) in all groups of treated male mice. This effect was not dose dependent. Relative kidney weight was increased and absolute brain weight and relative and absolute spleen weight were decreased in the surviving females at 500 mg/kg.

Increased incidences of hepatic necrosis (10/10), mild to moderate necrosis of the proximal tubular epithelium of the kidney (8/10), splenic lymphoid depletion (5/10), thymic lymphoid depletion (4/10) and thymic lymphoid necrosis (5/10) were noted in high dose males. Increased incidences of hepatic necrosis (1/10), hepatic degeneration (4/10), splenic lymphoid depletion (9/10), thymic lymphoid depletion (3/10) and thymic lymphoid necrosis (1/10) were noted in high dose females. All liver and thymic lesions observed at 750 mg/kg were graded as moderate to severe.

Increased incidences of hepatic necrosis (10/10), mild to moderate necrosis of the proximal tubular epithelium of the kidney (9/10), splenic lymphoid depletion (2/10), thymic lymphoid depletion (2/10) and thymic lymphoid necrosis (8/10) (similar changes as 750 mg/kg) were noted in males treated with 500 mg/kg. Increased incidences of hepatic necrosis (8/10), hepatic degeneration (9/10), bone marrow myeloid depletion (3/10), splenic lymphoid depletion (3/10), and minimal to mild myeloid depletion (4/10), and thymic lymphoid depletion (3/10) were noted in females treated with 500 mg/kg. All liver and thymic lesions observed at 500 mg/kg were graded as moderate to severe.

Increased incidences of hepatic necrosis (7/10), hepatic degeneration (2/10), mild to moderate regeneration of the proximal tubular epithelium of the kidney (4/10), minimal to mild bone marrow myeloid depletion (4/10), splenic lymphoid (4/10) and myeloid (4/10) depletion, and thymic lymphoid necrosis (4/10) were noted males treated with 250 mg/kg. Increased incidences of hepatic necrosis (10/10), mild to moderate regeneration of

the proximal tubular epithelium of the kidney (4/10), minimal to mild bone marrow myeloid depletion (2/10), splenic lymphoid (2/10) and myeloid (4/10) depletion, and thymic lymphoid necrosis (3/10) were noted in females treated with 250 mg/kg. All liver and thymic lesions observed at 250 mg/kg were graded as moderate to severe.

One male treated with 125 mg/kg and another treated with 60 mg/kg exhibited hepatic necrosis. There were no other changes observed in animals treated with 60 or 125 mg/kg. There were no abnormalities in controls.

The NOAEL was 125 mg/kg, as this dose only produced an effect in the liver in one male animal.

Test condition : Mice were 4-6 weeks old upon arrival and were observed for 2 weeks prior to treatment. Animals were provided with food and water ad libitum. Ten animals/sex/group (6-8 weeks old) were treated by gavage with corn oil vehicle or 60, 125, 250, 500 or 750 mg/kg/day test material, 5 days/week for 13 weeks. Animals were observed 2 times/day for condition. Body weights and cage food consumption (5 rats/cage) were determined once weekly. Urine was collected for 24 hours one week before termination from mice housed (N = 3-6) in metabolism cages. Urine volume, pH, protein, glucose, ketones, bilirubin, specific gravity, creatinine and occult blood were measured. Urine was applied to an anion-exchange column for separation of porphyrins. Eluates were assayed for uroporphyrins and coproporphyrins by spectrofluometry.

Blood samples were taken one day prior to euthanization (via orbital bleeding) for hematologies (hemoglobin, packed cell volume, mean corpuscular volume, and total and differential white blood cell, red blood cell, platelet, and reticulocyte counts), and on day of termination (via cardiac puncture) for clinical chemistries (alkaline phosphatase, glutamic pyruvic transaminase, gamma glutamyl transpeptidase, bilirubin, cholesterol, glucose, triglyceride, urea nitrogen (BUN), total protein and globulins). Samples of liver were homogenized and analyzed for porphyrins.

All surviving animals were euthanized at the end of the 13 week exposure period. Body, lung, liver, heart, spleen, thymus, brain, kidney (right), and testis (right) or uterus weights were taken at necropsy. All animals were given a complete gross examination. Mandibular lymph node, salivary gland, femur, thyroid, parathyroids, small intestine, colon, liver, prostate, testis, ovary, lungs and bronchi, heart, esophagus, stomach, uterus, brain, thymus, trachea, pancreas, spleen, kidneys, adrenals, urinary bladder, pituitary, gall bladder and mammary gland were removed, fixed and sectioned. All tissues were examined microscopically for the control, 250, 500 and 750 mg/kg/day animals and from all animals that died during the study. Tissues from other dose groups were examined if lesions were identified at the next highest dose. Frozen sections of liver were stained with Oil Red to determine the presence of lipids.

All clinical chemistry, hematology and organ weight data were analyzed with Dunnett's multiple comparison test. The level of significance was $p < 0.05$.

Test substance : Test material was determined to be > 99.9% pure by gas chromatography.

Reliability : (1) valid without restriction

Flag : Key study for endpoint

27.11.2001 (23) (32)

Species	:	dog
Sex	:	male/female
Strain	:	Beagle
Route of admin.	:	other: oral administration via capsule
Exposure period	:	93 d
Frequency of treatment	:	5 days/week for 13 weeks
Post obs. period	:	no
Doses	:	0.025, 0.05, 0.25 ml/kg/day
Control group	:	yes
NOAEL	:	= .05 ml/kg bw
Method	:	other
Year	:	1967
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Using a density of 1.09, doses can be converted to 27.25, 54.5 or 272.5 mg/kg/day. The NOAEL on a mg/kg basis is 54.5 mg/kg/day.
Result	:	There was no mortality in any of the groups except the high dose. Two dogs in the high dose group died and two were euthanized in moribund condition. Clinical signs before death in these animals included decreased appetite and activity, anorexia, body weight loss, cachexia and coma. Alterations at the high dose included a low hemogram, an increased number of immature white blood cells, low blood sugar, slightly to markedly increased values for alkaline phosphatase, serum glutamic-pyruvic transaminase, total bilirubin, and total cholesterol, increased urinary acetone and moderate to marked increased in urinary bilirubin. There were gross and /or microscopic changes in the liver, kidney, GI tract and hematopoetic tissue of the high dose dogs that died and, less extensively, in the high dose dogs that were euthanized at the end of the exposure period. The four high dose dogs that died or were euthanized during the study showed increased relative liver, kidney, adrenal and heart weight (N=3) and slightly increased relative thyroid weight (N=2). There were no consistent signs of toxicity at the intermediate and low dose levels.
Test condition	:	Sixteen male and 16 female purebred beagles (5.3 kg to 12.0 kg) were used. They were given 0, 0.025, 0.050, or 0.250 ml/kg/day test material orally (by gelatin capsule) five days/week for 13 weeks. The compound was placed into capsules just prior to administration. Animals were observed daily for clinical signs, and body weight and food consumption were monitored weekly. Blood was taken initially and at one and 3 months for analysis of hematocrit, hemoglobin, erythrocyte counts, total and differential leukocyte counts, blood sugar, blood urea nitrogen, alkaline phosphatase, and serum glutamic-pyruvic transaminase. Total bilirubin and total cholesterol were measured in the control and high dose dogs at one month only. Urine was collected initially and at 1 and 3 months for urinalysis. Gross necropsies were performed on all dogs that died or were euthanized during the study or at termination (13 weeks). Thyroid, heart, liver, spleen, kidneys, adrenals and testes were weighed. A standard set of tissues was preserved and analyzed microscopically.
Reliability	:	(2) valid with restrictions. Purity of test compound was assumed to be 100%, but was not analytically verified.

27.11.2001 (24)

Species	:	dog
Sex	:	male/female
Strain	:	Beagle
Route of admin.	:	inhalation
Exposure period	:	six months
Frequency of treatment	:	6 hr/day, 5 days/wk (total of 128 exposures)
Post obs. period	:	
Doses	:	0.79, 1.59 and 2.06 mg/l
Control group	:	yes
NOAEL	:	= 2.06 mg/l
Method	:	other
Year	:	1980
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The study personnel did not perceive the increases in relative organ weight or decreases in relative adrenal weight seen at the mid and high dose to be adverse as there were no corresponding clinical chemistry changes or pathological lesions in these organs. They also considered emesis and loose stools as being nonspecific changes, since these were not supported by any abnormal hematological, clinical chemistry or pathological findings. Therefore, they established a NOEL of 2.06 mg/l. It should be noted that others have considered increased organ weights without pathological changes to be an adverse effect. Raw data were not supplied (only summaries)
Result	:	Clinical signs: There were no deaths. All test animals had abnormal stool (diarrhea with yellow, blood-tinged, soft or tarry stools) during weeks 7-11. This also was evident in controls at week 11. Emesis occurred in the mid and high dose groups during weeks 8-10 and 17 to 29. Hematology/clinical chemistry: None of the hematological parameters measured were significantly altered by treatment. High dose dogs showed sporadic increases in serum alkaline phosphatase midway through the study and increases in lactate dehydrogenase towards the end of the study. These elevations were slight and within the normal range for dogs, but were significantly different from control values. Urinalyses were unremarkable. Bone marrow examinations showed no significant differences in mean myeloid to erythroid ratios between test groups and controls. Organs: The relative liver weights in the mid and high dose females were increased relative to controls, and the relative adrenal weights in the mid and high dose males were decreased with respect to controls. No significant compound-related changes were observed upon gross and microscopic examination of any tissues.
Test condition	:	Male and female dogs were 8 and 6 months of age upon arrival, respectively. They were acclimated for 29 days, during which time they were vaccinated for prominent canine disease, tested for major parasitic infections, and examined by a veterinarian. Groups of beagles (6/sex) were exposed to air containing mean (+ SD) analytical concentrations of 0 (control), 0.79 +/- 0.04, 1.59 +/- 0.06, or 2.06 +/- 0.1 mg/l of test material 6 hr/day, 5 days per week, over a 6 month period (total of 128 exposures). Chamber concentrations were measured 4 times per day. Animals were weighed weekly and examined twice daily for signs of toxicity. Blood and urine samples were collected prior to test and during weeks 2, 4, 8, 10, 12,

20 and 26. Bone marrow specimens were obtained from all dogs one week prior to test, from 2/sex/dose at weeks 2, 4, 8, 12, 16, and 20, and from all animals at necropsy.

Gross and microscopic pathology was performed on 24 tissues or organs at necropsy. Adrenals, brain, heart, kidney, liver, pituitary and testes weights were determined.

Hematological parameters measured were white blood cell count, red blood cell count, hemoglobin, hematocrit, mean corpuscular hemoglobin and differential leukocyte count. Clinical chemistry parameters measured in blood were glucose, blood urea nitrogen, lactate dehydrogenase, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase, total protein, total bilirubin, sodium and potassium.

Test substance : Purity of test substance was determined to be 96 +/- 0.5%.
Reliability : (1) valid without restriction
27.11.2001 (33)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Ames test
System of testing : S. typhimurium strains TA98, TA100, TA1535, TA1537, TA1538
Concentration : 0.02 microliters to 1.28 microliters/plate
Cytotoxic conc. : 1.28 microliters (all strains)
Metabolic activation : with and without
Result : negative
Method : other
Year : 1983
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Remark : Thirty different chemicals were tested in this experiment (benzene, 4 fluorobenzenes (mono or di), chlorobenzene, 3 dichlorobenzenes (ortho, meta and para), nitrobenzene, 6 fluoronitrobenzenes (mono or di), 8 chloronitrobenzenes (mono, di or tri), 1 chloro-, fluorobenzene, 3 nitrobenzenes, 1 dinitrofluorobenzene and 1 dinitrochlorobenzene). Fifteen of the compounds containing nitro groups were mutagenic. All compounds without a nitro group showed no mutagenic activity.
Result : The average number of revertants in the DMSO controls for strains TA98, TA100, TA1535, TA1537 and TA1538 in the absence of S-9 were 28, 181, 32, 8, and 22, respectively. Addition of S-9 to controls did not significantly increase the frequency of mutations. The average number of revertants in all positive control cultures were as follows: ENNG, 1994 in TA100 and 2489 in TA1535; 2-NF, 1798 in TA98 and 1659 in TA1538; 9-AA, 1288 in 1537; and 2-AA, from 132 in TA1537 to 1549 in TA100. The test was valid based on these data.

The number of revertants induced by chlorobenzene was not increased from that of controls at any concentration (based on a visual review of the data). Metabolic activation did not appear to increase the potential for mutagenicity. The number of revertants observed in cultures treated with nontoxic concentrations of chlorobenzene (in the absence or presence of S-9) ranged from 25-35 in TA98, 163-231 in TA100, 30-43 in TA1535, 6-13 in TA1537, and 16-27 in TA1538. A concentration of 1.28 microliters/plate was toxic to all strains.
Test condition : All strains of bacteria were supplied by the same supplier (Dr. B. Ames). Test material was dissolved in sterile dimethylsulfoxide. S-9 was prepared

from liver homogenate (25% in 0.15M KCl) from male Sprague-Dawley rats (100-200 g) that had been injected with PCB at a dose of 500 mg/kg 5 days before they were killed. S-9 mix contained per ml: S9(0.3 ml), MgCl2 (8 micromoles), KCl (33 micromoles), glucose 6 -phosphate (5 micromoles), NADH (4 micromoles), NADPH (4 micromoles), and sodium phosphate, pH 7.4 (100 micromoles).

Various concentrations (from 0.02 microliters/plate to the concentration that caused toxicity) of test compound (0.1 ml) were added to sterile test tubes containing 3-6 x 10E7 bacterial cells, 0.5 ml of S-9 mix (+ activation) or sodium phosphate buffer (pH 7.4) (- activation). This mixture was pre-incubated in a shaker water bath at 37 degrees C for 15 min, then added to 2 ml molten top agar (45 degrees C). The contents of each tube were mixed and immediately poured onto the surface of a minimal-agar plate. DMSO (0.05 ml) was added to plates containing each kind of bacteria (negative control). N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG; 2 or 10 micrograms/plate incubated with strains TA100 and TA 1535 without S-9, 2-nitrofluorene (2-NF; 2 or 5 micrograms/plate incubated with strains TA98 and TA1538 without S-9, 9-aminoacridine (9-AA; 100 micrograms/plate incubated with strain TA1537 without S-9); and 2-aminoanthracene (2-AA;5 micrograms/plate incubated with all strains with S-9 only) were used as positive controls. All tests were performed in duplicate and were repeated at least 3 times separately.

Plates were inverted and incubated at 37 degrees C in the dark for 3 days. Colonies of his+ revertants were counted after incubation. Chemicals inducing more than twice the number of revertant colonies as negative control plates were considered to be mutagenic. Tests without metabolic activation were carried out first. Tests with metabolic activation were only carried out only if results of the tests without activation were negative.

Two strains (TA98 and TA100) were checked routinely for the presence of the ampicillin resistance for the R factor. The background bacterial lawn was routinely checked by microscopy for thinning (evidence of toxicity) and contamination.

Statistical analyses were not performed.

Test substance : TS: Purity of the test substance was 98%.
Reliability : (2) valid with restrictions. This test was given a reliability of 2 because statistical analyses were not performed.
Flag : Key study for endpoint
27.11.2001 (37)

Type : Ames test
System of testing : S. typhimurium strains TA98, TA100, TA1535, TA1537
Concentration : 3.3 to 3333 microliters/plate
Cytotoxic conc. : 3333 microliters/plate
Metabolic activation : with and without
Result : negative
Method : other: preincubation modification
Year : 1985
GLP : no data
Test substance : as prescribed by 1.1 – 1.4
Reliability : (2) valid with restrictions. Purity of test material was not listed.
24.02.2002 (32)

Method : other

Type	:	Ames test
Type	:	Ames test
System of testing	:	S. typhimurium strains TA98, TA100, TA1535, TA1537, TA1538
Concentration	:	0.01 - 5 microliters/plate
Cytotoxic conc.	:	5 microliters/plate
Metabolic activation	:	with and without
Result	:	negative
Method	:	other
Year	:	1979
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Ortho, meta and para dichlorobenzene also tested negative. Using a density of 1.1, the dose range can be converted to 0.01 to 5.5 mg/plate.
Reliability	:	(2) valid with restrictions. Purity of test material was not listed.

27.11.2001 (38)

Type	:	Escherichia coli reverse mutation assay
System of testing	:	E. coli WP2(trp-, uvRA-)
Concentration	:	0.0005 to 1.0 micrograms/l
Cytotoxic conc.	:	1.0 micrograms/l
Metabolic activation	:	with and without
Result	:	negative
Method	:	other
Year	:	1979
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	1,2-, 1,3- and 1,4-dichlorobenzene also tested negative in this study.
Reliability	:	(2) valid with restrictions. Purity of test substance was not given.

19.11.2001 (38)

Type	:	Chromosomal aberration test
System of testing	:	Chinese Hamster Ovary Cells
Concentration	:	125 to 500 micrograms/ml in separate experiments
Cytotoxic conc.	:	500 micrograms/ml
Metabolic activation	:	with and without
Result	:	negative
Method	:	other
Year	:	1985
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	1,2,3- and 1,2,4- trichlorobenzene also tested negative in this study. Article is in Japanese.
Result	:	There was no test-material related increase in the frequency of aberrant cells in either experiment
Test condition	:	Two different tests were done with the test material. In the first, cells were incubated for 24 or 48 hours with DMSO vehicle, and 125, 250 and 500 micrograms/ml test material. 500 micrograms/ml was toxic at both time points. In the second experiment, cells were incubated with DMSO or 200, 300 or 400 micrograms/ml test material and with DMSO, 300, 400, and 500 micrograms test material in the presence of S9. In this experiment, 500 micrograms/ml was not toxic.
Reliability	:	(2) valid with restrictions. Reference was not translated in total.

27.11.2001 (39)

Type	:	Sister chromatid exchange assay
System of testing	:	Chinese Hamster Ovary Cells
Concentration	:	100 to 1000 micrograms/ml
Metabolic activation	:	without
Result	:	positive
Method	:	other: BrdUrd/dye technique
Year	:	1989
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4. Purity was 99+ %
Source	:	Bayer AG Leverkusen
Reliability	:	(4) unassignable. Study was not reviewed. Data came from a IUCLID document from the European Chemicals Bureau, creation date 10-FEB-2000.

27.11.2001 (26)

5.6 GENETIC TOXICITY 'IN VIVO'

Type	:	Micronucleus assay
Species	:	mouse
Sex	:	male
Strain	:	B6C3F1
Route of admin.	:	i.p.
Exposure period	:	3 days
Doses	:	128.8, 257.5, 515 mg/kg/day (total dose 386.4, 772.5, 1545 mg/kg)
Result	:	negative
Method	:	other
Year	:	1993
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	The % PCE in animals treated with 0, 128.8, 257.5 or 515 mg/kg/day was 57.8, 51.1, 52.8, and 48.0, respectively. The incidences of MN-PCE/1000 (mean +/- SE) in pooled samples from 5-6 animals treated with 0, 128.8, 257.5 or 515 mg/kg/day were 3.70 +/- 0.58, 2.80 +/- 0.64, 2.10 +/- 0.33, and 3.42 +/- 0.57, respectively. The test was negative and was not repeated. Solvent (corn oil) data were scored as 2.12 +/- 0.70 and 2.38 +/- 0.93 MN-PCE/1000 PCE (mean +/- SD) by two separate labs (not significantly different). These values are slightly lower than those reported in the test. The data for the positive control DMBA were 6.93 +/- 2.59 and 7.93 +/- 1.69 MN-PCE/1000 PCE (mean +/- SD) in the two labs. The data for the positive control MMC were 6.82 +/- 1.24 and 6.85 +/- 2.26 MN-PCE/1000 PCE (mean +/- SD) in the two labs (no significant difference).
Test condition	:	Male mice between 9 and 14 weeks of age between 23 and 35 g were used. Test material was mechanically suspended in corn oil and was administered within 30 minutes of preparation. Five mice/group were dosed with 0 (corn oil control), 128.8, 257.5 or 515 mg/kg test material, or a weakly active dose of the positive control chemicals 7,12-dimethyl benzanthracene (DMBA; 12.5 mg/kg) or mitomycin C (MMC; 0.2 mg/kg) by i.p. injection on three consecutive days (volume 0.4 ml). The doses tested were based on results of toxicity/mortality in a preliminary study. Animals were monitored 2 times/day.

Mice were killed 48 hours after the third treatment. Bone marrow and peripheral blood smears (two slides/tissue/mouse) were prepared by a direct technique (Tice et al. 1990. Effect of treatment protocol and sample time on frequencies of micronucleated cells in mouse bone marrow and peripheral blood. Mutagen.5:313-321). Air-dried smears were fixed using absolute methanol and stained with acridine orange. Smears from each animal were evaluated at 1000 x magnification using epi-illuminated fluoresence microscopy (450-490 nm excitation; 520 nm emission) for the percentage of polychromatic erythrocytes (PCE) among 200 erythrocytes and the number of micronucleated PCE (MN-PCE) among 2000 PCE. Repeat tests were conducted if the results suggested a possible effect or if no toxicity was observed at the highest dose level.

The data were analyzed using the Micronucleus Assay Data Management and Statistical software package (version 1.4), which was designed specifically for in vivo micronucleus test data (ILS.1990. Integrated Laboratory Systems, P.O. Box 13501, Research Triangle Park, NC). The level of significance was set at $p < 0.05$. The numbers of MN-PCE at each dose group were pooled and analyzed by a one-tailed trend test. In the software package used, the trend test incorporates a variance inflation factor to account for excess animal variability. In the event that the increase in the dose response curve was non-monotonic, the software allowed for the data to be analyzed for a significant positive trend after data at the highest dose only had been excluded. In this event, the alpha level was adjusted to $p < 0.01$ to protect against false positives. The %PCE data were analyzed by an analysis of variance (ANOVA) test based on pooled data. Pairwise comparisons between each group and the solvent control were made using an unadjusted one-tailed Pearson chi-squared test, which incorporated the calculated variance inflation factor for the study. Solvent (corn oil) and positive control data were analyzed by two separate laboratories.

Reliability : (2) valid with restrictions. NTP study. Purity of test material was not noted.
Flag : Key study for endpoint
27.11.2001 (36)

Type : Micronucleus assay
Species : mouse
Sex : male
Strain : NMRI
Route of admin. : i.p.
Exposure period : 48 hours
Doses : 225, 450, 675, 900 mg/kg (in 2 doses administered 24 hours apart)
Result : positive
Method : other
Year : 1987
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Remark : Chlorobenzene, 1,2-, 1,3- and 1,4- dichlorobenzene, and 1,23- and 1,2,4-trichlorobenzenes also tested positive in this study
Result : The number of micronucleated cells/1000 PCE (mean +/- SD) in control, 225, 450, 675, and 900 mg/kg groups was 1.80 +/- 0.748, 3.10 +/- 0.830, 3.90 +/- 0.830, 4.90 +/- 0.943, and 7.20 +/- 0.871, respectively. All values were significantly different from control. The number of micronucleated cells/1000 PCE (mean +/- SD) in animals treated with 264, 528, 1056 (in a split dose) and 528 (in one dose) mg/kg benzene (positive control) was 4.40 +/- 0.800, 8.10 +/- 0.943, 12.40 +/- 1.356, 10.83 +/- 1.343, respectively.

Test condition : Eight week old male mice (5 per group) were given i.p. doses of test compound (total dose of 225, 450, 675 and 900 mg/kg) such that the highest dose did not exceed 70% of the reported LD50 (1355 mg/kg). Each dose was given in a divided dose 24 hours apart (doses given at each 24 hour injection were 112.5, 225, 337.5 and 450 mg/kg). The control group of 10 mice received corn oil only. Benzene was the positive control. Animals were killed 30 hours after the first injection. The femora were removed and the marrow was suspended in serum. Two smears per femur were prepared and coded. The smears were scored by two different people. One-thousand polychromatic erythrocytes per smear were examined for the presence of micronuclei. T-tests were used to compare data. No further test details are given.

Test substance : Purity of test substance (given by manufacturer) was 99.0 %.

Reliability : (4) not assignable. The use of multiple t-tests is an inappropriate means of determining the significance of the data. The study documentation is lacking in sufficient detail as to assess its validity

27.11.2001 (28)

Type : Cytogenetic assay
Species : mouse
Sex : male
Strain : B6C3F1
Route of admin. : i.p.
Exposure period : 17 or 36 hr
Doses : 0, 312.5, 625, 1250 mg/kg (17 hr protocol) and 0, 250, 500 and 1000 mg/kg (36 hour protocol)
Result : ambiguous
Method : other
Year : 1995
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Result : In the first experiment (17 hr), there was no effect of treatment on the percentage of cells with abnormalities. The results for 0, 312.5, 625 and 1250 mg/kg were 1.00 +/- 0.53, 3.25 +/- 0.92, 1.75 +/- 0.59, and 1.71 +/- 0.68 %.

In the second experiment (36 hr) there was a positive outcome (based on analysis by a trend test, P = 0.022). The results for 0, 250, 500 and 1000 mg/kg were 1.50 +/- 0.82, 2.67 +/- 1.12, 2.25 +/- 0.96, and 5.25 +/- 2.33 % (significantly different from control).

Test condition : Two experiments were performed. The first used a standard collection time of 17 hr and the second (conducted when there was no evidence of a positive effect at 17 hr) used a harvest time of 36 hr. In the first study, male mice (8 animals/dose group) received a single i.p. injection of corn oil, or 312.5, 625 or 1250 mg/kg test material in corn oil (volume = 0.4 ml). Mice were subcutaneously implanted with a BrdUrd tablet one hour before injection of test material. The mice received an i.p. injection of colchicine 2 hr prior to termination.

In the second experiment, male mice (8 animals/dose group) received a single i.p. injection of corn oil, or 250, 500 or 1000 mg/kg test material in corn oil (volume = 0.4 ml). They were implanted with a BrdUrd tablet and given an i.p. injection of colchicine 18 and 2 hours before termination (respectively) at 36 hours.

At termination, one or both femurs were removed and the marrow was flushed out with PBS. Cells were treated with a hypotonic salt solution,

fixed and dropped onto chilled slides. After a 24 hour drying period, cells were stained for differential chromatid counting. Fifty well-spread first-division metaphase cells from each animal per treatment group were scored for the presence of chromosomal aberrations.

The data were analyzed using the Micronucleus Assay Data Management and Statistical software package (version 1.4), which was designed specifically for in vivo micronucleus test data (ILS.1990. Integrated Laboratory Systems, P.O. Box 13501, Research Triangle Park, NC). The level of significance was set at $p < 0.05$. The numbers of MN-PCE at each dose group were pooled and analyzed by a one-tailed trend test. In the software package used, the trend test incorporates a variance inflation factor to account for excess animal variability. In the event that the increase in the dose response curve was non-monotonic, the software allowed for the data to be analyzed for a significant positive trend after data at the highest dose only has been excluded. In this event, the alpha level was adjusted to $p < 0.01$ to protect against false positives. The %PCE data were analyzed by an analysis of variance (ANOVA) test based on pooled data. Pairwise comparisons between each group and the solvent control were made using an unadjusted one-tailed Pearson chi-squared test which incorporated the calculated variance inflation factor for the study.

Reliability : (2) valid with restrictions. Purity of the test material was not listed.
27.11.2001 (35)

5.7 CARCINOGENITY

Species	:	rat
Sex	:	male/female
Strain	:	Fischer 344
Route of admin.	:	gavage
Exposure period	:	103 weeks
Frequency of treatment	:	5 days/week
Post. obs. period	:	no
Doses	:	60 or 120 mg/kg/day
Result	:	ambiguous
Control group	:	other: vehicle (corn oil) and untreated
Method	:	other
Year	:	1985
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	Throughout the study, mean body weights of treated males were similar to controls. During the second year of the study, mean body weights of females were greater than controls. No clinical signs of toxicity were observed. Positive titers of Kilham Rat Virus were detected at 24 months. The significance of this finding on the outcome of the study is unknown.

The survival rate of high dose males was significantly less than that of vehicle controls (41/50 vs. 49/50 at 78 weeks and 26/50 vs 39/50 at 103 weeks; $p = 0.033$), but not untreated controls (48/50 at 78 weeks and 34/50 at 103 weeks). The significance of these data is questionable, since there was no pathological evidence of marked toxic lesions or emaciation in these animals.

An apparent increase in the incidence of hepatocellular necrosis was

observed in treated animals. However, a blind review of all liver sections failed to detect an increase in this lesion in treated animals. Both pathologists generally graded the necrotic lesions as minimal to mild in severity. Therefore, the evidence for mild test-related liver cell necrosis is considered equivocal.

All statistical tests indicated a significant increase in neoplastic nodules in the livers of males in high dose group (120 mg/kg) compared to the vehicle control group (8/29 vs. 2/50) and pooled controls (8/49 vs. 6/100). The incidence of this lesion in untreated controls and rats treated with 60 mg/kg was similar (4/50 and 4/49, respectively). The only 2 hepatocellular carcinomas diagnosed in males were in vehicle controls. When both liver types were combined, the life table test was the only test that indicated a significant effect of treatment with 120 mg/kg versus the vehicle control, and 2 of the tests (life table and incidental tumor test) indicated a significant effect of treatment with 120 mg/kg versus pooled controls. There was no effect of treatment on the incidence of hepatic neoplasms in females.

Animals treated with 60 or 120 mg/kg test material had a statistically significant increase in the aspiration of foreign bodies into the lung in both sexes of rats versus vehicle controls. When the incidences of acute or chronic and focal granulomatous inflammation are combined, they are greater in all gavaged groups (including vehicle controls) than in controls that were not gavaged. These data indicate that gavage administration and not test material was the probable origin of this lesion.

Other tumors observed in treated animals were kidney tubular adenocarcinoma in one high dose female, and a transitional cell papilloma in the bladder in 1 male at each dose. The incidence of pituitary adenomas, carcinomas or carcinomas in males and adenomas in females was lower in high dose animals than controls and the incidence of endometrial stromal polyps was lower in the low dose group than controls.

The results show that interstitial cell tumors in the testes occurred with a significant positive trend by the life table test, and that the incidence in the high dose group was significantly higher by the life cycle test only. One of the tumors in the control group was malignant and none of them in dosed groups were malignant. This comment was reported in the NTP report and not in the Kluwe paper. The data listed in Tables in the NTP Report are not in agreement with some of the text. Data in tables indicate a significant negative trend for this lesion with treatment (47/50, 44/50, 43/49 and 43/50 in untreated control, vehicle control, 60 mg/kg and 120 mg/kg respectively). These data are suspect because of the high incidences. In conclusion, the testicular lesions are not considered to be treatment-related or as providing evidence of carcinogenicity.

Test condition : Rats were obtained at 4-6 weeks of age and were observed for 2 weeks before randomization. Rats (50/sex/group) were treated with 0 (corn oil vehicle), 60 or 120 mg/kg test material by gavage, 5 days/wk for 103 weeks. An additional control group of 50/sex was untreated. Groups of 15 rats of both sexes were left untreated and were used as sentinel animals for viral disease status at 6, 12, and 18 months (5/sex at each time). Food and water were supplied ad libitum. Animals were observed twice daily for morbidity and mortality. Those appearing moribund were terminated. Clinical signs were recorded monthly. Individual rat weights were recorded every week for the first 13 weeks and monthly thereafter.

All surviving animals were killed at the end of the 103-week exposure

period. All animals (including those that died during the study) were necropsied. The following set of organs was examined microscopically (unless precluded by autolysis or cannibalization): mandibular lymph node, salivary gland, femur, thyroid, parathyroids, small intestine, colon, liver, prostate, testis, ovary, lungs and bronchi, heart, esophagus, stomach, uterus, brain, thymus, trachea, pancreas, spleen, kidneys, adrenals, urinary bladder, pituitary, and mammary gland.

Body weight data were analyzed using Dunnett's multiple comparison test. Probabilities of survival were estimated by the product limit procedure and were analyzed for equality and dose-related trend. Site-specific tumor frequencies were analyzed between groups for dose-response effects and by pairwise comparisons with controls. Three different methods were used to analyze data. The first method of analysis (life table test) is a survival-correcting method most appropriate for fatal tumors. The second (incidental tumor test) is a survival-correcting method most appropriate for nonfatal tumors. The Fisher's exact test for pairwise comparisons and Cochran-Armitage linear trend test for dose-response trends also were conducted. These tests were based on the overall proportion of tumor-bearing animals. Except where noted, the 3 tests indicated similar results. Survival and incidence data were compared between groups using two- and one-tailed analyses, respectively.

All data and slides were sent to an independent quality assurance laboratory for verification. The final tumor diagnoses represented a consensus between the original pathologist, independent contractors, and the NTP Pathology Working Group. Sections of liver from all rats were reexamined in a blind fashion by an independent pathologist due to the equivocal nature of the non-neoplastic liver changes. The diagnoses of both pathologists were listed in the study documentation.

Test substance : Test material was determined to be > 99.9% pure by gas chromatography.
Reliability : (2) valid with restrictions. Testicular data are suspect.
27.11.2001 (23) (32)

Species : mouse
Sex : male/female
Strain : B6C3F1
Route of admin. : gavage
Exposure period : 103 weeks
Frequency of treatment : 5 days/week
Post. obs. period : none
Doses : males: 30 or 60 mg/kg/day; females 60 or 120 mg/kg/day
Result : negative
Control group : other: vehicle (corn oil) and untreated
Method : other
Year : 1985
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Result : The survival rate of treated males (both doses) was marginally less that that of either control. The absence of marked toxic lesions or emaciation in the animals that died early do not support a causal relationship between treatment and shortened survival. Several tumors commonly observed in aged mice (e.g. liver and lung) occurred at low frequencies in all groups. There was no effect of treatment on frequency of any tumor examined. No compound-related effects were observed with regard to body weight,

clinical condition, or organ toxicity.

Five untreated (10%), five corn oil treated (10%), and 4 low dose males (8%) exhibited focal necrosis of hepatocytes, and 3 low dose males (6%) exhibited coagulative necrosis of hepatocytes. The incidences of other liver lesions were 4% or less in treated and control males.

A high incidence of liver inflammation was noted in untreated control females (21%). Two untreated (4%), five low dose (10%), and 2 high dose females (4%) exhibited focal necrosis of hepatocytes, and 3 high dose females (6%) exhibited coagulative necrosis of hepatocytes (not significantly different from control). One vehicle control (3%), 4 low dose (11%) and 3 high dose (8%) females had anterior pituitary cell hyperplasia (not significantly different).

There were no treatment-related carcinogenic effects of the test material in male or female mice.

Test condition : Mice were obtained at 4-6 weeks of age and were observed for 2 weeks before randomization. Male mice (50/group) were treated with 0 (corn oil vehicle), 30 or 60 mg/kg test material and female mice (50/group) were treated with 0 (corn oil vehicle), 60 or 120 mg/kg test material by gavage, 5 days/wk for 103 weeks. An additional control group of 50/sex was untreated. Groups of 15 mice of both sexes were left untreated and were used as sentinel animals for viral disease status at 6, 12, and 18 months (5/sex at each time). Food and water were supplied ad libitum. Animals were observed twice daily for morbidity and mortality. Those appearing moribund were terminated. Clinical signs were recorded monthly. Individual mouse weights were recorded every week for the first 13 weeks and monthly thereafter.

All surviving animals were terminated at the end of the 103 week exposure period. All animals (unless precluded by autolysis or cannibalization) were necropsied. The following set of organs was examined microscopically (unless precluded by autolysis or cannibalization): mandibular lymph node, salivary gland, femur, thyroid, parathyroids, small intestine, colon, liver, gallbladder, prostate, testis, ovary, lungs and bronchi, heart, esophagus, stomach, uterus, brain, thymus, trachea, pancreas, spleen, kidneys, adrenals, urinary bladder, pituitary, and mammary gland.

Body weight data were analyzed using Dunnett's multiple comparison test. Probabilities of survival were estimated by the product limit procedure and were analyzed for equality and dose-related trend. Site-specific tumor frequencies were analyzed between groups for dose-response effects and by pairwise comparisons with controls. Three different methods were used to analyze data. The first method of analysis (life table test) is a survival-correcting method most appropriate for fatal tumors. The second (incidental tumor test) is a survival-correcting method most appropriate for nonfatal tumors. The Fisher's exact test for pairwise comparisons and Cochran-Armitage linear trend test for dose-response trends also were conducted. These tests were based on the overall proportion of tumor-bearing animals. Except where noted, the 3 tests indicated similar results. Survival and incidence data were compared between groups using two- and one-tailed analyses, respectively.

All data and slides were sent to an independent quality assurance laboratory for verification. The final tumor diagnoses represented a

consensus between the original pathologist, independent contractors, and the NTP Pathology Working Group. None of the tumors were considered to be treatment-related.

Test substance : Test material was determined to be > 99.9% pure by gas chromatography.
Reliability : (1) valid without restriction
27.11.2001 (23) (32)

5.8 TOXICITY TO REPRODUCTION

Type : Two generation study
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : inhalation
Exposure period : F0 and F1 generations during mating period, days 0-20 of gestation and days 4-21 of lactation
Frequency of treatment : 6 hrs/day, 7 days/week
Premating exposure period
Male : F0 : 10 weeks, F1:11 weeks
Female : F0 : 10 weeks, F1:11 weeks
Duration of test : until weaning of F2 pups
Doses : 50, 150 or 450 ppm (234, 702 or 2105 mg/m3)
Control group : yes
NOAEL Parental : = 50 ppm
NOAEL F1 Offspr. : = 450 ppm
NOAEL F2 Offspr. : = 450 ppm
Method : other
Year : 1987
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Remark : The NOAELs listed for F1 and F2 offspring are for reproductive indices in F0 and F1 females and males and survival of F1 and F2 fetuses. The NOAEL for toxicity for F0 and F1 parental animals is 50 ppm (based on changes in liver and kidney in males exposed to higher doses).
Result : Cumulative mean (+SD) analytical exposure concentrations were 51 (+/- 5), 151 (+/-8) and 451 (+/- 25) ppm for the F0 generation, and 49 (+/- 4), 150 (+/- 11) and 454 (+/- 21) ppm for the F1 generation.

No mortalities were observed in the adult generations. Mean body weights and food consumption for adult male and females in both generations were comparable for all groups. Mating and fertility indices for males and females were unaffected by treatment. For the F0 generation, the percentage of females that were pregnant in the control, 50, 150 and 450 ppm groups was 90%, 100%, 93.1% and 86.7%, respectively. The percentage of F0 males that successfully impregnated females in the control, 50, 150 and 450 ppm groups was 92.3%, 100%, 93.1% and 89.7%, respectively. For the F1 generation, the percentage of females that were pregnant in the control, 50, 150 and 450 ppm groups was 80%, 100%, 79.3 % and 89.3%, respectively. The percentage of F1 males that successfully impregnated females in the control, 50, 150 and 450 ppm groups was 77.8%, 100%, 79.3% and 88.0%, respectively. The mean (+SD) number of days for F0 males and females to mate in the control, 50, 150 and 450 ppm groups was 4.2 +/- 4.6, 3.1 +/- 2.8, 2.8 +/- 2.0 and 3.1 +/- 2.9, respectively (not significantly different). The mean (+SD) number of days for F1 males

and females to mate in the control, 50, 150 and 450 ppm groups was 4.9 +/- 4.2, 3.2 +/- 2.3, 3.1 +/- 2.4 and 4.6 +/- 4.1, respectively. None of these values were significantly different from control.

In the F1 and F2 litters, pup and litter survival for all treated groups was comparable to controls. The pup viability index at birth for F1 offspring ranged from 96.1 +/- 12.0 in the 50 ppm group to 99.0 +/- 2.4 in the 150 ppm group, and for F2 offspring was 97-98% for all groups. Overall survival indices of F1 litters from F0 rats exposed to 0, 50, 150 or 450 ppm were 100%, 96.7%, 96.3% and 92.3%. Survival indices of F2 litters from F1 rats exposed to the aforementioned concentrations were 91.7%, 96.6%, 91.3% and 88.0%, respectively (not significantly different). In the F2 litters, a slight, nonsignificant decrease in pup survival index (Days 0-4) was seen in offspring from high dose animals. This was not considered to be treatment-related as this was predominantly due to loss of litters from two dams (one dam lost 12/15 pups and another lost all 10).

Significant increases in absolute and relative liver weight were observed in F0 and F1 adults exposed to 150 or 450 ppm. The relative liver weight of F1 males exposed to 50 ppm was also greater than control (3.73 +/- 0.36 vs. 3.47 +/- 0.32). An increase in the incidence of small flaccid testes and dilated renal pelvis was observed in high dose F0 and F1 males. For the 50, 150 and 450 ppm groups the incidence of small flaccid testes was 0, 1, and 3 for F0 males and 0, 1 and 5 for F1 males. For the 0, 50, 150 and 450 ppm males the incidence of dilated renal pelvis was 1, 1, 2 and 5 for F0 males (dose-related) and 1, 4, 6 and 4 for F1 males (not dose-related). In F0 females, the incidence of dilated renal pelvis in treated animals (4-6) was similar to control (5). In the F1 generation, 2 females treated with 150 or 450 ppm had dilated renal pelvis versus 0 in control.

Microscopic changes were observed in the liver and kidneys of treated male rats. Hepatocellular hypertrophy (graded minimal to mild) was noted in 5 mid and 14 high dose F0 males (none in control) and 3 mid and 7 high dose F1 males (2 in control). This lesion was observed in only one female (a high dose F0). The incidence of renal changes in male rats is shown in the Table below:

Table: Incidence of renal changes in male rats inhaling monochlorobenzene for 2 generations

	Group (ppm)							
	F_0 adults				F_1 adults			
	0	50	150	450	0	50	150	450
Total No. of animals	30	30	30	30	30	30	30	30
U/tubular dilation Eosinophilic material	0	3	2	3	4	4	6	6
B/tubular dilation Eosinophilic material	0	1	4	15	4	3	8	16
U/chronic interstitial nephritis	0	0	0	1	1	2	1	0
B/chronic interstitial nephritis	1	2	7	9	0	1	6	11
U/foci of regenerative epithelium	0	0	0	0	0	0	1	1
B/foci of regenerative epithelium	0	1	5	8	1	0	4	10

U/ = unilateral, B/ = bilateral
Lesions were not present in females

Two F0 and 3 F1 males in the 150 ppm group and 6 F1 males in the 450 ppm group exhibited unilateral degeneration, and 6 F0 males of the 450 ppm group exhibited bilateral degeneration of varying degrees in the germinal epithelium of the testes. This lesion was observed in 1 male control in each of the generations. In order to determine the effect of these

lesions on reproductive performance, the reproductive performance of all males that showed this lesion were reviewed. All controls, all affected F0 and 2/3 F1 males in the 150 ppm group, and 3/6 affected animals in the 2 generations treated with 450 ppm were successful in siring litters.

Test condition : Test article: Test article was administered by the inhalation route with animals exposed in 6m3 glass and stainless steel chambers. Targeted exposure levels were 50, 150 and 400 ppm; included in the study was a chamber exposed, sham-air control group. The chambers were operated dynamically at an air flow rate of at least 2140 l/min (one air change per 2.8 min). The test material was fed into an atomizing nozzle via an FMI fluid metering pump. The vaporized test material was diluted with preconditioned air prior to entry into the exposure chamber. Test concentrations were monitored hourly during exposures. A daily nominal concentration was determined by dividing the difference in weight of the generation apparatus and test material before and after exposure by the total volume of air delivered during the day.

Test conduct: Animals were acclimated for at least 13 days prior to exposure. They were 6 weeks old at time of exposure. F0 adult animals (30/sex/group) were exposed daily (6 hrs/day, 7 days/week) with 0 (filtered air), 50, 150 or 450 ppm for a 10 week pre-mating treatment period and during mating. Once mated (as evidenced by the presence of a copulatory plug), females were exposed (6 hrs/day) during gestation (Days 0-19) and lactation (Days 5-28) of the FI litters. F0 males continued to be treated daily during the post-mating period until termination. Similarly, F0 females continued to be treated daily post-weaning until euthanization after weaning of the last litter.

FI pups (30/sex/group) were exposed to comparable dose levels as the dams one week after weaning to at least 11 weeks prior to mating. Animals chosen to be the F1 parents were selected to maximize representation from the number of available litters. Exposure of F1 animals during the mating, gestation and lactation intervals of the F2 litters was similar to that of the F0 animals.

Animals were given free access to standard laboratory diet and water during all non-exposure periods, and water during lactation. All animals were observed twice daily for toxicity or mortality. Detailed physical examinations were performed weekly. Body weights and food consumption of F0 and F1 adults were generally measured weekly for most animals (with the exception of slightly different intervals for females during gestation and lactation). Litters were examined twice daily for death and general appearance. On day 4 of lactation, all litters with greater than 8 pups were culled to that number. The sex distribution within litters was equalized (if possible). Pup weights, the number of pups in each litter and pup sex distribution were determined on days 0, 4 (pre and post cull), 7, 14, and 21 of lactation. The mating index for males and females, pregnancy rate and fertility index for males were calculated for each of the two matings. Pup survival indices at various intervals during lactation were calculated.

All F0 and F1 adults were killed after all F1 pups and F2 pups were weaned, respectively. All F2 pups were killed at day 21 of lactation. Complete gross postmortem examinations were conducted on all F0 and F1 parents, all F1 weanlings not selected to become parents of the F2 generation, and all F2 weanlings. Liver and brain weights of F0 and F1 adults were recorded. Liver, kidneys, pituitary gland, and reproductive

organs (males- epididymes, seminal vesicle and prostate; females- vagina, uterus and ovaries) were examined microscopically for all F0 and F1 adult animals in the control and high dose groups. Liver, kidneys and testes of male rats in the low and mid-dose groups were examined histologically.

Statistical analyses: Mean body weights, food consumption, organ weights, organ to body weight ratios, gestation lengths, and numbers of offspring were evaluated for equal variance using Bartlett's test. Parametric methods (one way analysis of variance followed by a Dunnett's test) were performed on data if variances were equal. A Kruskal-Wallis test or Dunn's summed rank test was used to analyze nonparametric data. The nonparametric test for determining monotonic trend was the Jonckheere's test and standard linear regression was used for parametric data. Pup viability and survival indices were analyzed with the litter as the experimental unit, and these data were transformed using arcsine. Incidence data were analyzed using contingency tables. A standard chi-square analysis was performed on these data to determine if the proportion of incidences differed between the groups tested. Next, each treatment group was compared to the control group using a 2 x 2 Fischer exact test. The significance level was corrected using the Bonferroni inequality. An Armitage test for linear trend was performed.

Test substance : Purity of test material was 99.9%.

Conclusion : Exposure to male rats of 150 or 450 ppm caused hepatocellular hypertrophy and increased liver weight, degenerative and inflammatory lesions in the kidneys and degenerative testicular changes. The relationship between testicular damage and exposure to monochlorobenzene is unclear because although 3/6 affected high dose males in each generation did not sire litters, the overall incidence of males not siring litters in each generation of the high dose animals (4 F0 and 8 F1) and mid-dose animals (3 F0 and 7 F1) was not different from control (6 F0 and 9 F1). At the doses tested, chlorobenzene had no adverse effect on reproduction.

In the absence of microscopic changes, the increased liver weight in the low dose males and mid and high dose females was not considered to be indicative of an adverse effect.

Reliability : (1) valid without restriction

27.11.2001 (31)

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : rat
Sex : female
Strain : Fischer 344
Route of admin. : inhalation
Exposure period : day 6-15 of gestation
Frequency of treatment : 6 hours/day
Duration of test : to day 21 of gestation
Doses : 75, 210 or 590 ppm (351, 982 or 2760 mg/m3)
Control group : yes
NOAEL Maternalt. : = 210 ppm
NOAEL Teratogen : = 590 ppm
Method : other
Year : 1984

GLP : no data

Test substance : as prescribed by 1.1 - 1.4

Remark : The NOAEL for fetotoxicity is 210 ppm, as evidenced by increased incidences of some skeletal variations at 590 ppm.

Result : Maternal: No maternal deaths or abnormal clinical signs were noted in treated rats. High dose animals lost weight (2 grams) during the first three days of exposure. Other groups gained an average of 2-4 g. Absolute and relative liver weights of high dose rats were slightly, but significantly greater than control at necropsy, indicating slight maternal toxicity at the high dose.

The pregnancy rate was not altered by exposure to any concentration of test material (data not shown). No adverse effects were noted in mean litter size or incidence of implantations that underwent resorption.

Fetal: Fetal body measurements for exposed animals were similar to controls. The incidence of malformations was not altered by treatment (N = 4 in control, N= 1 at 76 ppm, N = 2 in two litters at 210 ppm and N = 3 in 3 litters at 590 ppm). With the exception of a cleft palate in one animal in the 75 ppm group, the malformations observed in litters from treated rats were similar to the study control and were at historical incidences for controls. Decreases in the incidence of focal necrosis in the liver were seen in litters from animals exposed to 210 (22 vs. 30 in control) and 590 ppm (19 vs. 30 in control). This was not considered to be of toxicological importance.

There was an increased incidence of some minor skeletal variants in fetuses from treated animals. A higher incidence of delayed ossification of centra of the cervical veterbrae was noted in offspring from rats exposed to 75 ppm (92 in 27 litters vs. 59 in 23 litters in controls) and 590 ppm (103 in 27 litters vs. 59 in 23 litters in controls), but not 210 ppm (73 in 23 litters). A higher incidence of bilobed centra of the thoracic vertebrae (12 in 11 litters vs. 8 in 5 litters in controls) and a lower incidence of cervical spurs (13 in 11 litters vs. 25 in 17 control litters) was noted in offspring from high dose animals. The skeletal changes observed in offspring from high dose animals were concluded to be indicative of a slight delay in skeletal development. None of the variants described were considered to be indicative of a specific teratogenic response.

Test condition : Test vapor generation: Temperature and relative humidity were controlled at 21 degrees C and 50%, respectively. Vapor was generated by metering the liquid test material at controlled rates into vaporization tubes. Vapors from the tubes were swept into the chamber inlet ducts with preheated compressed air (120 to 135 degrees C) where they were mixed and diluted with incoming air by turbulence. Concentrations of test material were determined 1-2 times per hour throughout the exposure by infrared spectrometry. The actual concentration in the exposure chamber was determined by interpolation from standard curves derived from vapor standards of known concentrations. At least one standard was run before each daily exposure. The nominal concentration (ratio of amount of test material vaporized/total amount of air through the chamber) was calculated for each chamber for each exposure day. The mean daily time weighted average analytical concentrations for each chamber were within 7-8 % of intended chamber concentrations. Mean daily nominal concentrations were in close agreement with the time weighted analytical concentrations, indicating that test material losses were negligible.

Study conduct: After a 2 week acclimation period, females (175 to 225 g) were bred to adult males of the same strain (one female to one male). The

day sperm were found in a vaginal smear was considered to be Day 0 of gestation. Groups of 30-32 bred rats were exposed to filtered room air (control), or 75, 210 or 590 ppm test material for 6 hr/day on Days 6 through 15 of gestation. Exposure levels were based on results of preliminary studies that showed that 1000 ppm produced maternal toxicity. Inhalation exposures were conducted in 4.3 m3 stainless steel and glass chambers under dynamic airflow conditions (800 liters air/min). Food and water were available ad libitum except during exposures. Treated and control animals were held in separate rooms.

Animals were observed daily throughout the experimental period for toxicity. Body weights were recorded on gestation days 6, 9, 12, 16 and 21. Food and water consumption was recorded at 3-day intervals beginning on Day 6 of gestation. Animals were killed on Day 21 of gestation. Maternal liver and kidney weights were recorded. The uterine horns were exteriorized and the following data were recorded: number and position of fetuses in utero, number of live and dead fetuses, number and position of resorption sites, number of corpora lutea, the sex, body weight and crown -rump length of each fetus, and any gross external alterations. The uteri of apparently nonpregnant animals were stained with a 10% solution of sodium sulfide and examined for implantation sites. These data were not used to calculate the incidence of resorptions. One-half of each litter was randomly selected for immediate examination under a dissection microscope for soft tissue abnormalities. The heads of these fetuses were removed, placed in Bouin's fixative and examined by serial sectioning. All fetuses were preserved in alcohol, eviscerated, stained with alizarin red-S and examined for skeletal alterations.

Statistical evaluations: Statistical evaluations of the frequencies of alterations and resorptions among litters and the fetal population were conducted by the Wilcoxon test as modified by Haseman and Hoel (J Statis. Comput. Simul. 3:117-135, 1974). Statistical analyses of the percentage of pregnancy and other incidence data were conducted by the Fisher exact probability test. Analyses of other data were made by parametric or nonparametric analysis of variance followed by the Dunnett's test (for parametric data) or the Wilcoxon test (for nonparametric data). The reported level of statistical significance was $p < 0.05$. Statistical outliers in feed and water consumption data were identified by a sequential outlier test and were not used when calculating mean values.

Test substance : Test material was 99.982 % pure. Impurities (% by weight) were benzene(< 0.005%), bromobenzene (0.018%) and water (0.0077%).

Conclusion : The authors concluded that the test material was not embryotoxic or teratogenic. Fetal effects (slight delays in skeletal development) were noted only at a concentration that produced maternal toxicity.

Reliability : (1) valid without restriction

Flag : Key study for endpoint

27.11.2001 (21)

Species : rabbit

Sex : female

Strain : New Zealand white

Route of admin. : inhalation

Exposure period : days 6 - 18 of gestation

Frequency of treatment : 6 hours/day

Duration of test : to day 29 of gestation

Doses : 75, 210, 590 ppm (first study), and 10, 30, 75 and 590 ppm (second study)

Control group	:	yes
NOAEL Maternalt.	:	= 75 ppm
NOAEL Teratogen	:	= 590 ppm
Method	:	other
Year	:	1984
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Since the 61% resorption rate in high dose animals in the second study was within the historical range (19-67%) from 21 studies conducted in the laboratory and was not observed in the first study (where 63% of the control litters had resorptions) it was not interpreted by the authors to be indicative of an embryotoxic effect. The increased incidence of extra ribs observed at the high dose in the first study also was not confirmed by the second study. Therefore, the NOAELs for embryotoxicity, teratogenicity and fetotoxicity are 590 ppm.
Result	:	In the first study, there were increased absolute and relative liver weights in rabbits exposed to 210 or 590 ppm. There was no effect of treatment on pregnancy rate, mean litter size or the incidence of resorption. However, exposure to 0.75, 210 or 590 ppm test material resulted in a variety of malformations in all groups at incidences slightly higher than historical controls. The incidence of malformations at these doses was 11 (in 6 litters), 8 (in 7 litters), 6 (in 5 liters) and 8 (in 7 litters). There was no effect of treatment on the incidence of any malformation or variation, except for an increased incidence of extra ribs (a variation) in the high dose animals (113 in 26 litters vs. 79 in 24 control litters). Head/facial abnormalities were present in one fetus from the 75 ppm group and another from the 590 ppm group, and heart defects were seen in 1 fetus from the 210 ppm group and 2 fetuses (in 2 litters) in the 590 ppm group. To determine if these malformations were true effects of treatment, the study was repeated. In the second group, exposure to 590 ppm produced an increase in liver weight of maternal animals. A significant increase in the percentage of implantations undergoing resorption was observed in the 590 ppm group (61% vs. 41% in controls). There was no effect of treatment on the number of bred females, % pregnant, number of litters, implantation sites/dam, number of fetuses/litter, % of implantations resorbed, fetal body weight or fetal crown-rump length. The incidence of malformations in fetuses from animals treated with 0, 10, 30, 75 or 590 ppm was 14 (in 11 litters), 3 (in 3 liters), 14 (in 8 litters), 7 (in 5 litters) and 14 (in 5 litters). Fetuses with external, soft tissue and skeletal malformations were observed among all groups (including controls). There were seven fetuses in one litter with ablepharia (missing eyelid) in the 590 ppm group. This anomaly was not observed in the first study. Heart abnormalities were observed at similar incidences in controls and treated animals (2 in controls and 0-2 in treated), and there was no dose-dependent effect. No head or facial abnormalities were observed in any group. Skeletal examinations revealed a significant increase in the incidence of extra ribs in the 10 ppm group (94 in 23 litters vs. 72 in 21 control litters). This alteration was considered a skeletal variant.
Test condition	:	Test vapor generation: Temperature and relative humidity were controlled at 21 degrees C and 50%, respectively. Vapor was generated by metering the liquid test material at controlled rates into vaporization tubes. Vapors from the tubes were swept into the chamber inlet ducts with preheated compressed air (120 to 135 degrees C) where they were mixed and diluted with incoming air by turbulence. Concentrations of test material were determined 1-2 times per hour throughout the exposure by infrared spectrometry. The actual concentration in the exposure chamber was

determined by interpolation from standard curves derived form vapor standards of known concentrations. At least one standard was run before each daily exposure. The nominal concentration (ratio of amount of test material vaporized /total amount of air through the chamber) was calculated for each chamber for each exposure day. The mean daily time weighted average analytical concentrations for each chamber were within 7-8 % of intended chamber concentrations. Mean daily nominal concentrations were in close agreement with the time weighted analytical concentrations indicating that test material losses were negligible.

Study conduct: After a 2 week acclimation period, rabbits (3.5 to 4.5 kg) were artificially inseminated. The day of insemination was considered gestation day 0. Groups of 30 inseminated rabbits were exposed to filtered room air (control), or 75, 210 or 590 ppm test material for 6 hr/day on Days 6 through 18 of gestation. Exposure levels were based on results of preliminary studies that showed that 1000 ppm produced maternal toxicity. Inhalation exposures were conducted in 4.3 m3 stainless steel and glass chambers under dynamic airflow conditions (800 liters air/min). Food and water were available ad libitum except during exposures. Treated and control animals were held in separate rooms.

Animals were observed daily throughout the experimental period for toxicity. Body weights were recorded on gestation days 6, 9, 12, 16 and 21. Food and water consumption was recorded at 3-day intervals beginning on Day 6 of gestation. Animals were euthanized on Day 21 of gestation. Maternal liver and kidney weights were recorded. The uterine horns were exteriorized and the following data were recorded: number and position of fetuses in utero, number of live and dead fetuses, number and position of resorption sites, number of corpora lutea, the sex, body weight and crown -rump length of each fetus, and any gross external alterations. The uteri of apparently nonpregnant animals were stained with a 10% solution of sodium sulfide and examined for implantation sites. These data were not used to calculate the incidence of resorptions. One-half of each litter was randomly selected for immediate examination under a dissection microscope for soft tissue abnormalities. The heads of these fetuses were removed, placed in Bouin's fixative and examined by serial sectioning. All fetuses were preserved in alcohol, eviscerated, stained with alizarin red-S and examined for skeletal alterations.

Statistical evaluations: Statistical evaluations of the frequencies of alterations and resorptions among litters and the fetal population were conducted by the Wilcoxon test as modified by Haseman and Hoel (J Statis. Comput. Simul. 3:117-135, 1974). Statistical analyses of the percentage of pregnancy and other incidence data were conducted by the Fisher exact probability test. Analyses of other data were made by parametric or nonparametric analysis of variance followed by the Dunnett's test (for parametric data) or the Wilcoxon test (for nonparametric data). The reported level of statistical significance was $p < 0.05$. Statistical outliers in feed and water consumption data were identified by a sequential outlier test and were not used when calculating mean values.

Repeat Study: Due to the finding of a variety of external and visceral malformations in the exposed animals, a second study was initiated. This study was initiated prior to the completion of the skeletal examinations in the first study to ascertain if the low incidences of head and facial anomalies and heart defects in the first study were true effects of treatment. In this study, groups of 30-32 inseminated rabbits were

exposed to filtered room air (control), or 10, 30, 75 or 590 ppm test material for 6 hr/day on Days 6 through 18 of gestation. A control group of 22 rabbits was exposed to filtered room air.

Test substance : Test material was 99.982 % pure. Impurities (% by weight) were benzene(< 0.005%), bromobenzene (0.018%) and water (0.0077%).

Conclusion : Maternal toxicity (as evidenced by increased liver weight) was observed in rabbits exposed to 210 or 590 ppm. Inhalation of up to 590 ppm test material was not teratogenic in rabbits. Malformations were evenly distributed among groups (including controls) and were not dose-related. The increase in incidence of resorptions at 590 ppm test material in the second study, although significantly increased from control, was within the historic range of 19-67% in controls from 21 teratology studies performed by the laboratory. Since an increase in resorptions did not occur in the first experiment, the increase in the second experiment was not interpreted to be indicative of an embryotoxic effect.

Reliability : (1) valid without restriction

Flag : Key study for endpoint

27.11.2001 (21)

5.10 OTHER RELEVANT INFORMATION

5.11 EXPERIENCE WITH HUMAN EXPOSURE

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7.1 END POINT SUMMARY

7.2 HAZARD SUMMARY

7.3 RISK ASSESSMENT

Robust Summaries and Repository of Knowledge for CAS No. 95-50-1

Existing Chemical	:	ID: 95-50-1
CAS No.	:	95-50-1
EINECS Name	:	1,2-dichlorobenzene
EINECS No.	:	202-425-9
TSCA Name	:	Benzene, 1,2-dichloro-
Molecular Formula	:	C6H4Cl2

Printing date	:	29.01.2003
Revision date	:	29.01.2003
Date of last Update	:	29.01.2003
Number of Pages	:	62
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 7

1.0.1 OECD AND COMPANY INFORMATION

1.0.2 LOCATION OF PRODUCTION SITE

1.0.3 IDENTITY OF RECIPIENTS

1.1 GENERAL SUBSTANCE INFORMATION

Substance type : organic
Physical status : liquid
Purity : = 99 - 99.5 % w/w
Reliability : (1) valid without restriction
29.11.2001 (43)

1.1.0 DETAILS ON TEMPLATE

1.1.1 SPECTRA

1.2 SYNONYMS

benzene, 1,2-dichloro
29.11.2001

benzene, o-dichloro
29.11.2001

o-dichlorobenzene
29.11.2001

o-dichlorobenzol
29.11.2001

ortho dichlorobenzene
29.11.2001

1.3 IMPURITIES

Test substance : as prescribed by 1.1-1.4
CAS-No : 106-46-7
EINECS-No : 203-400-5
EINECS-Name : 1,4-dichlorobenzene
Contents : = .5 - 1 % w/w
Reliability : (1) valid without restriction. Data were obtained from MSDS.
29.11.2001 (43)

1.4 ADDITIVES

1.5 QUANTITY

1.6.1 LABELLING

1.6.2 CLASSIFICATION

1.7 USE PATTERN

1.7.1 TECHNOLOGY PRODUCTION/USE

1.8 OCCUPATIONAL EXPOSURE LIMIT VALUES

1.9 SOURCE OF EXPOSURE

1.10.1 RECOMMENDATIONS/PRECAUTIONARY MEASURES

1.10.2 EMERGENCY MEASURES

1.11 PACKAGING

1.12 POSSIB. OF RENDERING SUBST. HARMLESS

1.13 STATEMENTS CONCERNING WASTE

1.14.1 WATER POLLUTION

1.14.2 MAJOR ACCIDENT HAZARDS

1.14.3 AIR POLLUTION

1.15 ADDITIONAL REMARKS

1.16 LAST LITERATURE SEARCH

1.17 REVIEWS

1.18 LISTINGS E.G. CHEMICAL INVENTORIES

2.1 MELTING POINT

Value	:	= -17 °C
Decomposition	:	no at °C
Sublimation	:	no
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4; purity 99.0-99.5% (0.5-1.0% 1,4-dichlorobenzene impurity)
Remark	:	The test method is not given. However, the value stated is most likely a measured value, since this substance is a well-known, basic chemical. Kirk Othmer Encyclopedia of Chemical Technology, 4th Edition, Volume 6, page 89, cites a melting point value of -16.97° C. Ulmann's Encyclopedia of Industrial Chemistry, 5th Ed., page 328, cites a value of -16.7° C.
Reliability	:	(2) valid with restrictions. Data were obtained from a Solutia Inc. MSDS.
Flag	:	Key study for endpoint

29.11.2001 (43)

Value	:	-17 °C
Test substance	:	as prescribed by 1.1-1.4
Source	:	Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Information came from IUCLID document created by NICNAS, creation date 23-AUG-2001

01.06.1994 (2)(4)

Value	:	-17.5 °C
Sublimation	:	
Method	:	other: not specified
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1-1.4
Source	:	ELF ATOCHEM S.A., France Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Information came from IUCLID document created by NICNAS, creation date 23-AUG-2001

10.05.1994 (16)

2.2 BOILING POINT

Value	:	= 180.5 °C at 1013 hPa
Decomposition	:	No
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4

Remark	:	The test method is not given. However, the value stated is most likely a measured value, since this substance is a well-known, basic chemical, that is purified by distillation. Kirk Othmer Encyclopedia of Chemical Technology, 4th Edition, Volume 6, page 89, cites a boiling point value of 180.4° C. Ulmann's Encyclopedia of Industrial Chemistry, 5th Ed., page 328, cites a value of 179° C.
Reliability	:	(2) valid with restrictions. Data were obtained from the MSDS.

29.11.2001 (43)

2.3 DENSITY

Type	:	density
Value	:	1.298 g/cm3 at 20° C
Test substance	:	as prescribed by 1.1 - 1.4
Source	:	Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Information came from IUCLID document created by NICNAS, creation date 23-AUG-2001

01.06.1994 (2)

Type	:	
Value	:	= 1.31 g/cm3 at 15° C
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Reliability	:	(2) valid with restrictions. Data were obtained from the MSDS

29.11.2001 (43)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value	:	= 1.3 hPa at 20° C
Decomposition	:	no
Method		other (measured)
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Decomposition	:	no
Remark	:	The test method is not given. However, the value stated is most likely a measured value, since this substance is a well-known, basic chemical.
Reliability	:	(2) valid with restrictions. Data were obtained from a MSDS
Flag	:	Key study for endpoint

29.11.2001 (43)

Value	:	1.3 hPa at 20° C
Test substance	:	as prescribed by 1.1-1.4
Source	:	Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Information came from IUCLID document

created by NICNAS, creation date 23-AUG-2001

01.06.1994 (2)

Value	:	1.4 hPa at 20° C
Decomposition	:	
Method		
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1-1.4
Remark	:	Method: not specified
Source	:	ELF ATOCHEM S.A., France Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Information came from IUCLID document created by NICNAS, creation date 23-AUG-2001

10.05.1994 (17)

2.5 PARTITION COEFFICIENT

Log pow	:	3.43 at ° C
Test substance	:	as prescribed by 1.1-1.4
Remark	:	experimentally measured
Source	:	Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Information came from BUA Report 53. Little study detail given
Flag	:	Key study for endpoint

31.01.1994 (12)(33)

Log pow	:	3.39 at ° C
Method	:	High pressure liquid chromatography
Test substance	:	as prescribed by 1.1-1.4
Remark	:	experimentally measured
Source	:	
Reliability	:	(2) valid with restrictions. Study predates GLP and OECD guidelines.
Flag	:	Supporting study

31.01.1994 (27)

Log pow	:	ca. 3.28 at 20° C
Method		other (calculated)
Year	:	2001
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The log Kow was calculated using the EPIWIN KOWWIN Program (v1.66). This program uses values previously determined for each bond fragment in the molecule and sums these values.
Reliability	:	(2) valid with restrictions. Data were obtained by modeling.

29.11.2001 (41)

2.6.1 WATER SOLUBILITY

Value	:	= .145 g/l at 20 ° C
Qualitative	:	
Pka	:	at 25 ° C
PH	:	= 7 at and ° C
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The test method is not given. However, the value stated is most likely a measured value, since this substance is a well-known, basic chemical. Ulmann's Encyclopedia of Industrial Chemistry, 5th Ed., page 328, cites water solubility values of 0.015 and 0.023 g/100g (0.15 and 0.23 g/l).
Reliability	:	(2) valid with restrictions. Data were obtained from a MSDS.
Flag	:	Key study for endpoint

29.11.2001 (43)

Value	:	.13 g/l at 20 ° C
Qualitative	:	
Pka	:	at 25 ° C
PH	:	at and ° C
Test substance	:	as prescribed by 1.1-1.4
Source	:	Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Information came from IUCLID document created by NICNAS, creation date 23-AUG-2001

31.01.1994 (3)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

Value	:	66 ° C
Type	:	other
Method	:	other: closed cup, DIN 51758
Year	:	
GLP	:	
Test substance	:	as prescribed by 1.1-1.4
Source	:	Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2)valid with restrictions. DIN is an acceptable method. Information came from IUCLID document created by NICNAS, creation date 23-AUG-2001

01.06.1994 (3)

Value	:	68 ° C
Type	:	closed cup
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1-1.4
Remark	:	Method: NFT 60-103
Source	:	ELF ATOCHEM S.A., France Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

Reliability : (2) valid with restrictions. Information came from IUCLID document created by NICNAS, creation date 23-AUG-2001

10.05.1994 (16)

2.8 AUTO FLAMMABILITY

Value : >= 600 ° C at
Method : other: DIN 51794
Year :
GLP :
Test substance : as prescribed by 1.1-1.4
Source : Bayer AG Leverkusen
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (2) valid with restrictions. DIN is an acceptable method. Information came from IUCLID document created by NICNAS, creation date 23-AUG-2001

01.06.1994 (4)

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

Result : other
Test substance : as prescribed by 1.1-1.4
Remark : Explosivity limits: 2 to 9 %v/v
Source : ELF ATOCHEM S.A., France
Bayer AG Leverkusen
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (2) valid with restrictions. Information came from IUCLID document created by NICNAS, creation date 23-AUG-2001

10.05.1994 (16)

Test substance : as prescribed by 1.1-1.4
Remark : explosive limits: upper: 12 % by vol.
lower: 2.2 % by vol.
Source : Bayer AG Leverkusen
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (2) valid with restrictions. Information came from IUCLID document created by NICNAS, creation date 23-AUG-2001

01.06.1994 (4)

2.11 OXIDIZING PROPERTIES

2.12 ADDITIONAL REMARKS

Test substance : as prescribed by 1.1-1.4
Remark : Thermal decomposition products: toxic chlorinated substances such as hydrogen chloride, phosgene
Source : ELF ATOCHEM S.A., France
Bayer AG Leverkusen

Reliability : EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
(2) valid with restrictions. Information came from IUCLID document created by NICNAS, creation date 23-AUG-2001

10.05.1994 (16)

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	Sun light
Light spect.	:	nm
Rel. intensity	:	based on Intensity of Sunlight
Direct photolysis		
Halflife t1/2	:	ca. 320 hour(s)
Degradation	:	% after
Quantum yield	:	
Indirect photolysis		
Sensitizer	:	OH
Conc. of sens.	:	1500000 molecule/cm3
Rate constant	:	ca. .0000000000004 cm3/(molecule*sec)
Degradation	:	% after
Deg. Product	:	
Method	:	other (calculated)
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The OH radical rate constant was estimated by EPIWIN [AOP Program (v1.90)] based on estimated reaction rate constants for hydroxyl radical abstractions at individual bonds in the molecule. These individual contributions were summed to obtain an overall rate constant. The half life was calculated assuming a constant concentration of OH radical, and assuming pseudo first order kinetics.
Reliability	:	(2) valid with restrictions. Data were obtained by modeling
Flag	:	Key study for endpoint

01.12.2001 (18)

Type	:	air
Light source	:	
Light spect.	:	nm
Rel. intensity	:	based on Intensity of Sunlight
Indirect photolysis		
Sensitizer	:	OH
Conc. of sens.	:	500000 molecule/cm3
Rate constant	:	.0000000000003 cm3/(molecule*sec)
Degradation	:	50 % after 53 day
Deg. Product	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1-1.3
Remark	:	Method: not specified
Source	:	Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(4) not assignable. Information came from IUCLID document created by NICNAS, creation date 23-AUG-2001

10.05.1994 (42)

Type	:	air
Light source	:	
Light spect.	:	nm

Rel. intensity	:	based on Intensity of Sunlight
Indirect photolysis		
Sensitizer	:	OH
Conc. of sens.	:	500000 molecule/cm3
Rate constant	:	cm3/(molecule*sec)
Degradation	:	% after
Deg. Product	:	
Method	:	other (calculated)
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1-1.3
Remark	:	Rate of Constant: 4.2 +/- 0.2 E-13 cm3/(molecule .sec) Degradation: 50 % after 38 +/- 2 day at 292 degree C
Source	:	Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Information came from IUCLID document created by NICNAS, creation date 23-AUG-2001

10.05.1994 (45)(46)

Type	:	water
Light source	:	
Light spect.	:	nm
Rel. intensity	:	based on Intensity of Sunlight
Indirect photolysis		
Sensitizer	:	OH
Conc. of sens.	:	
Rate constant	:	cm3/(molecule*sec)
Degradation	:	% after
Deg. Product	:	
Method	:	other (measured): Photochemical degradation in presence of H2O2 on indication with light of wave length > 290 nm
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1-1.4
Remark	:	Concentration of sensitizer: e-16 to e17 mol/l Rate of constant: 3.0e9 l/molxsec Degradation: 50 % after 642 - 6418 hour of sunshine According to the author degradation proceeds via intermediates (chlorobenzene, chlorophenol) as far as mineralization to CO2 and HCl
Source	:	Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Information came from IUCLID document created by NICNAS, creation date 23-AUG-2001

10.05.1994 (31) (32)

Type	:	water
Light source	:	
Light spect.	:	nm
Rel. intensity	:	based on Intensity of Sunlight
Indirect photolysis		
Sensitizer	:	OH
Conc. of sens.	:	
Rate constant	:	cm3/(molecule*sec)
Degradation	:	% after
Deg. Product	:	
Method	:	other (measured): water was sampled at a depth of 8 cm beneath the surface of the river Goldbach 440 m above sea level under cloudless sky

Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1-1.4
Remark	:	Conc. of sensitizer: 0.00000000000000001 mol/l Rate of Constant: no data Degradation: 50 % after 12.8 days (10 h sunshine/d)
Source	:	Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Information came from IUCLID document created by NICNAS, creation date 23-AUG-2001

11.05.1994 (12)

3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH3	:	35.5 days
t1/2 pH7	:	35.4 days
t1/2 pH11	:	45.4 days
Deg. Product	:	
Method	:	TSCA Guideline No. 796.3500
Year	:	1989
GLP	:	No data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Temperature was not listed, but is assumed to be room temperature.
Result	:	The rate constants at pH 3, 7, and 11 were 0.0195, 0.0196 and 0.0153 $days^{-1}$. The results indicate that the rate of hydrolysis does not change with acid or neutral pH, but the rate is decreased at high pH
Reliability	:	(4) not assignable. Methodological data were not available.
Flag	:	Key study for endpoint

29.11.2001 (19)

Type	:	abiotic
t1/2 pH4	:	at degree C
t1/2 pH7	:	at degree C
t1/2 pH9	:	at degree C
Deg. Product	:	
Method	:	other (calculated)
Year	:	2001
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	An attempt was made to determine the hydrolysis rate constant for o-dichlorobenzene using EPIWIN HYDROWIN Program (v1.67).
Result	:	The EPIWIN Program cannot estimate a hydrolysis rate constant for halobenzenes. It is a general long-term organic chemistry observation, however, that chlorobenzenes are highly resistant to hydrolysis in water. Hydrolysis is therefore an unimportant degradative pathway for o-dichlorobenzene.
Reliability	:	(4) not assignable

29.11.2001 (18)

Type	:	abiotic
t1/2 pH4	:	at degree C

t1/2 pH7	:	at degree C
t1/2 pH9	:	at degree C
Deg. Product	:	
Method	:	other: Hydrolysis
Year	:	
GLP	:	
Test substance	:	as prescribed by 1.1-1.4
Result	:	o-dichlorobenzene is stable in aqueous solution (15 mg/l) for 2 months at 4 degrees C. Hydrolysis of o-dichlorobenzene under conditions obtaining in the environment is improbable.
Source	:	Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Information came from IUCLID document created by NICNAS, creation date 23-AUG-2001

29.11.2001 (12) (28)

3.1.3 STABILITY IN SOIL

3.2 MONITORING DATA

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	:	fugacity model level III
Media	:	water - air
Air (level III)	:	12.4
Water (level III)	:	19
Soil (level I)	:	
Biota (level II / III)	:	.72
Soil (level II / III)	:	67.9
Method	:	other
Test Condition	:	Measured inputs to the EPIWIN model were melting point (-17°C), boiling point (180.5°C), vapor pressure (1 mm Hg), water solubility (140 mg/l) and Log Kow (3.39).
Year	:	2001
Test substance	:	as prescribed by 1.1-1.4
Remark	:	The fugacity model used a Henry's Law Constant of 0.00192 atm-m3/mole, a vapor pressure of 0.975 mm Hg, a log Kow of 3.43, and a soil Koc of 1.1e+3. The Henry's Law Constant was calculated using EPIWIN Henry Program (v3.10). The Log Kow was estimated using EPIWIN KOWWIN Program (v1.66). A Koc (soil/sediment coefficient of 443.1 was estimated using EPIWIN PCKOC Program (v1.66). A BCF (Bioconcentration Factor) was estimated to be 87.23 (log BCF 1.941) using EPIWIN BCF Program (v2.14). Level III fugacity calculations allow non-equilibrium conditions to exist between connected media at steady state.
Reliability	:	(2) valid with restrictions. Data were obtained by modeling.
Flag	:	Key study for endpoint

29.11.2001 (18)

Type	:	fugacity model level I
Media	:	water – air
Air (level I)	:	94.0
Water (level I)	:	
Soil (level I)	:	4.0

Biota (level II / III) :
Soil (level II / III) :
Method : Other
Year : 2001
Test substance : as prescribed by 1.1-1.4
Remark : Level I EQC modeling by Mackay assumes equilibrium, steady state conditions.
Reliability : (2) valid with restrictions. Data were obtained by modeling.
Flag : Key study for endpoint

(30)

Type : Adsorption
Media : other
Air (level I) :
Water (level I) :
Soil (level I) :
Biota (level II / III) :
Soil (level II / III) :
Method : other: adsorption to soil
Year :
Test substance : as prescribed by 1.1-1.4
Result : Experimental determination of soil/water coefficients in various soils and coefficients of soil sorption coefficient (KOC = carbon organic content) showed value ranging from 0.02 to 250 and 286 to 4654 respectively
Source : Bayer AG Leverkusen
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (2) valid with restrictions. Information came from IUCLID document created by NICNAS, creation date 23-AUG-2001

11.05.1994 (12)

Type : adsorption
Media : other
Air (level I) :
Water (level I) :
Soil (level I) :
Biota (level II / III) :
Soil (level II / III) :
Method : other: adsorption to soil
Year :
Test substance : as prescribed by 1.1-1.4
Result : The soil sorption coefficient KOC expressed in terms of the content of organic carbon was 383 for a surface soil after 24 h exposure at 22.5 +/- 1 degree C with an unadjusted pH of 6.3. The soil sorption coefficient was significantly reduced under basic conditions.
Source : Bayer AG Leverkusen
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (2) valid with restrictions. Information came from IUCLID document created by NICNAS, creation date 23-AUG-2001

17.05.1994 (12) (44)

Type : volatility
Media : water - air
Air (level I) :
Water (level I) :
Soil (level I) :
Biota (level II / III) :

Soil (level II / III) :
Method : other: Henry's constant
Year :
Test substance : as prescribed by 1.1-1.4
Result : experimental values:
H = 165 Pa m3/mol at 10 degree C
H = 145 Pa m3/mol at 15 degree C
H = 170 Pa m3/mol at 20 degree C
H = 159 Pa m3/mol at 25 degree C
H = 240 Pa m3/mol at 30 degree C
Source : Bayer AG Leverkusen
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (2) valid with restrictions. Some study detail is lacking.
10.05.1994 (1)(12)

Type : volatility
Media : water - air
Air (level I) :
Water (level I) :
Soil (level I) :
Biota (level II / III) :
Soil (level II / III) :
Method : other
Year :
Test substance : as prescribed by 1.1-1.4
Result : 19.82 and 85 % evaporation from aqueous solution after 2, 4 and 8 d at room temperature without aeration
Source : Bayer AG Leverkusen
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable. Information came from IUCLID document created by NICNAS, creation date 23-AUG-2001
10.05.1994 (14)

Type : volatility
Media : water - air
Air (level I) :
Water (level I) :
Soil (level I) :
Biota (level II / III) :
Soil (level II / III) :
Method : other
Year :
Test substance : as prescribed by 1.1-1.4
Result : In a closed system under addition of 2 % methanol to aqueous phase, 25 % of o-dichlorobenzene had evaporated within 25 min. and 90 % within 3.5 h without aeration
Source : Bayer AG Leverkusen
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable. Information came from IUCLID document created by NICNAS, creation date 23-AUG-2001
10.05.1994 (25)

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Remark : Static culture 5-10 mg/l biodegraded 20-45%, 59-66%, 32-4% AND 18-29%. ODCB reduced from 50 mg/l to 2-4 mg/l in 7 days of incubation using industrial/municipal sludge.

ODCD was found to be degradation resistant using the Japanese MITI test.

Source : DALTRADE LTD LONDON
Reliability : (4) not assignable. Information came from IUCLID document created by NICNAS, creation date 23-AUG-2001. No reference was listed.
Flag : Supportive study for endpoint
06.04.1998

Type : aerobic
Inoculum : predominantly domestic sewage, adapted
Concentration : 4 mg/l related to Test substance
related to
Contact time :
Degradation : 58 % after 20 day
Result :
Deg. Product :
Method : OECD Guide-line 301 D "Ready Biodegradability: Closed Bottle Test"
Year : 1977
GLP : no
Test substance : as prescribed by 1.1-1.4
Source : Bayer AG Leverkusen
Reliability : (2) valid with restrictions. Information came from EUROPEAN COMMISSION IUCLID data set.
Flag : Supportive study for endpoint
11.05.1994 (12)

Type : aerobic
Inoculum : activated sludge
Concentration : 100mg/l related to
related to
Contact time :
Degradation : 0 % after 28 day
Result : under test conditions no biodegradation observed
Deg. Product :
Method : other: see remarks
Year :
GLP :
Test substance : as prescribed by 1.1-1.4
Remark : "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). Sludge conc. : 30 mg/l
Source : Bayer AG Leverkusen
Reliability : (2) valid with restrictions. Information came from IUCLID document created by European Commission.
Flag : Supportive study for endpoint

01.02.1994 (6)

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	flow through
Species	:	Pimephales promelas (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	yes
LC50	:	m = 9.47
EC50	:	m = 4.38
Method	:	other: APHA (1980) Standard Methods for the examination of water and wastewater
Year	:	1986
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	The average (+/- SD) temperature, dissolved oxygen, hardness, alkalinity and pH of the water in the test chambers were 26.2 +/- 0.51 degrees C, 7.1 +/- 0.22 mg/l, 46.8 +/- 1.32 mg/l CaCO3, 43.9 +/- 1.99 mg/l CaCO3, and 7.80 +/- 0.04. It is not known whether these variables were affected by test material concentration. Average and ranges of analytical concentrations of chambers treated with 0, 6.34, 9.75, 15.0, 23.0 and 35.4 mg/l were <0.01, 2.10 (1.71 – 2.40), 3.34 (2.99 - 3.63), 5.89 (5.16 - 6.92), 7.81 (6.33 – 9.43) and 18.1 (15.5 – 19.4) mg/l. In most cases, concentrations of test material were slightly lower at time 0 than at other time points. When corrected for recovery (100.5 %), test material concentrations were 2.09, 3.32, 5.86, 7.77 and 18.0 mg/l. The mean length and weight (+/- SD) of the fish at study termination were 17.3 +/- 3.076 mm and 0.079 +/- 0.0370 g. All fish exposed to 18.0 mg/l died within 24 hours of treatment. Five fish exposed to 7.77 mg/l died by 96 hours, four exposed to 5.86 mg/l died by 96 hours, and one exposed to 2.09 mg/l died by 24 hours. The 96 hour LC50 value (with confidence limits) was 9.47 (8.23 – 10.9) mg/l. All fish exposed to 5.86, 7.77 and 18.0 mg/l , and one fish exposed to 2.09 mg/l exhibited signs of toxicity (including death). The 96 hour EC50 value was 4.38 mg/l (confidence limits were not relevant).
Test condition	:	A stock solution of test material (84 mg/l) was prepared in 18.9 l of test water. When the pH of the stock was outside of the 7 to 8 range, it was adjusted to pH 7.8 with either NaOH or concentrated HCl. Newly hatched minnows from adults reared in flow-through tanks were held at 25 degrees C in flowing water with a 16-hr photoperiod and were fed 40 to 48-hour brine shrimp nauplii two times/day (except once/day on weekends). They were cultured in filtered Lake Superior water or dechlorinated water from the city of Superior, WI (exact source not given) The two waters were similar in all measured chemical parameters. This water was used for test material dilution and all tests. Healthy fish (32 days old) were fasted for 24 hours before treatment. They were pooled together in one tank and randomly distributed among the exposure chambers. Tests were initiated by adding 20 fish per treatment (6.34, 9.75, 15.0, 23.0, and 35.4 mg/l) and control to test chambers

containing 1 liter of water. Fish loading was 1.58 g/l. The modified mini-diluter had a 0.65 dilution factor, a flow rate from the toxicant dilution cell of 29 ml/min, and a flow to each test chamber of 10 ml/min.

Observations of fish behavior and toxic signs were made at 2-8, 24, 48, 72 and 96 hr and recorded on checklists specifically formatted to convert observational data for approximately 100 endpoints into a numerically coded form. Unique behavior was recorded using a color video camera and 0.5 inch video tape recorder. Death was recorded at 24, 48, 72 and 96 hours. Dead fish were removed.

All test exposure chambers were sampled mid-depth at 0, 24, 48, 72 and 96 hours. Concentrations of test material from the exposure tanks were analyzed using gas-liquid chromatography. All analyses included one spike and one duplicate sample for every 6 to 12 water samples.

Five water quality parameters were routinely measured for each test: temperature, dissolved oxygen, total hardness, total alkalinity, and pH. The desired test temperature was 25 +/- 1 degrees C. Daily measurements of oxygen concentration and pH were taken in each treatment and the control exposure chambers if fish were present. The low, mid and high test concentration chambers were sampled once for total hardness and alkalinity.

At study termination, individual control fish were weighed (wet) and measured. Four surviving fish from the control, lowest dose group, and the dose group closest to the LC50 were preserved in 10% buffered formalin and kept for histopathological examination.

The estimated LC50 and EC50 values, with corresponding 95% confidence intervals were calculated using the corrected average of the analyzed tank concentrations and the Trimmed Spearman-Karber Method. The EC50 values were based on loss of equilibrium manifested by an inability of the fish to remain in an upright position when swimming. The mean concentrations used in the calculations were corrected for analytical recoveries of spiked water samples.

Test substance : Purity of test material was 98%.
Reliability : (1) valid without restriction
Flag : Key study for endpoint
29.11.2001 (23)

Type : static
Species : Salmo gairdneri (Fish, estuary, fresh water)
Exposure period : 24 hour(s)
Unit : mg/l
Analytical monitoring : yes
LC50 : m = 2.3
Method : other
Year : 1983
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Remark : The toxicity of monochlorobenzene, 1,4 -dichlorobenzene and 1,2,3- and 1,2,4- trichlorobenzenes also were tested in this study. The 24-hour LC50 values for these materials were 4.1, 1.18, 0.71, and 1.95 mg/l, respectively.

It is assumed that the conditions under which the test material was handled

were similar to the algal study reported below, since the study was performed in the same laboratory at a later date. The results are reported as 48 hour LC50 values in the study; however, the methods state that the experiment was only carried out for 24 hours. Therefore, the values are reported in this summary as 24 hour.

Result : The 24 hour EC50 value for immobilization (with confidence limits) was 2.3 (2.2 - 2.4) mg/l. The slope of the effect concentration curve was 1.12.

The concentrations used to calculate the LC50 value were nominal (not listed), as analytical concentrations were within 10% of nominal concentrations.

Test condition : An ISRA test (Quaderni dell'Instituto di Ricerca sulle Acgue, 11, Consiglio Nazionale delle Ricerche-Roma, 1973). was performed to establish the 24 hr LC50 value. Two closed bottles (10 l) containing 5 fish were used for each concentration (not stated). The test vessels were modified according to the method of Galassi and Vighi (Chemosphere 10: 1123, 1981) in order to maintain constant concentration and to allow for sampling of the medium without opening the vessel.

Water conditions during the experiment were: hardness 320 mg CaCO3/l, pH 7.4, oxygen not less than 70% saturation at the end of the test, and a temperature of 15 degrees.

Concentrations of test material in vessels were measured analytically (by gas chromatography) at the beginning and end of the tests. Dilute solutions were extracted with n-hexane before analysis. The limit of detection was 0.002 mg/l.

Mortality data were analyzed according to the method of Litchfield and Wilcoxon.

Test substance : Test material was analytical grade

Reliability : (2) valid with restrictions. Data conflict as to whether the value obtained is for 24 or 48 hours.

29.11.2001 (13)

Type : static
Species : Brachydanio rerio (Fish, fresh water)
Exposure period : 24 hour(s)
Unit : mg/l
Analytical monitoring : yes
LC50 : m = 6.8
Method : other
Year : 1983
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Remark : The toxicity of monochlorobenzene, 1,4 –dichlorobenzene, and 1,2,3- and 1,2,4- trichlorobenzenes also was tested in this study. The 24-hour LC50 values for these materials were 10.5, 4.25, 3.1 and 6.3 mg/l, respectively.

It is assumed that the conditions under which the test material was handled were similar to the algal study reported below, since the study was performed in the same laboratory at a later date.
The results are reported as 48 hour LC50 values in the study; however, the methods state that the experiment was only carried out for 24 hours. Therefore, the values are reported in this summary as 24 hour.

Result : The 24 hour LC50 value (with confidence limits) was 6.8 (5.2 - 8.9) mg/l. The slope of the effect concentration curve was 1.36.

The concentrations used to calculate the LC50 value were nominal (not listed), as analytical concentrations were within 10% of nominal concentrations.

Test condition : An ISRA test (Quaderni dell'Instituto di Ricerca sulle Acgue, 11, Consiglio Nazionale delle Ricerche-Roma, 1973). was performed to establish the 24 hr LC50 value. Two closed bottles (10 l) containing 5 fish were used for each concentration (not stated). The test vessels were modified according to the method of Galassi and Vighi (Chemosphere 10: 1123, 1981) in order to maintain constant concentration and to allow for sampling of the medium without opening the vessel.

Water conditions during the experiment were: hardness 320 mg CaCO3/l, pH 7.4, oxygen not less than 70% saturation at the end of the test, and a temperature of 23 degrees.

Concentrations of test material in vessels were measured analytically (by gas chromatography) at the beginning and end of the tests. Dilute solutions were extracted with n-hexane before analysis. The limit of detection was 0.002 mg/l.

Mortality data were analyzed according to the method of Litchfield and Wilcoxon.

Test substance : Test material was analytical grade

Reliability : (2) valid with restrictions. Data conflict as to whether the value obtained is for 24 or 48 hours.

29.11.2001 (13)

Type :
Species : other: freshwater fish
Exposure period : 96 hour(s)
Unit : mg/l
Analytical monitoring : no
LC50 : c = 5.379
Method : other: ECOSAR program (v 0.99g)
Year : 2002
GLP : no
Test substance : as prescribed by 1.1 - 1.4
Test condition : The LC50 value was estimated by the ECOSAR program (v0.99g), with a log Kow of 3.39 and water solubility of 140 mg/l entered by the user.
Reliability : (2) valid with restrictions. Data were obtained by modeling.
Flag : Supportive study for endpoint

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 24 hour(s)
Unit : mg/l
Analytical monitoring : yes
EC50 : m = .78
Method : other
Year : 1983
GLP : no data

Test substance : as prescribed by 1.1 - 1.4
Remark : The toxicity of monochlorobenzene, 1,4 -dichlorobenzenes (para), and 1,2,3- and 1,2,4- trichlorobenzenes also was tested in this study. The 24-hour EC50 values for these materials were 4.3, 1.6, 0.35, and 1.2 mg/l, respectively.

It is assumed that the conditions under which the test material was tested were similar to the algal study reported below, since the study was performed in the same laboratory at a later date
Result : The 24 hour EC50 value for immobilization was 0.78 mg/l. Confidence limits were not determined. The slope of the effect concentration curve was 1.33.

The concentrations used to calculate the EC50 were nominal (not listed), as analytical concentrations were within 10% of nominal concentrations.
Test condition : An AFNOR test (Norme Experimentale NFT, 90-301, 1974) was performed to determine the IC50 (concentration for 50% of animals to be immobilized) at 24 hours. The test vessels were modified according to the method of Galassi and Vighi (Chemosphere 10: 1123, 1981) in order to maintain constant concentration and to allow for sampling of the medium without opening the vessel.

Concentrations of test material in vessels were measured analytically (by gas chromatography) at the beginning and end of the tests. Dilute solutions were extracted with n-hexane before analysis. The limit of detection was 0.002 mg/l.

The EC50 value was taken from curves fitted by eye on log probability paper and not elaborated, being very close to the concentrations with 0 and 100% immobilized animals.
Reliability : (2) valid with restrictions. Purity of test material was not stated.
29.11.2001 (13)

Type :
Species : Daphnia
Exposure period : 48 hour(s)
Unit : mg/l
Analytical monitoring : no
LC50 : c = 6.345
Method : other: ECOSAR program (v 0.99g)
Year : 2002
GLP : no
Test substance : as prescribed by 1.1 - 1.4
Test condition : The LC50 value was estimated by the ECOSAR program (v0.99g), with a log Kow of 3.39 and water solubility of 140 mg/l entered by the user.
Reliability : (2) valid with restrictions. Data were obtained by modeling.
Flag : Supportive study for endpoint

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Selenastrum capricornutum (Algae)
Endpoint : growth rate
Exposure period : 96 hour(s)
Unit : mg/l

Analytical monitoring : yes
EC50 : m = 2.2
Method : other: modification of AAPBT
Year : 1981
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Remark : Culture medium and test conditions were similar to the AAPBT, with the exception that the temperature was 20 +/- 1 degrees C.

Chlorobenzene, para dichlorobenzene and 1,2,3- and 1,2,4-trichlorobenzene also were tested in this study. The 96-hour EC50 values for these materials were 12.5, 1.6, 0.9 and 1.4 mg/l, respectively. It was remarked that the EC50 values calculated for chlorobenzenes by this method are at least 2 fold lower than other methods that did not analytically control test material concentrations.

Result : Initial concentrations could not be measured due to high volatility of the test material. Within a few minutes of adding the test material to the flasks the concentration was very low compared to theoretical values. Therefore, the initial concentrations calculated from the dilution of the titrated stock solutions were assumed to be the initial concentrations. Equilibrium concentrations were calculated as the mean of the analytical concentrations in samples taken after the equilibrium period and at 48 and 96 hours. For initial concentrations of 4.75, 7.6, 14.25, 19.0, 33.25, 47.5 and 66.5 mg/l, equilibrium concentrations of 0.88, 2.75, 4.99, 6.38, 10.66, 17.55, and 21.19 were determined. The mean initial concentration/ equilibrium concentration (Ci/Ceq +SD) was 2.93 +/- 0.18. Henry's constant (H) can be calculated from the equation Ci = Ceq(H x air volume in the flask/culture medium volume + 1). The value calculated from this equation (0.09) was fairly close to the reported value (0.08), confirming the validity of the method for prediction of concentrations at equilibrium.

After the 24 hour equilibration period, the concentration of test material in the culture medium remained almost constant. Differences in the values obtained at equilibrium and after 48 or 96 hours were within the range of acceptable analytical variability.

The 96 hour EC50 value determined for 1,2 dichlorobenzene inhibition of algal fluorescence was 2.2 mg/l. The maximum tested concentration that produced no effect was < 0.9 mg/l and the minimum concentration that was 100% effective was 17.5 mg/l.

Test condition : A stock solution of test material was made by adding 1,2 dichlorobenzene at 10 times higher than the saturation solubility to distilled water in a closed vessel. The solution was stirred for 48 hours and decanted for 24 hours. The supernatant was filtered through paper filters and the concentration was measured. Final solutions were made by adding 10 ml of stock culture medium to different amounts of stock solution. Solutions were then diluted to 100 ml with distilled water and quickly transferred into the 2 liter spherical culture flasks. The medium to flask volume ratio (0.047) was low enough to avoid notable carbon dioxide deficiency. Flasks were closed by screw caps with both silicone rubber (4 mm thick) and teflon gaskets. The caps were pierced by a stainless steel needle dipped into the culture medium. Sampling for measurement of algal growth and toxicant concentrations was made through the needle by means of a syringe. The outer end of the needle was closed with Parafilm.

Capped flasks were shaken for 24 hours at 20 degrees C to let vapor and liquid phases equilibrate. The algal inoculum was then added at cell

concentration of 5 x 10E6 cells/l. Culture medium and test conditions were similar to the AAPBT, with the exception that the temperature was 20 +/- 1 degrees C.

Concentrations of test material in the flasks were measured by GC after the 24 hour equilibration period and 48 and 96 hours after the inoculum was added. Aqueous solutions (4 microliters) were injected directly into the GC with a flame ionization detector.

Algal growth was measured at 24, 48, and 96 hours by in vivo fluorescence (CJ Lorenzen, Deep Sea Res. 13:223-227, 1966). Results were expressed as a percentage of the growth in the control culture and the EC50 was interpolated from the data.

Reliability : (2) valid with restrictions. Purity of test material was not stated. The control response in terms of the increase in cell concentration, and the pH, dissolved oxygen concentration and temperatures were not provided.

19.11.2001 (22)

Type	:	
Species	:	other: green algae
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	no
EC50	:	c = 4.297
Method	:	other: ECOSAR program (v 0.99g)
Year	:	2002
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Test condition	:	The EC50 value was estimated by the ECOSAR program (v0.99g), with a log Kow of 3.39 and water solubility of 140 mg/l entered by the user.
Reliability	:	(2) valid with restrictions. Data were obtained by modeling.
Flag	:	Supportive study for endpoint

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6.1 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO OTHER NON-MAMM. TERRESTRIAL SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Species	:	guinea pig
Strain	:	other: albino
Sex	:	male/female
Number of animals	:	20
Vehicle	:	other: olive oil (by intubation as a 50% solution)
Value	:	> 800 mg/kg and < 2000 mg/kg bw
Method	:	other
Year	:	1958
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	All animals dosed with 800 mg/kg lived and all dosed with 2000 mg/kg died.
Test condition	:	Animals (10 of mixed sex/group) were dosed with a 50% solution of test material in olive oil and observed over 14 days.
Reliability	:	(2) valid with restrictions. The LD50 value was not determined. Details about the study design were not provided.
Test substance	:	The material used was redistilled 1,2-dichlorobenzene with a boiling point range of 179.5 -181.1 degrees C at 760 mm Hg . Based on this fact, the authors stated that the purity was > = 99%.

19.11.2001 (26)

Type	:	LD50
Species	:	rat or mouse
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Reliable studies in rats or mice were not located. Data from category members and surrogates (see below) are predictive of 1,2-dichlorobenzene acute oral toxicity in these species.
Reliability	:	(2) valid with restrictions

Type	:	LD50
Species	:	rat
Strain	:	Sherman
Sex	:	male/female
Number of animals	:	40
Vehicle	:	peanut oil
Value	:	3863 mg/kg (males), 3790 mg/kg (females)
Method	:	other
Year	:	1986
GLP	:	no data
Test substance	:	other TS
Result	:	The LD50 values (with 95% confidence limits) were 3863 (3561-4153) mg/kg in males and 3790 (3425-4277) mg/kg in females. The slope of the curve was 14.5 and 8.4 for males and females, respectively.
Test condition	:	Test material was dissolved in peanut oil. Test material was administered in a volume of 5 ml/kg body weight. Rats were 90 days old when tested. A minimum of 10 animals/group (not stated if evenly divided among sex) and 4 doses were tested (up to 16.7 ml/kg). Animals were observed for at least 14 days or until all survivors had recovered from signs of toxicity. LD50 values and related parameters were calculated using a computer-based implementation of Finney's maximum likelihood probit technique.
Test material	:	Test material was para dichlorobenzene (CAS No. 106-46-7)

Reliability : (2) valid with restrictions. Purity of test material was not given.
19.11.2001 (21)

Type : LD50
Species : rat
Strain : Sprague-Dawley
Sex : male/female
Number of animals : 20
Vehicle :
Value : = 1540 mg/kg bw
Method : other
Year : 1976
GLP : no
Test substance : other TS
Result : There were no deaths at 1260 mg/kg, 3/5 deaths (2 males, 1 female) at 1580 mg/kg, 3/5 deaths at 2000 mg/kg (1 male, 2 females) and 5/5 deaths with 2510 mg/kg. All deaths occurred within 3 days. The LD50 value was 1540 mg/kg and the 95% confidence interval was 1380 - 1710 mg/kg.
Test condition : Rats (220 - 250 g) were dosed orally (2-3 males and females/group) with 1260, 1580, 2000, and 2510 mg/kg and were observed over 14 days.
Test substance : Test material was chlorobenzene (CAS No.108-90-7).
Reliability : (2) valid with restrictions. Purity of test material is unknown. Test material is a related substance.
Flag :
27.11.2001 (7)

Type : LD50
Species : rat
Strain : Sprague-Dawley
Sex : male/female
Number of animals : 50
Vehicle :
Value : = 1100 mg/kg bw
Method : other
Year : 1980
GLP : no data
Test substance : other TS
Result : Initial average weights of males in the 631, 794, 1000, 1260 and 1580 mg/kg groups were 200, 195, 215, 205 and 205 g (respectively). Weights of females in these respective groups were 190, 190, 205, 195 and 205 g. All animals dosed with 1580 mg/kg died within one day. Two animals of each sex died within 1 to 2 days of being dosed with 1260 mg/kg. All males and one female dosed with 1000 mg/kg died within 2 to 4 days. Three females and no males dosed with 794 mg/kg died. One male and no females dosed with 631 mg/kg died within 4 days of treatment. Average weights of survivors in each dosage group were similar (with the exception of slightly lower body weights of males treated with 1260 mg/kg).

Signs of toxicity included lethargy, increasing weakness, ocular discharge, and collapse. Viscera of survivors appeared normal. Hemorrhagic lungs, liver discoloration, discoloration of kidneys and spleen (in some instances) and acute GI inflammation were observed in animals that died before study termination.

The LD50 values (with 95 % confidence limits in females and males) were

		1000 (740 - 1350) and 1200 (840-1720) mg/kg, respectively. The LD50 value for both sexes together (with 95 % confidence limits) was 1,100 mg/kg (900 - 1340). The slopes of the curves for females, males and males/females were 3.7, 3.7 and 3.8, respectively.
Test condition	:	Groups of 5 rats/sex (fasted) were dosed orally with 631, 794, 1000, 1260 and 1580 mg/kg test material. Animals were observed for death and toxic symptoms for 14 days. Survivors were weighed on days 7 and 14 and terminated on day 14.
Test substance	:	Test material is meta dichlorobenzene (CAS No. 541-73-1).
Reliability	:	(2) valid with restrictions. Purity of test material was not noted. Test material is a related substance
Flag	:	

18.11.2001 (8)

Type	:	LD50
Species	:	rat
Strain	:	CFE
Sex	:	male/female
Number of animals	:	
Vehicle	:	
Value	:	= 756 mg/kg bw
Method	:	other
Year	:	1969
GLP	:	no
Test substance	:	other TS
Result	:	The LD50 value was 756 mg/kg (556-939 mg/kg, 95% confidence limit). All deaths occurred within 5 days of exposure. Animals that died and survivors did not exhibit evidence of gross pathological lesions.
Test condition	:	Rats (150 - 250 g) were fasted overnight and were gavaged with various concentrations of test material (4/sex/group, doses not stated). Water and feed were freely available after dosing. Animals were observed for 10 days At the end of that time all survivors were weighed, examined and necropsied. Animals that died were also necropsied. The LD50 value and 95% confidence interval was calculated (method unknown).
Test substance	:	Test substance was 1,2,4-trichlorobenzene (CAS No. 120-82-1).
Reliability	:	(4) not assignable. The purity of test material is unknown. The doses tested, the number of deaths at each dose and the method used to calculate the LD50 value were not listed. A related test material was utilized.

27.11.2001 (11)

5.1.2 ACUTE INHALATION TOXICITY

Type	:	LC50
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male
Number of animals	:	
Vehicle	:	
Exposure time	:	6 hour(s)
Value	:	= 1532 ppm
Method	:	other
Year	:	1979
GLP	:	no

Result	:	The LC50 value with confidence limits was 1532 (1384 - 1730) ppm. The regression equation was y = 6.5 x + 15.8. The LC50 in mg/l is 9.38.
Test condition	:	Rats (130 -160 g) were exposed to concentrations ranging from 1000 to 2000 ppm over 6 hours (12 animals/dose group). Vapor was generated at 24 degrees C, 50 % relative humidity. Rats were observed for 14 days. A line of regression was plotted with the abscissa being the log of the concentration of test material and the ordinate being the probit of the percentage of corresponding deaths. The LC50 value was the concentration (x value) at which the y value was 50%.
Test substance	:	The test material was 99% pure
Reliability	:	(1) valid without restriction.
Flag	:	Key study for acute toxicity endpoint

19.11.2001 (9)

Type	:	LC50
Species	:	mouse
Strain	:	
Sex	:	female
Number of animals	:	
Vehicle	:	
Exposure time	:	6 hour(s)
Value	:	= 1236 ppm
Method	:	other
Year	:	1979
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4.
Result	:	The LC50 value with confidence limits was 1236 (1201 - 1279) ppm. The regression equation was y = 13.303x + 42870. The LC50 value in mg/l is 7.43.
Test condition	:	Female mice (21 g) were exposed in 200 l chambers to various concentrations of test material for 6 hours (25 animals/group). Vapor was generated at 24 degrees C, 50 % relative humidity and an air flow of 40 m3/hr. There were 60 air changes/hour. Animals were observed for 14 days. A line of regression was plotted with the abscissa being the log of the concentration of test material and the ordinate being the probit of the percentage of corresponding deaths. The LC50 value was the concentration (x value) at which the y value was 50%.
Test stubstance	:	The test material was 99% pure.
Reliability	:	(1) valid without restriction.
Flag	:	Key study for acute toxicity endpoint

19.11.2001 (9) (10)

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD50
Species	:	rat or rabbit
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Reliable studies in rats or rabbits were not located. Data from category members and surrogates (see below) are predictive of 1,2-dichlorobenzene acute dermal toxicity in these species.
Reliability	:	(2) valid with restrictions

Type	:	LD50
Species	:	rat
Strain	:	Sherman
Sex	:	male/female

Number of animals : 40
Vehicle : xylene
Value : > 6000 mg/kg in both sexes
Method : other
Year : 1986
GLP : no data
Test substance : other TS
Result : The LD50 values in males and females were greater than the highest dose given (6000 mg/kg).
Test condition : Test material was dissolved in xylene. Test material was administered to the shaved shoulder and back area in a volume of 1.6 ml/kg body weight. Rats were 90 days old when tested. A minimum of 10 animals/group (not stated if evenly divided among sex) and 4 doses were tested (up to 6 g/kg). Animals were observed for at least 14 days or until all survivors had recovered from signs of toxicity. LD50 values and related parameters were calculated using a computer-based implementation of Finney's maximum likelihood probit technique.
Test material : Test material was para dichlorobenzene (CAS No. 106-46-7)
Reliability : (2) valid with restrictions. Purity of test material was not given.
19.11.2001 (21)

Type : LD50
Species : rat
Strain : CFE
Sex : male/female
Number of animals :
Vehicle :
Value : = 6139 mg/kg bw
Method : other
Year : 1969
GLP : no
Test substance : other TS
Result : The LD50 value was 6139 mg/kg (4299-9056 mg/kg, 95% confidence limit). All deaths occurred within 5 days of exposure. Animals that died and survivors did not exhibit evidence of gross pathological lesions.
Test condition : Undiluted test material was placed on the shorn dorso-lumbar skin of rats (4/sex/group; doses and weights not stated). The area was bandaged with an impermeable dressing of aluminum foil and waterproof plaster. The dressing was kept in place for 24 hours. The treated site was then washed with tepid, dilute detergent solution. Water was freely available during the experiment but food was withdrawn over the 24 hour exposure period. Animals were observed for 10 days At the end of that time all survivors were weighed, examined and necropsied. Animals that died were also necropsied. The LD50 value and 95% confidence interval was calculated (method unknown).
Test substance : Test substance was 1,2,4-trichlorobenzene (CAS No. 120-82-1).
Reliability : (4) not assignable. The purity of test material is unknown. The doses tested, the number of deaths at each dose and the method used to calculate the LD50 value were not listed. A related test material was utilized.
27.11.2001 (11)

Type : LD50
Species : rabbit
Strain : New Zealand white
Sex : male/female

Number of animals	:	4
Vehicle	:	
Value	:	> 2000 mg/kg bw
Method	:	other
Year	:	1980
GLP	:	no data
Test substance	:	1,3-dichlorobenzene (CAS No. 541-73-1)
Remark	:	No signs of toxicity were noted.
Reliability	:	(4) not assignable. There are not enough details in study documentation to assign a reliability rating.

18.11.2001 (8)

Type	:	LD50
Species	:	rabbit
Strain	:	New Zealand white
Sex	:	male/female
Number of animals	:	3
Vehicle	:	
Value	:	> 7940 mg/kg bw
Method	:	other
Year	:	1976
GLP	:	no
Test substance	:	Monochlorobenzene (CAS No. 108-90-7)
Result	:	There were no deaths.
Test condition	:	One female (1.8 kg) was dosed with 5010 mg/kg and 2 males (2.0 and 2.2 kg) were dosed with 7940 mg/kg. Animals were observed over 14 days.
Reliability	:	(4) not assignable. Only 3 animals were tested.

27.11.2001 (7)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

5.2.2 EYE IRRITATION

5.3 SENSITIZATION

5.4 REPEATED DOSE TOXICITY

Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	90 days
Frequency of treatment	:	daily
Post obs. period	:	none
Doses	:	25, 100, 400 mg/kg/day

Control group	:	yes, concurrent vehicle
NOAEL	:	= 25 mg/kg bw
LOAEL	:	= 100 mg/kg bw
Method	:	other
Year	:	1991
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	All doses: There was no effect of treatment on any urinalysis parameter. All animals survived treatment.

400 mg/kg/day: Males treated with 400 mg/kg/day exhibited decreased mean body weight (489 +/- 54 g vs. 561 +/- 59 g in controls), increased absolute (4.15 +/- 0.68 g vs. 3.38 +/- 0.31 g in control) and relative (0.85 +/- 0.09 vs. 0.60 +/- 0.04 in control) kidney weight, increased absolute (17.6 +/- 2.3 g vs. 13.7 +/- 2.1 g in control) and relative (3.61 +/- 0.30 vs. 2.44 +/- 0.19 in control) liver weight, decreased absolute (0.61 +/- 0.11 g vs. 0.85 +/- 0.10 g in control) and relative (0.12 +/- 0.02 vs. 0.15 +/- 0.02 in control) spleen weight, increased relative heart weight (0.31 +/- 0.04 vs. 0.27 +/-0.03 in control), increased relative lung weight (0.37 +/- 0.03 vs. 0.34 +/-0.03 in control), increased relative brain weight (0.44 +/- 0.04 vs. 0.39 +/-0.04 in control), and increased relative testes weight (1.06 +/- 0.13 vs. 0.93 +/-0.11 in control). A significant increase in erythrocytes versus control was noted in high dose males (9.3+/- 0.3 10E6/ml vs. 8.9 +/- 0.4 10E6/ml in controls). High dose males also had increased serum ALT (42 +/- 15 IU/l vs. 26 +/- 4 IU/l in control), increased BUN (15 +/- 4.1 mg/dl vs. 12 +/- 1.7 mg/dl in controls) and increased serum total bilirubin (0.1 +/- 0.07 mg/dl vs. 0.0 +/- 0.05 mg/dl in controls).

There was a significant increase in total food consumption for female rats treated with 400 mg/kg/day during weeks 11 to 13 compared to controls. Females treated with this dose exhibited increased absolute (2.17 +/- 0.15 g vs. 1.84 +/- 0.15 g in control) and relative (0.80 +/- 0.05 vs. 0.67 +/- 0.08 in control) kidney weight, increased absolute (11.30 +/- 0.90 g vs. 7.00 +/- 0.40 g in control) and relative (4.20 +/- 0.39 vs. 2.54 +/- 0.19 in control) liver weight, and decreased absolute spleen weight (0.43 +/- 0.06 g vs. 0.52 +/- 0.09 g in control). High dose females exhibited increased serum T-bilirubin (0.1 +/- 0.05 mg/dl vs. 0.0 +/- 0.05 mg/dL in controls).

Degeneration, hypertrophy and necrosis of liver cells was observed in 10/10, 9/10, and 7/10 high dose males and 8/10, 10/10 and 5/10 high dose females. No lesions were observed in controls. Hepatocellular degeneration generally occurred concurrently with hypertrophy, and together they were characterized by karyomegaly, cytomegaly, karyorrhexis, reduced cytoplasmic basophilia, and dissolution of the cytoplasm. Single cell necrosis, characterized by pyknotic cells surrounded by a few inflammatory cells, occurred exclusively within zones of hepatocellular degeneration. The severity of these lesions was not stated.

100 mg/kg/day: Males treated with 100 mg/kg/day exhibited increased absolute (15.90 +/- 1.60 g vs. 13.7 +/- 2.1 g in control) and relative (2.81 +/- 0.18 vs. 2.44 +/- 0.19 in control) liver weight and increased ALT (53 +/- 36 IU/l vs. 26 +/- 4 IU/l in control). Females treated with 100 mg/kg/day had increased absolute (8.20 +/- 0.70 g vs. 7.00 +/- 0.40 g in

control) and relative (2.86 +/- 0.25 vs. 2.54 +/- 0.19 in control) liver weight, and increased absolute kidney weight (2.03 +/- 0.12 g vs. 1.84 +/- 0.15 g in control). No other effects of treatment were noted.

25 mg/kg/day: No effects of treatment were noted.

Test condition : Test animals: Test animals (forty per sex) were obtained at 44 days of age. Animals were randomized into 4 groups after a 10 day quarantine. They were housed 2/cage in polycarbonate cages containing hardwood chip bedding. The animal room was maintained at 21 to 24 degrees C and 40 to 60 % relative humidity with a 12 hr light/dark cycle. They were given Purina certified Rodent Chow 5002 and distilled water ad libitum.

Test conduct: Groups of 10 animals per sex were given 0 (corn oil), 25, 100 or 400 mg/kg/day test material daily by gavage for 90 days. All animals were observed daily for overt signs of toxicity. Physical examinations were performed at initiation, and weekly thereafter. Mortality/moribundity checks were performed twice daily. Individual body weights were recorded prior to randomization, at study initiation, and weekly thereafter. Food consumption was recorded (times not stated). Opthalmoscopic examinations were performed prior to treatment and during the last week of the study. Pupils were dilated prior to examination using 1% Mydriacil.

Urinalyses: Urine was collected during the final week of the study (16 hours overnight) in stainless steel metabolism cages from all rats and samples were analyzed for pH, glucose, protein, bilirubin, urobilinogen, and occult blood using reagent test strips.

Hematology and clinical chemistries: Animals were fasted overnight after 90 days of treatment, and blood samples were collected via the orbital sinus while animals were under ketamine anesthesia. Hematological parameters measured included hemoglobin, hematocrit, red and white blood cell counts, mean cell volume, platelet count, differential count and cell morphology. Serum clinical chemistry parameters measured included alanine aminotransferase (ALT) , aspartate aminotransferase (AST), lactate dehydrogenase, cholesterol, phosphorus, calcium, glucose, blood urea nitrogen (BUN), creatinine, sodium, potassium, total protein, albumin, total bilirubin (TB), and alkaline phosphatase.

Necropsy: The brain, liver, spleen, lungs, thymus, kidneys, adrenal glands, heart and testes or ovaries were grossly examined and weighed at necropsy. Gross lesions, skin, mammary glands, clitoral or preputial glands, mandibular and mesenteric lymph nodes, thigh muscle, sciatic nerve, sternebrae, mandibular and mesenteric lymph nodes, thigh muscle, sciatic nerve, sternebrae, femur, duodenum, ileum, jejunum, salivary gland, colon, cecum, rectum, pancreas, urinary bladder, seminal vesicles, prostate, nasal cavity (with turbinates), pituitary, and Zymbal's gland of each animal were examined grossly. All collected tissues were preserved in 10% neutral formalin. Histopathological evaluations were performed on the liver, kidneys, spleen, adrenal glands, thymus, brain, heart, testes/ovaries, and lungs of each animal in the high dose group and 5 randomly selected animals per sex in the control group.

Statistical analyses: Data for males and females were analyzed separately. Analysis of variance (ANOVA) was used to analyze normally distributed measures. When a treatment effect was denoted by the F test ($p < 0.05$), differences between the control and treated groups were

determined using a Dunnett's t-test. Data that were not normally distributed were tested for homogeneity of variance by the Levene test. If found to be homogeneous, they were subjected to ANOVA. If data were heterogeneous, they were transformed by log 10 (liver to body weight ratio), square, square root, reciprocal, arcsine, or rank (food consumption, ovary weight, AST and potassium in females and ALT in both sexes) before being subjected to ANOVA. Histopathological findings were analyzed for statistical significance using Fisher's Trend Test for incidence of lesions, and the logrank test for severity.

Data are presented as mean +/- SD. Relative organ weights are expressed at % of body weight.

Test substance : Test substance was specified as 99% pure by the vendor. Mass spectrometry analysis showed no impurities present within the detectable limits of the instrumentation (less than 0.1%)

Reliability : (1) valid without restriction

Flag : Supportive study for endpoint

19.11.2001 (36)

Species : rat
Sex : male/female
Strain : other: F344/N
Route of admin. : gavage
Exposure period : 13 weeks
Frequency of treatment : 5 days/week
Post obs. period : no
Doses : 30, 60, 125, 250, 500 mg/kg/day
Control group : yes, concurrent vehicle
NOAEL : = 60 mg/kg bw
LOAEL : = 125 mg/kg bw
Method : other
Year : 1985
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Remark : Changes observed in uroporphyrin and coporporphyrin were not considered to be indicative of porphyria because there were no lesions in the liver and kidney indicative of porphyria and the concentration of total porphyrins in the liver was not altered by test material at any dose level.

Result : Two females receiving 500 mg/kg died (one at week 6 and the other at Week 9). One male each from the control, 30 mg/kg and 125 mg/kg groups died.

500 mg/kg only: Males treated with the high dose had lower body weights than controls at the end of the study (-19%). There were significant increases in relative lung, kidney and brain weights and significant decreases in the absolute and relative thymus, and absolute hear, spleen and testicle weights in high dose males. High dose females had decreased absolute and relative uterus weights. Minimal decreases in hematocrit, hemoglobin and mean corpuscular volume were observed in high dose animals of both sexes and a decrease in the number of red blood cells and lymphocytes, and a small increase in segmented neutrophils was observed in high dose males. High dose males also had decreased serum triglycerides and increased bilirubin. High dose males and females had increased serum alpha a beta globulin. Twenty four hour urine volume was increased 57% over control in high dose males. The urinary concentration of uroporohyrin and coproporphyrin was 3-5 times higher in high dose

males and females compared to controls. The two high dose rats that died early had a moderate degree of centrilobular hepatocellular necrosis. Most of the surviving high dose rats (7/8 females and 8/10 males) had liver lesions. Renal tubular degeneration and thymic lymphoid depletion was found in 6/10 and 4/10 high dose males, respectively.

Dose/related changes: Significant increases in relative liver weight were noted in males and females treated with 125, 250 and 500 mg/kg. The number of platelets was increased at 60, 125 and 500 mg/kg in female, but not male rats. This change was not considered to be significant. There was a slight, dose related increase in serum cholesterol at 30, 125 250 and 500 mg/kg (in males) and 125-500 mg/kg (in females). Dose-related increases in total serum protein were noted in males treated with 250 and 500 mg/kg and females treated with 30-500 mg/kg. Serum glucose was increased in females treated with 30 and 125-500 mg/kg. All serum chemistry changes were relatively small. Renal and thymic lesions were not observed at the 125 and 250 mg/kg doses. Hepatocellular necrosis occurred in 4/9 males and 5/10 females treated with 250 mg/kg, and 1 female rat treated with 125 mg/kg. The effects observed at doses less than 125 mg/kg were not considered to be adverse.

Test condition : Rats were 4 weeks old upon arrival and were observed for 2 weeks prior to treatment. Ten animals/sex/group were treated with corn oil vehicle or 30, 60, 125, 250 or 500 mg/kg/day by gavage for 13 weeks. Animals were checked two times a day for mortality. A clinical exam was conducted weekly, and body weights were determined once weekly. Urinalyses (including uroporphyrins and coproporphyrins) were performed on urine from control and high dose animals collected one week before termination. Blood samples were taken one day prior to termination (via orbital bleeding) for standard hematological parameters and on day of termination (via cardiac puncture) for clinical chemistries (including globulins). Total liver porphyrins were measured for all animals. Standard organs were taken at necropsy for organ weights (all groups) and histology (control and high doses only).

Test substance : Purity was > 99%
Reliability : (1) valid without restriction
Flag : Supportive study for endpoint
19.11.2001 (35)

Species : rat
Sex : male/female
Strain : no data
Route of admin. : inhalation
Exposure period : 6 to 7 months
Frequency of treatment : 7 hr/day, 5 days/week
Post obs. period : no
Doses : Nominal: 65 and 106 ppm; actual 49 ppm (ca. 0.3 mg/l) and 93 ppm (ca. 0.57 mg/l)
Control group : other: air exposed control (for 93 ppm only)
NOAEL : = 49 ppm
LOAEL : = 93 ppm
Method : other
Year : 1958
GLP : no
Test substance : as prescribed by 1.1 - 1.4
Result : The only effect of the test material was decreased average body weight of males exposed to 93 ppm. There were no effects of treatment on organ

weight, gross appearance, behavior, growth, mortality and gross and microscopic examination of tissues.

Test condition : Groups of 20 rats/sex were exposed to 106 or 65 ppm test material via inhalation for 6 to 7 months. Concentrations were determined analytically to be 93 or 49 ppm.

Test substance : Purity was at least 99%

Reliability : (2) valid with restrictions

Flag : Supportive study for endpoint

19.11.2001 (26)

Species : mouse

Sex : male/female

Strain : B6C3F1

Route of admin. : gavage

Exposure period : 13 weeks

Frequency of treatment : 5 days/wk

Post obs. period : no

Doses : 30, 60, 125, 250, 500 mg/kg/day

Control group : yes, concurrent vehicle

NOAEL : = 125 mg/kg bw

LOAEL : = 250 mg/kg bw

Method : other

Year : 1985

GLP : no data

Test substance : as prescribed by 1.1 - 1.4

Remark : The apparent increase in white blood cell counts in males may have been due to abnormally low white blood cell count (WBC) in male controls (3.4 +/- 0.8 x 103/mm3). Previous laboratories from other NTP contract labs have reported WBC cell counts in control male mice at 4.3 +/- 1.8 x 103/mm3 (N= 10). An additional lab reported counts of 8.5 +/- 1.8 x 103/mm3. Therefore, it was concluded that the increase in white blood cell count was unlikely to be biologically significant.

The increase in WBC cell count also was not dose-dependent. Similar values were obtained at all doses. The largest value (7.2 +/- 1.3 x 103/mm3) was observed in males given 60 mg/kg and the smallest value (5.4 +/- 1.0 x 103/mm3) was observed in males given 250 mg/kg.

Result : All doses: There was a decreased relative spleen weight in females given any dose. However, since the decrease was similar in all groups of treated animals, it is likely not to be related to test material administration. There was an increased in white blood cell count in males at all doses. The relative number of lymphocytes increased in high dose males and decreased in females treated with 250 mg/kg. Corresponding decreases (males) and increases (females) occurred in the relative number of neutrophils.

500 mg/kg: Four of ten males and 3/10 females given 500 mg/kg died. Weights and weight gains of high dose males and females were depressed. There was increased relative liver weight in high dose males and females. The urinary concentration of coproporphyrin and the concentration of liver porphyrins were 3 and 2 times higher in high dose females than controls (respectively). No effects on these parameters were noted in males. Centrilobular necrosis, necrosis of individual hepatocytes, or hepatocellular degeneration were observed in 9/10 high dose males and females. Multiple foci of mineralization of myocardial fibers were noted in hearts of 3/10 and 8/10 high dose males and females, respectively. Some

necrosis, myositis and mineralization in skeletal muscle was observed (sex and dose not stated, but presumed to be 500 mg/kg). Lymphoid depletion was observed in the thymus (2/10 of each sex dosed with 500 mg/kg) and spleen (2/10 high dose females and 4/10 high dose males). Necrosis of lymphocytes in the spleen was observed in one high dose female mouse. Hemosiderin deposits were found in livers of 4/10 high dose males and 2/10 high dose females. The severity of the lesions was not reported.

250 mg/kg: The only compound-related lesions observed were necrosis of individual hepatocytes (2/10 males), hepatocellular degeneration (1/10 males) and pigment deposition (1/10 males). Myocardial dystrophic mineralization and lymphoid depletion of the thymus and spleen were not observed at this dose. No lesions were noted in females at this dose.

The LOAEL was based on a dose that caused minimal effects in the liver. Effects seen at lower doses cannot be definitively linked to treatment.

Test condition : Mice were 6 weeks old upon arrival and were observed for 2 weeks prior to treatment. Ten animals/sex/group were treated with corn oil vehicle or 30, 60, 125, 250 or 500 mg/kg/day by gavage for 13 weeks. A clinical exam was conducted weekly. Body weights were determined once weekly. Urinalyses (including uroporphyrins and coproporphyrins) were performed on control and high dose animals one week before termination. Blood samples were taken one day prior to termination (via orbital bleeding) for hematologies and on day of termination (via cardiac puncture) for clinical chemistries (including globulins). Total liver porphyrins were performed on all animals. Necropsies were performed on all animals not autolyzed or cannibalized. Standard organs were taken at necropsy for organ weights (all groups) and histology (control and high doses only). Additional histological examinations of thymus, spleen, heart and thigh muscle were performed on mice given 250 mg/kg, and liver in mice given 125 or 250 mg/kg.

Test substance : Purity was > 99%

Reliability : (1) valid without restriction

Flag : Supportive study for endpoint

19.11.2001 (35)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Ames test

System of testing : S. typhimurium strains TA98, TA100, TA1535, TA1537, TA1538

Concentration : 0.02 to 2.56 microliters/plate

Cytotoxic conc. : 1.28 microliters/plate (except 0.32 microliters/plate for TA1537)

Metabolic activation : with and without

Result : negative

Method : other

Year : 1983

GLP : no data

Test substance : as prescribed by 1.1 - 1.4

Remark : Thirty different chemicals were tested in this experiment (benzene, 4 fluorobenzenes (mono or di), chlorobenzene, 3 dichlorobenzenes (ortho, meta and para), nitrobenzene, 6 fluoronitrobenzenes (mono or di), 8 chloronitrobenzenes (mono, di or tri), 1 chloro-, fluorobenzene, 3 nitrobenzenes, 1 dinitrofluorobenzene and 1 dinitrochlorobenzene). Fifteen of the compounds containing nitro groups were mutagenic. All compounds without a nitro group showed no mutagenic activity.

Result : The average number of revertants in the DMSO controls for strains TA98, TA100, TA1535, TA1537 and TA1538 in the absence of S-9 were 28, 181, 32, 8, and 22, respectively. Addition of S-9 to controls did not significantly increase the frequency of mutations. The average number of revertants in all positive control cultures were as follows: ENNG, 1994 in TA100 and 2489 in TA1535; 2-NF, 1798 in TA98 and 1659 in TA1538; 9-AA, 1288 in 1537; and 2-AA, from 132 in TA1537 to 1549 in TA100. The test was valid based on these data.

The number of revertants induced by o-dichlorobenzene was not increased from that of controls at any concentration (based on a visual review of the data). Metabolic activation did not appear to significantly increase the number of mutations observed at each concentration. The number of revertants observed in cultures treated with nontoxic concentrations of m-dichlorobenzene (in the presence or absence of S-9) ranged from 18-31 in TA98, 141-233 in TA100, 15-36 in TA1535, 7-13 in TA1537, and 6-33 in TA1538. A concentration of 1.28 microliters/plate was toxic to most strains. A higher concentration (2.56 microliters/plate) was required to produce toxicity to strains TA98, TA1537, and TA100 in the presence of S9. A concentration of 0.32 microliters/plate was toxic to strain TA1537 (in the absence, but not presence of S-9).

Test condition : All strains of bacteria were supplied by the same supplier (Dr. B. Ames). Test material was dissolved in sterile dimethylsulfoxide. S-9 was prepared from liver homogenate (25% in 0.15M KCl) from male Sprague-Dawley rats (100-200 g) that had been injected with PCB at a dose of 500 mg/kg 5 days before they were killed. S-9 mix contained per ml: S9(0.3 ml), MgCl2 (8 micromoles), KCl (33 micromoles), glucose 6 -phosphate (5 micromoles), NADH (4 micromoles), NADPH (4 micromoles), and sodium phosphate, pH 7.4 (100 micromoles).

Various concentrations (from 0.02 microliters/plate to the concentration that caused toxicity) of test compound (0.1 ml) were added to sterile test tubes containing 3-6 x 10E7 bacterial cells, 0.5 ml of S-9 mix (+ activation) or sodium phosphate buffer (pH 7.4) (- activation). This mixture was preincubated in a shaker water bath at 37 degrees C for 15 min, then added to 2 ml molten top agar (45 degrees C). The contents of each tube were mixed and immediately poured onto the surface of a minimal-agar plate. DMSO (0.05 ml) was added to plates containing each kind of bacteria (negative control). N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG; 2 or 10 micrograms/plate incubated with strains TA100 and TA 1535 without S-9), 2-nitrofluorene (2-NF; 2 or 5 micrograms/plate incubated with strains TA98 and TA1538 without S-9), 9-aminoacridine (9-AA; 100 micrograms/plate incubated with strain TA1537 without S-9) and 2-aminoanthracene (2-AA;5 micrograms/plate incubated with all strains with S-9 only) were used as positive controls. All tests were performed in duplicate and were repeated at least 3 times separately.

Plates were inverted and incubated at 37 degrees C in the dark for 3 days. Colonies of his+ revertants were counted after incubation. Chemicals inducing more than twice the number of revertant colonies as negative control plates were considered to be mutagenic. Tests without metabolic activation were carried out first. Tests with metabolic activation were carried out only if results of the tests without activation were negative.

Two strains (TA98 and TA100) were checked routinely for the presence of the ampicillin resistance for the R factor. The background bacterial lawn was routinely checked by microscopy for thinning (evidence of toxicity) and

contamination.

Statistical analyses were not performed.

Test substance : Purity of the test material was 98%
Reliability : (2) valid with restrictions. Statistical analyses were not performed.
Flag : Key study for endpoint
19.11.2001 (40)

Type : Ames test
System of testing : S. typhimurium strains TA98, TA100, UTH8413, UTH8414
Concentration : 50, 100, 500, 1000 and 2000 micrograms/plate
Cytotoxic conc. : not listed
Metabolic activation : with and without
Result : negative
Method : other
Year : 1985
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Reliability : (2) valid with restrictions. Only 4 strains were tested.
19.11.2001 (15)

Type : Ames test
System of testing : S. typhimurium strains TA98, TA100, TA1535, TA1537
Concentration : 3.3, 10, 33, 100 or 333 micrograms per plate; also 1000, 10000 or 13000 micrograms/plate in TA100
Cytotoxic conc. : 333 micrograms/plate in most; 100 micrograms/plate in TA1537, 1000 micrograms/plate in TA100
Metabolic activation : with and without
Result : Negative
Method : other
Year : 1985
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Remark : Negative (DMSO), but not positive controls were tested. Two separate tests (in triplicate) were performed 2 weeks apart. The chemical was tested in systems containing S-9 from Arochlor-1254 induced rats and hamsters.
Result : Purity of test substance was 95%
Reliability : (2) valid with restrictions. There were no positive controls
19.11.2001 (35)

Type : Ames test
System of testing : S. typhimurium strains TA98, TA100, TA1535, TA1537, TA1538
Concentration : 0.0005 to 1.0 microliters/plate (0.00065 to 1.3 mg/plate)
Cytotoxic conc. : 0.5 microliters/plate (TA100), 1.0 microliters/plate (other strains)
Metabolic activation : with and without
Result : Negative
Method : other
Year : 1979
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Test condition : The test material was protected from light
Reliability : (2) valid with restrictions. The purity of the test material was not noted.
19.11.2001 (41)

Type : Ames test
System of testing : S. typhimurium strains TA98, TA100, TA1535, TA1537
Concentration : 10 mg (spot test); 0.6, 2.4, 12, 60, 180, 600 micrograms/plate (plate

incorporation test)
Cytotoxic conc. : 600 micrograms/plate (plate incorporation test)
Metabolic activation : with and without
Result : Negative
Method : other
Year : 1978
GLP : No
Test substance : as prescribed by 1.1 - 1.4
Remark : Material was tested with S-9 from rats and mice. Tests with DMSO (vehicle) and positive controls were performed. Plate tests were performed in triplicate.
Reliability : (2) valid with restrictions. Tests were performed in only 4 strains
19.11.2001 (20)

Type : Escherichia coli reverse mutation assay
System of testing : E. coli WP2(trp-, uvRA-)
Concentration : 0.0005 to 1.0 micrograms/l
Cytotoxic conc. : 1.0 micrograms/l
Metabolic activation : with and without
Result : negative
Method : other
Year : 1979
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Remark : Monochlorobenzene and 1,3- and 1,4-dichlorobenzene also tested negative in this study.
Reliability : (2) valid with restrictions. Purity of test material was not stated.
19.11.2001 (41)

Type : Chromosomal aberration
System of testing : Chinese hamster ovary cells
Concentration : 75, 100 or 143 micrograms/ml
Cytotoxic conc. :
Metabolic activation : with and without
Result : negative
Method :
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4. Purity 99.7%
Source : Bayer AG Leverkusen
Reliability : (4) not assignable. The study was not available for review. Data came from a IUCLID document created by the European Chemicals Bureau, creation date 11-FEB-2000.
19.11.2001 (5)

Type : Sister chromatid exchange assay
System of testing : Chinese hamster ovary cells
Concentration : 5.9, 19.7 or 59 micrograms/ml (without metabolic activation) and 19.7, 59 or 197 micrograms/ml in one test (with metabolic activation) and 300, 400 or 500 micrograms/ml in another test (with metabolic activation).
Cytotoxic conc. : Unknown
Metabolic activation : with and without
Result : Ambiguous
Method : other
Year : 1990
GLP : no data

Test substance : as prescribed by 1.1 – 1.4

Remark : The results of this study were difficult to read. Standard errors or deviations were not stated. Although the two experiments with S-9 met the criterion for a positive result, the incidence of SCE's in either experiment was not concentration-dependent. The incidence of SCE's in the control for the second experiment was as high as the incidence of SCE's in the treated groups of the first experiment. Furthermore, although the concentrations listed as being positive were denoted as such in Appendix 12, the test in the presence of S-9 was denoted as being negative in the summary table.

Result : The test for sister chromatid exchange (SCE) was negative in the absence of S9. In the first experiment with S-9, concentrations of 19.7, 59.0 and 197.0 micrograms/ml induced 8.64, 8.98 and 8.90 SCE per cell (compared to 7.26 in control). In a second experiment with S-9, concentrations of 300, 400 and 500 micrograms/ml induced 10.68, 10.38 and 10.06 SCE per cell (compared to 8.46 in control). The results in this test were positive at 300 and 400 micrograms/ml. The positive control CPA induced 13.82 and 14.9 SCE per cell at 0.4 micrograms/ml and 35.3 and 40.3 SCE per cell at 2.5 micrograms/ml in the two experiments. The test for chromosomal aberrations was negative both in the presence and absence of S-9.

Test condition : Chinese hamster ovary (CHO) cells were obtained at their fifth passage level after cloning. Cells were tested regularly for mycoplasma contamination. They were not used beyond the fifteenth passage after cloning. Stocks of CHO cells were maintained at 37 degrees C in McCoy's A (modified) medium buffered with 20 mM HEPES and supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 50 IU/ml penicillin and 50 micrograms/ml streptomycin. Test cultures were set up in 75 cm2 flasks 24 hours before treatment at a density of 1.25 x 10E6 cells/flask.

Stock solutions of 1,2 dichlorobenzene were prepared at 500 mg/ml (or the limit of solubility). A series of dilutions were made from the stock solution to achieve 10 test concentrations in a half-log series (covering a range of 5 logs). The highest dose used was that which allowed for a sufficient number of cells to be scored at time of harvest.

Cells were exposed to test material, vehicle, or positive control agent (mitomycin C at a level known to induce an 20-80% increase in sister chromatid exchanges (SCEs) and another to induce a greater than 100% increase in SCEs) in the absence or presence of S-9 for 2 hours before addition of bromodeoxyuridine (BrDU; 10E-5 M). In cells that were incubated with S-9, test medium did not contain serum. Flasks were sealed. Cultures were incubated for 24 additional hours with BrDU. The chemical and BrDU were then removed and cells were rinsed twice with PBS. Fresh medium with BrdU and colcemid were added and the cells were incubated at 37 degrees C for an additional 2-2.5 hr. Cells were then examined for toxicity (% confluency of monolayer). Cells were then harvested, treated for 12 min at 37 degrees C with hypotonic buffer, and resuspended in 3 volumes of fixative. Slides were prepared and examined with fluorescence microscopy to determine the frequency of metaphase cells that had completed one or two cell cycles in BrDU (M1 or M2 cells). Fifty second division M2 cells from each of the top 3 test concentrations were scored for SCEs.

Chromosomal aberrations in CHO cells were determined by incubating cells at 37 degrees C (1.75 x 10E6 cells/75 cm2 flask) with test chemical for 8 hours (without S-9) or incubating cells for 2 hrs in serum free medium (with S-9) and then an additional 8 (without S-9), washing cells, and

incubating them with colcemid 2 -2.5 hr before cell harvest. The total durations of the nonactivated and activated experiments were 10 and 12 hrs, respectively. Cells were harvested, stained with Giemsa and scored for aberrations. Two tests were performed in the presence of S-9.

All slides (except those from the high dose positive control) were coded and a complete experiment was scored by the same person. A trend test of SCEs per chromosome vs. log of concentration was used to examine data. A 20% increase in sister chromatid exchange at 2 doses was considered positive.

Test substance : Purity of test material was 99.4%.
Reliability : (4) unassignable. The results are ambiguous.
19.11.2001 (29)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Micronucleus assay
Species : mouse
Sex : male
Strain : B6C3F1
Route of admin. : i.p.
Exposure period : 3 days
Doses : 50, 100, and 200 mg/kg/day (total dose of 150, 300, 600 mg/kg) (experiment 1) ; and 150, 250 mg/kg/day (total dose of 450 and 750 mg/kg) (experiment 2)
Result : negative
Method : other
Year : 1993
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Result : The % PCE in animals treated with 0, 50, 100 or 200 mg/kg/day was 62.5, 56.4, 62.2, and 64.2, respectively. The incidences of MN-PCE/1000 (mean +/- SE) in pooled samples from 5 animals treated with 0, 50, 100 or 200 mg/kg/day were 1.70 +/- 0.49, 1.90 +/- 0.49, 2.40 +/- 0.49, and 2.70 +/- 0.72, respectively. This test was barely positive based on trend analysis (p = 0.049), but no dose group was positive. The test was repeated to 250 mg/kg and was found to be negative by trend analysis (p= 0.358). Because of the relatively small increased in MN-PCE in the initial test and the lack of reproducibility, the overall result was considered negative.

Solvent (corn oil) data were scored as 2.12 +/- 0.70 and 2.38 +/- 0.93 MN-PCE/1000 PCE (mean +/- SD) by two separate labs (not significantly different). These values are slightly higher than those reported in the test. The data for the positive control DMBA were 6.93 +/- 2.59 and 7.93 +/- 1.69 MN-PCE/1000 PCE (mean +/- SD) in the two labs. The data for the positive control MMC were 6.82 +/- 1.24 and 6.85 +/- 2.26 MN-PCE/1000 PCE (mean +/- SD) in the two labs (no significant difference).
Test condition : Male mice between 9 and 14 weeks of age and a mean weight of 23 and 35 g were used. Test material was mechanically suspended in corn oil and was administered within 30 minutes of preparation. Five mice/group were dosed with 0 (corn oil control), 50, 100 or 200 mg/kg test material, or a weakly active dose of the positive control chemicals 7,12-dimethyl benzanthracene (DMBA; 12.5 mg/kg) or mitomycin C (MMC; 0.2 mg/kg) by i.p. injection on three consecutive days (volume 0.4 ml). The doses tested were based on results of toxicity/mortality in a preliminary study. Animals were monitored 2 times/day.

Mice were killed 48 hours after the third treatment. Bone marrow and peripheral blood smears (two slides/tissue/mouse) were prepared by a direct technique (Tice et al. 1990. Effect of treatment protocol and sample time on frequencies of micronucleated cells in mouse bone marrow and peripheral blood. Mutagen.5:313-321). Air-dried smears were fixed using absolute methanol and stained with acridine orange. Smears from each animal were evaluated at 1000 x magnification using epi-illuminated fluoresence microscopy (450-490 nm excitation; 520 nm emission) for the percentage of polychromatic erythrocytes (PCE) among 200 erythrocytes and the number of micronucleated PCE (MN-PCE) among 2000 PCE. Repeat tests were conducted if the results suggested a possible effect or if no toxicity was observed at the highest dose level. Since the result of the experiment suggested a possible effect, the experiment was repeated at 0, 150 and 250 mg/kg/day.

The data were analyzed using the Micronucleus Assay Data Management and Statistical software package (version 1.4), which was designed specifically for in vivo micronucleus test data (ILS.1990. Integrated Laboratory Systems, P.O. Box 13501, Research Triangle Park, NC). The level of significance was set at $p < 0.05$. The number of MN-PCE at each dose group were pooled and analyzed by a one-tailed trend test. In the software package used, the trend test incorporates a variance inflation factor to account for excess animal variability. In the event that the increase in the dose response curve was nonmonotonic, the software allowed for the data to be analyzed for a significant positive trend after data at the highest dose only had been excluded. In this event, the alpha level was adjusted to $p < 0.01$ to protect against false positives. The %PCE data were analyzed by an analysis of variance (ANOVA) test based on pooled data. Pairwise comparisons between each group and the solvent control were made using an unadjusted one-tailed Pearson chi-squared test which incorporated the calculated variance inflation factor for the study. Solvent (corn oil) and positive control data were analyzed by two separate laboratories.

Reliability : (2) valid with restrictions. Purity of test material was not noted.
Flag : Key study for endpoint
19.11.2001 (39)

Type : Micronucleus assay
Species : mouse
Sex : male
Strain : NMRI
Route of admin. : i.p.
Exposure period : 30 hours
Doses : 187, 375, 562, 750 mg/kg in 2 doses given 24 hours apart
Result : positive
Method : other
Year : 1987
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Remark : Chlorobenzene, 1,2-, 1,3- and 1,4- dichlorobenzene, and 1,2,3- and 1,2,4-trichlorobenzenes also tested positive in this study.
Result : The number of micronucleated cells/1000 PCE (mean +/- SD) in control, 187, 375, 562, and 750 mg/kg groups was 1.80 +/- 0.748, 3.90 +/- 0.943, 4.50 +/- 1.204, 5.44 +/- 1.257, and 5.90 +/- 1.135, respectively. The number of micronucleated cells/1000 PCE (mean +/- SD) in animals treated with 264, 528, 1056 (in a split dose) and 528 (in one dose) mg/kg

benzene (positive control) was 4.40 +/- 0.800, 8.10 +/- 0.943, 12.40 +/- 1.356, and 10.83 +/- 1.343, respectively.

The test was judged positive.

Test condition : Eight week old male mice (5 per group) were given i.p. doses of test compound (total dose of 187, 375, 562 and 750 mg/kg) such that the highest dose did not exceed 70% of the reported LD50 value (1228 mg/kg). Each dose was given in a divided dose 24 hours apart (doses given at each 24 hour injection were 93.5, 187.5, 281 and 375 mg/kg). The control group of 10 mice received corn oil only. Benzene was the positive control. Animals were killed 30 hours after the first injection. The femora were removed and the marrow was suspended in serum. Two smears per femur were prepared and coded. The smears were scored by two different people. One-thousand polychromatic erythrocytes per smear were examined for the presence of micronuclei. T-tests were used to compare data. No further test details are given.

Test substance : Purity of test substance was 99%.

Reliability : (4) not assignable. The use of multiple t-tests is an inappropriate means of determining the significance of the data. The study documentation is lacking in sufficient detail as to assess its validity.

19.11.2001 (34)

Type : Cytogenetic assay
Sex : Male/female
Species : rat
Strain : Sprague-Dawley
Route of admin. : i.p.
Exposure period : The animals received a single i.p. injection and were terminated 6, 12 or 24 hours later
Doses : 150, 300 or 600 mg/kg bw (6 hr assay) and 135, 270 or 540 mg/kg bw (12 and 24 hr assays)
Result : negative
Method :
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4. Purity 99.7%
Result : The test material did not cause an increase in the frequency of chromosomal breaks or aberrations in the bone marrow cells.
Source : Bayer AG Leverkusen
Reliability : (4) not assignable. Study was not reviewed. Data came from a IUCLID data set for CAS No. 95-50-1 created by NICNAS, creation date 23-AUG-2001.

19.11.2001 (5)

5.7 CARCINOGENITY

Species : mouse
Sex : male/female
Strain : B6C3F1
Route of admin. : gavage
Exposure period : 103 weeks
Frequency of treatment : 5 days/week
Post. obs. period : no
Doses : 60, 120 mg/kg (dose volume: 5 ml/kg)
Result : negative
Control group : yes, concurrent vehicle

Method : other
Year : 1985
GLP : no
Test substance : as prescribed by 1.1 - 1.4
Remark : Results were QA audited
Result : Mean body weights of doses and control rats were similar throughout the study. No significant differences in survival were observed between groups.

Non-neoplastic lesions: There was a dose-related trend in tubular regeneration of the kidney in male mice (control, 17%, low dose 24%, high dose 35%). No other increases were observed in nonneoplastic lesions in the liver, bone marrow, spleen, or other organs as a result of test material administration.

Neoplastic: Malignant histiocytic lymphomas occurred in male and female mice with statistically significant positive trends. A significantly negative trend was observed in male mice for malignant lymphocytic lymphomas. The combined incidence of all types of lymphomas was not significantly greater than that of controls. Therefore, the findings were dismissed.

There was a significant, dose-related decrease in the incidence of hepatocellular adenomas in dose male mice. The incidence of this lesion was significantly lower in high dose males than controls. (high dose = 2/46, control 8/50). The combined incidence of male mice with liver adenoma or carcinoma decreased with increasing concentration (according to the life table test). The significance of this finding was not discussed.

Alveolar/bronchiolar carcinomas occurred in males with a significant positive trend (control = 4/50, mid dose = 2/50, high dose = 10/50) in the Cochran-Armitage test only (p = 0.037), but the more appropriate combined incidence of male mice with alveolar/bronchiolar adenomas or carcinomas was not statistically significant in any of the test groups (control = 8/50, low dose = 8/50, high dose = 13/50)

Test condition : Mice were obtained at 4.5 weeks old and were observed for 17-18 days before randomization. Mice (50/sex/group) were treated with 0 (corn oil vehicle), 60 or 120 mg/kg test material by gavage, 5 days/wk for 103 weeks. Food and water were supplied ad libitum. Animals were observed 2 times daily for morbidity and mortality. Clinical signs were observed intermittently until month 18, and monthly thereafter. Body weights by cage (5 mice) were recorded every week for the first 13 weeks and monthly thereafter. Mean body weight was calculated as the total weight of animals in the group/ number of survivors in the group.

All animals (except precluded by autolysis or cannibalization) were necropsied. The following set of organs was examined microscopically: tissue masses, abnormal lymph nodes, mammary gland, salivary gland, bone marrow, sternebrae, femur or vertebrae, thymus, trachea, lungs and bronchi, heart, thyroid, parathyroid, esophagus, stomach, small intestine, colon, liver, pancreas, spleen, kidneys, adrenals, urinary bladder, prostate/testes or ovaries/uterus, brain, and pituitary. Eyes, thigh muscle and spinal cord were examined grossly and were examined microscopically if were found to be grossly abnormal.

Neoplastic nodules were classified according to the recommendations of Squire and Levitt (1975) and the National Academy of Sciences (1980).

All data and slides were sent to an independent quality assurance laboratory for verification. The final tumor diagnoses represented a consensus between the original pathologist, independent contractors, and the NTP Pathology Working Group.

Tumor incidence data were analyzed by two different methods. The first method of analysis (life table test) assumed that all tumors of a given type observed in animals dying before the end of the study were fatal. For this test, the proportions of tumor-bearing animals in the dosed and control groups were compared at each point in time at which an animal died with a tumor of interest. The denominators of these proportions were the total number of animals at risk in each group. The second (incidental tumor test) assumed that all tumors of a given test observed in animals dying before the end of the study were unrelated to cause of death. For this test, the proportions of animals found to have tumors in each group were compared in each of 5 time intervals: 0-52 weeks, 53-78 weeks, 79-82 weeks, and 93 weeks to the week before termination, and the terminal kill period. The denominators of these proportions were the number of animals on which autopsies were performed during the time interval. The Fisher's exact test for pairwise comparisons and Cochran-Armitage linear trend test for dose-response trends also were performed. These tests were based on the overall proportion of tumor-bearing animals. When differing results were obtained, the final interpretation of the data depended on the extent to which the tumor was regarded as being the cause of death.

Test substance : Purity of test substance was > 99%
Conclusion : The test material was not considered to be carcinogenic in mice at the doses administered.

Reliability : (1) valid without restriction
19.11.2001 (35)

Species : rat
Sex : male/female
Strain : other: F344/N
Route of admin. : gavage
Exposure period : 103 wk
Frequency of treatment : 5 days/wk
Post. obs. period : no
Doses : 60, 120 mg/kg /day(dose volume: 5 ml/kg bw)
Result : negative
Control group : yes, concurrent vehicle
Method : other
Year : 1985
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Remark : Results were QA audited
Result : Survival of high dose rats was significantly shorter than controls. However, it was concluded that gavage error may have been the cause.

There was no increase in non-neoplastic lesions of the liver, kidney, bone marrow, spleen, thymus or other organ.

The incidence of pheochromocytomas in the low dose males was increased when compared to controls (16/50 vs. 9/50 in controls). This increase was significant by the life table test but not by the incidental tumor

test. The incidence of this lesion was not increased in high dose males (6/49 vs. 9/50 in controls). No malignant pheochromocytomas were observed in either of the test groups.

Interstitial-cell tumors of the testes in male rats occurred with a significant positive trend when analyzed by the life-table test, but with a significant negative trend when analyzed by the Cochran-Armitage test. Since this tumor is not considered to be life-threatening, the increase detected by the life-table test was discounted.

The incidence of undifferentiated leukemia was significantly lower than control in low dose females (3/50 vs. 12/50), and equal to control in high dose females. There were no differences between the incidence of female rats with all types of leukemia in the control versus low- and high-dose groups (13/50, 6/50 and 12/50, respectively). No differences were noted between the incidence of leukemia in controls or low or high-dose males (10/50, 7/50 and 5/50, respectively).

Test condition : Rats were obtained at 4 weeks old and were observed for 17-18 days before randomization. Rats (50/sex/group) were treated with 0 (corn oil vehicle), 60 or 120 mg/kg test material by gavage, 5 days/wk for 103 weeks. Food and water was supplied ad libitum. Rats were observed 2 times daily for morbidity and mortality. Clinical signs were observed intermittently until month 18, and monthly thereafter. Body weights by cage (5 rats) were recorded every week for the first 13 weeks and monthly thereafter. Mean body weight was calculated as the total weight of animals in the group/ number of survivors in the group.

All animals (except those precluded by autolysis or cannibalization) were necropsied. The following set of organs was examined microscopically: tissue masses, abnormal lymph nodes, mammary gland, salivary gland, bone marrow, sternebrae, femur or vertebrae, thymus, trachea, lungs and bronchi, heart, thyroid, parathyroid, esophagus, stomach, small intestine, colon, liver, pancreas, spleen, kidneys, adrenals, urinary bladder, prostate/testes or ovaries/uterus, brain, and pituitary. Eyes, thigh muscle and spinal cord were examined grossly and were examined microscopically if were found to be grossly abnormal.

Neoplastic nodules were classified according to the recommendations of Squire and Levitt (1975) and the National Academy of Sciences (1980).

All data and slides were sent to an independent quality assurance laboratory for verification. The final tumor diagnoses represented a consensus between the original pathologist, independent contractors, and the NTP Pathology Working Group.

Tumor incidence data were analyzed by two different methods. The first method of analysis (life table test) assumed that all tumors of a given type observed in animals dying before the end of the study were fatal. For this test, the proportions of tumor-bearing animals in the dosed and control groups were compared at each point in time at which an animal died with a tumor of interest. The denominators of these proportions were the total number of animals at risk in each group. The second (incidental tumor test) assumed that all tumors of a given test observed in animals dying before the end of the study were unrelated to cause of death. For this test, the proportions of animals found to have tumors in each group were compared in each of 5 time intervals: 0-52 weeks, 53-78 weeks, 79-82 weeks, and 93 weeks to the week before terminal kill, and the terminal kill period. The

denominators of these proportions were the number of animals on which autopsies were performed during the time interval. The Fisher's exact test for pairwise comparisons and Cochran-Armitage linear trend test for dose-response trends also were performed. These tests were based on the overall proportion of tumor-bearing animals. When differing results were obtained, the final interpretation of the data depended on the extent to which the tumor was regarded as being the cause of death.

Test substance : Purity of test substance was > 99%

Conclusion : The test material was not considered to be carcinogenic in rats at the doses administered.

Reliability : (1) valid without restriction

19.11.2001 (35)

5.8 TOXICITY TO REPRODUCTION

Type : Two generation study
Species : rat
Sex : male/female
Strain : other: Charles River CD (Sprague-Dawley derived)
Route of admin. : inhalation
Exposure period :
Frequency of treatment : 6 hr/day, 7 days/week during times specified under test condition
Premating exposure period
Male : 10 weeks
Female : 10 weeks
Duration of test : to weaning of F1 and F2 generations
Doses : 50, 150, 400 ppm (0.305, 0.915, or 2.44 mg/l)
Control group : yes
NOAEL Parental : < 50 ppm
NOAEL F1 Offspr. : = 50 ppm
NOAEL F2 Offspr. : = 150 ppm
Method : other
Year : 1989
GLP : yes
Test substance : as prescribed by 1.1 - 1.4
Remark : Study personnel concluded that in the absence of significant mortality in all other litters but one high dose litter during the day 0-4 interval, the decrease in pup survival index for this group (while statistically significant compared to control) was not indicative of a treatment-related effect.

The reduced postnatal weights of pups from high dose animals (both F1 and F2) and mid dose animals (F1) are indicative of slight fetotoxicity.

Result : Mean analytical concentrations for the F0 and FI generation animals were similar to targeted exposure levels. For the F0 generation; the mean analytical concentrations (+ S.D.) for the low-, mid- and high-exposure groups were 50 +/- 3, 150 +/- 5 and 397 +/- 18 ppm, respectively. In the FI, these mean analytical concentrations were 51 +/- 3, 151 +/- 8 and 391 +/- 25 respectively, for these same groups.

Some mortality was seen among the control (1/sex in F0; 1 male in F1) and treated adult animals in each generation (1 mid dose F0 female on day 24 during delivery; 1 high dose male F1, one low dose female F1 during lactation, 1 high dose F1 female killed moribund); however, no adverse effect of treatment was indicated.

The only effect of treatment seen in the low-exposure group was a slight increase in absolute and relative liver weight in both the F0 and FI adult animals. No adverse effect of treatment at the low-exposure level was evident from growth of the adult animals, reproductive performance, fertility, gestation length or litter size data. Pups delivered and weaned to females in this group showed comparable growth and survival rates to weaning as control animals. A slight, but significant increase in mean gestation length was seen in low-dose females; however this was not considered to be relevant as it was not observed at higher dose levels.

In the mid-exposure group, mean weights of adults were lower than control at several weekly intervals early in the pre-mating period of the F0 and throughout this same interval in the F1; however, mean weight gain over the entire pre-mating interval for both generations was comparable to control data. No adverse effect of treatment in the mid-exposure group was evident from reproductive performance or fertility indices, litter size or gestation length or maternal weight gain data during gestation/lactation intervals in either generation. In the F1 litters, mean pup weight at Day 0 (birth) for the mid-exposure group was statistically significantly lower than control; however, pup weight data for the remaining weighing intervals of the FI litters and for all weighing intervals of the F2 litters were comparable to control data. Pup survival indices in the mid-exposure group for each litter interval were comparable to control data. Mean liver weights (absolute and relative to body or brain weights) for the mid-exposure group were higher than control in the F0 while in the FI, only relative liver weights were increased. Additionally, relative kidney weights were increased in the mid-exposure males from both generations.

In the high-exposure group, no adverse effect of treatment was evident from reproductive performance or fertility indices for either generation. Maternal weight gain during gestation/lactation intervals, gestation length, litter size data and pups survival indices were generally comparable to control data for both generations. However, the pup survival index for the day 0-4 lactation interval for F2 pups was lower than control (94.3% vs. 98.1% for control). This was largely attributed to the loss of all pups within a single litter (one high dose female delivered a litter of 14 live pups and 2 dead pups, but none survived to day 4). Excluding data for this litter, the pup survival index for the day 0-4 interval was 98.4 % (317/322 pups), which was not significantly different from control. Pup survival for the high dose group over the day 4-21 lactation interval was comparable to control. Mean weekly weights for F0 and FI adults in the high-exposure group during the pre-mating treatment intervals were lower than control,and mean weights continued to be depressed for these animals through to termination.

Mean weight gains over the entire pre-mating intervals were lower than control for males in both generations and for F0 females. Excessive salivation was seen in high dose F0 and F1 males and females at some time points. F0 and F1 adult males and females in the high-exposure group had increased liver weights (absolute and relative) and males (F0, FI) also had increased relative kidney weights. Mean pup weights in the high-exposure group were statistically significantly lower than control on Days 0, 14, 21 and 28 of the FI litters and on Days 14 and 21 of the F2 litters. Growth and food consumption in unselected F1 high dose pups were similar to control.

No adverse effect of treatment was evident from the gross postmortem evaluations of adults and offspring. Treatment-related morphologic abnormalities in the liver and kidneys were seen in the F0 and Fl adults.

Liver: Hypertrophy of the central lobular hepatocytes was found in almost all F0 and Fl adult males and females in the high-exposure group and numerous males and several females from the mid-exposure group. This effect was not seen in the liver of F0 and F1 males and females from the control group or in the liver of females from the low-exposure group.

Kidneys: Dilated tubular lumens with intraluminal granular casts were seen predominantly at the cortico-medullary junctions in several F0 and Fl adult males from the mid- and high-exposure groups. This effect was seen most frequently in the high-exposure group.

Intracytoplasmic granules/droplets in the proximal convoluted tubular epithelium were seen in almost all F0 and Fl adult males for which the kidneys were examined microscopically. Based on mean severity this kidney effect was most pronounced in F0 and F1 adult males from the high-exposure group followed by the mid- and low- exposure groups. It was least pronounced in the control group. The granules/droplets were eosinophilic in the hematoxylin and eosin stained sections and stained positive with the Mallory Heidenhain stain in the specially stained sections.

Other postmortem findings seen in the F0 and Fl adults, gross and microscopically, either occurred with comparable incidence and severities in the treated and control animals or they occurred sporadically and were not considered to be related to the test article.

Several F1 generation animals in all groups were noted on week 37 to have findings suggestive of Sialodacryoadenitis viral (SDAV) infection. All males were noted as free of SDAV infection by week 41. Females were generally free of symptoms of SDAV infection at week 41. The presence of SDAV was confirmed by analyses of serum from affected males for SDAV antibodies. The presence of SDAV did not appear to have adversely affected the study.

Test condition : Test article: Test article was administered by the inhalation route with animals exposed in 6m3 glass and stainless steel chambers. Targeted exposure levels were 50, 150 and 400 ppm; included in the study was a chamber exposed, sham-air control group. Appropriate amounts of the test material were placed into a 2 l erlenmeyer flask connected to a fluid metering pump. The metering pump settings were varied to provide the target exposure levels. The test article was fed from the flask directly into the liquid inlet of an air atomizing nozzle via Teflon tubing. House-supply air was delivered through Teflon tubing from a regulator and flowmeter with a backpressure gauge to the air inlet of the atomizer via tygon tubing to generate the aerosol. The aerosol was directed into the side inlet of the air inlet pipe where it volatized in the chamber airflow stream. This stream entered the exposure chambers. Test concentrations were monitored hourly during exposures.

Test conduct: Each study group consisted of 60 CD rats (30/sex/generation). F0 adult animals (41 days old at treatment) were exposed daily (6 hrs/day) for a 10 week pre-mating treatment period and during mating. Once mated, females were exposed (6 hrs/day) during gestation (Days 0-19) and lactation (Days 5-28) of the Fl litters. F0 males continued to be treated daily during the post-mating period until

termination. Similarly, F0 females continued to be treated daily post-weaning until termination after weaning of the last litter. FI pups (2/sex/litter) randomly selected at Day 28 (weaning) became a pool of animals from which the FI adult generation was selected. These animals were exposed at 29 days of age to comparable dose levels as the dams. Once the FI adult generation was chosen, these animals received an 11-week pre-mating treatment period. Exposure of animals during the mating, gestation and lactation intervals of the F2 litters was similar to that for the F0 animals. Animals were given free access to standard laboratory diet during all non-exposure periods, and water during exposure and non-exposure periods. All animals were observed twice daily for toxicity or mortality. Detailed physical examinations were performed for the F0 and F1 adult generation animals and unselected F1 high dose animals (see below). Body weights and food consumption of F0 and F1 adults were generally conducted weekly for most animals (with the exception of slightly different intervals for females during gestation and lactation). Pup weights were determined on days 0, 4 (pre and post cull), 7 (F2 litter only), 14, 21 and 28 of lactation (F1 litters only). They were sexed at each examination.

F0 and FI adult males were terminated as a group three to four weeks post-mating; F0 and FI females were killed as a group after the last litters, FI and F2, respectively, were weaned. Each adult generation animal was given a gross postmortem examination and liver, kidneys, pituitary gland and reproductive tissues were saved in 10% formalin. Liver, kidney, testes and brain weights were recorded at termination and absolute and relative liver, kidney and testes weight data were evaluated. Initially, microscopic evaluations were restricted to tissues for the control and high-dose groups, both generations; however, these evaluations were extended to include the livers of all F0 and F1 adults in the low- and mid-dose groups and the kidneys of all F0 and FI low- and mid-dose males. FI and F2 pups were given a gross external and internal examination and discarded; only abnormal tissues were saved in 10% formalin. FI pups were terminated either at weaning (Day 28) or at the time of selection for the FI adult generation. F2 pups were terminated at Day 21 of lactation.

If, following weaning of the F1 last litters more than 30 pups/sex were present for a particular group, the excess were culled so that each litter was represented in the parental generation by at least one pup per sex. In the control, low and mid dose groups, the excess pups were culled and given a gross external and internal examination. In the high-exposure group, the excess pups were retained on study. They were removed from the exposure regimen and were maintained on basal diet over an 11-week period that corresponded to the pre-mating treatment period. These animals (unselected F1 high-dose pups) were then terminated and given a gross postmortem examination. Only abnormal tissues from these animals were saved.

Data from treated groups were compared to control (methods for statistical tests were not available).

Test substance : The purity of the test material was > 99.2%.
Reliability : (1) valid without restriction
19.11.2001 (38)

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : rat

Sex	:	
Strain	:	Fischer 344
Route of admin.	:	inhalation
Exposure period	:	days 6 through 15 of gestation
Frequency of treatment	:	6 hr/day
Duration of test	:	Day 21 of gestation
Doses	:	100, 200, 400 ppm (0.6, 1.2, 2.4 mg/l)
Control group	:	other: filtered room air
NOAEL Maternalt.	:	< 100 ppm
NOAEL Teratogen	:	= 400 ppm
Method	:	other
Year	:	1985
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The NOAEL for fetotoxicity is 200 ppm, based on an increased occurrence of delayed ossification of cervical vertebral centra at 400 ppm.
Result	:	Maternal observations: A slight to moderate degree of urine soaking of the perineal area was observed in 8/32 rats in the high dose group. Mean body weights of high dose females were reduced from gestation days 6 though 30, and mean body weight gains of all groups were depressed from days 6-8, 12-15, and 6-20 of gestation. Food consumption was slightly depressed during the first three exposure days (data not shown). A significant increase in both absolute (10.25 +/- 0.69 g versus 9.71 +/- 0.61 g in control) and relative liver weight (3.98 +/- 0.23 vs. 3.62 +/- 0.19 in control) was seen in rats exposed to 400 ppm and relative liver weight in rats exposed to 100 ppm (3.79 +/- 0.24 vs. 3.62 +/- 0.19 in control). There were no significant differences in the % pregnant, number of litters, number of corpora lutea/dam, number of implantation sites/dam, number of fetuses/litter, % of implantation sites resorbed, % litters with resorption, litters totally resorbed, the ratio of resorptions/litters with resorptions, sex ratio, fetal body weight or fetal crown-rump length between groups. Fetal alterations: The incidence of major malformations, when considered individually or collectively, was not significantly increased in treated groups. The incidence of major malformations in the control, 100, 200 and 400 ppm treatment groups was 5 in 2 litters, 3 in three litters, 1 in 1 litter and 5 in three litters, respectively. Four fetuses in one control litter were missing one pair of ribs and a single thoracic vertebra; two of these fetuses had cervical ribs. Three fetuses in three litters in the 100 ppm group had cervical ribs. One fetus in the 200 ppm group had a cervical rib. In the 400 ppm group, the malformations included single cases of coarcted and retroesophageal aortic arch, unilateral testicular agenesis, polydactyly, cervical ribs and microphthalmia. Skeletal evaluation of the fetuses revealed a significant increase in the occurrence of spurs on the first lumbar vertebra (6 in 6 litters vs. 1 in 1 control litter) and delayed ossification of sternebrae in the 200 ppm group (84 in 27 litters vs. 66 in 22 litters in control). These variations were not considered to be related to treatment, as their incidence was not increased in the 400 ppm group. The occurrence of delayed ossification of cervical vertebral centra was significantly increased with respect to controls in the 400 ppm group (79 in 25 litters vs. 60 in 22 litters). This variation was not considered to be a major malformation.
Test condition	:	Test animals: Male and female F344 rats were acclimated for at least 2 weeks prior to breeding. Adult virgin females (175-220 g at breeding) were

bred to adult males (one female to one male). The day sperm were found in a vaginal smear was considered Day 0 of gestation. Animals were randomly assigned to test groups according to their day 0 of gestation. Food and water were available ad libitum except during test material exposure.

Test vapor generation: Temperature and relative humidity were controlled at 21 degrees C and 50%, respectively. Vapor was generated by metering the liquid test material at controlled rates into vaporization tubes. Vapors from the tubes were swept into the chamber inlet ducts with preheated compressed air (120 to 135 degrees C) where they were mixed and diluted with incoming air by turbulence. Concentrations of test material were determined 1-2 times per hour throughout the exposure by infrared spectrometry. The actual concentration in the exposure chamber was determined by interpolation from standard curves derived form vapor standards of known concentrations. At least one standard was run before each daily exposure. The nominal concentration (ratio of amount of test material vaporized /total amount of air through the chamber) was calculated for each chamber for each exposure day. The mean daily time weighted average analytical concentrations for each chamber were within 1% of intended chamber concentrations. Mean daily nominal concentrations were in close agreement with the time weighted analytical concentrations indicating that test material losses were negligible.

Study conduct: Groups of 30-32 bred rats were exposed to filtered room air (control), or 100, 200 or 400 ppm test material for 6 hr/day on Days 6 through 15 of gestation. Exposure levels were based on results of preliminary studies that showed that 400 ppm produced maternal toxicity. Inhalation exposures were conducted in 4.3 m3 stainless steel and glass chambers under dynamic airflow conditions (800 liters air/min). Animals were observed daily throughout the experimental period for toxicity. Body weights were recorded on gestation days 6, 9, 12, 16 and 21. Food and water consumption was recorded at 3-day intervals beginning on Day 6 of gestation. Animals were killed on Day 21 of gestation. Maternal liver and kidney weights were recorded. The uterine horns were exteriorized and the following data were recorded: number and position of fetuses in utero, number of live and dead fetuses, number and position of resorption sites, number of corpora lutea, the sex, body weight and crown -rump length of each fetus, and any gross external alterations. The uteri of apparently nonpregnant animals were stained with a 10% solution of sodium sulfide and examined for implantation sites. These data were not used to calculate the incidence of resorptions. One-half of each litter was randomly selected for immediate examination under a dissection microscope for soft tissue abnormalities. The heads of these fetuses were removed, placed in Bouin's fixative and examined by serial sectioning. All fetuses were preserved in alcohol, eviscerated, stained with alizarin red-S and examined for skeletal alterations.

Statistical evaluations: Body weights and absolute and relative organ weights were evaluated by Bartlett's test for equality of variance. A parametric or nonparametric analysis of variance (ANOVA) was then conducted (as needed) followed by analysis with Dunnett's test (parametric data) or the Wilcoxon rank-sum test with Bonferroni's correction (nonparametric data) if the ANOVA was significant. Statistical outliers for food and water consumption data were identified using a sequential outlier test and excluded from analysis. Frequency of fetal alterations and resorption data among litters and the fetal population were analyzed using

a sensored Wilcoxon test. Other incidence data were analyzed with the Fisher exact probability test. The litter was considered the basic unit of analysis. The final interpretation of numerical data considered statistical analyses along with other factors such as dose-response relationships and whether the results were significant in the light of other biologic and pathological findings.

Test substance : Test material was analyzed by gas chromatography and found to be 98.81 % pure.

Conclusion : The only developmental treatment-related effect (small increase in occurrence of delayed ossification of cervical vertebral centra) occurred at a dose (400ppm) that clearly produced maternal toxicity. 1,2, dichlorobenzene was not teratogenic or embryotoxic.

Reliability : (1) valid without restriction

Flag : Key study for endpoint

19.11.2001 (24)

Species : rabbit
Sex : female
Strain : New Zealand white
Route of admin. : inhalation
Exposure period : days 6 through 18 of gestation
Frequency of treatment : 6 hr/day
Duration of test : to day 29 of gestation
Doses : 100, 200, 400 ppm (0.6, 1.2, 2.4 mg/l)
Control group : other: filtered room air
NOAEL Maternalt. : < 100 ppm
NOAEL Teratogen : = 400 ppm
Method : other
Year : 1985
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Remark : The majority of malformations observed in the study have occurred historically among rabbits in the laboratory and have been reported by others to occur spontaneously. The NOAEL listed above for teratogenicity (400 ppm) is also the NOAEL for fetotoxicity.

Result : Maternal: Rabbits exposed to all concentrations of test material lost weight during the first 3 days of exposure. Average body weight gains of controls, 100, 200 and 400 ppm groups on gestation days 6-8 were 21 +/- 62 g, -54 +/ 67 g, -44 +/- 50 g, and -62 +/- 54 g. Total weight gain over days 6-28 was less in treated animals than controls (but significant only for the 100 ppm group). There were no effects of treatment on absolute or relative liver or kidney weights. There were no significant differences in the % pregnant, number of litters, number of corpora lutea/dam, number of implantation sites/dam, number of fetuses/litter, % of implantation sites resorbed, % litters with resorptions, litters totally resorbed, the ratio of resorptions/liters with resorptions, fetal body weight or fetal crown-rump length between groups. The ratio of male/female offspring was significantly different from a 50:50 distribution in the 200 ppm group (61 males:39 females). It was not significantly altered in the 400 ppm group.

Fetal alterations: The incidence of major malformations, when considered individually or collectively, was not significantly increased in treated groups. The incidence of major malformations in the control, 100, 200 and 400 ppm treatment groups was 4 in 4 litters, 4 in 4 litters, 2 in 2 litters and 8 in 7 litters, respectively. One fetus from the control group exhibited anencephaly (failure of formation of the brain), aprosopia (missing facial

structures), and forelimb flexure. Examination of the cephalic tissue indicated that they also exhibited agnathia (missing jaw) and epitheliogenesis imperfecta (focal lack of formation of skin and adnexa). Another fetus from the control group had forelimb flexure, another had calloused ribs, and an additional control fetus had multiple malformations. In the 100 ppm group, a single fetus had a dilated renal pelvis, single fetuses from 2 litters had forked ribs, and one fetus from another litter had multiple malformations. In the 200 ppm group, one fetus had coarctation of the aortic arch and a fetus from a second litter was missing a vertebra. In the 400 ppm group, one fetus exhibited anencephaly, one had forelimb flexure and a rotated hind limb, and another had a rotated hind limb. One fetus from another litter had multiple malformations. A second fetus from this litter had a missing vertebra and fused ribs. Other skeletal malformations in the 400 ppm group included single cases of fused ribs, missing vertebra and hemivertebra. None of the malformations in treated animals were at a higher statistical frequency than controls.

Test condition : Test animals: Rabbits (3.5 to 4.5 kg) were artificially inseminated. The day of insemination was considered day 0 of gestation. Animals were randomly assigned to test groups. Food and water were available ad libitum except during test material exposure.

Test vapor generation: Temperature and relative humidity were controlled at 21 degrees C and 50%, respectively. Vapor was generated by metering the liquid test material at controlled rates into vaporization tubes. Vapors from the tubes were swept into the chamber inlet ducts with preheated compressed air (120 to 135 degrees C) where they were mixed and diluted with incoming air by turbulence. Concentrations of test material were determined 1-2 times per hour throughout the exposure by infrared spectrometry. The actual concentration in the exposure chamber was determined by interpolation from standard curves derived form vapor standards of known concentrations. At least one standard was run before each daily exposure. The nominal concentration (ratio of amount of test material vaporized/total amount of air through the chamber) was calculated for each chamber for each exposure day. The mean daily time weighted average analytical concentrations for each chamber were within 1% of intended chamber concentrations. Mean daily nominal concentrations were in close agreement with the time weighted analytical concentrations indicating that test material losses were negligible.

Study conduct: Groups of 28-30 inseminated rabbits were exposed to filtered room air (control), or 100, 200 or 400 ppm test material for 6 hr/day on Days 6 through 18 of gestation. Exposure levels were based on results of preliminary studies that showed that 500 ppm produced maternal toxicity. Inhalation exposures were conducted in 4.3 m3 stainless steel and glass chambers under dynamic airflow conditions (800 liters air/min). Animals were observed daily throughout the experimental period for toxicity. Body weights were recorded on gestation days 6, 9, 12, 15, 19 and 29. Animals were killed on Day 29 of gestation. Maternal liver and kidney weights were recorded. The uterine horns were exteriorized and the following data were recorded: number and position of fetuses in utero, number of live and dead fetuses, number and position of resorption sites, number of corpora lutea, the sex, body weight and crown -rump length of each fetus, and any gross external alterations. The uteri of apparently nonpregnant animals were stained with a 10% solution of sodium sulfide and examined for implantation sites. These data were not used to calculate the incidence of resorptions. One-half of each litter was randomly selected for immediate examination under a dissection microscope for soft

tissue abnormalities. The heads of these fetuses were removed and examined using a free-hand sectioning technique. All fetuses were preserved in alcohol, eviscerated, stained with alizarin red-S and examined for skeletal alterations.

Statistical evaluations: Body weights and absolute and relative organ weights were evaluated by Bartlett's test for equality of variance. A parametric or nonparametric analysis of variance (ANOVA) was then conducted (as needed) followed by analysis with Dunnett's test (parametric data) or the Wilcoxon rank-sum test with Bonferroni's correction (nonparametric data) if the ANOVA was significant. Statistical outliers for food and water consumption data were identified using a sequential outlier test and excluded from analysis. Frequency of fetal alterations and resorption data among litters and the fetal population were analyzed using a sensored Wilcoxon test. Other incidence data were analyzed with the Fisher exact probability test. The litter was considered the basic unit of analysis. The final interpretation of numerical data considered statistical analyses along with other factors such as dose-response relationships and whether the results were significant in the light of other biologic and pathological findings.

Test substance : Test material was analyzed by gas chromatography and found to be 98.81 % pure.
Conclusion : Test material was not embryotoxic or teratogenic at the doses used.
Reliability : (1) valid without restriction
Flag : Key study for endpoint
19.11.2001 (24)

Species : rat
Sex : female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : days 6 to 15 of gestation
Frequency of treatment : daily (presumed)
Duration of test :
Doses : 50, 100, 200 mg/kg
Control group : no data specified
NOAEL Maternal Tox : = 200 mg/kg bw
NOAEL Teratogen : = 200 mg/kg bw
Method : other
Year : 1983
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Remark : Meta and para dichlorobenzene also tested negative in this study.
Result : Treatment was not associated with any teratological effect. Maternal toxicity was not mentioned.
Test condition : Test material was given by gavage at 50, 100 and 200 mg/kg to pregnant rats (number not stated) on days 6 - 15 of gestation. Maternal weight gain, changes in microscopic examination and 15 biochemical parameters (types not stated) were used to evaluate maternal toxicity. Changes in litter size, fetal weight, deciduoma, skeletal and visceral examination, residue analysis and microscopic pathology were used to evaluate fetal toxicity.
Reliability : (4) not assignable. There is not enough information given in the abstract to determine the validity of the study or the NOAEL for fetotoxicity.
19.11.2001 (37)

5.10 OTHER RELEVANT INFORMATION

5.11 EXPERIENCE WITH HUMAN EXPOSURE

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7.1 END POINT SUMMARY

7.2 HAZARD SUMMARY

7.3 RISK ASSESSMENT

Robust Summaries and Repository of Knowledge for CAS No. 87-61-6

Existing Chemical	:	ID: 87-61-6
CAS No.	:	87-61-6
EINECS Name	:	1,2,3-trichlorobenzene
EINECS No.	:	201-757-1
TSCA Name	:	Benzene, 1,2,3-trichloro-
Molecular Formula	:	C6H3Cl3

Printing date	:	29.01.2003
Revision date	:	29.01.2003
Date of last Update	:	29.01.2003
Number of Pages	:	63
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 7

1.0.1 OECD AND COMPANY INFORMATION

1.0.2 LOCATION OF PRODUCTION SITE

1.0.3 IDENTITY OF RECIPIENTS

1.1 GENERAL SUBSTANCE INFORMATION

1.1.0 DETAILS ON TEMPLATE

1.1.1 SPECTRA

1.2 SYNONYMS

1.3 IMPURITIES

1.4 ADDITIVES

1.5 QUANTITY

1.6.1 LABELLING

1.6.2 CLASSIFICATION

1.7 USE PATTERN

1.7.1 TECHNOLOGY PRODUCTION/USE

1.8 OCCUPATIONAL EXPOSURE LIMIT VALUES

1.9 SOURCE OF EXPOSURE

1.10.1 RECOMMENDATIONS/PRECAUTIONARY MEASURES

1.10.2 EMERGENCY MEASURES

1.11 PACKAGING

1.12 POSSIB. OF RENDERING SUBST. HARMLESS

1.13 STATEMENTS CONCERNING WASTE

1.14.1 WATER POLLUTION

1.14.2 MAJOR ACCIDENT HAZARDS

1.14.3 AIR POLLUTION

1.15 ADDITIONAL REMARKS

1.16 LAST LITERATURE SEARCH

1.17 REVIEWS

1.18 LISTINGS E.G. CHEMICAL INVENTORIES

2.1 MELTING POINT

Value	:	= 52.6 ° C
Sublimation	:	
Method	:	other
Year	:	2001
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remarks	:	The test method is not given. However, the value stated is most likely a measured value, since this substance is a well-known, basic chemical. Kirk Othmer Encyclopedia of Chemical Technology, 4th Edition, Volume 6, page 89, cites a melting point value of 53.5° C, as also does Ulmann's Encyclopedia of Industrial Chemistry, 5th Ed., page 328.
Reliability	:	(2) valid with restrictions. Data were obtained from a MSDS.

04.12.2001 (42)

2.2 BOILING POINT

Value	:	= 221 ° C at 1013 hPa
Decomposition	:	
Method	:	other
Year	:	2001
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remarks	:	The test method is not given. However, the value stated is most likely a measured value, since this substance is a well-known, basic chemical, that is purified by distillation. Kirk Othmer Encyclopedia of Chemical Technology, 4th Edition, Volume 6, page 89, cites a boiling point value of 218.5° C, as also does Ulmann's Encyclopedia of Industrial Chemistry, 5th Ed., page 328.
Reliability	:	(2) valid with restrictions. Data were obtained from a MSDS.

04.12.2001 (42)

2.3 DENSITY

Type	:	density
Value	:	= 1.69 g/cm3 at 25° C
Method	:	other
Year	:	2001
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Reliability	:	(2) valid with restrictions. Data were obtained from a MSDS.

04.12.2001 (42)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value	:	= 1.33 hPa at 40° C
Decomposition	:	
Method		other (measured)
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remarks	:	The test method is not given. However, the value stated is most likely a measured value, since this substance is a well-known, basic chemical.
Reliability	:	(2) valid with restrictions. Data were obtained from a MSDS.

04.12.2001 (42)

2.5 PARTITION COEFFICIENT

Log pow	:	ca. 3.93 at ° C
Method		other (calculated)
Year	:	2001
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	EPIWIN KOWWIN Program (v1.66) was used to estimate Log Pow. EPIWIN KOWWIN uses previously determined individual bond fragment values for Log Kow and sums them up to obtain an overall value for the molecule.
Reliability	:	(2) valid with restrictions. Data were obtained by modeling

01.12.2001 (15)

2.6.1 WATER SOLUBILITY

Method	:	other
Year	:	2001
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	No measured value available. MSDS sheet states that substance is insoluble in water.
Reliability	:	(2) valid with restrictions. Data were obtained from a MSDS.

04.12.2001 (42)

Value	:	17.38 mg/l
Method	:	other: calculated using EPIWIN
Year	:	2002
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	EPIWIN WSKOW Program (v1.40) was used to estimate water solubility.
Reliability	:	(2) valid with restrictions. Data were obtained by modeling.

04.12.2001

2.6.2 SURFACE TENSION

2.7 FLASH POINT

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	Sun light
Light spect.	:	nm
Rel. intensity	:	based on Intensity of Sunlight
Direct photolysis		
Halflife t1/2	:	ca. 37.7 day (12 hour days)
Degradation	:	% after
Quantum yield	:	
Indirect photolysis		
Sensitizer	:	OH
Conc. of sens.	:	1500000 molecule/cm3
Rate constant	:	ca. .0000000000002817 cm3/(molecule*sec)
Degradation	:	% after
Deg. Product	:	
Method	:	other (calculated)
Year	:	2001
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The photolysis half life and hydroxyl rate constant for 1,2,4-trichlorobenzene (CAS No. 120-82-1) are 37.967 days and 0.2817E-12 cm3/molecule-sec, respectively. The photolysis half life and hydroxyl rate constant for 1,4-dichlorobenzene (CAS No. 106-46-7) are 26.708 days and 0.4005E-12 cm3/molecule-sec, respectively. These values were estimated in the same manner as for 1,2,3-trichlorobenzene.
Source	:	The photolysis half-life and hydroxyl rate constants were estimated using EPIWIN AOP Program (v1.90).
Reliability	:	(2) valid with restrictions. Data were obtained by modeling.

01.12.2001 (15)

3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH4	:	at degree C
t1/2 pH7	:	at degree C
t1/2 pH9	:	at degree C
Deg. Product	:	
Method	:	other (calculated)
Year	:	2001
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	The EPIWIN HYDROWIN Program (v1.67) was used (unsuccessfully) to estimate hydrolysis half-life. EPIWIN HYDROWIN cannot estimate a hydrolysis rate constant for chlorobenzenes. It has long been generally recognized that halobenzenes are highly resistant to water hydrolysis. Hydrolysis is likely therefore to be an unimportant degradative process for this substance.
Reliability	:	(4) not assignable

01.12.2001 (15)

3.1.3 STABILITY IN SOIL

3.2 MONITORING DATA

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	:	fugacity model level III
Media	:	water - air
Air (level I)	:	6.45
Water (level I)	:	11.9
Soil (level I)	:	
Biota (level II / III)	:	2.55
Soil (level II / III)	:	79.1
Method	:	calculated using EPIWIN. Inputs to the EPIWIN model were measured values for melting point (52.6°C), boiling point (221°C), and vapor pressure (1 mm Hg).
Year	:	2001
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The Level III Fugacity Model assumes a Henry's Law Constant of 0.00125 atm-m3/mole obtained by EPIWIN HENRY (v3.10), a vapor pressure of 0.0718 mm Hg (Modified Grain Method), an exp Log Kow of 4.05, and a soil sediment constant (Koc) of 4.6e+3. The EPIWIN BCF Program (v2.14) estimates a BCF (bioconcentration factor) of 262.1 (Log BCF = 0.77) using the equation Log BCF = 0.77 log Kow - 0.70 + correction. Henry's Law Constants estimated by EPIWIN for 1,2,4-trichlorobenzene (CAS No. 120-82-1) and 1,4-dichlorobenzene (CAS No. 106-46-7) are 0.00142 and 0.00241 atm-m^3/mole respectively.
Source	:	The EPIWIN Level III Fugacity Model, using the MacKay methodology, was used to calculate environment compartment parameters.
Reliability	:	(2) valid with restrictions. Data were obtained by modeling.
Flag	:	Key study for endpoint

01.12.2001 (15)

Type	:	fugacity model level I
Media	:	water – air
Air (level I)	:	79.7
Water (level I)	:	18.2
Soil (level I)	:	
Biota (level II / III)	:	
Soil (level II / III)	:	
Method	:	Other
Year	:	2001
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	For Comparison, fugacity model level I results for 1,2,4-trichlorobenzene (CAS No. 120-82-1) are 81.8% in air and 16.3% in water. Level I results for 1,4-dichlorobenzene (CAS No. 106-46-7) are 91.0% in air and 6.0% in water.
Reliability	:	(2) valid with restrictions. Data were obtained by Mackay Level I EQC modeling.
Flag	:	Key study for endpoint

01.12.2001 (42)

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type	:	aerobic
Test substance	:	as prescribed by 1.1-1.4
Remark	:	No biodegradation studies are available for CAS No. 87-61-6. Endpoint is filled using data from other category members and surrogates (see below).
Reliability	:	(2) valid with restrictions. Acceptable method. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.

Type	:	aerobic
Inoculum	:	activated sludge
Contact time	:	
Degradation	:	15 % after 28 day
Result	:	
Deg. Product	:	
Method	:	Directive 84/449/EEC, C.7 "Biotic degradation - modified MITI test"
Year	:	1991
GLP	:	yes
Test substance	:	Monochlorobenzene (CAS No. 108-90-7): chemically pure
Source	:	Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Acceptable method. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.

(1)

Type	:	aerobic
Inoculum	:	predominantly domestic sewage
Contact time	:	
Degradation	:	50 - 60 % after 20 day
Result	:	
Deg. Product	:	
Method	:	OECD Guide-line 301 D "Ready Biodegradability: Closed Bottle Test"
Year	:	1973
GLP	:	no
Test substance	:	Monochlorobenzene (CAS No. 108-90-7)
Source	:	Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Acceptable method. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.

(11)

Type	:	aerobic
Inoculum	:	other: sludge samplings from different sewage plants, rivers, bays and a lake

Concentration	:	100mg/l related to related to
Contact time	:	
Degradation	:	% after 28 day
Result	:	under test conditions no biodegradation observed
Deg. Product	:	
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	Monochlorobenzene (CAS No. 108-90-7)
Remark	:	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, the Minister of Health and Welfare, the MITI No. 1). This guideline corresponds to "301C, Ready Biodegradability: Modified MITI Test I" stipulated in the OECD Guideline for Testing of Chemicals (May 12, 1981) Sludge conc. : 30 mg/l
Source	:	Bayer AG Leverkusen EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Acceptable method. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000. (2)

Type	:	aerobic
Inoculum	:	activated sludge, industrial, non-adapted
Contact time	:	
Degradation	:	> 90 % after 15 day
Result	:	
Kinetic of test substance	:	5 day = 30 % 10 day = 70 % 15 day > 90 % % %
Deg. Product	:	
Method	:	other: Respirometrischer Test mit Sapromat [Respirometer test sludge]
Year	:	1982
GLP	:	no
Test substance	:	Monochlorobenzene (CAS No. 108-90-7)
Source	:	Hoechst AG Frankfurt/Main Bayer AG Leverkusen Hoechst AG Frankfurt/Main Clariant GmbH Frankfurt am Main EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000. (20)

Contact time	:	
Degradation	:	76.7 % after 2 month
Result	:	
Deg. Product	:	
Method	:	
Year	:	1987
GLP	:	no data

Test substance : Monochlorobenzene (CAS No. 108-90-7)
Remark : Concentration: 400 ppb, was added to a ground-water microcosm, incubated at 22 deg C. 76.7% was removed after 8 weeks.
Source : Petrasol B.V. Gorinchem
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.

(22)

Deg. Product :
Method :
Year :
GLP : no data
Test substance : Monochlorobenzene (CAS No. 108-90-7)
Remark : A large number of bacteria and fungi found in the environment are capable of degrading chlorobenzene and mineralizing it. 2- and 4-chlorophenol are products of this biodegradation. Degradation is generally slow in water and soil, but may be significant in some situations. Acclimation of the degrading microorganisms in an important factor.
Source : Petrasol B.V. Gorinchem
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable. Information came from IUCLID document created by European Chemicals Bureau, creation date 10-FEB-2000.

(22)

Type : aerobic
Inoculum : predominantly domestic sewage, adapted
Concentration : 4mg/l related to Test substance
related to
Contact time :
Degradation : 58 % after 20 day
Result :
Deg. Product :
Method : OECD Guide-line 301 D "Ready Biodegradability: Closed Bottle Test"
Year : 1977
GLP : no
Test substance : 1,2-dichlorobenzene (CAS No. 95-50-1)
Source : Bayer AG Leverkusen
Reliability : (2) valid with restrictions. OECD study. Information came from IUCLID document created by NICNAS, creation date 23-AUG-2001

11.05.1994

(10)

Type : aerobic
Inoculum : activated sludge
Concentration : 100mg/l related to
related to
Contact time :
Degradation : 0 % after 28 day
Result : under test conditions no biodegradation observed
Deg. Product :
Method : other: see remarks
Year :
GLP :
Test substance : 1,2-dichlorobenzene (CAS No. 95-50-1)
Remark : "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). Sludge conc. : 30 mg/l

Source : Bayer AG Leverkusen
Reliability : (2) valid with restrictions. Information came from IUCLID document created by NICNAS, creation date 23-AUG-2001

01.02.1994 (2)

Test substance : 1,2-dichlorobenzene (CAS No. 95-50-1)
Remark : Reduced from 50 mg/l to 2-4 mg/l in 7 days of incubation (92-96%) using industrial/municipal sludge.

Source : DALTRADE LTD LONDON
Reliability : (4) not assignable. Information came from IUCLID document created by NICNAS, creation date 23-AUG-2001, No reference was listed.

06.04.1998

Type : aerobic
Inoculum : activated sludge, non-adapted
Concentration : 100mg/l related to Test substance
related to
Contact time :
Degradation : = 0 % after 28 day
Result : under test conditions no biodegradation observed
Deg. Product :
Method : OECD Guide-line 301 C "Ready Biodegradability: Modified MITI Test (I)"
Year :
GLP : no data
Test substance : 1,3-dichlorobenzene (CAS No. 541-73-1)
Source : Hoechst AG Frankfurt/Main
Clariant GmbH Frankfurt am Main
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (2) valid with restrictions. OECD guideline study. Data were obtained from a IUCLID document prepared by the European Chemicals Bureau, 11-FEB-2000.

(2)

Type : aerobic
Inoculum : domestic sewage
Concentration : 10mg/l related to Test substance
related to
Contact time :
Degradation : = 58 % after 7 day
Result :
Deg. Product :
Method : other: Closed flask test according to Bunch und Chambers, J. Water. Poll. Contr. Fed. 39, 181-187 (1967)
Year :
GLP : No
Test substance : 1,3-dichlorobenzene (CAS No. 541-73-1)
Remark : 67% breakdown in Subculture 1 in 7 days
31% breakdown in Subculture 2 in 7 days
33% breakdown in Subculture 3 in 7 days
Source : Hoechst AG Frankfurt/Main
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

Test condition	:	Addition of yeast abstract 5 mg/l, temperature 25 degrees C, use of an emulsifying agent (triglyceride) for solubilization
Reliability	:	(2) valid with restrictions.

(38)(39)

Type	:	Aerobic
Inoculum	:	Pseudomonas sp. (Bacteria)
Concentration	:	200mg/l related to Test substance related to
Contact time	:	
Degradation	:	ca. 100 % after 96 hour(s)
Result	:	
Deg. Product	:	
Method	:	other: Respirometertest
Year	:	
GLP	:	No
Test substance	:	1,3-dichlorobenzene (CAS No. 541-73-1)
Remark	:	100% breakdown was achieved after 28 hours by using a mutated inoculum produced by irradiation
Source	:	Hoechst AG Frankfurt/Main Hoechst AG Frankfurt/Main Clariant GmbH Frankfurt am Main EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Test condition	:	The bacteria were obtained from an industrial waste sludge adapted to 1,3-diclorobenzene. The incubation temperature was at 30 degrees C.
Reliability	:	(2) valid with restrictions.

(26)

Type	:	Aerobic
Inoculum	:	Activated sludge, industrial, non-adapted
Degradation	:	= 20% after 15 day
Result	:	5 day = 5% 10 day = 15% 15 day = 20% 20 day = 20%
Method	:	OECD Guideline 302B "Inherent biodegradability: modified Zahn-Wallens test"
Year	:	1982
GLP	:	No
Test substance	:	1,4-dichlorobenzene (CAS No. 106-46-7)
Source	:	Hoechst AG Frankfurt/Main Hoechst AG Frankfurt/Main Clariant GmbH Frankfurt am Main
Reliability	:	(2) valid with restrictions. Study was not available. Information came from IUCLID data set created by European Chemicals Bureau, creation date 10-FEB-2000.

(19)

Type	:	aerobic
Inoculum	:	activated sludge
Concentration	:	100 mg/l
Degradation	:	0% after 14 day
Method	:	Other
Year	:	
GLP	:	no data

Test substance	:	1,2, 4-trichlorobenzene (CAS No. 120-82-1)
Remark	:	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 conc: 30 mg/l
Source	:	Bayer AG Leverkusen
Reliability	:	(2) valid with restrictions. Recognized method. Study was not available. Information came from IUCLID data set created by European Chemicals Bureau, creation date 11-FEB-2000.

(2)

Type	:	aerobic
Inoculum	:	activated sludge
Degradation	:	= 56% after 15 day
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	1,2, 4-trichlorobenzene (CAS No. 120-82-1)
Remark	:	After 5 days, 56% converted to CO2, 23% converted to polar metabolites, 7% evaporated. Radiolabeled compound used, degeneration rate measured by evolution of radiolabelled CO2.
Source	:	Bayer AG Leverkusen
Reliability	:	(4) not assignable. Study was not available. Information came from IUCLID data set created by European Chemicals Bureau, creation date 11-FEB-2000.

(33)

Type	:	aerobic
Inoculum	:	Other: Acinetobacter and Pseudomonas from industrial waste water treatment plants
Result	:	Other: oxidative dehalogenation
GLP	:	no
Test substance	:	1,2, 4-trichlorobenzene (CAS No. 120-82-1)
Remark	:	1,2, 4-trichlorobenzene (TCB) could be oxidatively dehalogenated with a quantitative release of inorganic chloride from organic bound chlorine.
Source	:	Bayer AG Leverkusen
Reliability	:	(4) not assignable. Study was not available. Information came from IUCLID data set created by European Chemicals Bureau, creation date 11-FEB-2000.

(35)

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	static
Species	:	Brachydanio rerio (Fish, fresh water)
Exposure period	:	24 hour(s)
Unit	:	mg/l
Analytical monitoring	:	yes
LC50	:	m = 3.1
Method	:	other
Year	:	1983
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The toxicity of monochlorobenzene, 1,2 and 1,4 -dichlorobenzenes (ortho and para), and 1,2,4- trichlorobenzene also were tested in this study. The 24-hour LC50 values for these materials were 10.5, 6.8, 4.25, and 6.3 mg/l, respectively. It is assumed that the conditions under which the test material was tested were similar to the algal study reported below, since the study was performed in the same laboratory at a later date. The results are reported as 48 hour LC50 values in the study; however, the methods state that the experiment was only carried out for 24 hours. Therefore, the values are reported in this summary as 24 hour.
Result	:	The 24 hour LC50 value (with confidence limits) was 3.1 (2.3 - 4.1) mg/l. The slope of the effect concentration curve was 1.37. The concentrations used to calculate the LC50 value were nominal (not listed), as analytical concentrations were within 10% of nominal concentrations.
Test condition	:	An ISRA test (Quaderni dell'Instituto di Ricerca sulle Acgue, 11, Consiglio Nazionale delle Ricerche-Roma, 1973). was performed to establish the 24 hr LC50 value. Two closed bottles (10 l) containing 5 fish were used for each concentration (not stated). The test vessels were modified according to the method of Galassi and Vighi (Chemosphere 10: 1123, 1981) in order to maintain constant concentration and to allow for sampling of the medium without opening the vessel. Water conditions during the experiment were: hardness 320 mg CaCO3/l, pH 7.4, oxygen not less than 70% saturation at the end of the test, and a temperature of 23 degrees. Concentrations of test material in vessels were measured analytically (by gas chromatography) at the beginning and end of the tests. Dilute solutions were extracted with n-hexane before analysis. The limit of detection was 0.002 mg/l. Mortality data were analyzed according to the method of Litchfield and Wilcoxon.
Test substance	:	Test material was analytical grade
Reliability	:	(2) valid with restrictions. It is unclear whether results are for 24 or 48 hours.

29.11.2001 (12)

Type	:	flow through
Species	:	Pimephales promelas (Fish, fresh water)

Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	yes
NOEC	:	m = .69
LC50	:	m = 2.4
LC100	:	m = 3.5
Method	:	EPA OTS 797.1400
Year	:	1988
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Based on EPA criteria the test material is classified as moderately toxic to the flathead minnow.
Result	:	The water quality measurements remained within acceptable ranges throughout the test and were unaffected by test concentration. The pH ranged from 6.9 to 7.2 at all time points, the dissolved O2 concentration ranged from 7.9 - 10.0 mg/l, and temperature ranged from 22-23 degrees C. The system that delivered test solutions to the exposure aquaria functioned properly, as evidenced by consistent results of test concentration measurements during the preliminary test. During the test, a small amount of precipitate was observed in the mixing chamber (doses not stated). However, there were no signs of insolubility in the test aquariums at any of the test concentrations. Measured concentrations of test material in aquariums treated with 1.1, 1.6, 2.5, 3.9 and 6.0 mg/l averaged (+/-SD) 0.69 +/- 0.022, 0.96 +/- 0.29, 1.5 +/- 0.10, 2.2 +/- 0.43, and 3.5 +/- 0.33 mg/l. Measured concentrations in controls were less than the limits of detection. In general, there was good agreement between values obtained at 0 and 96 hours. There was also good agreement between replicates (with the exception of one replicate of the second to lowest concentration). The mean measured concentrations were 56 to 63% of nominal concentrations. Analyses of the quality assurance samples resulted in measured concentrations that were consistent with the predetermined recovery range. Exposure to a measured concentration of 3.5 mg/l produced an average of 25% (20 and 30%) mortality at 24 hours, 95% (90 and 100%) at 48 hours and 100% at 72 hours. Exposure to a measured concentration of 2.2 mg/l caused no mortality at 24 hours, 5% (0 and 10%) at 48 hours, 15% (10 and 20 %) at 72 hours, and 30% (20 and 40%) at 96 hours. No deaths were observed at 1.5 mg/l, however; this dose produced toxicity as evidenced by partial loss of equilibrium, darkened pigmentation and lethargy. Several fish treated with 0.96 mg/l also exhibited lethargy and darkened pigmentation. There were no adverse effects seen in the 0.69 mg/l or either of the control groups. The LC50 values and 95% confidence intervals (as calculated by probit analysis for 48 hours and by nonlinear interpolation and binomial probability for 72 and 96 hours) were > 3.5 mg/l at 24 hours, 2.8 (2.5 - 3.1) mg/l at 48 hours, 2.6 (2.2-3.5) mg/l at 72 hours, and 2.4 (1.5-3.5) mg/l at 96 hours. The no observed effect concentration through 96 hours was 0.69 mg/l.
Test condition	:	The protocol followed closely followed the EPA/OTS guideline for the acute toxicity to freshwater fish, 40CFR, section 797.1400. Test organisms and water: Fish (mean wet weight of 0.46 g and length of

36 mm) were obtained from brood cultures maintained at the test site. They were kept in a 500 l fiberglass tank for a 14 day holding period under a regulated photoperiod of 16 hours of light and 8 hours darkness. The well water that flowed into the tank had a total hardness and alkalinity as CaCO3 of 27-34 mg/l and 24-27 mg/l, respectively, a pH of 6.4 - 7.3, a dissolved oxygen concentration of 67-85% of saturation, a temperature of 19-21 degrees C and a specific conductance range of 100-110 micromhos/cm. The flow rate was 9 tank volume replacements/day. All fish were fed a dry commercial pelleted food ad libitum except during the 48 hours prior to testing.

The dilution water was from the same source as holding tank water and was characterized as having a total hardness and alkalinity as CaCO3 of 30-32 mg/l and 28-34 mg/l, respectively, a pH of 7.0 - 7.1, and a specific conductance range of 110-120 micromhos/cm. Water quality was biologically monitored by assessing the survival and reproduction of stock cultures of Daphnia maintained in it. Routine analytical analyses of pesticides and PCBs in the water were performed. None of these compounds were detected in the water (detection limit of 0.1 mg/l).

Test material: A stock solution of 13 mg test material/ml was prepared by diluting 13.04 grams of 1,2,3-trichlorobenzene with acetone to a total volume of 1000 ml. A peristaltic pump was used to inject the stock solution into the exposure system's mixing cell. The mixing cell was partially submerged in an ultrasonicating bath to promote mixing of the test material in the dilution water. Each test aquarium measured 39 x 20 x 25 cm with a 19.5 cm high standpipe which maintained a constant test water volume of 15 l. The flow rate of exposure solutions to each test aquarium provided for 6.5 volume additions per 24 hours.

Test condition: The test was conducted using an exposure system consisting of a modified proportional diluter, a temperature controlled water bath and a set of 14 test aquaria. Five concentrations of the test material (1.1, 1.6, 2.5, 3.9 and 6.0 mg/l), a water control and a solvent control (the maximum amount of acetone in any test concentration, 460 microliters/l) were tested. Concentrations chosen for use were based on results of a preliminary study that showed 100% lethality at 6 mg/l, 40% mortality at 3.9 mg/l and no mortality at 2.5 mg/l. All tests were performed in duplicate. Test aquaria were labeled to identify the test sample concentration. Light intensity of the test area ranged from 20-100 footcandles.

Each test aquarium contained two retention chambers that housed the fish during the exposure period. Test aquaria were positioned in a water bath containing heated (22 +/- 1 degrees C), circulating water. All aquariums were sampled from the midpoint of the aquarium and analyzed for test material prior to the start of exposure to determine if sufficient quantities of test material were being delivered and maintained in the aquariums.

The test was initiated by randomly adding 10 fish per retention chamber (maximum loading concentration 0.047 g/l of solution. Mortalities, biological observations and observations of the physical characteristics of the test solutions were recorded at test initiation and in 24 hour intervals. Dead organisms were removed at each observation. The criteria used for determining death were lack of gill movement and a reaction to gentle prodding. Dissolved oxygen, pH, and temperature were measured once daily in each replicate.

Samples of the exposure solutions were collected 96 hours after the initiation of the test. Three blind samples were prepared at each sampling time (at concentrations of test material unknown to the analyst) and were maintained with the exposure solution samples through analysis. All samples were analyzed for test material using a gas chromatograph. A method validation study conducted prior to the test established an average recovery of test material of 92.6 +/- 9.19% from freshwater.

Statistical analyses: Three statistical methods were available in the computer program that was used to analyze data: moving average angle analysis, probit analysis, and nonlinear interpolation with 95% confidence intervals calculated by binomial probability. Moving average angle and probit analyses were valid only if at least two concentrations produced a mortality of between 0 and 100%. If two or more statistical methods produced credible results, then the method which yielded the smallest 95% confidence interval was selected for determination of the LC50 and no observable effect values.

Test substance : Purity of test substance was 99.7%
Reliability : (1) valid without restriction
18.11.2001 (36)

Type : static
Species : Salmo gairdneri (Fish, estuary, fresh water)
Exposure period : 24 hour(s)
Unit : mg/l
Analytical monitoring : yes
LC50 : m = .71
Method : other
Year : 1983
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Remark : The toxicity of monochlorobenzene, 1,2 and 1,4 -dichlorobenzenes (ortho and para), and 1,2,4- trichlorobenzene also were tested in this study. The 24-hour LC50 values for these materials were 4.1, 2.3, 1.18, and 1.95 mg/l, respectively.

It is assumed that the conditions under which the test material was tested were similar to the algal study reported below, since the study was performed in the same laboratory at a later date.

The results are reported as 48 hour LC50 values in the study; however, the methods state that the experiment was only carried out for 24 hours. Therefore, the values are reported in this summary as 24 hour.

Result : The 24 hour LC50 value (with confidence limits) was 0.71 (0.65-0.77) mg/l. The slope of the effect concentration curve was 1.15.

The concentrations used to calculate the LC50 were nominal (not listed), as analytical concentrations were within 10% of nominal concentrations.

Test condition : An ISRA test (Quaderni dell'Instituto di Ricerca sulle Acgue, 11, Consiglio Nazionale delle Ricerche-Roma, 1973). was performed to establish the 24 hr LC50 value. Two closed bottles (10 l) containing 5 fish were used for each concentration (not stated). The test vessels were modified according to the method of Galassi and Vighi (Chemosphere 10: 1123, 1981) in order to maintain constant concentration and to allow for sampling of the medium without opening the vessel.

Water conditions during the experiment were: hardness 320 mg CaCO3/l,

pH 7.4, oxygen not less than 70% saturation at the end of the test, and a temperature of 15 degrees.

Concentrations of test material in vessels were measured analytically (by gas chromatography) at the beginning and end of the tests. Dilute solutions were extracted with n-hexane before analysis. The limit of detection was 0.002 mg/l.

Mortality data were analyzed according to the method of Litchfield and Wilcoxon.

Test substance : Test material was analytical grade
Reliability : (2) valid with restrictions
29.11.2001 (12)

Type :
Species : other: freshwater fish
Exposure period : 96 hour(s)
Unit : mg/l
Analytical monitoring : no
LC50 : c = 2.063
Method : other: ECOSAR program (v 0.99g)
Year : 2002
GLP : no
Test substance : as prescribed by 1.1 - 1.4
Test condition : The LC50 value was estimated by the ECOSAR program (v0.99g), with a log Kow of 3.93 and water solubility of 17.91 mg/l calculated by EPIWIN.
Reliability : (2) valid with restrictions. Data were obtained by modeling.
Flag : Supportive study for endpoint

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 24 hour(s)
Unit : mg/l
Analytical monitoring : yes
EC50 : m = .35
Method : other
Year : 1983
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Remark : The toxicity of monochlorobenzene, 1,2- and 1,4 -dichlorobenzenes (ortho and para), and 1,2,4- trichlorobenzene also was tested in this study. The 24-hour EC50 values for these materials were 4.8, 0.78, 1.6, and 1.2 mg/l, respectively.

It is assumed that the conditions under which the test material was tested were similar to the algal study reported below, since the study was performed in the same laboratory at a later date.

Result : The 24 hour EC50 value for immobilization was 0.35 mg/l. The confidence interval was not determined. The slope of the effect concentration curve was 1.16.

The concentrations used to calculate the EC50 were nominal (not listed),

as analytical concentrations were within 10% of nominal concentrations

Test condition : An AFNOR test (Norme Experimentale NFT, 90-301, 1974) was performed to determine the IC50 (concentration for 50% of animals to be immobilized) at 24 hours. The test vessels were modified according to the method of Galassi and Vighi (Chemosphere 10: 1123, 1981) in order to maintain constant concentration and to allow for sampling of the medium without opening the vessel.

Concentrations of test material in vessels were measured analytically (by gas chromatography) at the beginning and end of the tests. Dilute solutions were extracted with n-hexane before analysis. The limit of detection was 0.002 mg/l.

The EC50 value was taken from curves fitted by eye on log probability paper and not elaborated, being very close to the concentrations with 0 and 100% immobilized animals.

Reliability : (2) valid with restrictions

29.11.2001 (12)

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
Analytical monitoring : no
EC50 : m = 2.71
Method : other
Year : 1983
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Remark : Concentrations of test material were not confirmed analytically.
Using a molecular weight of 181.44981, a value of 2.722 mg/l can be calculated.

Result : Dissolved oxygen concentration dropped from 9 to 5 mg/l and pH from 7 to 6 during the 48 hour exposure period. The LC50 of the phenol control was 30 mg/l, which fell between reported 48-hour LC50 values (21 to 100 mg/l).

The LC50 value determined for 1,2,3-trichlorobenzene was 15 mmol/m3.

Test condition : A saturated aqueous solution of test material was made by adding an excess quantity to distilled water in 250 ml glass flasks with glass stoppers. The solution was stirred gently for 24 hours and was then allowed to settle at room temperature for at least 48 hours.

Stock cultures of Daphnia were fed trout chow and algae and were kept in distilled water with a pH of 6.5. Daphnia of similar size and age (1.5 mm in length, 4-6 days old) were used in the test.

Tests were performed in 24 ml glass vials of known mass with Teflon-lined screw on caps. Ten animals were tested per concentration, with 2-4 replicates per test concentration. Daphnia (number not stated) were placed in the vials, followed by varying amounts of distilled water. The saturated test solution was carefully withdrawn with a syringe so as to not disturb precipitated material and was added to the test vials to the point of overflow. Vials were weighed before and after test material addition. Vials were immediately closed tightly and shaken gently for a few minutes. All solutions were prepared in situ and no analysis was done since it was assumed that there was negligible loss of test material from the saturated solution by volatilization. The concentrations of test material added were

determined from the mass of the solution added and available literature solubility data. Control tests were conducted by adding water up to the point of overflow (and not adding any amount of saturated test solution). A test with a positive control (phenol) also was conducted.

Temperatures were maintained at 23 +/- 2 degrees C. The vials had no air spaces and were not aerated. Daphnia were not fed during the test. Daphnia mortalities were observed at 48 hours. Death was defined as immobility characterized by lying on the bottom of the vial, with no visible movement of antennae, thoracic appendages or postabdomen, after tapping or rotating of the vial. LC50 values and their 95% confidence intervals were calculated using interpolation from log concentration-mortality curves. Tests were considered valid if the average control mortality did not exceed 10%. Dissolved oxygen concentrations and pH were measured in control vials at the beginning and end of the experiment.

Test substance : Purity of the test material was at least 97%.
Reliability : (2) valid with restrictions
18.11.2001 (6)

Type : flow through
Species : Mysidopsis bahia (Crustacea)
Exposure period : 96 hour(s)
Unit : mg/l
Analytical monitoring : yes
NOEC : m = .21
EC50 : m = .35
Method : EPA OTS 797.1930
Year : 1988
GLP : yes
Test substance : as prescribed by 1.1 - 1.4
Remark : The toxicity of 1,2,4-trichlorobenzene (CAS No. 120-82-1) to Mysodopsis bahia was determined in a similar study (Springborn Life Sciences, Inc., SLS Report 88-4-2704, Dated June 28, 1988). The NOEC, 24-, 48-, 72-, and 96-hour LC50 values were 0.19, 0.72, 0.60, 0.51 and 0.49 mg/l.
Result : The water quality measurements remained within acceptable ranges throughout the test and were unaffected by test concentration. The pH ranged from 7.7 to 7.8 at all time points, the dissolved O2 concentration was not less than 6.1 mg/l (86% saturation), and salinity remained at 30 °/oo. Temperature of the control solution that was continuously monitored ranged from 24-25 degrees C.

The system that delivered test solutions to the exposure aquaria functioned properly, as evidenced by consistent results of test concentration measurements during the preliminary test. During the test, a precipitate was observed in both replicates of the highest test concentration. This was removed from the test vessels. Afterward, no precipitate was observed for the remainder of the test. There were no signs of insolubility at the other test concentrations.

Measured concentrations of test material in aquariums treated with 0.89, 1.4, 2.1, 3.3 and 5.0 mg/l averaged (+/-SD) 0.12 +/- 0.018, 0.13 +/- 0.056, 0.21 +/- 0.101, 0.35 +/- 0.161, and 0.57 +/- 0.16 mg/l. Measured concentrations in controls were less than the limits of detection. There was fairly good agreement between values obtained at 48 and 96 hours. In general, concentrations of test material that were measured at the beginning of the study were less than those determined at 48 and 96 hours. Although the mean measured concentrations were generally only

11% of nominal, the expected concentration gradient (65% dilution factor) was maintained. Analyses of the quality assurance samples resulted in measured concentrations that were consistent with the predetermined recovery range.

Exposure to a measured concentration of 0.57 mg/l produced an average of 10% mortality at 24 hours, 75% at 48 (70 and 80%) and 72 hours (70 and 80%), and 85% (70 and 100%) at 96 hours. Exposure to a measured concentration of 0.35 mg/l caused no mortality at 24 hours, 40% (30 and 50%) at 48 hours, 50% (40 and 60 %) at 72 hours, and 55% (50 and 60%) at 96 hours. Exposure to measured concentrations of 0.21 and 0.12 mg/l produced 10% lethality in one aquarium and 0% in the other at 96 hours. No mortality occurred at 0.13 mg/l or in controls.

The LC50 values with 95% confidence intervals (as calculated by moving average angle analysis) were > 0.57 mg/l at 24 hours, 0.41 (0.36 - 0.49) mg/l at 48 hours, 0.39 (0.34-0.46) at 72 hours, and 0.35 (0.30-0.42) at 96 hours. The no observed effect concentration through 96 hours was 0.21 mg/l.

Test condition : The protocol followed closely followed the EPA/OTS guideline for the "Mysid shrimp acute toxicity test", 40CFR, section 797.1930.

Test organisms and water: Mysids were obtained from laboratory cultures maintained at the test site. They were kept under a regulated photoperiod of 16 hours of light (70-110 footcandles) and 8 hours darkness. They were fed brine shrimp nauplii two times daily and Hatchfry Encapsulon three times weekly. Culture and dilution water was prepared by filtering natural seawater collected from the Cape Cod Canal, Bourne Massachusetts. Seawater was transferred by a pump (fiberglass reinforced thermoplastic housing) though a PVC pipe and transported to the laboratory in a 3400 l tank. There it was filtered through a series of polypropylene core filters (20- and 5-micron) and then recirculated within an epoxy-lined concrete reservoir prior to use. Before use it was pumped under constant pressure through an activated carbon filter and a polypropylene heat exchanger system. Water quality was biologically monitored by assessing the survival of stock cultures of mysids maintained in it. Routine analytical analyses of pesticides and PCBs in the water were performed. None of these compounds were detected in the water (detection limit of 0.1 micrograms/l).

Test material: A stock solution of 77 mg test material/ml was prepared by diluting 15.4 grams of 1,2,3-trichlorobenzene with acetone to volume in a 200 ml volumetric flask. A syringe pump was used to inject the stock solution into the exposure system's mixing cell. Each test aquarium measured 39 x 20 x 25 cm with a self-starting siphon attached to the drain. The flow rate of exposure solutions to each test aquarium was equivalent to 7 volume additions per 24 hours. Aquarium volume fluctuated between 4 and 7 l and ensured a solution exchange within the organism retention chamber.

Test condition: The test was conducted using an exposure system consisting of a modified proportional diluter, a temperature controlled water bath and a set of 14 test aquaria. Five concentrations of the test material (0.89, 1.4, 2.1, 3.3 and 5.0 mg/l), a seawater control and a solvent control (the maximum amount of acetone in any test concentration, 65 microliters/l) were tested. Concentrations chosen for use were based on results of a preliminary study that showed 100% lethality at 10 mg/l and no mortality at 1 mg/l. All tests were performed in duplicate. Test aquaria were labeled to

identify the test sample concentration. Light intensity of the test area ranged from 10-110 footcandles.

Each test aquarium contained two mysid retention chambers which housed the organisms during the exposure period. Test aquaria were positioned in a water bath containing heated (25 +/- 1 degrees C), circulating water. All aquariums were sampled from the midpoint of the aquarium and analyzed for test material prior to the start of exposure to determine if sufficient quantities of test material were being delivered and maintained in the aquariums.

The test was initiated by randomly adding 5 shrimp (< = 24 hours old) per retention chamber (maximum loading concentration < 3 mg/l of solution). Mortalities, biological observations and observations of the physical characteristics of the test solutions were recorded at test initiation and in 24 hour intervals. Dead organisms were removed at each observation. The criteria used for determining death were absence of mobility and failure to respond to gentle prodding. Live brine shrimp naplii were added twice daily to each retention chamber containing live test organisms. Dissolved oxygen, pH, salinity and temperature were measured once daily in each replicate. Temperature in one replicate of the solvent control was also continuously monitored.

Samples of the exposure solutions were collected 48 and 96 hours after the initiation of the test. Three blind samples were prepared at each sampling time (at concentrations of test material unknown to the analyst) and were maintained with the exposure solution samples through analysis. All samples were analyzed for test material using a gas chromatograph. A method validation study conducted prior to the test established an average recovery of test material of 93.1 +/- 4.86% from seawater.

Statistical analyses: Three statistical methods were available in the computer program that was used to analyze data: moving average angle analysis, probity analysis, and nonlinear interpolation with 95% confidence intervals calculated by binomial probability. Moving average angle and probit analyses were valid only if at least two concentrations produced a mortality of between 0 and 100%. If two or more statistical methods produced credible results, then the method which yielded the smallest 95% confidence interval was selected for determination of the LC50 and no observable effect values.

Test substance : Purity of test substance was 99.7%
Conclusion : Based on EPA criteria, the test material would be classified as highly toxic to Mysidopsis bahia.
Reliability : (1) valid without restriction
29.11.2001 (37)

Type :
Species : Daphnia
Exposure period : 48 hour(s)
Unit : mg/l
Analytical monitoring : no
LC50 : c = 2.526
Method : other: ECOSAR program (v 0.99g)
Year : 2002
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Test condition : The LC50 value was estimated by the ECOSAR program (v0.99g), with a log Kow of 3.93 and water solubility of 17.91 mg/l calculated by EPIWIN.
Reliability : (2) valid with restrictions. Data were obtained by modeling.
Flag : Supportive study for endpoint

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Selenastrum capricornutum (Algae)
Endpoint : growth rate
Exposure period : 96 hour(s)
Unit : mg/l
Analytical monitoring : yes
EC50 : m = .9
Method : other: modification of AAPBT
Year : 1981
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Remark : Culture medium and test conditions were similar to the AAPBT, with the exception that the temperature was 20 +/- 1 degrees C.

Chlorobenzene, 1,2- dichlorobenzene, 1,4- dichlorobenzene and 1,2,4 trichlorobenzene also were tested in this study. The 96-hour EC50 values for these materials were 12.5, 2.2, 1.6, and 1.4 mg/l, respectively. It was remarked that the EC50 values calculated for chlorobenzenes by this method are at least 2 fold lower than other methods that did not analytically control test material concentrations.

Result : Initial concentrations could not be measured due to high volatility of the test material. Within a few minutes of adding the test material to the flasks the concentration was very low compared to theoretical values. Therefore, the initial concentrations calculated from dilution of the titrated stock solutions were assumed to be the initial concentrations. Equilibrium concentrations were calculated as the mean of the analytical concentrations in samples taken after the equilibrium period and at 48 and 96 hours. For initial concentrations of 0.58, 1.15, 2.30, 3.46, 4.61,
5.76 and 8.06 mg/l, equilibrium concentrations of 0.26, 0.45, 1.09, 1.5, 1.79, 1.95 and 2.94 were determined. The mean initial concentration/equilibrium concentration (Ci/Ceq +/- SD) was 2.49 +/- 0.3. Henry's constant (H) can be calculated from the equation Ci = Ceq(H x air volume in the flask/culture medium volume + 1). The value calculated from this equation (0.07) was fairly close to x [Note: we need to add a value from EPIWIN].

After the 24 hour equilibration period, the concentration of test material in the culture medium remained almost constant. Differences in the values obtained at equilibrium and after 48 or 96 hours were within the range of acceptable analytical variability.

The 96 hour EC50 value determined for 1,2,3-trichlorobenzene inhibition of algal fluorescence was 0.9 mg/l. The maximum tested concentration that produced no effect was 0.22 mg/l and the minimum concentration that was 100% effective was 3.0 mg/l.

Test condition : A stock solution of test material was made by adding test material at 10 times higher than the saturation solubility to distilled water in a closed vessel. The solution was stirred for 48 hours and decanted for 24 hours. The supernatant was filtered through paper filters and the concentration was measured. Final solutions were made by adding 10 ml of stock culture

medium to different amounts of stock solution. Solutions were then diluted to 100 ml with distilled water and quickly transferred into the 2 liter spherical culture flasks. The medium to flask volume ratio (0.047) was low enough to avoid notable carbon dioxide deficiency. Flasks were closed by screw caps with both silicone rubber (4 mm thick) and teflon gaskets. The caps were pierced by a stainless steel needle dipped into the culture medium. Sampling for measurement of algal growth and toxicant concentrations was made through the needle by means of a syringe. The outer end of the needle was closed with Parafilm.

Capped flasks were shaken for 24 hours at 20 degrees C to let vapor and liquid phases equilibrate. The algal inoculum was then added at cell concentration of 5 x 10E6 cells/l. Culture medium and test conditions were similar to the AAPBT, with the exception that the temperature was 20 +/- 1 degrees C.

Concentrations of test material in the flasks were measured by GC after the 24 hour equilibration period and 48 and 96 hours after the inoculum was added. Aqueous solutions (4 microliters) were injected directly into the GC with a flame ionization detector.

Algal growth was measured at 24, 48, and 96 hours by in vivo fluorescence (CJ Lorenzen, Deep Sea Res. 13:223-227, 1966). Results were expressed as a percentage of the growth in the control culture, and the EC50 was interpolated from the data.

Test substance : Purity of test material was not stated.
Reliability : (2) valid with restrictions. Purity of test material was not stated. The control response in terms of the increase in cell concentration, and the pH, dissolved oxygen concentration and temperatures were not provided.

19.11.2001 (17)

Type :
Species : other: green algae
Exposure period : 96 hour(s)
Unit : mg/l
Analytical monitoring : no
EC50 : c = 1.765
Method : other: ECOSAR program (v 0.99g)
Year : 2002
GLP : no
Test substance : as prescribed by 1.1 - 1.4
Test condition : The EC50 value was estimated by the ECOSAR program (v0.99g), with a log Kow of 3.93 and water solubility of 17.91 mg/l calculated by EPIWIN.
Reliability : (2) valid with restrictions. Data were obtained by modeling.
Flag : Supportive study for endpoint

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6.1 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO OTHER NON-MAMM. TERRESTRIAL SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Test substance	:	No acute oral toxicity studies were available on CAS No. 87-61-6. Data from other category members and surrogates are used to fill this endpoint (see below).
Reliability	:	(2) valid with restrictions.

Type	:	LD50
Species	:	rat
Strain	:	CFE
Sex	:	male/female
Number of animals	:	
Vehicle	:	
Value	:	= 756 mg/kg bw
Method	:	other
Year	:	1969
GLP	:	no
Test substance	:	other TS
Result	:	The LD50 value was 756 mg/kg (556-939 mg/kg, 95% confidence limit). All deaths occurred within 5 days of exposure. Animals that died and survivors did not exhibit evidence of gross pathological lesions.
Test condition	:	Rats (150 - 250 g) were fasted overnight and were gavaged with various concentrations of test material (4/sex/group, doses not stated). Water and feed were freely available after dosing. Animals were observed for 10 days At the end of that time all survivors were weighed, examined and necropsied. Animals that died were also necropsied. The LD50 value and 95% confidence interval was calculated (method unknown).
Test substance	:	Test substance was 1,2,4-trichlorobenzene (CAS No. 120-82-1).
Reliability	:	(4) not assignable. The purity of test material is unknown. The doses tested, the number of deaths at each dose and the method used to calculate the LD50 value were not listed. A related test material was utilized.

27.11.2001 (9)

Type	:	LD50
Species	:	mouse
Strain	:	CF No 1.
Sex	:	male/female
Number of animals	:	
Vehicle	:	
Value	:	= 766 mg/kg bw
Method	:	other
Year	:	1969
GLP	:	no
Test substance	:	other TS
Result	:	The LD50 value was 766 mg/kg (601-979 mg/kg, 95% confidence limit). All deaths occurred within 3 days of exposure. Animals that died and survivors did not exhibit evidence of gross pathological lesions.
Test condition	:	Mice (18 - 23 g) were fasted overnight and were gavaged with various concentrations of test material (4/sex/group, doses not stated). Water and feed were freely available after dosing. Animals were observed for 10 days At the end of that time all survivors were weighed, examined and

necropsied. Animals that died were also necropsied. The LD50 value and 95% confidence interval was calculated (method unknown).

Test substance : Test substance was 1,2,4-trichlorobenzene (CAS No. 120-82-1).

Reliability : (4) not assignable. The purity of test material is unknown. The doses tested, the number of deaths at each dose and the method used to calculate the LD50 value were not listed. A related test material was utilized.

27.11.2001 (9)

Type : LD50
Species : rat
Strain : Sherman
Sex : male/female
Number of animals : 40
Vehicle : peanut oil
Value : 3863 mg/kg (males), 3790 mg/kg (females)
Method : other
Year : 1986
GLP : no data
Test substance : other TS
Result : The LD50 values (with 95% confidence limits) were 3863 (3561-4153) mg/kg in males and 3790 (3425-4277) mg/kg in females. The slope of the curve was 14.5 and 8.4 for males and females, respectively.

Test condition : Test material was dissolved in peanut oil. Test material was administered in a volume of 5 ml/kg body weight. Rats were 90 days old when tested. A minimum of 10 animals/group (note stated if evenly divided among sex) and 4 doses were tested (up to 16.7 ml/kg). Animals were observed for at least 14 days or until all survivors had recovered from signs of toxicity. LD50 values and related parameters were calculated using a computer-based implementation of Finney's maximum likelihood probit technique.

Test material : Test material was para dichlorobenzene (CAS No. 106-46-7)

Reliability : (2) valid with restrictions. Purity of test material was not given.

19.11.2001 (16)

Type : LD50
Species : rat
Strain : Sprague-Dawley
Sex : male/female
Number of animals : 20
Vehicle :
Value : = 1540 mg/kg bw
Method : other
Year : 1976
GLP : no
Test substance : other TS
Result : There were no deaths at 1260 mg/kg, 3/5 deaths (2 males, 1 female) at 1580 mg/kg, 3/5 deaths at 2000 mg/kg (1 male, 2 females) and 5/5 deaths with 2510 mg/kg. All deaths occurred within 3 days. The LD50 value was 1540 mg/kg and the 95% confidence interval was 1380 - 1710 mg/kg.

Test condition : Rats (220 - 250 g) were dosed orally (2-3 males and 2-3 females/group; 5 males and females/group) with 1260, 1580, 2000, and 2510 mg/kg and were observed over 14 days.

Test substance : Test material was chlorobenzene (CAS No.108-90-7). Purity was 96.7%.

Reliability : (2) valid with restrictions. No details about the study design or statistical analyses were given. Test material is a related substance.

27.11.2001 (3)

Type	:	LD50
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	50
Vehicle	:	
Value	:	= 1100 mg/kg bw
Method	:	other
Year	:	1980
GLP	:	no data
Test substance	:	other TS
Result	:	Initial average weights of males in the 631, 794, 1000, 1260 and 1580 mg/kg groups were 200, 195, 215, 205 and 205 g (respectively). Weights of females in these respective groups were 190, 190, 205, 195 and 205 g. All animals dosed with 1580 mg/kg died within one day. Two animals of each sex died within 1 to 2 days of being dosed with 1260 mg/kg. All males and one female dosed with 1000 mg/kg died within 2 to 4 days. Three females and no males dosed with 794 mg/kg died. One male and no females dosed with 631 mg/kg died within 4 days of treatment. Average weights of survivors in each dosage group were similar (with the exception of slightly lower body weights of males treated with 1260 mg/kg). Signs of toxicity included lethargy, increasing weakness, ocular discharge, and collapse. Viscera of survivors appeared normal. Hemorrhagic lungs, liver discoloration, discoloration of kidneys and spleen (in some instances) and acute GI inflammation were observed in animals that died before study termination. The LD50 values (with 95 % confidence limits in females and males) were 1000 (740 - 1350) and 1200 (840-1720) mg/kg, respectively. The LD50 value for both sexes together (with 95 % confidence limits) was 1,100 mg/kg (900 - 1340). The slopes of the curves for females, males and males/females were 3.7, 3.7 and 3.8, respectively.
Test condition	:	Groups of 5 rats/sex (fasted) were dosed orally with 631, 794, 1000, 1260 and 1580 mg/kg test material. Animals were observed for death and toxic symptoms for 14 days. Survivors were weighed on days 7 and 14 and killed on day 14.
Test substance	:	Test material is meta dichlorobenzene (CAS No. 541-73-1).
Reliability	:	(2) valid with restrictions. Purity of test material was not noted. The method used to calculate the LD50 value was not listed. Test material is a related substance.

18.11.2001 (4)

Type	:	LD50
Species	:	guinea pig
Strain	:	other: albino
Sex	:	male/female
Number of animals	:	20
Vehicle	:	other: olive oil (by intubation as a 50% solution)
Value	:	> 800 mg/kg and < 2000 mg/kg bw
Method	:	other
Year	:	1958
GLP	:	no

Test substance	:	other TS; ortho dichlorobenzene (CAS No. 95-50-1).
Result	:	All animals dosed with 800 mg/kg lived and all dosed with 2000 mg/kg died.
Test condition	:	Animals (10 of mixed sex/group) were dosed with a 50% solution of test material in olive oil and observed over 14 days.
Test substance	:	The material used was redistilled 1,2 DCB with a boiling point range of 179.5-181.1 degrees C at 760 mm Hg. Based on this fact, the authors stated that the purity was > = 99%.
Reliability	:	(2) valid with restrictions. Details about the study design were not provided. Test material is a related substance.

19.11.2001 (21)

5.1.2 ACUTE INHALATION TOXICITY

Type	:	LD50
Test substance	:	No acute inhalation toxicity studies were available on CAS No. 87-61-6. Data from other category members and surrogates are used to fill this endpoint (see below).
Reliability	:	(2) valid with restrictions.

Type	:	LC50
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male
Number of animals	:	
Vehicle	:	
Exposure time	:	6 hour(s)
Value	:	= 2965 ppm
Method	:	other
Year	:	1982
GLP	:	no
Test substance	:	Monochlorobenzene (CAS No. 108-90-7), 99% pure
Result	:	The LC50 value was 2965 (2787 - 3169) ppm. The regression equation was y = 10.9x + 33. The LC50 value in mg/l is 13.9.
Test condition	:	Rats (130 -160 g) were exposed to concentrations ranging from 2000 to 3500 ppm over 6 hours (12/group). Vapor was generated at 24 degrees C, 50 % relative humidity. Rats were observed for 14 days. A line of regression was plotted with the abscissa being the log of the concentration of test material and the ordinate being the probit of the percentage of corresponding deaths. The LC50 value was the concentration (x value) at which the y value was 50%.
Reliability	:	(2) valid with restrictions. Test material was a related chemical

27.11.2001 (7)

Type	:	LC50
Species	:	mouse
Strain	:	
Sex	:	female
Number of animals	:	
Vehicle	:	
Exposure time	:	6 hour(s)
Value	:	= 1886 ppm
Method	:	other
Year	:	1979
GLP	:	no

Test substance	:	Monochlorobenzene (CAS No. 108-90-7); 99% pure.
Result	:	The LC50 value was calculated as 1886 ppm (1781-1980) with a regression equation of y = 6.734 x + 17.06. The value in mg/l is 8.8.
Test condition	:	Female mice (21 g) were exposed in 200 l chambers to 1400 to 3000 ppm for 6 hours (25/group). Vapor was generated at 24 degrees C, 50 % relative humidity and an air flow of 40 m3/hr. There were 60 air changes/hour. Animals were observed for 14 days. A line of regression was plotted with the abscissa being the log of the concentration of test material and the ordinate being the probit of the percentage of corresponding deaths. The LC50 value was the concentration (x value) at which the y value was 50%.
Reliability	:	(2) valid with restrictions. Test material was a related chemical.

27.11.2001 (7)(8)

Type	:	LC50
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male
Number of animals	:	
Vehicle	:	
Exposure time	:	6 hour(s)
Value	:	= 1532 ppm
Method	:	other
Year	:	1979
GLP	:	no
Test substance	:	1,2-dichlorobenzene (CAS No. 95-50-1). The test material was 99% pure
Result	:	The LC50 value with confidence limits was 1532 (1384 - 1730) ppm. The regression equation was y = 6.5 x + 15.8. The LC50 in mg/l is 9.38.
Test condition	:	Rats (130 -160 g) were exposed to concentrations ranging from 1000 to 2000 ppm over 6 hours. Vapor was generated at 24 degrees C, 50 % relative humidity. Rats were observed for 14 days. A line of regression was plotted with the abscissa being the log of the concentration of test material and the ordinate being the probit of the percentage of corresponding deaths. The LC50 value was the concentration (x value) at which the y value was 50%.
Reliability	:	(2) valid with restrictions. Test material was a related chemical.

19.11.2001 (7)

Type	:	LC50
Species	:	mouse
Strain	:	
Sex	:	female
Number of animals	:	
Vehicle	:	
Exposure time	:	6 hour(s)
Value	:	= 1236 ppm
Method	:	other
Year	:	1979
GLP	:	no
Test substance	:	1,2-dichlorobenzene (CAS No. 95-50-1). The test material was 99% pure.
Result	:	The LC50 value with confidence limits was 1236 (1201 - 1279) ppm. The regression equation was y = 13.303x + 42870. The LC50 value in mg/l is 7.43.
Test condition	:	Female mice (21 g) were exposed in 200 l chambers to various concentrations of test material for 6 hours. Vapor was generated at 24 degrees C, 50 % relative humidity and an air flow of 40 m3/hr. There were

60 air changes/hour. Animals were observed for 14 days. A line of regression was plotted with the abscissa being the log of the concentration of test material and the ordinate being the probit of the percentage of corresponding deaths. The LC50 value was the concentration (x value) at which the y value was 50%.

Reliability : (2) valid with restrictions. Test material was a related chemical.
19.11.2001 (7)(8)

Type : LC50
Species : rat
Strain : Sprague-Dawley
Sex : male/female
Number of animals : 10
Vehicle :
Exposure time : 4 hour(s)
Value : > 6.0 mg/l (997 ppm)
Method : other
Year : 1990
GLP : yes
Test substance : 1,4-dichlorobenzene (CAS No. 106-46-7). The test substance was analyzed as 103% 1,4-dichlorobenzene, 0.0435% 1,3-dichlorobenzene, and 0.0368% 1,2-dichlorobenzene.
Result : All animals survived the exposure. The LC50 value was greater than the amount tested (6.0 mg/l). Based on a molecular weight of 147, rats were expoed to 997 ppm.

Animals exhibited lacrimation, salivation, nasal discharge, labored breathing, gasping, tremors, ano-genital staining and chromodacryorrhea.

Test condition : Rats (5/sex, 202-236 g) were exposed to vapor (target concentration of 5.0 mg/l) by inhalation for 4 hours. The exposure concentration (6.0 mg/l)was analyzed by infrared spectroscopy. An aerodynamic particle sizer was used to confirm that the material did not aerosolize. Rats were observed for toxicity every 15 min for the first hour and hourly for the remainder of the study. All animals received detailed physical observations just prior to exposure, hourly for the first two hours of exposure, and once daily thereafter. Animals were weighed just prior to exposure and on Days 2, 3, 5, 8 and 15. Animals were euthanized on Day 15. Complete gross postmortem examinations were performed on all animals.

Reliability : (2) valid with restrictions. Test material was a related chemical.
19.11.2001 (27)

5.1.3 ACUTE DERMAL TOXICITY

Type : LD50
Test substance : No acute dermal toxicity studies were available on CAS No. 87-61-6. Data from other category members and surrogates are used to fill this endpoint (see below).

Type : LD50
Species : rat
Strain : CFE
Sex : male/female
Number of animals :
Vehicle :

Value	:	= 6139 mg/kg bw
Method	:	other
Year	:	1969
GLP	:	no
Test substance	:	other TS
Result	:	The LD50 value was 6139 mg/kg (4299-9056 mg/kg, 95% confidence limit). All deaths occurred within 5 days of exposure. Animals that died and survivors did not exhibit evidence of gross pathological lesions.
Test condition	:	Undiluted test material was placed on the shorn dorso-lumbar skin of rats (4/sex/group; doses and weights not stated). The area was bandaged with an impermeable dressing of aluminum foil and waterproof plaster. The dressing was kept in place for 24 hours. The treated site was then washed with tepid, dilute detergent solution. Water was freely available during the experiment, but food was withdrawn over the 24 hour exposure period. Animals were observed for 10 days. At the end of that time all survivors were weighed, examined and necropsied. Animals that died were also necropsied. The LD50 value and 95% confidence interval was calculated (method unknown).
Test substance	:	Test substance was 1,2,4-trichlorobenzene (CAS No. 120-82-1).
Reliability	:	(4) not assignable. The purity of test material is unknown. The doses tested, the number of deaths at each dose and the method used to calculate the LD50 value were not listed. A related test material was utilized.

27.11.2001 (9)

Type	:	LD50
Species	:	rabbit
Strain	:	New Zealand white
Sex	:	male/female
Number of animals	:	4
Vehicle	:	
Value	:	> 2000 mg/kg bw
Method	:	other
Year	:	1980
GLP	:	no data
Test substance	:	1,3-dichlorobenzene (CAS No. 541-73-1)
Remark	:	No signs of toxicity were noted.
Reliability	:	(4) not assignable. There are not enough details in study documentation to assign a reliability rating.

18.11.2001 (4)

Type	:	LD50
Species	:	rabbit
Strain	:	New Zealand white
Sex	:	male/female
Number of animals	:	3
Vehicle	:	
Value	:	> 7940 mg/kg bw
Method	:	other
Year	:	1976
GLP	:	no
Test substance	:	Monochlorobenzene (CAS No. 108-90-7)
Result	:	There were no deaths.
Test condition	:	One female (1.8 kg) was dosed with 5010 mg/kg and 2 males (2.0 and 2.2 kg) were dosed with 7940 mg/kg. Animals were observed over 14 days.

Reliability	:	(4) not assignable. Only 3 animals were used. Test material was a related substance.

27.11.2001 (3)

Type	:	LD50
Species	:	rat
Strain	:	Sherman
Sex	:	male/female
Number of animals	:	40
Vehicle	:	xylene
Value	:	> 6000 mg/kg in both sexes
Method	:	other
Year	:	1986
GLP	:	no data
Test substance	:	other TS
Result	:	The LD50 values in males and females were greater than the highest dose given (6000 mg/kg).
Test condition	:	Test material was dissolved in xylene. Test material was administered to the shaved shoulder and back area in a volume of 1.6 ml/kg body weight. Rats were 90 days old when tested. A minimum of 10 animals/group (note stated if evenly divided among sex) and 4 doses were tested (up to 6 g/kg). Animals were observed for at least 14 days or until all survivors had recovered from signs of toxicity. LD50 values and related parameters were calculated using a computer-based implementation of Finney's maximum likelihood probit technique.
Test material	:	Test material was para dichlorobenzene (CAS No. 106-46-7)
Reliability	:	(2) valid with restrictions. Purity of test material was not given. Test material was a related substance.

19.11.2001 (16)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

5.2.2 EYE IRRITATION

5.3 SENSITIZATION

5.4 REPEATED DOSE TOXICITY

Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	oral diet
Exposure period	:	90 days
Frequency of treatment	:	daily

Post obs. period	:	none
Doses	:	1, 10, 100, 1000 ppm (0.08, 0.78, 7.6 and 78 mg/kg/day in males and 0.13, 1.3, 12 and 113 mg/kg/day in females, for average of 0.105, 1.04, 9.8 and 95.5 mg/kg/day)
Control group	:	yes, concurrent vehicle
NOAEL	:	= 100 ppm (9.8 mg/kg/day)
LOAEL	:	= 1000 ppm (95.5 mg/kg/day)
Method	:	other
Year	:	1988
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The toxicity of 1,2,4 trichlorobenzene at 1, 10, 100 and 1000 ppm (0.07, 0.78, 7.8 and 82 mg/kg/day in males and 0.11, 1.4, 15 and 101 mg/kg/day in females) also was tested in this study. There was no effect of treatment with 1,2,4 trichlorobenzene on body weight. Relative liver and kidney weights of males were increased with respect to control only at the highest dose. Animals treated with 1000 ppm 1,2,4-trichlorobenzene had marked changes in the liver characterized by aggregated basophilia and widespread midzonal vacuolization due to fatty infiltration. Mild to moderate changes in the thyroid were also observed (similar to 1,2,3-trichlorobenzene). Higher activities of hepatic microsomal aniline hydroxylase and aminopyrene demethylase (AMD) were also observed in animals treated with 1000 ppm 1,2,4 trichlorobenzene.
Result	:	There were no signs of clinical toxicity (except reduced weight gains in some groups). There was no effect of treatment on any hematological or urinary parameter. 1000 ppm: Male rats exhibited decreased weight gain. The liver to body weight and kidney to body weight ratios of males were greater than control. Mild to moderate histopathological changes in the liver and thyroid were observed in males and females, with effects being more pronounced in males. Microscopic changes in the liver were mild to moderate increases in cytoplasmic volume and anisokaryosis of hepatocytes. Changes in the thyroid included reduction in follicular size and colloid density. Thyroid changes were also rated as mild to moderate. 100 ppm: Changes observed at this concentration were not considered to be attributable to treatment. 10 ppm: Males exhibited reduced weight gains and increased kidney/bw ratios. Changes observed at this concentration were not considered to be attributable to treatment. 1ppm: Males exhibited increased kidney/bw ratios. Changes observed at this concentration were not considered to be attributable to treatment.
Test condition	:	Test diet was prepared by blending diet with corn oil solutions containing appropriate amount of test material to give dietary levels of 1, 10, 100 or 1,000 ppm. Fresh diets were made very fourth week throughout the study and were kept in air-tight steel containers. Animals were acclimated for one week before treatment. Animals (10/sex/group) received either corn oil-treated diet, or diet that had been treated with 1, 10, 100 or 1000 ppm test material. Test diets were replaced on a weekly basis. Body weight was measured weekly. Food consumption was recorded at weeks 1, 4, 8, and 12 for 5 animals/sex/group. Urinalysis (pH, protein and nitrite) was carried out on 5 rats/sex/group at weeks 4, 8, and 12. Clinical examinations were performed daily on all animals.

Animals were killed at the end of week 13 and were examined grossly. The brain, heart, liver, spleen and kidneys were weighed. Blood samples were collected and analyzed for hemoglobin, packed cell volume, erythrocyte count, total and differential leukocyte counts, platelet count, and prothrombin time. Mean corpuscular hemoglobin concentration and hemoglobin values were calculated. Serum samples were analyzed for sodium, potassium, inorganic phosphate, total bilirubin, alkaline phosphatase, aspartate aminotransferase, total protein, calcium, cholesterol, glucose, uric acid, and lactate dehydrogenase. Hepatic microsomal aniline hydroxylase and aminopyrene demethylase also were measured. Fatty change in frozen sections of liver also was analyzed.
A section of femoral bone marrow was stained for cytological evaluation.
Standard organs (including reproductive) were examined histologically.

Data were analyzed by one-way analysis of variance followed by Duncan's multiple range test.

Test substance : Purity of the test material was > 99%.
Reliability : (2) valid with restrictions. Test diets were not analyzed to determine the actual concentration of test material in them at the beginning and end of each week.

18.11.2001 (14)

Species : rat
Sex : male/female
Strain : other: F344/N
Route of admin. : gavage
Exposure period : 13 weeks
Frequency of treatment : 5 days/week
Post obs. period : no
Doses : 37.5, 75, 150, 300, 600 mg/kg/day
Control group : yes, concurrent vehicle
NOAEL : = 300 mg/kg bw
LOAEL : = 600 mg/kg bw
Method : other
Year : 1987
GLP : no data
Test substance : other TS
Result : No compound-related deaths occurred. Body weight gains of dosed animals were similar to controls. An increase in the incidence and severity of kidney cortical tubular degeneration was observed in males receiving 600 mg/kg of 1,4-dichlorobenzene (vehicle control, 7/10 mild; 150 mg/kg, 5/10, mild-moderate; 600 mg/kg, 9/10, moderate). No compound-related histopathologic effects were observed in female rats.
Test condition : Rats were 4 weeks old upon arrival and were observed for 17 days prior to treatment. Ten animals/sex/group were treated with corn oil vehicle or 37.5, 75, 150, 300 or 600 mg/kg/day by gavage for 13 weeks (5 days/week). Animals were checked two times a day for mortality. A clinical exam was conducted weekly, and body weights were determined once weekly. Standard organs were taken at necropsy for organ weights (all groups) and histology (control and 3 highest dose groups)
Test substance : Test material was para dichlorobenzene (CAS No. 106-46-7). Purity was > 99%
Reliability : (1) valid without restriction

19.11.2001 (28)

Species	:	mouse
Sex	:	male/female
Strain	:	other: B6C3F1
Route of admin.	:	gavage
Exposure period	:	13 weeks
Frequency of treatment	:	5 days/week
Post obs. period	:	no
Doses	:	84.4, 168.8, 337.5, 675, 900 mg/kg/day
Control group	:	yes, concurrent vehicle
NOAEL	:	= 337.5 mg/kg bw
LOAEL	:	= 675 mg/kg bw
Method	:	other
Year	:	1987
GLP	:	no data
Test substance	:	other TS
Result	:	No compound-related deaths occurred. There was no significant change in body weight gain. Final mean body weights of male mice were 1-8% greater than vehicle controls. Centrilobular to midzonal hepatocytomegaly was observed in male and female mice that received 675 or 900 mg/kg (8/10 male and 4/10 females at 675 mg/kg and 9/10 males and 10/10 females at 900 mg/kg. This was not observed in controls or animals treated with 337.5 mg/kg. The severity of the lesion was minimal to mild at 675 mg/kg and mild to moderate at 900 mg/kg.
Test condition	:	Mice were 5 weeks old upon arrival and were observed for 14 days prior to treatment. Ten animals/sex/group were treated with corn oil vehicle or 84.4 168.8, 337.5, 675 or 900 mg/kg/day by gavage for 13 weeks (5 days/week). Animals were checked two times a day for mortality. A clinical exam was conducted weekly, and body weights were determined once weekly. Standard organs were taken at necropsy for organ weights (all groups) and histology (control and 3 highest dose groups)
Test substance	:	Test material was para dichlorobenzene (CAS No. 106-46-7). Purity was > 99%
Reliability	:	(1) valid without restriction

19.11.2001 (28)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Ames test
System of testing	:	S. typhimurium strains TA98, TA100, TA1535, TA1537
Concentration	:	3.3 to 333.3 micrograms/plate
Cytotoxic conc.	:	100 micrograms/plate (absence of S-9), 333.3 micrograms/plate (presence of S-9).
Metabolic activation	:	with and without
Result	:	negative
Method	:	other
Year	:	1983
GLP	:	no data
Test substance	:	as prescribed by 1.1-1.4
Remark	:	This test was given a reliability of 2 because statistical analyses were not performed. Two hundred fifty chemicals were tested in 3 different laboratories. 1,2-, 1,3- and 1,4 – dichlorobenzene and 1,2,4-trichlorobenzene also tested negative.
Result	:	The average number of revertants in the DMSO controls for strains TA98,

TA100, TA1535, and TA1537 in the absence of S-9 were 25, 124, 25, and 11. In the presence of rat and hamster liver S-9, the average number of revertants in the DMSO controls for strains TA98, TA100, TA1535, and TA1537 were 22 and 35, 138 and 131, 12 and 14, and 20 and 26, respectively. Incidences in the positive control cultures ranged from 163 (in 1537 without S-9) to 934 (in TA98 with hamster S-9).

The number of revertants induced by test material was not increased from that of controls at any concentration and tended to decrease or remain steady with increasing concentration (based on a visual review of the data). Metabolic activation with rat or hamster S-9 did not appear to significantly increase the number of mutations observed at each concentration. The number of revertants observed in cultures treated with nontoxic concentrations of test material in the absence of S9 ranged from 10-20 in strain TA98, 91-134 in TA100, 17-20 in TA1535, and 6-12 in TA1537. In the presence of rat or hamster S-9, the number of revertants observed in cultures treated with nontoxic concentrations of test material ranged from 23-32 in strainTA98, 91-135 in TA100, 8-16 in TA1535, and 11-22 in TA1537. Concentrations of 100 and 333.3 micrograms/plate were toxic to all strains in the absence and presence of S-9, respectively.

Test condition : Each chemical was coded and tested as an unknown. All strains of bacteria were supplied by the same supplier (Dr. B. Ames). Test material was dissolved in sterile dimethylsulfoxide. S-9 was prepared from liver homogenate from male Sprague-Dawley rats and male Syrian hamsters that had been injected with Aroclor 1254 (500 mg/ml) 5 days before they were killed.

Five concentrations of test compound (3.3, 10, 33.3, 100 and 333.3 micrograms/plate) were added to sterile test tubes containing bacterial cells, 0.5 ml of S-9 mix (+ activation) or phosphate buffer (- activation). The highest dose used exhibited some toxicity to strain TA100 in a preliminary experiment. This mixture was preincubated in a water bath at 37 degrees C for 20 min, then added to 2 ml molten top agar (45 degrees C). The contents of each tube were mixed and immediately poured onto the surface of a minimal-agar plate. DMSO (0.05 ml) also was added to plates containing each kind of bacteria (negative control). The positive control @-aminoanthracene was tested on all strains in the presence of rat and hamster S-9. 4-nitro-o-phenylenediamine was tested on TA98, sodium azide was tested on TA100 and TA1535, and 9-aminoacridine was tested on TA1537 without S-9. All tests were performed in triplicate and were repeated no less than one week after the initial test.

Plates were inverted and incubated at 37 degrees C for 48 hr. Colonies of his+ revertants were counted after incubation. The data were evaluated by an analysis based on the model described by Margolin et al., 1981.

Test substance : The purity of the test material was 97%.
Reliability : (2) valid with restrictions. Only four strains of bacteria were used.
18.11.2001 (18)

Type : Chromosomal aberration test
System of testing : Chinese Hamster Ovary Cells
Concentration : 15.7 to 125 micrograms/ml in separate experiments
Cytotoxic conc. : > 125 micrograms/ml
Metabolic activation : with and without
Result : negative

Method	:	other
Year	:	1985
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Monochlorobenzene and 1,2,4- trichlorobenzene also tested negative in this study. Article is in Japanese
Result	:	There was no test-material related increase in the frequency of aberrant cells in either experiment.
Test condition	:	Two different tests were done with the test material. In the first, cells were incubated for 24 or 48 hours with DMSO vehicle, and 15.7, 31.3 and 62.5 micrograms/ml test material. In the second experiment, cells were incubated with DMSO or 100 or 125 micrograms/ml test material in the presence and absence of S9.
Reliability	:	(2) valid with restrictions. Reference was not translated in total.

29.11.2001 (34)

5.6 GENETIC TOXICITY 'IN VIVO'

Type	:	Micronucleus assay
Species	:	mouse
Sex	:	male
Strain	:	NMRI
Route of admin.	:	i.p.
Exposure period	:	48 hours
Doses	:	250, 500, 750, 1000 mg/kg (one-half of dose each 24 hours)
Result	:	positive
Method	:	other
Year	:	1987
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Chlorobenzene, 1,2-, 1,3- and 1,4- dichlorobenzene, and 1,23- and 1,2,4- trichlorobenzenes also tested positive in this study. The use of multiple t-tests is an inappropriate means of determining the significance of the data. The study documentation is lacking in sufficient detail as to assess its validity.
Result	:	The number of micronucleated cells/1000 PCE (mean +/- SD) in control, 250, 500, 750, and 1000 mg/kg groups was 1.80 +/- 0.748, 4.10 +/- 0.943, 5.20 +/- 1.249, 6.80 +/- 0.979, and 7.30 +/- 1.005, respectively. The number of micronucleated cells/1000 PCE (mean +/- SD) in animals treated with 264, 528, 1056 (in split doses) and 528 (in one dose) mg/kg benzene (positive control) was 4.40 +/- 0.800, 8.10 +/- 0.943, 12.40 +/- 1.356, and 10.83 +/- 1.343, respectively.
Test condition	:	Eight week old male mice (5 per group) were given i.p. doses of test compound (total dose of 250, 500, 750 and 1000 mg/kg) such that the highest dose did not exceed 70% of the reported LD50 (1390 mg/kg). Each dose was given in a divided dose 24 hours apart (doses given at each 24 hour injection were 125, 250, 375 and 500 mg/kg). The control group of 10 mice received corn oil only. Benzene was the positive control. Animals were killed 30 hours after the first injection. The femora were removed and the marrow was suspended in serum. Two smears per femur were prepared and coded. The smears were scored by two different people. One-thousand polychromatic erythrocytes per smear were examined for the presence of micronuclei. T-tests were used to compare data. No further test details are given.

Test substance : Purity of test substance (given by manufacturer) was 99.0 %.
Conclusion : The test material was positive in the test.
Reliability : (4) unassignable
18.11.2001 (24)

Type : Micronucleus assay
Species : mouse
Sex : male
Strain : B6C3F1
Route of admin. : i.p.
Exposure period : 3 days
Doses : chlorobenzene: 128.8, 257.5, 515 mg/kg/day (total dose 386.4, 772.5, 1545 mg/kg); ortho dichlorobenzene: 50, 100, 200 mg/kg/day (total dose 150, 300 and 600 mg/kg) (experiment 1) ; and 150, 250 mg/kg/day (total dose of 450 and 750 mg/kg) (experiment 2)
Result : negative
Method : other
Year : 1993
GLP : no data
Test substance : other TS
Result : Chlorobenzene: The % PCE in animals treated with 0, 128.8, 257.5 or 515 mg/kg/day was 57.8, 51.1, 52.8, and 48.0, respectively. The incidences of MN-PCE/1000 (mean +/- SE) in pooled samples from 5-6 animals treated with 0, 128.8, 257.5 or 515 mg/kg/day were 3.70 +/- 0.58, 2.80 +/- 0.64, 2.10 +/- 0.33, and 3.42 +/- 0.57, respectively. The test was negative and was not repeated.

Ortho dichlorobenzene: The % PCE in animals treated with 0, 50, 100 or 200 mg/kg/day was 62.5, 56.4, 62.2, and 64.2, respectively. The incidences of MN-PCE/1000 (mean +/- SE) in pooled samples from 5 animals treated with 0, 50, 100 or 200 mg/kg/day were 1.70 +/- 0.49, 1.90 +/- 0.49, 2.40 +/- 0.49, and 2.70 +/- 0.72, respectively. This test was barely positive based on trend analysis (p = 0.049), but no dose group was positive. The test was repeated to 250 mg/kg and was found to be negative by trend analysis (p= 0.358). Because of the relatively small increase in MN-PCE in the initial test and
the lack of reproducibility, the overall result was considered negative.

Control: Solvent (corn oil) data were scored as 2.12 +/- 0.70 and 2.38 +/- 0.93 MN-PCE/1000 PCE (mean +/- SD) by two separate labs (not significantly different). These values are slightly lower than those reported in the test. The data for the positive control DMBA were 6.93 +/- 2.59 and 7.93 +/- 1.69 MN-PCE/1000 PCE (mean +/- SD) in the two labs. The data for the positive control MMC were 6.82 +/- 1.24 and 6.85 +/- 2.26 MN-PCE/1000 PCE (mean +/- SD) in the two labs (no significant difference).
Test condition : Male mice between 9 and 14 weeks of age between 23 and 35 g were used. Test material was mechanically suspended in corn oil and was administered within 30 minutes of preparation. Five mice/group were dosed with 0 (corn oil control), 128.8, 257.5 or 515 mg/kg chlorobenzene, 50, 100 or 200 mg/kg ortho dichlorobenzene, or a weakly active dose of the positive control chemicals 7,12-dimethyl benzanthracene (DMBA; 12.5 mg/kg) or mitomycin C (MMC; 0.2 mg/kg) by i.p. injection on three consecutive days (volume 0.4 ml). The doses tested were based on results of toxicity/mortality in a preliminary study. Animals were monitored 2 times/day.

Mice were killed 48 hours after the third treatment. Bone marrow and peripheral blood smears (two slides/tissue/mouse) were prepared by a direct technique (Tice et al. 1990. Effect of treatment protocol and sample time on frequencies of micronucleated cells in mouse bone marrow and peripheral blood. Mutagen.5:313-321). Air-dried smears were fixed using absolute methanol and stained with acridine orange. Smears from each animal were evaluated at 1000 x magnification using epi-illuminated fluoresence microscopy (450-490 nm excitation; 520 nm emission) for the percentage of polychromatic erythrocytes (PCE) among 200 erythrocytes and the number of micronucleated PCE (MN-PCE) among 2000 PCE. Repeat tests were conducted if the results suggested a possible effect or if no toxicity was observed at the highest dose level. Since the result of the experiment with ortho dichlorobenzene suggested a possible effect, the experiment was repeated at 0, 150 and 250 mg/kg /day.

The data were analyzed using the Micronucleus Assay Data Management and Statistical software package (version 1.4), which was designed specifically for in vivo micronucleus test data (ILS.1990. Integrated Laboratory Systems, P.O. Box 13501, Research Triangle Park, NC). The level of significance was set at $p < 0.05$. The numbers of MN-PCE at each dose group were pooled and analyzed by a one-tailed trend test. In the software package used, the trend test incorporates a variance inflation factor to account for excess animal variability. In the event that the increase in the dose response curve was nonmonotonic, the software allowed for the data to be analyzed for a significant positive trend after data at the highest dose only had been excluded. In this event, the alpha level was adjusted to $p < 0.01$ to protect against false positives. The %PCE data were analyzed by an analysis of variance (ANOVA) test based on pooled data. Pairwise comparisons between each group and the solvent control were made using an unadjusted one-tailed Pearson chi-squared test which incorporated the calculated variance inflation factor for the study. Solvent (corn oil) and positive control data were analyzed by two separate laboratories.

Test substance : Test materials were chlorobenzene (CAS No. 108-90-7) and ortho dichlorobenzene (CAS No. 95-50-1).

Reliability : (2) valid with restrictions. NTP study. Purity of test material was not noted.
27.11.2001 (31)(32)

Type : Micronucleus assay
Species : mouse
Strain : NMRI
Route of admin. : oral unspecified
Exposure period : single application
Doses : 100, 330 or 1000 mg/kg bw
Result : negative
Method : Other: OECD Guideline 474 and Directive 84/449/EEC, B.12
Year : 1983
GLP : yes
Test substance : other TS
Test condition : Test material was given in polyethylene glycol 200 vehicle
Test substance : Test material was 1,2,4- trichlorobenzene (CAS No. 120-82-1). Purity of test substance was 99.6 %.
Source : Bayer Leverkusen
Reliability : (2) valid with restrictions. OECD guideline study. The reference was not consulted. Information came from IUCLID file for CAS No. 120-82-1, creation date 11-FEB-2000.

(13)

Type	:	Micronucleus assay
Species	:	mouse
Strain	:	NMRI
Route of admin.	:	oral gavage, and i.p. (similar to conditions of Mohtashampir et al.)
Doses	:	2500 mg/kg (oral), 2 x 177.5 and 2 x 355 mg/kg (i.p.)
Result	:	negative
Method	:	Other
Year	:	2000
GLP	:	no data
Test substance	:	other TS
Remark	:	The metabolite 2,5, dichlorophenol also tested negative in the oral test. The average number of micronucleated cells/1000 PCE for cyclophosphamide in 87 experiments was used as the positive control in the i.p. test (16.8 +/- 4.2). Because a positive control was not tested in this test, it is not valid.
Result	:	Oral : The % PCE in animals treated with 0 (24 hr) or 2500 mg/kg dichlorobenzene (24, 48 and 72 hrs) was 51.87, 48.59, 38.40 and 34.23 (significantly different from control), respectively. The incidences of MN-PCE/1000 (mean +/- SD) in these same groups was 1.2+/- 0.9, 1.6 +/- 1.1, 1.6 +/- 1.1 and 1.2 +/- 0.6, respectively (no significant difference). The positive control induced 12.0 +/- 5.0 MN-PCE/1000 PCE. i.p.: The % PCE in animals treated with 0 or 2x 177.5 or 2 x 355 mg/kg test material was 48.26, 45.79, and 43.35 (significantly different from control) respectively. The incidences of MN-PCE/1000 (mean +/- SD) in these same groups was 1.6+/- 1.1, 1.3 +/- 1.1, and 2.1 +/- 2.4, respectively. There was no significant difference between treated animals and control
Test condition	:	Oral : Ten NMRI mice (five per sex, 8-12 weeks of age) per group were dosed with vehicle (corn oil), or 2500 mg/kg 1,4-dichlorobenzene, 1500 mg/kg 2,5 –dichlorophenol or 20 mg/kg Cyclophosphamide (positive control, dissolved in water). The materials were administered by stomach tube in a volume of 5 ml/kg (2,5, -dichlorophenol) or 10 ml/kg (1,3-dichlorobenzene). Vehicle control groups received the same volume of corn oil (volume of positive control was not stated). Animals were killed 24, 48 or 72 hours after treatment. i.p.: 1,4-dichlorobenzene in corn oil was administered twice (2 x 177.5 or 2 x 355 m/kg) at an interval of 24 hours. Corn oil vehicle was also given twice. The volume administered was 5 ml/kg. Animals were killed 6hr after the second injection. Femoral bone marrow cells were flushed out with fetal bovine calf serum and the cellular suspension was centrifuged at 100 rpm for 5 min. Smears were prepared from the pellet. 1000 polychromatic erythrocytes (PCEs) per animal were scored for micronuclei. The number of normochromatic erythrocytes per 1000 PCEs was counted. Data were analyzed using Wilcoxon's nonparametric rank sum test. A P value of <= 0.05 was the criterion for significance.
Test substance	:	Test material was 1,4- dichlorobenzene (CAS No. 106-46-7). Purity was > = 99.5%.
Reliability	:	(1) valid without restriction (oral test), invalid (i.p. test). A positive control was not used in the i.p. experiment.

(40)

5.7 CARCINOGENITY

5.8 TOXICITY TO REPRODUCTION

Type	:	examination of reproductive organs from 90 day study
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	oral feed
Exposure period	:	91 days
Frequency of treatment	:	continuously
Doses	:	1, 10, 100, 1000 ppm (0.08, 0.78, 7.6 and 78 mg/kg/day in males and 0.13, 1.3, 12 and 113 mg/kg/day in females, for average of 0.105, 1.04, 9.8 and 95.5 mg/kg/day)
Control group	:	vehicle control
NOAEL Parental	:	= 100 ppm (9.8 mg/kg/day)
NOAEL Reproductive organs	:	= 1000 ppm (95.5 mg/kg/day)
Method	:	other
Year	:	1988
GLP	:	no data
Test substance	:	as prescribed by 1.1-1.4
Result	:	There was no effect of treatment on histology or weights of the ovaries, uterus, vagina, testes, prostate, or epididymes Treatment with 1000 ppm caused histologic changes in the liver, increased relative kidney and liver weight (males) and decreased body weight (males).
Test condition	:	Test diet was prepared by blending diet with corn oil solutions containing appropriate amount of test material to give dietary levels of 1, 10, 100 or 1,000 ppm. Fresh diets were made very fourth week throughout the study and were kept in air-tight steel containers. Animals were acclimated for one week before treatment. Animals (10/sex/group) received either corn oil-treated diet, or diet that had been treated with 1, 10, 100 or 1000 ppm test material. Test diets were replaced on a weekly basis. Body weight was measured weekly. Food consumption was recorded at weeks 1, 4, 8, and 12 for 5 animals/sex/group. Urinalysis (pH, protein and nitrite) was carried out on 5 rats/sex/group at weeks 4, 8, and 12. Clinical examinations were performed daily on all animals. Animals were killed at the end of week 13 and were examined grossly. The brain, heart, liver, spleen and kidneys were weighed. Blood samples were collected and analyzed for standard hematological and biochemical parameters. Standard organs (including reproductive) were examined histologically. Data were analyzed by one-way analysis of variance followed by Duncan's multiple range test.

Test substance : Purity of test material was >= 99%.
Reliability : (2) valid with restrictions. The effect on mating was not characterized. Test diets were not analyzed to determine the actual concentration of test material in them at the beginning and end of each week.

18.11.2001 (14)

Type : Two generation study
Species : rat
Sex : female
Strain : no data
Route of admin. : drinking water
Exposure period : from birth of F0 generation through weaning of F2 generation
Frequency of treatment : continuously
Premating exposure period
Male : F0: 90 days; F1:90 days
Female : F0: 90 days, F1:90 days
Duration of test : until day 32 of F2 generation
Doses : 25, 100 or 400 ppm
Control group : other: two controls (no treatment and vehicle treatment)
NOAEL Parental : = 100 ppm
NOAEL F1 Offspr. : = 400 ppm
Method : other
Year : 1981
GLP : no data
Test substance : other TS
Remark : Results of an additional study with i.p. dosing confirmed the effect of test material on adrenal weight and also showed that the test material had no effect on ovarian weight and decreased (rather than increased) uterine weight. This showed that the test compound did not have any estrogenic activity.
Result : The fertility of the F0 and F1 animals was not affected by treatment. The number of females delivering/number of females bred ranged from 17/24 to 22/24 in treated F0 and F1 females and 19/24 to 22/24 in Tween treated controls. Litter size was slightly lower in the F2 than the F1 generation in all treatment groups (ranged from 9.8 to 12.8 in F2 vs. 12.8 to 14.5 in F1), but did not differ between treatment groups. Percent mortality to day 12 was lower in the F2 generation than the F1 generation (ranged from 3.7 to 9.3 in F2 vs. 13.1 to 21.8 in F1), but was unaffected by treatment. Percent mortality from d12 to weaning was low in all groups (ranged from 1.0 to 4.3 in F1 and F2) and was not affected by treatment. The treated F2 females also did not differ from controls in the time of vaginal opening.

An increase in food intake was noted in F0 high dose males at 29 days and a decrease in water intake of high dose males and females at 83 days of age. There were no changes in food or water consumption of F1 animals treated with test material. There was no effect of treatment on locomotor activity.

Slight increases in the weight of the left adrenal gland (the only one weighed) of males and females treated with 400 ppm test material for 90 days were observed. The values obtained in high dose F0 and F1 males were 31.8 +/- 2.43 mg and 29.6 +/- 1.66 mg vs. 28.6 +/- 1.09 mg and 28.0 +/- 1.57 mg in their respective Tween- treated controls. The values obtained in high dose F0 and F1 females were 41.5 +/- 1.99 mg and 38.5

+/- 1.89 mg vs. 36.8 +/- 1.14 mg and 37.0 +/- 1.36 mg in their respective Tween- treated controls. No histological damage was found in livers or kidneys of F1 generation animals examined at 95 days. No dose-related changes were found in blood chemistries.

Test condition : One hundred 90 day old, timed pregnant animals received regular tap water and food ad libitum until birth of the F0 generation. At birth, the litters (17-23) were randomly reduced to 4 males and 4 females per litter. These F0 animals were randomly distributed into 5 treatment groups (25, 100 or 400 ppm test material plus 0.125% Tween 20 as a solubilizer in drinking water, 0.125% Tween 20 in drinking water, or normal drinking water). No test material precipitated from these preparations over at least a 1 week period. Animals were given freshly prepared solutions at least twice weekly and were allowed to drink ad libitum.

After 90 days on treatment, 12 breeding cages were established, each containing 2 F0 females and 1 F0 male (within treatment nonsiblings). After 2 weeks of cohabitation, males were euthanized and the females were housed individually. Litters were weaned at 25 days of age and were housed as unisexual littermates until breeding or euthanization. F1 litters (reduced to 3 males and 4 females at weaning) received similar treatment as their F0 parents and were similarly bred. The experiment was terminated when the F2 animals were 32 days old, at which time the females were examined for vaginal opening.

Maternal weights, litter size, neonate sex and weights and 24 hour food and water intake of test animals were recorded on days 1, 8, 15, and 22 for the F0 generation and days 1 and 12 for the F1 and F2 generations. Body weights and 24 hour food and water intake were measured on days 29, 35, 43, 50 and 83 for the F0 generation and on days 25, 53 and 80 for the F1 generation. Individual locomotor activity was measured for 12-20 animals per treatment group on days 16, 27, 48, and 90 for the F0 generation, days 31 and 90 in the F1 generation and at 26 days in the F2 generation by placing animals in a residential maze. Blood and organs were obtained from 10 males and females per treatment group (approximately 1 animal per litter was used) at 37 and 95 days of age for the F0 generation and 95 days only for the F1 generation. The liver, lungs, kidneys, adrenals, gonads and seminal vesicles were removed and weighed. Blood samples were collected by cardiac puncture. Serum was analyzed for glucose, blood urea nitrogen, creatinine, sodium, potassium, chloride, uric acid, calcium, phosphorus, cholesterol, triglyceride, bilirubin, alkaline phosphatase, glutamic-pyruvic transaminase, glutamic oxaloacetic transaminase, creatinine phosphokinase, lactic dehydrogenase, total protein, albumin, and globulin. The livers and kidneys from Tween 20 and 400 ppm test material treated animals in the F1 generation were preserved in neutral buffered formalin and examined histologically.

Since the vehicle was found to have a significant effect on kidney weight, the Tween 20 control group was used as the 0 dosage group in all statistical comparisons. Data were analyzed by analysis of variance, and when results were significant, by linear regression. When a significant dose response effect was found, Dunn's procedure was used to determine the significance of pairwise comparisons. The organ weight and blood chemistry data in the F0 and F1 generations at 95 days were analyzed by regression procedures as a 2 x 2 x 4 (sex, generation, dose) factorial design.

The calculated mean dosages for F0 females administered 25, 100 or 400

ppm test material (based on water intake) were 8.3, 28.0 and 133 mg/kg at 29 days of age and 3.7, 14.8 and 53.6 mg/kg at 83 days of age. The calculated mean dosages for F0 males administered 25, 100 or 400 ppm test material (based on water intake) were 8.5, 27.6 and 133.6 mg/kg at 29 days of age and 2.5, 8.9 and 33.0 mg/kg at 83 days of age.

Conclusion : At the concentrations tested, the test material did not affect fertility, growth, viability, locomotor activity or blood chemical analyses of two generations of rats

Test substance : Test material was 1,2,4-trichlorobenzene (CAS No. 120-82-1).

Reliability : (2) valid with restrictions. Purity of test material was not stated. The test material was a related substance.

18.11.2001 (29)

Type : Two generation study
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : inhalation
Exposure period : F0 and F1 generations during mating period, days 0-20 of gestation and days 4-21 of lactation
Frequency of treatment : 6 hrs/day, 7 days/week
Premating exposure period
Male : F0 : 10 weeks, F1:11 weeks
Female : F0 : 10 weeks, F1:11 weeks
Duration of test : until weaning of F2 pups
Doses : 50, 150 or 450 ppm (234, 702 or 2105 mg/m3)
Control group : yes
NOAEL Parental : = 50 ppm
NOAEL F1 Offspr. : = 450 ppm
NOAEL F2 Offspr. : = 450 ppm
Method : other
Year : 1987
GLP : no data
Test substance : other TS
Remark : The NOAELs listed for F1 and F2 offspring are for reproductive indices in F0 and F1 females and males and survival of F1 and F2 fetuses. The NOAEL for toxicity for F0 and F1 parental animals is 50 ppm (based on changes in liver and kidney in males exposed to higher doses).

Result : Cumulative mean (+SD) analytical exposure concentrations were 51 (+/- 5), 151 (+/-8) and 451 (+/- 25) ppm for the F0 generation, and 49 (+/- 4), 150 (+/- 11) and 454 (+/- 21) ppm for the F1 generation.

No mortalities were observed in the adult generations. Mean body weights and food consumption for adult male and females in both generations were comparable for all groups. Mating and fertility indices for males and females were unaffected by treatment. For the F0 generation, the percentage of females that were pregnant in the control, 50, 150 and 450 ppm groups was 90%, 100%, 93.1% and 86.7%, respectively. The percentage of F0 males that successfully impregnated females in the control, 50, 150 and 450 ppm groups was 92.3%, 100%, 93.1% and 89.7%, respectively. For the F1 generation, the percentage of females that were pregnant in the control, 50, 150 and 450 ppm groups was 80%, 100%, 79.3 % and 89.3%, respectively. The percentage of F1 males that successfully impregnated females in the control, 50, 150 and 450 ppm groups was

77.8%, 100%, 79.3% and 88.0%, respectively. The mean (+SD) number of days for F0 males and females to mate in the control, 50, 150 and 450 ppm groups was 4.2 +/- 4.6, 3.1 +/- 2.8, 2.8 +/- 2.0 and 3.1 +/- 2.9, respectively (not significantly different). The mean (+SD) number of days for F1 males and females to mate in the control, 50, 150 and 450 ppm groups was 4.9 +/- 4.2, 3.2 +/- 2.3, 3.1 +/- 2.4 and 4.6 +/- 4.1, respectively. None of these values were significantly different from control.

In the F1 and F2 litters, pup and litter survival for all treated groups was comparable to controls. The pup viability index at birth for F1 offspring ranged from 96.1 +/- 12.0 in the 50 ppm group to 99.0 +/- 2.4 in the 150 ppm group, and for F2 offspring was 97-98% for all groups. Overall survival indices of F1 litters from F0 rats exposed to 0, 50, 150 or 450 ppm were 100%, 96.7%, 96.3% and 92.3%. Survival indices of F2 litters from F1 rats exposed to the aforementioned concentrations were 91.7%, 96.6%, 91.3% and 88.0%, respectively (not significantly different). In the F2 litters, a slight, nonsignificant decrease in pup survival index (Days 0-4) was seen in offspring from high dose animals. This was not considered to be treatment-related as this was predominantly due to loss of litters from two dams (one dam lost 12/15 pups and another lost all 10).

Significant increases in absolute and relative liver weight were observed in F0 and F1 adults exposed to 150 or 450 ppm. The relative liver weight of F1 males exposed to 50 ppm was also greater than control (3.73 +/- 0.36 vs. 3.47 +/- 0.32). An increase in the incidence of small flaccid testes and dilated renal pelvis was observed in high dose F0 and F1 males. For the 50, 150 and 450 ppm groups the incidence of small flaccid testes was 0, 1, and 3 for F0 males and 0, 1 and 5 for F1 males. For the 0, 50, 150 and 450 ppm males the incidence of dilated renal pelvis was 1, 1, 2 and 5 for F0 males (dose-related) and 1, 4, 6 and 4 for F1 males (not dose-related). In F0 females, the incidence of dilated renal pelvis in treated animals (4-6) was similar to control (5). In the F1 generation, 2 females treated with 150 or 450 ppm had dilated renal pelvis versus 0 in control.

Microscopic changes were observed in the liver and kidneys of treated male rats. Hepatocellular hypertrophy (graded minimal to mild) was noted in 5 mid and 14 high dose F0 males (none in control) and 3 mid and 7 high dose F1 males (2 in control). This lesion was observed in only one female (a high dose F0). The incidence of renal changes in male rats is shown in the Table below:

Table: Incidence of renal changes in male rats inhaling monochlorobenzene for 2 generations

	Group (ppm)							
	F_0 adults				F_1 adults			
	0	50	150	450	0	50	150	450
Total No. of animals	30	30	30	30	30	30	30	30
U/tubular dilation Eosinophilic material	0	3	2	3	4	4	6	6
B/tubular dilation Eosinophilic material	0	1	4	15	4	3	8	16
U/chronic interstitial nephritis	0	0	0	1	1	2	1	0
B/chronic interstitial nephritis	1	2	7	9	0	1	6	11
U/foci of regenerative epithelium	0	0	0	0	0	0	1	1
B/foci of regenerative epithelium	0	1	5	8	1	0	4	10

U/ = unilateral, B/ = bilateral
Lesions were not present in females

Two F0 and 3 F1 males in the 150 ppm group and 6 F1 males in the 450 ppm group exhibited unilateral degeneration, and 6 F0 males of the 450 ppm group exhibited bilateral degeneration of varying degrees in the germinal epithelium of the testes. This lesion was observed in 1 male control in each of the generations. In order to determine the effect of these lesions on reproductive performance, the reproductive performance of all males that showed this lesion were reviewed. All controls, all affected F0 and 2/3 F1 males in the 150 ppm group, and 3/6 affected animals in the 2 generations treated with 450 ppm were successful in siring litters.

Test condition : Test article: Test article was administered by the inhalation route with animals exposed in 6m3 glass and stainless steel chambers. Targeted exposure levels were 50, 150 and 400 ppm; included in the study was a chamber exposed, sham-air control group. The chambers were operated dynamically at an air flow rate of at least 2140 l/min (one air change per 2.8 min). The test material was fed into an atomizing nozzle via an FMI fluid metering pump. The vaporized test material was diluted with preconditioned air prior to entry into the exposure chamber. Test concentrations were monitored hourly during exposures. A daily nominal concentration was determined by dividing the difference in weight of the generation apparatus and test material before and after exposure by the total volume of air delivered during the day.

Test conduct: Animals were acclimated for at least 13 days prior to exposure. They were 6 weeks old at time of exposure. F0 adult animals (30/sex/group) were exposed daily (6 hrs/day, 7 days/week) with 0 (filtered air), 50, 150 or 450 ppm for a 10 week pre-mating treatment period and during mating. Once mated (as evidenced by the presence of a copulatory plug), females were exposed (6 hrs/day) during gestation (Days 0-19) and lactation (Days 5-28) of the FI litters. F0 males continued to be treated daily during the post-mating period until termination. Similarly, F0 females continued to be treated daily post-weaning until killed after weaning of the last litter.

FI pups (30/sex/group) were exposed to comparable dose levels as the dams one week after weaning to at least 11 weeks prior to mating. Animals chosen to be the F1 parents were selected to maximize representation from the number of available litters. Exposure of F1 animals during the mating, gestation and lactation intervals of the F2 litters was similar to that of the F0 animals.

Animals were given free access to standard laboratory diet and water during all non-exposure periods, and water during lactation. All animals were observed twice daily for toxicity or mortality. Detailed physical examinations were performed weekly. Body weights and food consumption of F0 and F1 adults were generally measured weekly for most animals (with the exception of slightly different intervals for females during gestation and lactation). Litters were examined twice daily for death and general appearance. On day 4 of lactation, all litters with greater than 8 pups were culled to that number. The sex distribution within litters was equalized (if possible). Pup weights, the number of pups in each litter and pup sex distribution were determined on days 0, 4 (pre and post cull), 7, 14, and 21 of lactation. The mating index for males and females, pregnancy rate and fertility index for males were calculated for each of the two matings. Pup survival indices at various intervals during lactation were calculated.

All F0 and F1 adults were killed after all F1 pups and F2 pups were

weaned, respectively. All F2 pups were killed at day 21 of lactation. Complete gross postmortem examinations were conducted on all F0 and F1 parents, all F1 weanlings not selected to become parents of the F2 generation, and all F2 weanlings. Liver and brain weights of F0 and F1 adults were recorded. Liver, kidneys, pituitary gland, and reproductive organs (males- epididymes, seminal vesicle and prostate; females- vagina, uterus and ovaries) were examined microscopically for all F0 and F1 adult animals in the control and high dose groups. Liver, kidneys and testes of male rats in the low and mid-dose groups were examined histologically.

Statistical analyses: Mean body weights, food consumption, organ weights, organ to body weight ratios, gestation lengths, and numbers of offspring were evaluated for equal variance using Bartlett's test. Parametric methods (one way analysis of variance followed by a Dunnett's test) were performed on data if variances were equal. A Kruskal-Wallis test or Dunn's summed rank test was used to analyze nonparametric data. The nonparametric test for determining monotonic trend was the Jonckheere's test and standard linear regression was used for parametric data. Pup viability and survival indices were analyzed with the litter as the experimental unit, and these data were transformed using arcsine. Incidence data were analyzed using contingency tables. A standard chi-square analysis was performed on these data to determine if the proportion of incidences differed between the groups tested. Next, each treatment group was compared to the control group using a 2 x 2 Fischer exact test. The significance level was corrected using the Bonferroni inequality. An Armitage test for linear trend was performed.

Conclusion : Exposure to male rats of 150 or 450 ppm caused hepatocellular hypertrophy and increased liver weight, degenerative and inflammatory lesions in the kidneys and degenerative testicular changes. The relationship between testicular damage and exposure to monochlorobenzene is unclear because although 3/6 affected high dose males in each generation did not sire litters, the overall incidence of males not siring litters in each generation of the high dose animals (4 F0 and 8 F1) and mid-dose animals (3 F0 and 7 F1) was not different from control (6 F0 and 9 F1).

In the absence of microscopic changes, the increased liver weight in the low dose males and mid and high dose females was not considered to be indicative of an adverse effect. Chlorobenzene was not a reproductive toxicant.

Test substance : Test substance was chlorobenzene (CAS No. 108-90-7). The purity was 99.9%.

Reliability : (1) valid without restriction

27.11.2001 (25)

Type : Two generation study
Species : rat
Sex : male/female
Strain : other: Charles River CD (Sprague-Dawley derived)
Route of admin. : inhalation
Exposure period :
Frequency of treatment : 6 hr/day, 7 days/week during times specified under test condition
Premating exposure period
Male : 10 weeks

Female	:	10 weeks
Duration of test	:	to weaning of F1 and F2 generations
Doses	:	50, 150, 400 ppm (0.305, 0.915, or 2.44 mg/l)
Control group	:	yes
NOAEL Parental	:	< 50 ppm
NOAEL F1 Offspr.	:	= 50 ppm
NOAEL F2 Offspr.	:	= 150 ppm
Method	:	other
Year	:	1989
GLP	:	yes
Test substance	:	other TS
Remark	:	Study personnel concluded that in the absence of significant mortality in all other litters but one high dose litter during the day 0-4 interval, the decrease in pup survival index for this group (while statistically significant compared to control) was not indicative of a treatment-related effect. The reduced postnatal weights of pups from high dose animals (both F1 and F2) and mid dose animals (F1) are indicative of slight fetotoxicity.
Result	:	Mean analytical concentrations for the F0 and FI generation animals were similar to targeted exposure levels. For the F0 generation; the mean analytical concentrations (+ S.D.) for the low-, mid- and high-exposure groups were 50 +/- 3, 150 +/- 5 and 397 +/- 18 ppm, respectively. In the FI, these mean analytical concentrations were 51 +/- 3, 151 +/- 8 and 391 +/- 25 respectively, for these same groups. Some mortality was seen among the control (1/sex in F0; 1 male in F1) and treated adult animals in each generation (1 mid dose F0 female on day 24 during delivery; 1 high dose male F1, one low dose female F1 during lactation, 1 high dose F1 female killed moribund); however, no adverse effect of treatment was indicated. The only effect of treatment seen in the low-exposure group was a slight increase in absolute and relative liver weight in both the F0 and FI adult animals. No adverse effect of treatment at the low-exposure level was evident from growth of the adult animals, reproductive performance, fertility, gestation length or litter size data. Pups delivered and weaned to females in this group showed comparable growth and survival rates to weaning as control animals. A slight, but significant increase in mean gestation length was seen in low-dose females; however this was not considered to be relevant as it was not observed at higher dose levels. In the mid-exposure group, mean weights of adults were lower than control at several weekly intervals early in the pre-mating period of the F0 and throughout this same interval in the F1; however, mean weight gain over the entire pre-mating interval for both generations was comparable to control data. No adverse effect of treatment in the mid-exposure group was evident from reproductive performance or fertility indices, litter size or gestation length or maternal weight gain data during gestation/lactation intervals in either generation. In the F1 litters, mean pup weight at Day 0 (birth) for the mid-exposure group was statistically significantly lower than control; however, pup weight data for the remaining weighing intervals of the FI litters and for all weighing intervals of the F2 litters were comparable to control data. Pup survival indices in the mid-exposure group for each litter interval were comparable to control data. Mean liver weights (absolute and relative to body or brain weights) for the mid-exposure group were higher than control in the F0 while in the FI, only relative liver weights were increased. Additionally, relative kidney

weights were increased in the mid-exposure males from both generations.

In the high-exposure group, no adverse effect of treatment was evident from reproductive performance or fertility indices for either generation. Maternal weight gain during gestation/lactation intervals, gestation length, litter size data and pups survival indices were generally comparable to control data for both generations. However, the pup survival index for the day 0-4 lactation interval was lower than control (94.3% vs. 98.1% for control). This was largely attributed to the loss of all pups within a single litter (one high dose female delivered a litter of 14 live pups and 2 dead pups, but none survived to day 4). Excluding data for this litter, the pup survival index for the day 0-4 interval was 98.4 % (317/322 pups), which was not significantly different from control. The pup surivival for the high dose group over the day 4-21 lactation interval was comparable to control. Mean weekly weights for F0 and FI adults in the high-exposure group during the pre-mating treatment intervals were lower than control, and mean weights continued to be depressed for these animals through to termination.

Mean weight gain over the entire pre-mating intervals were lower than control for males in both generations and for F0 females. Excessive salivation was seen in high dose F0 and F1 males and females at some time points. F0 and F1 adult males and females in the high-exposure group had increased liver weights (absolute and relative), and males (F0, FI) also had increased relative kidney weights. Mean pup weights in the high-exposure group were statistically significantly lower than control on Days 0, 14, 21 and 28 of the FI litters and on Days 14 and 21 of the F2 litters. Growth and food consumption in unselected F1 high dose pups were similar to control.

No adverse effect of treatment was evident from the gross postmortem evaluations of adults and offspring. Treatment-related morphologic abnormalities in the liver and kidneys were seen in the F0 and FI adults.

Liver: Hypertrophy of the central lobular hepatocytes was found in almost all F0 and FI adult males and females in the high-exposure group and numerous males and several females from the mid-exposure group. This effect was not seen in the liver of F0 and F1 males and females from the control group or in the liver of females from the low-exposure group.

Kidneys: Dilated tubular lumens with intraluminal granular casts were seen predominantly at the cortico-medullary junctions in several F0 and FI adult males from the mid- and high-exposure groups. This effect was seen most frequently in the high-exposure group.

Intracytoplasmic granules/droplets in the proximal convoluted tubular epithelium were seen in almost all F0 and FI adult males for which the kidneys were examined microscopically. Based on mean severity this kidney effect was most pronounced in F0 and F1 adult males from the high-exposure group followed by the mid- and low- exposure groups. It was least pronounced in the control group. The granules/droplets were eosinophilic in the hematoxylin and eosin stained sections and they stained positive with the Mallory Heidenhain stain in the specially stained sections.

Other postmortem findings seen in the F0 and FI adults, gross and microscopically, either occurred with comparable incidence and severities in the treated and control animals or they occurred sporadically and were

not considered to be related to the test article.

Several F1 generation animals in all groups were noted on week 37 to have findings suggestive of Sialodacryoadenitis viral (SDAV) infection. All males were noted as free of SDAV infection by week 41. Females were generally free of symptoms of SDAV infection at week 41. The presence of SDAV was confirmed by analyses of serum from affected males for SDAV antibodies. The presence of SDAV did not appear to have adversely affected the study.

Test condition : Test article: Test article was administered by the inhalation route with animals exposed in 6m3 glass and stainless steel chambers. Targeted exposure levels were 50, 150 and 400 ppm; included in the study was a chamber exposed, sham-air control group. Appropriate amounts of the test material were placed into a 2 l erlenmeyer flask connected to a fluid metering pump. The metering pump settings were varied to provide the target exposure levels. The test article was fed from the flask directly into the liquid inlet of an air atomizing nozzle via Teflon tubing. House-supply air was delivered through Teflon tubing from a regulator and flowmeter with a backpressure gauge to the air inlet of the atomizer via tygon tubing to generate the aerosol. The aerosol was directed into the side inlet of the air inlet pipe where it volatized in the chamber airflow stream. This stream entered the exposure chambers. Test concentrations were monitored hourly during exposures.

Test conduct: Each study group consisted of 60 CD rats (30/sex/generation). F0 adult animals (41 days old at treatment) were exposed daily (6 hrs/day) for a 10 week pre-mating treatment period and during mating. Once mated, females were exposed (6 hrs/day) during gestation (Days 0-19) and lactation (Days 5-28) of the FI litters. F0 males continued to be treated daily during the post-mating period until termination. Similarly, F0 females continued to be treated daily post-weaning until termination after weaning of the last litter. FI pups (2/sex/litter) randomly selected at Day 28 (weaning) became a pool of animals from which the FI adult generation was selected. These animals were exposed at 29 days of age to comparable dose levels as the dams. Once the FI adult generation was chosen, these animals received an 11-week pre-mating treatment period. Exposure of animals during the mating, gestation and lactation intervals of the F2 litters was similar to that for the F0 animals. Animals were given free access to standard laboratory diet during all non-exposure periods, and water during exposure and non-exposure periods. All animals were observed twice daily for toxicity or mortality. Detailed physical examinations were performed for the F0 and F1 adult generation animals and unselected F1 high dose animals (see below). Body weights and food consumption of F0 and F1 adults were generally conducted weekly for most animals (with the exception of slightly different intervals for females during gestation and lactation). Pup weights were determined on days 0, 4 (pre and post cull), 7 (F2 litter only), 14, 21 and 28 of lactation (F1 litters only). They were sexed at each examination.

F0 and FI adult males were terminated as a group three to four weeks post-mating; F0 and FI females were killed as a group after the last litters, FI and F2, respectively, were weaned. Each adult generation animal was given a gross postmortem examination, and liver, kidneys, pituitary gland and reproductive tissues were saved in 10% formalin. Liver, kidney, testes and brain weights were recorded at termination, and absolute and relative liver, kidney and testes weight data were evaluated. Initially, microscopic evaluations were restricted to tissues for the control and high-dose groups,

both generations; however, these evaluations were extended to include the livers of all F0 and F1 adults in the low- and mid-dose groups and the kidneys of all F0 and FI low- and mid-dose males. FI and F2 pups were given a gross external and internal examination and discarded; only abnormal tissues were saved in 10% formalin. FI pups were killed either at weaning (Day 28) or at the time of selection for the FI adult generation. F2 pups were killed at Day 21 of lactation.

If, following weaning of the F1 last litters more than 30 pups/sex were present for a particular group, the excess were culled so that each litter was represented in the parental generation by at least one pup per sex. In the control, low and mid dose groups, the excess pups were culled and given a gross external and internal examination. In the high-exposure group, the excess pups were retained on study. They were removed from the exposure regimen and were maintained on basal diet over an 11-week period that corresponded to the pre-mating treatment period. These animals (unselected F1 high-dose pups) were then killed and given a gross postmortem examination. Only abnormal tissues from these animals were saved.

Data from treated groups were compared to control (methods for statistical tests were not available).

Test substance : The test material was orthodichlorobenzene (CAS No. 95-50-1). The purity of the test material was > 99.2%.

Reliability : (1) valid without restriction

19.11.2001 (30)

Type : Two generation study
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : inhalation
Exposure period :
Frequency of treatment : 6 hr/day, 7 days/week during times specified under test condition
Premating exposure period
Male : 10 weeks
Female : 10 weeks
Duration of test : to weaning of F1 and F2 generations
Doses : 50, 150, 450 ppm
Control group : yes
NOAEL Parental : < 66.3 ppm (< 50 ppm nominal)
NOAEL F1 Offspr. : 211 ppm (150 ppm nominal)
NOAEL F2 Offspr. : 211 ppm (150 ppm nominal)
Method : other
Year : 1989
GLP : yes
Test substance : other TS
Remark : The fact that the high dose had no effect on any reproductive parameter but caused a significant effect on the number of liveborn and stillborn pups suggests that this dose of material was fetotoxic.
Result : The mean analytical concentrations (+ S.D.) for the low-, mid- and high-exposure groups were 66.3 +/- 8.47, 211 +/- 18.0 and 538 +/- 50.5 ppm, respectively. These were calculated using the charcoal tube values obtained from days 172-282, as these were in better agreement with the

values obtained from the direct syringe method than from the syringe/stainless steel tubing sampling method used from Days 1-171. Samples obtained from the syringe/stainless steel method underestimated test concentrations by as mush as 37% (presumably due to condensation problems). The charcoal tube method was successful because the vapor did not contact any surfaces before reaching the charcoal bed. Within each chamber, vapor concentrations were uniformly distributed.
The maximum amount of test material detected in the control atmosphere was 3 ppm (once on day 194). All other readings in the control atmospheres were at or below the limit of detection (1 ppm). In this summary, the target concentrations will be used when stating results.

All groups: There were no treatment-related gross lesions observed in necropsies of F1 or F2 pups that died during lactation, F1 or F2 weanlings, or F0 or F1 adults. There was no effect of treatment on reproductive parameters (mating or fertility indices of males or females, the gestational index, the 7-, 14-, 21- or 28-day survival index, the lactation index, or the number of copora lutea or implantation sites (including resorptions and live conceptuses)) in either generation. Five adult animals died or were killed in moribund condition during the study (one control F0 male, three F0 females (one each in control, 150 and 450 ppm groups), and one F1 female at 50 ppm). The cause of death of the control F0 male was urinary tract inflammation/obstruction. The cause of death of the other animals was not determined.

450 ppm: F0 and F1 males and F1 females exhibited consistently reduced body weights and weight gains throughout the experiment. F0 females had reduced body weights for the first week of exposure prior to mating, and on gestational day 20. Gestational weight or weight gain of F0 and F1 dams was reduced for gestational days 0-20. Lactational weights of F1 females were reduced for postnatal days 0, 4, and 7. Nonexposed male and female F1 recovery animals exhibited consistently reduced body weights. Food consumption was reduced in F1 males for 5 of the 11 exposure weeks, and was reduced in F0 and F1 females and F0 males during the first week and third week (F0 females only) of exposure. Food consumption was reduced in recovery animals during the first two weeks of the recovery period. Clinical signs observed in F0 and F1 animals included unkempt appearance, tremors, twitches, hypoactivity, ataxia, salivation, and periocular and perioral encrustation. F0 and F1 females had an increased incidence of urogenital wetness. There were no significant clinical observations in recovery animals at 450 ppm. Liver and kidney weights of F0 males and females were increased (as well as relative liver, brain, testes and kidney weights of F0 males and relative liver and kidney weights of F0 females). An increased incidence of hepatocellular hypertrophy was observed in F0 males and females, and increased incidences of hydronephrosis, hyaline droplet nephropathy, tubular proteinosis, granular cast formation, renal tubular cell hyperplasia and interstitial nephritis were observed in F0 males. Exposure-related histologic findings in F1 adults were similar to those of F0 adults.

The mean number of F2 live born pups per litter was significantly reduced in the 450 ppm group. F1 and F2 litter size (but not sex ratio) was reduced on lactational day 4 at 450 ppm. Pups from F1 and F2 litters exhibited reduced body weights per litter during lactation. There was an increase in the incidence of stillborn pups (F2) and postnatal deaths from days 0-4 (both F1 and F2 pups).

150 ppm: Sporadic reductions in body weights and weight gains of F0 and F1 animals were observed at this concentration. F0 and F1 males had increased absolute and/or relative kidney and liver weights, and F0 females had increased relative liver weights. An increased incidence of nephrosis was observed in F0 and F1 males. Histologic findings in the liver were similar to those of males treated with 450 ppm (with the exception of no renal cell hyperplasia and the additional finding of renal interstitial fibrosis). There were no effects of treatment on the pups.

50 ppm: Absolute and/or relative kidney weights of F0 and F1 males and liver weights of F0 males were increased. There was an increased incidence of nephrosis in F0 males, with similar histological findings as in the 150 ppm males. F1 males did not exhibit nephrosis, but had an increased incidence of hyaline droplet formation in the kidneys. No histological alterations were noted in the liver. There were no effects of treatment on the pups.

Test condition : Test article: Test article was administered by the inhalation route with animals exposed in stainless steel chambers approximately 4320 liters in volume. Targeted exposure levels were 50, 150 and 450 ppm; included in the study was a chamber exposed, sham-air control group. Vapor was generated by metering in-house and filtered compressed air at a flow rate of 0.6 – 3.5 L/min (depending on the target concentration) into a heated (130 – 136 degrees C) 12-Liter stainless steel pot containing test material (1.8 – 2.8 kg). The resulting vapor was introduced into the exposure chamber through a heated tube. Chamber airflow was 1000 L/min (at least 14 air changes per hour). Chamber temperature, relative humidity and airflow were monitored. Concentrations of test material in the chambers were measured using gas chromatography with flame ionization detection. Samples were taken with gas-tight syringes up to day 171. Coconut-based charcoal sorbent tubes (placed within the breathing zone) were used for sampling from days 172-282. At least 6 samples were taken daily from each exposure from days 1-171, and 3 were taken from days 172-282. On at least 3 study days, samples were taken with syringes from different positions in the chamber to determine if the concentration of vapor varied throughout the chamber.

Test conduct: Rats (177 virgin females and 140 virgin males) were 28 days old upon receipt (males 75-100 g, females 50-75 g). They were housed 2/same sex for a quarantine period of approximately 2 weeks. Feed was available ad libitum except during exposure periods. Water was available ad libitum in the chambers (except for pre-mating exposure periods).

One hundred twelve healthy rats/sex (F0 rats) were weighed just prior to exposure (avg. Weights of males and females were 199.2 – 200.2 and 139.2 – 140.5 g, respectively) and randomly distributed into 4 treatment groups 28/sex/group). Forty additional females (10/group) were randomly selected for a special mating (satellite) study. Study animals and satellite females were housed individually and exposed to target concentrations of 0, 50, 150 or 450 ppm test material, 6 hrs/day, 7 days/week for 10 consecutive weeks. Animals were examined twice daily for mortality and once daily for clinical signs of toxicity during exposure. Animal weights and food consumption were measured weekly. Clinical observations, body weights or food consumption were not monitored in satellite females.

After the 10-week exposure, animals within each treatment group were mated for a period of 21 days (one male to one female). Exposure

continued during mating to gestation day 19. Exposure was discontinued from gestation day 20 through the fifth day postpartum. Beginning on postnatal day 5, mothers were removed from their offspring and exposed to test material (or air) as before, through postnatal day 27. Dams were returned to their offspring after each daily exposure (6 hr/day).

The day a copulation plug or sperm in the vaginal smears was found was considered day 0 of gestation. F0 males that did not show evidence of successful mating within 10 days were removed from the mating cages used in a special mating study with satellite females, which was initiated approximately 14 days later. Satellite females and F0 males were exposed throughout this mating period until scheduled termination. F0 males were euthanized and necropsied following the satellite mating period. Satellite females were killed on gestation day 15, and examined for evidence of pregnancy.

The corresponding F0 females that did not exhibit evidence of copulation were paired a second time for 11 days with proven males from the same concentration group. For F0 animals that showed no successful mating within 21 days, the last scheduled mating day was designated gestation day 0 for that female and the animals were treated accordingly for subsequent events. Mated study females were weighed on gestation days 0, 4, 7, 14, 21, and 28. Litters were weaned on day 28 postpartum ,and dams were necropsied. When the last F1 litter reached day 28 postpartum, 28 male and female pups per treatment group were randomly selected to produce the F2 generation. Each litter was represented at least once per sex (if possible). Brother/sister matings were avoided. Following selection of the F1 parents, ten female F1 pups were selected as satellite females for special mating, and 20 animals per sex from the control and 450 ppm groups were selected as recovery animals. The remaining F1 pups were euthanized and necropsied.

F1 animals selected to be parents of the F2 generation and F1 satellite females (approximate age of 4-7 weeks) were exposed to test material (or control) for at least 11 weeks as described for F0 animals. The mean weight for F1 males was 192.2-230.6 g, and for F1 females was 143.5 – 172.1 g at the start of exposure. Animals were mated at the end of 11 weeks, and continued to be exposed during mating and gestation day 19 (as described above). F1 males that did not successfully mate within 10 days were mated with F1 satellite females and unsuccessful F1 females were mated with proven F1 males (as described above).

F1 recovery animals were retained for the first 5 weeks of the F1 exposure period. Recovery animals were not exposed but had food and water withheld for 6 hrs/ day during exposure periods of the F1 animals. They were monitored daily for clinical signs of toxicity and weekly for body weights, body weight gains, and food consumption. The animals were euthanized and necropsied after the 5 week period.

All pups from the F1 and F2 generations were sexed and examined on the day of birth. Litters were evaluated twice daily for survival. Each litter was culled to yield 4 pups/sex /litter (if possible). Survival indices, sex, and weight of the pups were determined at days 0, 4, 7, 14 and 21 days after birth and at weaning (postnatal day 28). All pups were examined for physical abnormalities at birth and throughout the pre-weaning period.

All F0 and F1 parental animals in all groups and F1 recovery animals were

euthanized and subjected to a gross necropsy. Tissues from the control and 450 ppm groups (pituitary, liver, kidneys (2), vagina, uterus, ovaries, testes, epididymes, seminal vesicles, prostate and any tissues with gross lesions) were examined histopathologically. Kidney sections from all groups of F) and F1 animals were examined for the presence of alpha2μ globulin protein droplets. The number of copora lutea, implantation sites (including resorptions and live conceptuses) in F0 and F1 satellite females were recorded. Any apparently nonpregnant uteri were stained with 10% ammonium sulfide for confirmation of pregnancy status. Satellite females were not subjected to gross necropsies. A complete necropsy and histopathologic examination was conducted on any parental animals dying on test. Absolute organ weights for testes, ovaries, brain, liver and kidneys were recorded and organ to body and brain weight ratios were determined for all animals scheduled for termination. A gross internal examination was performed on any pup appearing abnormal or that died on test, and all remaining F1 and F2 pups.

Statistical Analyses: The unit of comparison was the male, female, or litter. Body weight, food consumption and organ weight data were tested for homogeneity using Levene's test for equal variances. When variances were homogeneous, data were compared using analysis of variance and pooled t-tests (for pairwise comparisons). An analysis of variance for unequal variances, followed by the separate variance t-test for pairwise comparisons was used when data were not homogenous. The significance levels for the t-test comparisons were corrected by the Bonferroni method.

Nonparametric data were evaluated using the Kruskal-Wallis test, followed by the Mann-Whitney U test for pairwise comparisons (when appropriate). Frequency data were compared using the Fisher's exact test. For all tests, the fiducial limit of 0.05 (two-tailed) was used as the criterion for statistical significance.

Test substance : The test material was para dichlorobenzene (CAS No. 106-46-7). The purity of the test material was approximately 100%.

Reliability : (1) valid without restriction
19.11.2001 (41)

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rat
Sex	:	female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	days 6 to 15 of gestation
Frequency of treatment	:	daily
Duration of test	:	to day 22 of gestation
Doses	:	150, 300 and 600 mg/kg
Control group	:	yes, concurrent vehicle
NOAEL Maternalt.	:	= 150 mg/kg bw
NOAEL Teratogen	:	= 600 mg/kg bw
Method	:	other
Year	:	1988
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The NOAEL listed for teratogenicity is also the NOAEL for fetoxicity.

Teratogenicity of 75, 150 and 300 mg/kg 1,2,4-trichlorobenzene was tested in this study. This material induced similar changes in dams as 1,2,3-trichlorobenzene (although at lower concentrations). The maternal NOAEL for 1,2,4-trichlorobenzene was 75 mg/kg. Decreased hemoglobin was seen at 150 mg/kg; and decreased hemoglobin, thyroid toxicity (mild), and increased liver weight were observed at 300 mg/kg.

The results suggested that neither 1,2,3- nor 1,2,4-trichlorobenzene was embryotoxic, fetotoxic or teratogenic.

Result : One control animal and one treated with 300 mg/kg test material died over the course of the study. None of the tested animals displayed clinical signs of toxicity. A significant increase in relative liver weight was observed in rats treated with 600 mg/kg (5.8 +/-0.26%). APDM activity was significantly increased in high dose animals (27.03 +/- 0.9 nanomoles formaldehyde/hr/mg protein vs. 22.8 +/- 0.6 in control). Hemoglobin concentrations were slightly decreased in animals dosed with 300 or 600 mg/kg test material (11.4 +/- 0.2 and 11.3 +/- 0.2 g/dl vs. 12.0 +/- 0.3 g/dl in control, respectively). Mild changes in the thyroid were noted in animals dosed with 300 and 600 mg/kg. The changes consisted of a reduction of follicle size which was often accompanied by angular collapse.

Test material residue was only found in fat tissue of the dams treated with 300 and 600 mg/kg (0.02 and 0.4 ppm, respectively, with a detection limit of 0.01 ppm). No test material residue was found in the fetus.

There was no effect of treatment on pregnancy rate (ranged from 10-11/13 treated vs. 12/14 in control), number of resorptions + dead fetuses (ranged from 0.4 +/- 0.1 to 0.5 +/- 0.2 in treated vs. 0.7 +/- 0.2 in control), litter size (ranged from 11.5 +/-1.1 to 13.3 +/- 0.6 in treated vs. 11.5 +/- 1.2 in control), or fetal weight (ranged from 5.1 +/- 0.2 to 5.4 +/- 0.1 in treated vs. 5.4 +/- 0.1 in control). No visceral or skeletal abnormalities were found in the 27-37 pups/group examined for visceral changes and the 49-67 pups/group examined for skeletal changes. The incidence of sternal anomalies was 11 in 3 litters of control animals, 1 each in the 150 and 300 mg/kg groups, and 13 in 3 litters in the 600 mg/kg group. Wavy ribs were found in one fetus/group (except the 150 mg/kg group), and a short 13th rib was found in one animal in the 150 mg/kg group. Based on these data, the test material was concluded not to be embryotoxic, fetotoxic or teratogenic.

Test condition : Rats (122-175 g) were acclimatized one week prior to mating. Two females were placed in a cage overnight with a male. Vaginal swabs were checked for evidence of mating. The day on which sperm was detected was designated as day 1 of pregnancy. Test material was dissolved in corn oil and was administered to groups of animals (N=13/group) at 150, 300 and 600 mg/kg by gavage from days 6 to 15 of gestation. Controls (N =14) received an equal volume of corn oil. Dams were weighed on day 22 of gestation and killed. The uterus was transected and removed with the ovaries. Dams were reweighed and the liver, kidney, spleen, heart and brain were removed and weighed. The heart, brain, pituitary, eye, thyroid, parathyroid, trachea, bronchi, lung, thymus, stomach, small and large intestine, pancreas, liver, kidney, spleen, adrenal, skeletal muscle, peripheral nerve, skin, bone marrow, ovary, uterus and bladder were examined histologically.

Hemoglobin concentration, hematocrit, erythrocyte count, total and differential leukocyte count, mean corpuscular volume, and mean corpuscular hemoglobin concentration of maternal blood were determined. Serum from each dam was analyzed for sodium, potassium, inorganic

phosphorus, total bilirubin, alkaline phosphatase, glutamic oxaloacetic transaminase, total protein, calcium, cholesterol, glucose, uric acid and lactate dehydrogenase. Liver homogenate from each dam was analyzed for aniline hydroxylase, aminopyrene-N-demethylase (ADPM) and protein. Portions of kidney, brain, spleen, heart, liver and perirenal fat were analyzed for trichlorobenzene residue analysis. The concentration of trichlorobenzene in hexane extracts of tissues was measured using gas chromatography.

The fetuses were removed and weighed individually. Live fetuses were examined grossly at necropsy for birth defects. Two thirds of each litter were examined for skeletal abnormalities by examining the cleared and stained skeletons stereoscopically. The remainder were fixed for visceral examination (using dissection and razor sectioning). The amount of residual test material in one fetus/litter and the liver and brain of a litter mate was determined as described above.

The data from organ weight, body weights, hematology and biochemical parameters were analyzed using a one way analysis of variance. Duncan's multiple range test was used to compare means.

Test substance : Test material was 99.5% pure.
Reliability : (1) valid without restriction
18.11.2001 (5)

5.10 OTHER RELEVANT INFORMATION

5.11 EXPERIENCE WITH HUMAN EXPOSURE

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(22) HSDB, on-line via STN

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7.1 END POINT SUMMARY

7.2 HAZARD SUMMARY

7.3 RISK ASSESSMENT

Robust Summaries and Repository of Knowledge for CAS No. 541-73-1

Existing Chemical	:	ID: 541-73-1
CAS No.	:	541-73-1
EINECS Name	:	1,3-dichlorobenzene
EINECS No.	:	208-792-1
TSCA Name	:	Benzene, 1,3-dichloro-
Molecular Formula	:	C6H4Cl2

Company	:	PCA Services, Inc.
Creation date	:	08.11.2001

Printing date	:	29.01.2003
Revision date	:	29.01.2003
Date of last Update	:	29.01.2003

Number of Pages	:	74

Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 7

1.0.1 OECD AND COMPANY INFORMATION

1.0.2 LOCATION OF PRODUCTION SITE

1.0.3 IDENTITY OF RECIPIENTS

1.1 GENERAL SUBSTANCE INFORMATION

1.1.0 DETAILS ON TEMPLATE

1.1.1 SPECTRA

1.2 SYNONYMS

1,3-dichlorobenzol
29.11.2001

benzene, 1,3-dichloro
29.11.2001

benzene, m-dichloro
29.11.2001

m-dichlorobenzene
29.11.2001

m-dichlorobenzol
29.11.2001

meta dichlorobenzene
29.11.2001

29.11.2001

1.3 IMPURITIES

1.4 ADDITIVES

1.5 QUANTITY

1.6.1 LABELLING

1.6.2 CLASSIFICATION

1.7 USE PATTERN

1.7.1 TECHNOLOGY PRODUCTION/USE

1.8 OCCUPATIONAL EXPOSURE LIMIT VALUES

1.9 SOURCE OF EXPOSURE

1.10.1 RECOMMENDATIONS/PRECAUTIONARY MEASURES

1.10.2 EMERGENCY MEASURES

1.11 PACKAGING

1.12 POSSIB. OF RENDERING SUBST. HARMLESS

1.13 STATEMENTS CONCERNING WASTE

1.14.1 WATER POLLUTION

1.14.2 MAJOR ACCIDENT HAZARDS

1.14.3 AIR POLLUTION

1.15 ADDITIONAL REMARKS

1.16 LAST LITERATURE SEARCH

1.17 REVIEWS

1.18 LISTINGS E.G. CHEMICAL INVENTORIES

2.1 MELTING POINT

Value : = -25.5 ° C
Source : Hoechst AG Frankfurt/Main
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Test condition : Erstarrungspunkt [Solidification point]
Test substance : as prescribed by 1.1-1.4. Technisches trockenes Produkt [Technical dried product]
Remark : The test method is not given. However, the value stated is most likely a measured value, since this substance is a well-known, basic chemical. Kirk Othmer Encyclopedia of Chemical Technology, 4th Edition, Volume 6, page 89, cites a melting point value of -24.7° C. Ulmann's Encyclopedia of Industrial Chemistry, 5th Ed., page 328, cites a value of -26.3° C.
Reliability : (2) valid with restrictions. Data were obtained from a IUCLID document prepared by the European Chemicals Bureau, 11-FEB-2000.
Flag : Key study for endpoint

(21) (24)

Value : = -24.5 ° C
Source : Hoechst AG Frankfurt/Main
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Test condition : Erstarrungspunkt [Solidification point]
Test substance : as prescribed by 1.1-1.4. Destilliertes Produkt [Distilled product]
Reliability : (2) valid with restrictions. Data were obtained from a IUCLID document prepared by the European Chemicals Bureau, 11-FEB-2000.

(21)

2.2 BOILING POINT

Value : = 173° C at 1013 hPa
Decomposition :
Method :
Year :
GLP :
Test substance : as prescribed by 1.1-1.4
Remark : The test method is not given. However, the value stated is most likely a measured value, since this substance is a well-known, basic chemical, and because this material is purified by distillation. Kirk Othmer Encyclopedia of Chemical Technology, 4th Edition, Volume 6, page 89, cites a boiling point value of 173° C. Ulmann's Encyclopedia of Industrial Chemistry, 5th Ed., page 328, also cites a value of 173° C.
Source : Hoechst AG Frankfurt/Main
Clariant GmbH Frankfurt am Main
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (2) valid with restrictions. Data were obtained from a IUCLID document prepared by the European Chemicals Bureau, 11-FEB-2000.

(2) (25)

2.3 DENSITY

Type : density
Value : = 1.29 g/cm3 at 20° C

Test substance	:	as prescribed by 1.1-1.4
Source	:	Hoechst AG Frankfurt/Main Clariant GmbH Frankfurt am Main EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Data were obtained from a IUCLID document prepared by the European Chemicals Bureau, 11-FEB-2000.

(2) (25)

+

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value	:	= 1.8 hPa at 20° C
Test substance	:	as prescribed by 1.1-1.4
Source	:	Hoechst AG Frankfurt/Main Clariant GmbH Frankfurt am Main EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Remark	:	The test method is not given. However, the value stated is most likely a measured value, since this substance is a well-known, basic chemical.
Reliability	:	(2) valid with restrictions. Data were obtained from a IUCLID document prepared by the European Chemicals Bureau, 11-FEB-2000.

(2) (25)

2.5 PARTITION COEFFICIENT

Log pow	:	ca. 3.28 at ° C
Method		other (calculated)
Year	:	2001
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	EPIWIN KOWWIN (v1.66) estimates log Kow by the method of Hansch, C et al. 1995. Standard contribution values for each fragment of the molecule are summed up to calculate an overall value.
Reliability	:	(2) valid with restrictions. Data were obtained by modeling.
Flag	:	

30.11.2001 (12)

Log pow	:	= 3.38 at 25° C
Method	:	other (measured): Saeulenchromatographische Methode [Column chromatography method]
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1-1.4
Source	:	Hoechst AG Frankfurt/Main Hoechst AG Frankfurt/Main Clariant GmbH Frankfurt am Main EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Data were obtained from a IUCLID document prepared by the European Chemicals Bureau, 11-FEB-2000.

(30)

Log pow	:	3.39
Method	:	other: internal company protocol (measured)

Year	:	1988
GLP	:	yes
Test substance	:	Other TS. Test material is analytical grade 1,4-dichlorobenzene (99+%). (CAS No. 106-46-7).
Result	:	The mean measured partition coefficient of p-dichlorobenzene was 2460 (log Pow = 3.39)
Test condition	:	An aliquot of 14C labeled p-dichlorobenzene in octanol was partitioned with water. The ratio of activity in the octanol phase of the samples to the activity in the aqueous phase was used to calculate the partition coefficient. Deionized water was filtered through a 0.22 micron filter to remove any particulate matter present. Water was saturated with octanol prior to use. Octanol used was reagent grade. The octanol was saturated with filtered deionized water before use. Radiolabeled test compound was received as a solution in ethanol. Radiochemical Purity was >=99%. A standard solution of radiolabeled material was made up in reagent grade octanol. Radiochemical purity was rechecked during the study and found to be 95.8%. All measurements of radioactivity were made using liquid scintillation counting. Analyses of study samples were made using high performance liquid chromatography.
Reliability	:	(1) valid without restriction.
Flag	:	Key study for SIDS endpoint

(9)

2.6.1 WATER SOLUBILITY

Value	:	= .1 g/l at 20 ° C
Qualitative	:	
Pka	:	at 25 ° C
PH	:	at and ° C
Test substance	:	as prescribed by 1.1-1.4
Source	:	Hoechst AG Frankfurt/Main Clariant GmbH Frankfurt am Main EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Remark	:	The test method is not given. However, the value stated is most likely a measured value, since this substance is a well-known, basic chemical. Ulmann's Encyclopedia of Industrial Chemistry, 5th Ed., page 328, cites water solubility values of 1.1 and 2.0 g/100g (0.15 and 0.23 g/l).
Reliability	:	(2) valid with restrictions. Data were obtained from a IUCLID document prepared by the European Chemicals Bureau, 11-FEB-2000.
Flag	:	Key study for endpoint

(2) (25)

Value	:	= 200 mg/l at 60 ° C
Qualitative	:	
Pka	:	at 25 ° C
PH	:	at and ° C
Test substance	:	as prescribed by 1.1-1.4
Source	:	Hoechst AG Frankfurt/Main Hoechst AG Frankfurt/Main Clariant GmbH Frankfurt am Main EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Data were obtained from a IUCLID document prepared by the European Chemicals Bureau, 11-FEB-2000.

(2)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

Value	:	= 65 ° C
Type	:	
Test substance	:	as prescribed by 1.1-1.4
Source	:	Hoechst AG Frankfurt/Main EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Data were obtained from a IUCLID document prepared by the European Chemicals Bureau, 11-FEB-2000.

(2) (23) (24)

2.8 AUTO FLAMMABILITY

Value	:	> 500 ° C at
Remark	:	Ignition temperature
Test substance	:	as prescribed by 1.1-1.4
Source	:	Hoechst AG Frankfurt/Main EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Data were obtained from a IUCLID document prepared by the European Chemicals Bureau, 11-FEB-2000.

(23) (24)

2.9 FLAMMABILITY

Result	:	Other
Test substance	:	as prescribed by 1.1-1.4
Remark	:	m-dichlorobenzene is ignitable only with difficulty
Source	:	Hoechst AG Frankfurt/Main Clariant GmbH Frankfurt am Main EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Data were obtained from a IUCLID document prepared by the European Chemicals Bureau, 11-FEB-2000.

(2)

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	
Light spect.	:	nm
Rel. intensity	:	based on Intensity of Sunlight
Indirect photolysis		
Sensitizer	:	OH
Conc. of sens.	:	500000 molecule/cm3
Rate constant	:	ca. .00000000000069 cm3/(molecule*sec)
Degradation	:	= 50 % after 16 day
Deg. Product	:	
Method	:	other (measured): Resonanz-Fluoreszens-Technik [Resonance-fluorescent technique]
Year	:	
GLP	:	no data
Test substance	:	other TS
Source	:	Hoechst AG Frankfurt/Main Hoechst AG Frankfurt/Main Clariant GmbH Frankfurt am Main EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Test substance	:	Reinheitsgrad >99 % [Purity > 99%]
Reliability	:	(2) valid with restrictions. Data were obtained from a IUCLID document prepared by the European Chemicals Bureau, 11-FEB-2000.
Flag	:	Key study for endpoint

(51)

Type	:	air
Light source	:	
Light spect.	:	nm
Rel. intensity	:	based on Intensity of Sunlight
Indirect photolysis		
Sensitizer	:	OH
Conc. of sens.	:	500000 molecule/cm3
Rate constant	:	= .0000000000003 cm3/(molecule*sec)
Degradation	:	= 50 % after 37 day
Deg. Product	:	
Method	:	other (calculated): Hendry, Kenley (1979); described in: Nitrogeneous Air Pollutants (Grosjean, D., ed.), Ann Arbor Science, Michigan, 137-148
Year	:	
GLP	:	no data
Test substance	:	As prescribed by 1.1-1.4
Source	:	Hoechst AG Frankfurt/Main EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Data were obtained from a IUCLID document prepared by the European Chemicals Bureau, 11-FEB-2000.

(44)

Type	:	air
Light source	:	
Light spect.	:	nm
Rel. intensity	:	based on Intensity of Sunlight
Indirect photolysis		
Sensitizer	:	OH

Conc. of sens.	:	500000 molecule/cm3
Rate constant	:	= .00000000000072 cm3/(molecule*sec)
Degradation	:	ca. 50 % after 22.3 day
Deg. Product	:	
Method	:	other (measured): Experimental data of Atkinson
Year	:	
GLP	:	
Test substance	:	as prescribed by 1.1-1.4
Source	:	Hoechst AG Frankfurt/Main Clariant GmbH Frankfurt am Main EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Test condition	:	Room temperature
Reliability	:	(2) valid with restrictions. Data were obtained from a IUCLID document prepared by the European Chemicals Bureau, 11-FEB-2000. Study is from a recognized institute according to standard laboratory procedures. (1)

Type	:	Air
Light source	:	Sun light
Light spect.	:	Nm
Rel. intensity	:	based on Intensity of Sunlight
Direct photolysis		
Halflife t1/2	:	ca. 11.1 day
Degradation	:	% after
Quantum yield	:	
Indirect photolysis		
Sensitizer	:	OH
Conc. of sens.	:	1500000 molecule/cm3
Rate constant	:	ca. .00000000000072 cm3/(molecule*sec)
Degradation	:	% after
Deg. Product	:	
Method	:	other (calculated)
Year	:	2001
GLP	:	No
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The overall Hydroxyl rate constant is calculated by the EPIWIN AOP Program (v1.90), which identifies reactions of hydroxyl radical with individual bonds in the molecule and sums up the rate constants for these individual bond reactions to obtain an overall value. The half life was calculated by assuming a constant OH radical concentration and assuming pseudo first order kinetics.
Reliability	:	(2) valid with restrictions. Data were obtained by modeling

01.12.2001 (12)

Type	:	Air
Light source	:	
Light spect.	:	Nm
Rel. intensity	:	based on Intensity of Sunlight
Indirect photolysis		
Sensitizer	:	OH
Conc. of sens.	:	1500000 molecule/cm3
Rate constant	:	= .000000000010051 cm3/(molecule*sec)
Degradation	:	ca. 50 % after 10.6 day
Deg. Product	:	

Method	:	other (calculated): ATMOSPHERIC OXIDATION PROGRAMM, Version 1.51 of 13.03.94, Syracuse Research Corporation, according to Atkinson (1987 und 1988)
Year	:	
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4
Source	:	Hoechst AG Frankfurt/Main EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(2) valid with restrictions. Data were obtained from a IUCLID document prepared by the European Chemicals Bureau, 11-FEB-2000.

(22)

3.1.2 STABILITY IN WATER

Type	:	Abiotic
t1/2 pH4	:	at degree C
t1/2 pH7	:	at degree C
t1/2 pH9	:	at degree C
Deg. Product	:	
Method	:	other (calculated)
Year	:	2001
GLP	:	No
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	EPIWIN HYDROWIN (v1.67) cannot estimate a hydrolysis rate constant for halobenzenes. It has long been recognized in basic organic chemistry, however, that haloaromatics, such as m-dichlorobenzene, are highly resistant to hydrolysis. Water hydrolysis, therefore is not an important degradative pathway.
Flag	:	Key study for endpoint

30.11.2001 (12)

Type	:	Abiotic
t1/2 pH4	:	at degree C
t1/2 pH7	:	at degree C
t1/2 pH9	:	at degree C
Deg. Product	:	
Method	:	other: no data
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Because of the electronegative effect of halogen substituents, a nucleophilic attack of hydroxyl anion will be hindered. Therefore no significant hydrolysis is to be expected
Source	:	Hoechst AG Frankfurt/Main Hoechst AG Frankfurt/Main Clariant GmbH Frankfurt am Main EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability	:	(4) unassignable. Data were obtained from a IUCLID document prepared by the European Chemicals Bureau, 11-FEB-2000.

30.11.2001 (8)

3.1.3 STABILITY IN SOIL

3.2 MONITORING DATA

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	:	fugacity model level III
Media	:	water – air
Air (level III)	:	13.1
Water (level III)	:	20.2
Soil (level I)	:	
Biota (level II / III)	:	0.75
Soil (level II / III)	:	66
Method	:	Calculated (Estimated by model). Inputs to the EPIWIN model were measured values for melting point (-25.5°C), boiling point (173°C), vapor pressure (1.4 mm Hg), water solubility (100 mg/l) and Log Kow (3.38).
Year	:	2001
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The EPIWIN Level III Fugacity Model assumes a Henry's Law Constant of 0.00263 atm-m3/mole, a vapor pressure of 0 mm Hg, a log Kow of 3.53 and a soil Koc of 1.39E+3. The EPIWIN HENRY (V3.10) program was used to calculate the Henry's Law Constant. The EPIWIN PCKOC (V1.66) program was used to estimate the Koc(soil-sediment partition constant). Level III fugacity calculations allow non-equilibrium conditions to exist between connected media at steady state. The EPIWIN BCF (v2.14) program was used to estimate a BCF (bioconcentration factor) of 104.3 or a log BCF of 2.018.
Reliability	:	(2) valid with restrictions. Data were obtained using EPIWIN Fugacity Level III modeling.
Flag	:	Key study for endpoint

01.12.2001 (12)

Type	:	fugacity model level I
Media	:	water – air
Air (level I)	:	96
Water (level I)	:	
Soil (level I)	:	3
Biota (level II / III)	:	
Soil (level II / III)	:	
Method	:	Other
Year	:	2001
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Level I EQC modeling by Mackay assumes equilibrium, steady state conditions.
Reliability	:	(2) valid with restrictions. Data were obtained by modeling
Flag	:	Key study for endpoint

(46)

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type	:	Aerobic
Inoculum	:	Pseudomonas sp. (Bacteria)
Concentration	:	200mg/l related to Test substance related to
Contact time	:	
Degradation	:	ca. 100 % after 96 hour(s)
Result	:	
Deg. Product	:	
Method	:	other: Respirometer test
Year	:	
GLP	:	No
Test substance	:	no data
Remark	:	100% breakdown was achieved after 28 hours by using a mutated inoculum produced by irradiation. The original European IUCLID document stated "no data" under test substance (see above). Because it is listed under the test condition that the bacteria were adapted to 1,3 dichlorobenzene, it is obvious that this was the material tested.
Source	:	Hoechst AG Frankfurt/Main Hoechst AG Frankfurt/Main Clariant GmbH Frankfurt am Main EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Test condition	:	The bacteria were obtained from an industrial waste sludge adapted to 1,3-dichlorobenzene. The incubation temperature was at 30 degrees C.
Reliability	:	(4) not assignable. Data were obtained from a IUCLID document prepared by the European Chemicals Bureau, 11-FEB-2000.
Key	:	Supportive study for endpoint (34) (52)

Type	:	Aerobic
Inoculum	:	domestic sewage
Concentration	:	10mg/l related to Test substance related to
Contact time	:	
Degradation	:	= 58 % after 7 day
Result	:	
Deg. Product	:	
Method	:	other: Closed Flask test according to the method of Bunch und Chambers, J. Water. Poll. Contr. Fed. 39, 181-187 (1967)
Year	:	
GLP	:	No
Test substance	:	no data
Remark	:	67% breakdown in Subculture 1 in 7 days 31% breakdown in Subculture 2 in 7 days 33% breakdown in Subculture 3 in 7 days
Source	:	Hoechst AG Frankfurt/Main EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Test condition	:	Addition of yeast abstract 5 mg/l, temperature 25 degrees C, use of an emulsifying agent (trigylceride) for solubilization
Reliability	:	(4) not assignable. Data were obtained from a IUCLID document prepared

by the European Chemicals Bureau, 11-FEB-2000.

Key : Supportive study for endpoint

(46) (47)

Type : Aerobic
Inoculum : activated sludge, non-adapted
Concentration : 100mg/l related to Test substance
related to
Contact time :
Degradation : = 0 % after 28 day
Result : under test conditions no biodegradation observed
Deg. Product :
Method : OECD Guide-line 301 C "Ready Biodegradability: Modified MITI Test (I)"
Year :
GLP : no data
Test substance :
Source : Hoechst AG Frankfurt/Main
Clariant GmbH Frankfurt am Main
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (2) valid with restrictions. OECD guideline study. Data were obtained from a IUCLID document prepared by the European Chemicals Bureau, 11-FEB-2000.
Key : Supportive study for endpoint

(31)

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	flow through
Species	:	Pimephales promelas (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	yes
LC50	:	m = 8.03
ec50	:	m = 4.89
Method	:	other: APHA (1980) Standard Methods for the examination of water and wastewater
Year	:	1986
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	The average (+/- SD) temperature, dissolved oxygen, hardness, alkalinity and pH of the water in the test chambers were 26.0 +/- 0.30 degrees C, 7.5 +/- 0.10 mg/l, 45.8 +/- 0.50 mg/l CaCO3, 44.1 +/- 3.54 mg/l CaCO3, and 7.88 +/- 0.01. It is not known whether these variables were affected by test material concentration. Average and ranges of analytical concentrations of chambers treated with 0. 5.54, 8.52, 13.1, 20.1 and 30.9 mg/l were <0.01, 1.71 (1.32 - 1.98), 3.37 (2.68 - 3.82), 4.62 (3.68 - 5.23), 5.76 (5.38 - 6.38) and 14.4 (11.5 - 15.8) mg/l. In most cases, concentrations of test material remained fairly steady from 0 to 72 hours, and decreased at 96 hours. When corrected for recovery (96.5 %), test material concentrations were 1.8, 3.5, 4.78, 5.97 and 14.9 mg/l. The mean length and weight (+/- SD) of the fish at study termination were 15.8 +/- 1.251 mm and 0.054 +/- 0.0148 g. All fish exposed to 14.9 mg/l died within 5 hours of treatment. Four fish exposed to 5.97 mg/l died by 72 hours, two exposed to 4.78 mg/l died by 48 hours, two exposed to 3.5 mg/l died by 72 hours, and one exposed to 1.8 mg/l died at 96 hours. Three controls were dead at 96 hours. The 96 hour LC50 value (with confidence limits) was 8.03 (6.95 - 9.28) mg/l. A total of 20 fish exposed to 14.9 mg/l, 15 fish exposed to 5.97 mg/l, 8 exposed to 4.78 mg/l, 4 exposed to 3.5 mg/l, 2 exposed to 1.8 mg/l and 3 controls exhibited signs of toxicity (including death). The 96 hour EC50 value (with confidence limits) was 4.89 (4.06 - 5.89) mg/l.
Test condition	:	A stock solution of test material (75 mg/l) was prepared in 18.9 l of test water. When the pH of the stock was outside of the 7 to 8 range, it was adjusted to pH 7.8 with either NaOH or concentrated HCl. Newly hatched minnows from adults reared in flow-through tanks were held at 25 degrees C in flowing water with a 16-hr photoperiod and were fed 40 to 48-hour brine shrimp nauplii two times/day (except once/day on weekends). They were cultured in filtered Lake Superior water or dechlorinated water from the city of Superior, WI (exact source not given) The two waters were similar in all measured chemical parameters. This water was used for test material dilution and all tests. Healthy fish (28 days old) were fasted for 24 hours before treatment. They were pooled together in one tank and randomly distributed among the

exposure chambers. Tests were initiated by adding 20 fish per treatment (5.54, 8.52, 13.1, 20.1, and 30.9 mg/l) and control to test chambers containing 1 liter of water. Fish loading was 1.08 g/l. The modified mini-diluter had a 0.65 dilution factor, a flow rate from the toxicant dilution cell of 29 ml/min, and a flow to each test chamber of 10 ml/min.

Observations of fish behavior and toxic signs were made at 2-8, 24, 48, 72 and 96 hr and recorded on checklists specifically formatted to convert observational data for approximately 100 endpoints into a numerically coded form. Unique behavior was recorded using a color video camera and 0.5 inch video tape recorder. Death was recorded at 24, 48, 72 and 96 hours. Dead fish were removed.

All test exposure chambers were sampled mid-depth at 0, 24, 48, 72 and 96 hours. Concentrations of test material from the exposure tanks were analyzed using gas-liquid chromatography. All analyses included one spike and one duplicate sample for every 6 to 12 water samples.

Five water quality parameters were routinely measured for each test: temperature, dissolved oxygen, total hardness, total alkalinity, and pH. The desired test temperature was 25 +/- 1 degrees C. Daily measurements of oxygen concentration and pH were taken in each treatment and the control exposure chambers if fish were present. The low, mid and high test concentration chambers were sampled once for total hardness and alkalinity.

At study termination, individual control fish were weighed (wet) and measured. Four surviving fish from the control, lowest dose group, and the dose group closest to the LC50 were preserved in 10% buffered formalin and kept for histopathological examination.

The estimated LC50 and EC50 values, with corresponding 95% confidence intervals were calculated using the corrected average of the analyzed tank concentrations and the Trimmed Spearman-Karber Method. The EC50 values were based on loss of equilibrium manifested by an inability of the fish to remain in an upright position when swimming. The mean concentrations used in the calculations were corrected for analytical recoveries of spiked water samples.

Test substance : Purity of test material was 98%.
Reliability : (1) valid without restriction
29.11.2001 (16)

Type :
Species : other: freshwater fish
Exposure period : 96 hour(s)
Unit : mg/l
Analytical monitoring : no
LC50 : c = 5.497
Method : other: ECOSAR program (v 0.99g)
Year : 2002
GLP : no
Test substance : as prescribed by 1.1 - 1.4
Test condition : The LC50 value was estimated by the ECOSAR program (v0.99g), with a Log Kow of 3.38 and water solubility of 100 mg/l entered by the user.
Reliability : (2) valid with restrictions. Data were obtained by modeling.
Flag : Supportive study for endpoint

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
Analytical monitoring	:	yes
EC50	:	m = 4.2
LC50	:	m = 7.2
Method	:	other: ASTM (1980)
Year	:	1983
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The toxicity of 1,2,4- trichlorobenzene also was tested in this study. The test conduct was the same, with the exception that the column was coated with a different material (1.5% OV-17 plus 1.95 % QF-1). The 48 hr LC50 values in unfed (calculated with Probit method) and fed organisms (calculated with moving average method) for 1,2,4- trichlorobenzene were 2.1 (1.8-2.6) and 1.7 (1.5-1.9) mg/l, respectively. The EC50 values were not determined. Chronic toxicity (renewal life cycle over 28 days) also was determined in this test (ASTM method with minor variations to control loss due to volatization). The 28 day NOEL and LOEL based on either reproduction or length was 0.69 mg/l and 1.5 mg/l, respectively. Daphnia exposed to 1.5 mg/l had 93 +/- 30 (SD) young produced per adult (versus 165 +/- 23 in control) and the length of Daphnia treated with 1.5 mg/L was 3.5 +/- 0.2 mm (versus 4.2 +/- 0.1 mm in controls).
Result	:	Water hardness and alkalinity (means and ranges were 44.7 (43.5 - 47.5) mg/l CaCO3, and 41.5 (37.0 - 45.5) mg/l CaCO3, respectively. Dissolved O2 concentrations (ranges) for unfed and fed tests were 7.9- 9.9 and 4.1 - 8.4 mg/l. PH ranged from 7.1-7.7 and 7.0 - 7.5 for unfed and fed tests, respectively. There was no mortality among controls. The LC50 values for treated, unfed and fed Daphnia (using the Probit method) were 7.4 (6.3 - 8.8) and 7.2 (6.1 - 8.5),respectively, and the EC50 values for unfed and fed organisms (using the Binomial method) were 4.2 (3.3 - 5.9) and 6.0 (4.9 - 9.5), respectively. There was no difference in the values for fed and unfed organisms.
Test condition	:	Test Organisms: Adult Daphnids were obtained from laboratory stock reared at the U.S. Environmental Protection Agency, Duluth, MN. Culturing and testing systems were maintained in an enclosed water bath (20 +/- 1 degrees C). Daphnia were maintained on a 16L:8D photoperiod coupled with a 15 min transition period between light (344 lumens at the air/water interface)and dark phases. Brood cultures of 25 animals in 1 liter beakers were fed a slurry of trout chow and yeast (30 mg/l dry weight) and water three times each week. First instar Daphnids (less than 24 hr old) from brood animals (approximately 3 weeks old) were used in tests. Test Water: Culturing and testing were done with Lake Superior water which was passed through a 5 micron fiber filter, heated to 20 degrees C, and aerated with filtered air. Water hardness and alkalinity measurements were made in accordance with procedures described by APHA. Oxygen content was measured with either a Beckman Model 0260 oxygen analyzer

or by Winkler titration. pH measurements were made with a Corning Model 12 pH meter. These measurements were generally made at a low, medium and high concentration of test material (both new and old samples).

Test Material: Stock concentrations were made by saturating lake water with the test chemical on a magnetic stirrer plate. Because of high volatility, standards and solutions used for spike recoveries were refrigerated at 4 degrees C when not in use.

Test Conduct: Tests were performed according to ASTM standards. To determine if feeding would influence toxicity, experiments were performed on both fed (20 mg/l dry weight trout chow and yeast) and unfed animals. Test containers (200 ml erlenmeyer flasks) were filled to 200 or 160 ml for unfed and fed tests (respectively). Flasks were stoppered with foil wrapped, neoprene stoppers. Four replicates of 5 animals were untreated (control) or treated with six different concentrations of test material (concentrations not listed). Each concentration tested was 60% of the next higher one.

Acute toxicity values calculated were the 48 hr median effective concentration (EC50) based on complete immobilization, and the 48 hr median lethal concentration (LC50) based on death as defined by cessation of heart beat and gut movement. Immobilization and death were determined with a 30 x dissection scope at the end of exposure. EC50 and LC50 values were derived by the measured mean toxicant concentrations (by gas chromatography, see below) at the beginning and end of the experiment, and were calculated by probit, moving average, or binomial formulae depending on the characteristics of the data. In those cases where the data could be analyzed by more than one method, the results were not significantly different.

Analysis of test concentrations: Samples (75 ml) of exposure water at the beginning and end of each test were taken from some test flasks and were transferred to 100 ml volumetric flasks containing 25 ml of hexane or isohexane and stirred rigorously for 1 hour. When necessary, samples were stored in a refrigerator for a maximum of 3 days. A duplicate and spiked sample of lake water were analyzed with each set of samples. Extraction recoveries ranged from 91 to 100%. A Hewlett-Packard 5710A gas chromatograph with an autosampler, and 63Ni pulsed electron capture detector was used for analyses. The column (2.1 m x 2mm glass packed with 80/100 mesh Gas Chrom Q coated with 4% SE 30/6 OC-210), injection port and detector were maintained at 110, 200 and 300 degrees C, respectively. The carrier gas was 5% methane in argon with a flow rate of 41.7 m/min.

Test substance : The purity of the test material was at least 95 %.
Reliability : (1) valid without restriction
18.11.2001 (36)

Type :
Species : Daphnia
Exposure period : 48 hour(s)
Unit : mg/l
Analytical monitoring : no
LC50 : c = 6.479
Method : other: ECOSAR program (v 0.99g)
Year : 2002
GLP : no

Test substance	:	as prescribed by 1.1 - 1.4
Test condition	:	The LC50 value was estimated by the ECOSAR program (v0.99g), with a Log Kow of 3.38 and water solubility of 100 mg/l entered by the user.
Reliability	:	(2) valid with restrictions. Data were obtained by modeling.
Flag	:	Supportive study for endpoint

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Skeletonema costatum (algae)
Endpoint	:	other: no data
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	= 7.3
Method	:	other: no data
Test substance	:	as prescribed by 1.1-1.4
Year	:	
Source	:	Hoechst AG Frankfurt/Main Clariant GmbH Frankfurt am Main
Test condition	:	Static
Remark	:	Since the key study was not available, data from other category members and surrogates are used to support it.
Reliability	:	(4) unassignable. Data were obtained from a IUCLID document prepared by the European Chemicals Bureau, 11-FEB-2000.
Flag	:	Key study for endpoint

18.11.2001 (50)

Species	:	Selenastrum capricornutum (Algae)
Endpoint	:	growth rate
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	Yes
EC50	:	12.5 (chlorobenzene), 2.2 (1,2- dichlorobenzene), 1.6 (1,4-dichlorobenzene), 0.9 (1,2,3- trichlorobenzene), 1.4 (1,2,4-trichlorobenzene)
Method	:	other: modification of AAPBT batch test
Year	:	1981
GLP	:	no data
Test substance	:	other TS
Remark	:	Culture medium and test conditions were similar to the AAPBT, with the exception that the temperature was 20 +/- 1 degrees C. It was remarked that the EC50 values calculated for chlorobenzenes by this method are at least 2 fold lower than other methods that did not analytically control test material concentrations.
Result	:	All materials : Initial concentrations could not be measured due to high volatility of the test material. Within a few minutes of adding the test material to the flasks the concentration was very low compared to theoretical values. Therefore, the initial concentrations calculated from the dilution of the titrated stock solutions were assumed to be the initial concentrations. Equilibrium concentrations were calculated as the mean of the analytical concentrations in samples taken after the equilibrium period and 48 and 96 hours. After the 24 hour equilibration period, the concentration of test material in the culture medium remained almost constant. Differences in the values obtained at equilibrium and after 48 or 96 hours were within the range of

acceptable analytical variability.

Chlorobenzene: For initial concentrations of 31.6, 63.2, 94.8, 126.4, 158.0, 221.2 and 284.4 mg/l, equilibrium concentrations of 6.5, 14.3, 23.3, 29.6, 37.8, 45.0 and 63.0 mg/l were determined. The mean initial concentration/ equilibrium concentration (Ci/Ceq +SD) was 4.5 +/- 0.3. Henry's constant (H) to be calculated from the equation Ci = Ceq (H x air volume in the flask/culture medium volume + 1). The value calculated from this equation (0.16) was fairly close to the reported value (0.11), confirming the validity of the method for prediction of concentrations at equilibrium.

The 96 hour EC50 value calculated for chlorobenzene inhibition of algal fluorescence was 12.5 mg/l. The maximum tested concentration that produced no effect was < 6.8 mg/l and the minimum concentration that was 100% effective was 46.3 mg/l.

1,2- dichlorobenzene: For initial concentrations of 4.75, 7.6, 14.25, 19.0, 33.25, 47.5 and 66.5 mg/l, equilibrium concentrations of 0.88, 2.75, 4.99, 6.38, 10.66, 17.55, and 21.19 were determined. The mean initial concentration/ equilibrium concentration (Ci/Ceq +SD) was 2.93 +/- 0.18. The Henry's constant (H) can be calculated from the equation Ci = Ceq (H x air volume in the flask/culture medium volume + 1). The value calculated from this equation (0.09) was fairly close to the reported value (0.08), confirming the validity of the method for prediction of concentrations at equilibrium.

The 96 hour EC50 value determined for 1,2 dichlorobenzene inhibition of algal fluorescence was 2.2 mg/l. The maximum tested concentration that produced no effect was < 0.9 mg/l and the minimum concentration that was 100% effective was 17.5 mg/l.

1,4- dichlorobenzene: For initial concentrations of 2.45, 3.92, 7.35, 12.25, 19.6, 29.4 and 39.2 mg/l, equilibrium concentrations of 0.57, 0.90, 2.66, 2.56, 4.70, 8.27, and 9.79 mg/l were determined. The mean initial concentration/ equilibrium concentration (Ci/Ceq +SD) was 4.01 +/- 0.65. The Henry's constant (H) can be calculated from the equation Ci = Ceq(H x air volume in the flask/culture medium volume + 1). The value calculated from this equation (0.14) was fairly close to the reported value (0.10), confirming the validity of the method for prediction of concentrations at equilibrium.

The 96 hour EC50 value determined for 1,4 dichlorobenzene inhibition of algal fluorescence was 1.6 mg/l. The maximum tested concentration that produced no effect was < 0.6 mg/l and the minimum concentration that was 100% effective was 9.8 mg/l.

1,2,3- trichlorobenzene: For initial concentrations of 0.58, 1.15, 2.30, 3.46, 4.61, 5.76 and 8.06 mg/l, equilibrium concentrations of 0.26, 0.45, 1.09, 1.5, 1.79, 1.95 and 2.94 mg/l were determined. The mean initial concentration/equilibrium concentration (Ci/Ceq +/- SD) was 2.49 +/- 0.3. The Henry's constant (H) can be calculated from the equation Ci = Ceq(H x air volume in the flask/culture medium volume + 1). The value calculated from this equation (0.07) was fairly close to the value calculated by EPIWIN (0.00125).

The 96 hour EC50 value determined for 1,2,3-trichlorobenzene inhibition of algal fluorescence was 0.9 mg/l. The maximum tested concentration that

produced no effect was 0.22 mg/l and the minimum concentration that was 100% effective was 3.0 mg/l.

1,2,4- trichlorobenzene: For initial concentrations of 1.66, 3.32, 6.64, 9.96, 13.28, 16.60, and 23.24 mg /l, equilibrium concentrations of 0.40, 0.62, 1.52, 2.29, 2.39, 2.79, and 4.68 mg/l were determined. The mean initial concentration/equilibrium concentration (Ci/Ceq +/- SD) was 4.72 +/- 0.67. The Henry's constant (H) can be calculated from the equation Ci = Ceq(H x air volume in the flask/culture medium volume + 1). The value calculated from this equation (0.17) was fairly close to the reported value (0.16), confirming the validity of the method for prediction of concentrations at equilibrium.

The 96 hour EC50 value determined for 1,2,4-trichlorobenzene inhibition of algal fluorescence was 1.4 mg/l. The maximum tested concentration that produced no effect was 0.37 mg/l and the minimum concentration that was 100% effective was 4.9 mg/l.

Test condition : A stock solution of test material was made at 10 times higher than the saturation solubility in distilled water in a closed vessel. The solution was stirred for 48 hours and decanted for 24 hours. The supernatant was filtered through paper filters and the concentration was measured. Final solutions were made by adding 10 ml of stock culture medium to different amounts of stock solution. Solutions were then diluted to 100 ml with distilled water and quickly transferred into the 2 liter spherical culture flasks. The medium to flask volume ratio (0.047) was low enough to avoid notable carbon dioxide deficiency. Flasks were closed by screw caps with both silicone rubber (4 mm thick) and teflon gaskets. The caps were pierced by a stainless steel needle dipped into the culture medium. Sampling for measurement of algal growth and toxicant concentrations was made through the needle by means of a syringe. The outer end of the needle was closed with Parafilm.

Capped flasks were shaken for 24 hours at 20 degrees C to let vapor and liquid phases equilibrate. The algal inoculum was then added at cell concentration of 5 x 10E6 cells/l. Culture medium and test conditions were similar to the AAPBT, with the exception that the temperature was 20 +/- 1 degrees C.

Concentrations of test material in the flasks were measured by GC after the 24 hour equilibration period and 48 and 96 hours after the inoculum was added. Aqueous solutions (4 microliters) were injected directly into the GC with a flame ionization detector.

Algal growth was measured at 24, 48, and 96 hours by in vivo fluorescence (CJ Lorenzen, Deep Sea Res. 13:223-227, 1966). Results were expressed as a percentage of the growth in the control culture and the EC50 was interpolated from the data.

Test substance : Chlorobenzene (CAS No 108-90-7), ortho dichlorobenzene (CAS No. 95-50-1), para dichlorobenzene (CAS No. 106-46-7), 1,2,3- trichlorobenzene (CAS No. 87-61-6), and 1,2,4- trichlorobenzene (CAS No. 120-82-1)

Reliability : (2) valid with restrictions. Purity of test material was not stated. The control response in terms of the increase in cell concentration, and the pH, dissolved oxygen concentration and temperatures were not provided.

Flag : Supportive study for endpoint.

27.11.2001 (14)

Type :

Species	:	other: green algae
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	no
LC50	:	c = 4.386
Method	:	other: ECOSAR program (v 0.99g)
Year	:	2002
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Test condition	:	The EC50 value was estimated by the ECOSAR program (v0.99g), with a Log Kow of 3.38 and water solubility of 100 mg/l entered by the user.
Reliability	:	(2) valid with restrictions. Data were obtained by modeling.
Flag	:	Supportive study for endpoint

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6.1 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO OTHER NON-MAMM. TERRESTRIAL SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	50
Vehicle	:	
Value	:	= 1100 mg/kg bw
Method	:	other
Year	:	1980
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	Initial average weights of males in the 631, 794, 1000, 1260 and 1580 mg/kg groups were 200, 195, 215, 205 and 205 g (respectively). Weights of females in these respective groups were 190, 190, 205, 195 and 205 g. All animals dosed with 1580 mg/kg died within one day. Two animals of each sex died within 1 to 2 days of being dosed with 1260 mg/kg. All males and one female dosed with 1000 mg/kg died within 2 to 4 days. Three females and no males dosed with 794 mg/kg died. One male and no females dosed with 631 mg/kg died within 4 days of treatment. Average weights of survivors in each dosage group were similar (with the exception of slightly lower body weights of males treated with 1260 mg/kg). Signs of toxicity included lethargy, increasing weakness, ocular discharge, and collapse. Viscera of survivors appeared normal. Hemorrhagic lungs, liver discoloration, discoloration of kidneys and spleen (in some instances) and acute GI inflammation were observed in animals that died before study termination. The LD50 values (with 95 % confidence limits in females and males) were 1000 (740 - 1350) and 1200 (840-1720) mg/kg, respectively. The LD50 value for both sexes together (with 95 % confidence limits) was 1,100 mg/kg (900 - 1340). The slopes of the curves for females, males and males/females were 3.7, 3.7 and 3.8, respectively.
Test condition	:	Groups of 5 rats/sex (fasted) were dosed orally with 631, 794, 1000, 1260 and 1580 mg/kg test material. Animals were observed for death and toxic symptoms for 14 days. Survivors were weighed on days 7 and 14 and killed on day 14. The LD50 value was calculated according to the method of deBeer (J Pharmacol Exper Ther 85:1, 1945).
Reliability	:	(2) valid with restrictions. Purity of test material was not noted.
Flag	:	Key study for endpoint

18.11.2001 (4)

5.1.2 ACUTE INHALATION TOXICITY

Type	:	LC50
Species	:	Rat or mouse
Test substance	:	As prescribed by 1.1-1.4
Remark	:	Adequate acute inhalation studies were not located. Data from category members and surrogates are used to fill this endpoint.
Reliability	:	(2) valid with restrictions

Type	:	LC50
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male
Number of animals	:	
Vehicle	:	
Exposure time	:	6 hour(s)
Value	:	= 2965 ppm
Method	:	other
Year	:	1982
GLP	:	no
Test substance	:	Monochlorobenzene (CAS No. 108-90-7).
Result	:	The LC50 value was 2965 (2787 - 3169) ppm. The regression equation was y = 10.9x + 33. The LC50 value in mg/l is 13.9.
Test condition	:	Rats (130 -160 g) were exposed to concentrations ranging from 2000 to 3500 ppm over 6 hours (12/group). Vapor was generated at 24 degrees C, 50 % relative humidity. Rats were observed for 14 days. A line of regression was plotted with the abscissa being the log of the concentration of test material and the ordinate being the probit of the percentage of corresponding deaths. The LC50 value was the concentration (x value) at which the y value was 50%.
Test substance	:	The test material was 99% pure.
Reliability	:	(2) valid with restrictions. Test material was a related chemical

27.11.2001 (6)

Type	:	LC50
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male
Number of animals	:	6
Vehicle	:	
Exposure time	:	3.75 hour(s)
Value	:	< 39.7 mg/l
Method	:	other
Year	:	1976
GLP	:	no
Test substance	:	Monochlorobenzene (CAS No. 108-90-7)
Result	:	All animals died within 3.75 hours. Signs of toxicity were tremors, labored breathing and collapse. Slight lung congestion was noted at autopsy.
Test condition	:	Six male rats (initial weight not stated) were exposed to 39.7 mg/l test material for 3.75 hours at ambient temperature. The chamber humidity was 80% and the volume was 35 l. The air flow rate was 4.0 l/min.
Test substance	:	The material was technical grade, which has a purity of 99%.
Reliability	:	(2) valid with restrictions. The test material was a related chemical.

27.11.2001 (3)

Type	:	LC50
Species	:	mouse
Strain	:	
Sex	:	female
Number of animals	:	
Vehicle	:	
Exposure time	:	6 hour(s)
Value	:	= 1886 ppm
Method	:	other
Year	:	1979

GLP	:	no
Test substance	:	Monochlorobenzene (CAS No. 108-90-7), 99% pure
Result	:	The LC50 value was calculated as 1886 ppm (1781-1980) with a regression equation of y = 6.734 x + 17.06. The value in mg/l is 8.8.
Test condition	:	Female mice (21 g) were exposed in 200 l chambers to 1400 to 3000 ppm for 6 hours (25/group). Vapor was generated at 24 degrees C, 50 % relative humidity and an air flow of 40 m3/hr. There were 60 air changes/hour. Animals were observed for 14 days. A line of regression was plotted with the abscissa being the log of the concentration of test material and the ordinate being the probit of the percentage of corresponding deaths. The LC50 value was the concentration (x value) at which the y value was 50%.
Reliability	:	(2) valid with restrictions. Test material was a related chemical.

27.11.2001 (6)(7)

Type	:	LC50
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male
Number of animals	:	
Vehicle	:	
Exposure time	:	6 hour(s)
Value	:	= 1532 ppm
Method	:	other
Year	:	1979
GLP	:	no
Test substance	:	1,2-dichlorobenzene (CAS No. 95-50-1). The test material was 99% pure
Result	:	The LC50 value with confidence limits was 1532 (1384 - 1730) ppm. The regression equation was y = 6.5 x + 15.8. The LC50 in mg/l is 9.38.
Test condition	:	Rats (130 -160 g) were exposed to concentrations ranging from 1000 to 2000 ppm over 6 hours. Vapor was generated at 24 degrees C, 50 % relative humidity. Rats were observed for 14 days. A line of regression was plotted with the abscissa being the log of the concentration of test material and the ordinate being the probit of the percentage of corresponding deaths. The LC50 value was the concentration (x value) at which the y value was 50%.
Reliability	:	(2) valid with restrictions. Test material was a related chemical.

19.11.2001 (6)

Type	:	LC50
Species	:	mouse
Strain	:	
Sex	:	female
Number of animals	:	
Vehicle	:	
Exposure time	:	6 hour(s)
Value	:	= 1236 ppm
Method	:	other
Year	:	1979
GLP	:	no
Test substance	:	1,2-dichlorobenzene (CAS No. 95-50-1). The test material was 99% pure.
Result	:	The LC50 value with confidence limits was 1236 (1201 - 1279) ppm. The regression equation was y = 13.303x + 42870. The LC50 value in mg/l is 7.43.
Test condition	:	Female mice (21 g) were exposed in 200 l chambers to various concentrations of test material for 6 hours. Vapor was generated at 24

degrees C, 50 % relative humidity and an air flow of 40 m3/hr. There were 60 air changes/hour. Animals were observed for 14 days. A line of regression was plotted with the abscissa being the log of the concentration of test material and the ordinate being the probit of the percentage of corresponding deaths. The LC50 value was the concentration (x value) at which the y value was 50%.

Reliability : ((2) valid with restrictions. Test material was a related chemical.
19.11.2001 (6)(7)

Type : LC50
Species : rat
Strain : Sprague-Dawley
Sex : male/female
Number of animals : 10
Vehicle :
Exposure time : 4 hour(s)
Value : > 6.0 mg/l (997 ppm)
Method : other
Year : 1990
GLP : yes
Test substance : 1,4-dichlorobenzene (CAS No. 106-46-7).Test material was analyzed as 103% 1,4-dichlorobenzene, 0.0435 % 1,3- dichlorobenzene and 0.0368% 1,2- dichlorobenzene.
Result : All animals survived the exposure. The LC50 value was greater than the amount tested (6.0 mg/l). Based on a molecular weight of 147, rats were exposed to 997 ppm.

Animals exhibited lacrimation, salivation, nasal discharge, labored breathing, gasping, tremors, ano-genital staining and chromodacryorrhea.

Test condition : Rats (5/sex, 202-236 g) were exposed to vapor (target concentration of 5.0 mg/l) by inhalation for 4 hours. The exposure concentration (6.0 mg/l)was analyzed by infrared spectroscopy. An aerodynamic particle sizer was used to confirm that the material did not aerosolize. Rats were observed for toxicity every 15 min for the first hour and hourly for the remainder of the study. All animals received detailed physical observations just prior to exposure, hourly for the first two hours of exposure, and once daily thereafter. Animals were weighed just prior to exposure and on Days 2, 3, 5, 8 and 15. Animals were euthanized on Day 15. Complete gross postmortem examinations were performed on all animals.

Reliability : (2) valid with restrictions. Test material was a related chemical.
19.11.2001 (35)

5.1.3 ACUTE DERMAL TOXICITY

Type : LD50
Species : rabbit
Strain : New Zealand white
Sex : male/female
Number of animals : 4
Vehicle :
Value : > 2000 mg/kg bw
Method : other
Year : 1980
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark	:	No signs of toxicity were noted.
Reliability	:	(4) not assignable. There are not enough details in study documentation to assign a reliability rating.

18.11.2001 (4)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

5.2.2 EYE IRRITATION

5.3 SENSITIZATION

5.4 REPEATED DOSE TOXICITY

Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	90 days
Frequency of treatment	:	daily
Post obs. period	:	none
Doses	:	9, 37, 147, 588 mg/kg/day
Control group	:	yes, concurrent vehicle
NOAEL	:	= 37 mg/kg bw
LOAEL	:	= 147 mg/kg bw
Method	:	other
Year	:	1995
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Effects noted on the pituitary, liver and thyroid at 9 mg/kg are minimal. The pathological significance of the effects in the thyroid and pituitary were not discussed in the reference. There is no indication in the study that the pathology evaluation was blinded. A subjective evaluation of a change defined as minimal to mild cannot be considered a statistically significant effect. A decrease in LDH is not considered to be indicative of toxicity. While the AST is increased above control at 9 mg/kg, it is within normal range of levels reported for Sprague Dawley males and is not elevated at 37 mg/kg. Therefore, it is not considered to be a significant toxicological effect. The increase in serum cholesterol at this concentration, while statistically significant, is minor. According to USEPA guidance, changes in serum chemistry values are not considered to be adverse effects unless they can be clearly identified as a precursor to adverse effects. Therefore, 9 mg/kg should be the NOAEL, rather than the LOAEL. The values reported for alkaline phosphatase are suspect. Historical values for Sprague Dawley rats are approximately 50-100 U/l in females and 100 - 200 U/l in males. The values reported in this study are in the

single digits. These values seem more relevant for phosphorus, which is normally less than 10 mg/dl in Sprague-Dawley rats. The abbreviation used in the Tables and text for alkaline phosphatase is "phos", which could easily be interpreted as "phosphorus" by study personnel.
The reliability of this study is rated a 2 because the study may not have been evaluated blindly, and some laboratory values reported in tables do not seem accurate.

Oral toxicity of 37, 147, 368 and 735 mg/kg/day over 10 days was studied in a preliminary study. The NOAEL in this study was 147 mg/kg/day. Effects noted at higher doses were decreased body weight in males treated with 735 mg/kg/day, increased liver weight and serum cholesterol in both sexes treated with 365 and 735 mg/kg/day, and centrilobular hepatocellular degeneration at 368 mg/kg/day in males and 735 mg/kg/day in females.

Result : All doses: There were not compound-related deaths or clinical signs of toxicity in treated animals. Average daily food consumption was not different from controls in any of the test groups.

588 mg/kg: Average daily water consumption of both sexes was increased by 18% over control. Males and females treated with this dose had lower final body weights than controls (males 392 +/- 47 g vs. 517 +/- 47 g in controls; females 249 +/- 13 g vs. 277 +/- 22 g in controls). The weight loss was progressive over the course of the study (data not shown). Females had increased relative (g/100 g body weight) kidney (0.87 +/- 0.10 vs. 0.72 +/- 0.07 in controls) and liver weights (5.11 +/- 0.35 vs. 2.93 +/- 0.33 in controls) and males had significantly increased relative brain (0.50 +/- 0.07 vs. 0.41 +/- 0.04 in controls), testes (0.85 +/- 0.14 vs. 0.68 +/- 0.08 in controls), kidney (0.96 +/- 0.14 vs. 0.71 +/- 0.06 in controls) and liver weights (5.20 +/- 0.78 vs. 2.81 +/- 0.27 in controls). High dose females and males exhibited an increased white blood cell count (6400 +/- 1000 vs. 4100 +/- 1000 in controls) and red blood cell count (7.9 +/- 0.3 x 10E6 vs. 7.4 +/- 0.3 x 10E6 in controls), respectively. There was decreased plasma BUN (13.1 +/- 2.0 vs. 19.4 +/- 3.5 mg/dl in control) and increased cholesterol (152.6 +/- 2.6 vs. 68.2 +/- 1.7 mg/dl in control) and calcium (12.1 +/- 0.5 vs. 10.8 +/- 0.3 mg/dl) in females, and decreased plasma LDH (735 +/- 287.7 vs. 1762.0 +/- 765.2 U/l in control) and BUN (15.8 +/- 2.3 vs. 20.0 +/- 3.0 mg/dl in control) and increased calcium (12.0 +/- 0.5 vs. 11.3 +/- 0.7 mg/dl), cholesterol (89.5 +/- 1.5 vs. 73.5 +/- 1.4 mg/dl in control) and AST (82.8 +/- 13.8 vs. 43.7 +/- 37.7 U/l in control) in males. Pathological findings in this group included a depletion of colloid density in the thyroid (most graded as mild to moderate) in 16/17 animals graded, hepatic inflammation (grade and incidence not stated), hepatocellular cytoplasmic alterations (minimal to moderate in 14/18 examined), hepatocellular necrosis (minimal to moderate in 10/18 examined), and moderate cytoplasmic vacuolization in the pars distalis of the pituitary (in 100% of males but no females). The control incidences in reduction in colloid density in the thyroid and vacuolization of the pars distalis of the pituitary were 3/20 animals and 2/10 males, respectively). Other changes observed were not considered to be related to treatment.

147 mg/kg: There was no effect of treatment on body weight. Decreased relative brain (0.67 +/- 0.03 vs. 0.74 +/- 0.07 in controls) and increased relative liver (3.86 +/- 0.28 vs. 2.93 +/- 0.33 in controls) weight were noted in females, and increased relative kidney (0.99 +/- 0.08 vs. 0.71 +/- 0.06 in controls) and liver (4.24 +/- 0.25 vs. 2.18 +/- 0.27 in controls) weights were found in males. Males dosed with 147 mg/kg had an increased white blood cell count (8700 +/- 2100 vs. 5600 +/- 900 in controls). There were

increased cholesterol (approximately 158 mg/dl vs. approximately 70 mg/dl in controls) and calcium levels (similar to those at the 588 mg/kg dose) in males and females, and males had significantly decreased LDH (777.8 +/- 529.9 vs. 1762 +/- 765.2 U/l in control) and increased AST values (88.0 +/- 23.3 vs. 43.7 +/- 37.7 U/l in control). Pathological findings in this group included a depletion of colloid density in the thyroid (graded as minimal to moderate) in 17/19 animals graded, hepatic inflammation (grade and incidence not stated), hepatocellular cytoplasmic alterations (minimal to mild in 6/10 males and 1/10 females), hepatocellular necrosis (minimal in 5/20 examined), and minimal to moderate cytoplasmic vacuolization in the pars distalis of the pituitary (in 100% of males but no females). The control incidences in reduction in colloid density in the thyroid and vacuolization of the pars distalis of the pituitary were 3/20 animals and 2/10 males, respectively). Other changes observed were not considered to be related to treatment.

37 mg/kg: There was no effect on body weight, relative organ weights, or hematology parameters. Serum cholesterol (approximately 110 mg/dl vs. 70 mg/dl in controls) and calcium (similar to that of 588 mg/kg dose in males and slightly less than 588 mg/kg in females) increased in both sexes. Increased alkaline phosphatase levels were observed in females (8.0 +/- 0.7 U/l vs 6.9 +/- 1.0 U/l in controls) and decreased LDH levels (797.8 +/- 237.6 vs. 1762.0 +/- 762.2 U/l in control) in males. Pathological findings in this group included a depletion of colloid density in the thyroid (graded as mild to moderate) in 18/20 animals graded, hepatic inflammation (grade and incidence not stated), and minimal to moderate cytoplasmic vacuolization in the pars distalis of the pituitary (in 6/10 males but no females). The control incidences in reduction in colloid density in the thyroid and vacuolization of the pars distalis of the pituitary were 3/20 animals and 2/10 males, respectively). Other changes observed were not considered to be related to treatment.

9 mg/kg: There was no effect on body weight, relative organ weights, or hematology parameters. There were decreased LDH (623.0 +/- 466.3 vs. 1762.0 +/- 765.2 U/l in control) and increased AST (87.6 +/- 24.7 vs. 43.7 +/- 37.7 U/l in control) and cholesterol levels (96.6 +/- 1.7 vs. 73.5 +/- 1.4 mg/dL in control) in males. There were no effects on clinical chemistries in females. Pathological findings in this group included a depletion of colloid density in the thyroid (graded as minimal to mild) in 13/20 animals graded, hepatic inflammation (grade and incidence not stated), hepatocellular cytoplasmic alterations (minimal in 4/20 examined), mild necrotic hepatocellular foci (in 2 males) and minimal to mild cytoplasmic vacuolization in the pars distalis of the pituitary (6/10 males but no females). The control incidences in reduction in colloid density in the thyroid and vacuolization of the pars distalis of the pituitary were 3/20 animals and 2/10 males, respectively). Other changes observed were not considered to be related to treatment.

Test condition : Rats were 70 days old when received. Males weighed 300-325 g and females weighed 225-250 g. Animals were quarantined for 10 days before treatment. They were allowed free access to food and water. One hundred animals were divided into 5 experimental groups (10/sex/group). Animals were gavaged daily for 90 consecutive days with 0 (corn oil vehicle), 9, 37, 147 or 588 mg/kg. Individual doses were determined weekly from individual body weights. Each rat received a volume of 0.1 ml per 100 g body weight. Dosing solutions were prepared weekly from a stock solution.

All rats were observed daily for physiological and behavioral responses and for mortality. Body weights and food and water consumption were recorded weekly throughout the study. Blood samples were collected via cardiac puncture at necropsy for hematological and serum chemistry measurements. The tissues (brain, liver, spleen, lungs (with lower half of trachea), thymus, kidneys, adrenal glands, heart and gonads) from all animals in the high dose, and half the animals in the control group were weighed and grossly examined at necropsy. The skin, mandibular and mesenteric lymph nodes, mammary gland, thigh muscle, sciatic nerve, sternebrae, thymus, esophagus, stomach, duodenum, jejunum, tongue, salivary gland, ileum, colon, cecum, rectum, pancreas, urinary bladder, seminal vesicle, prostate, uterus, nasal cavity and turbinates, pituitary, preputial or clitoral gland, Zymbal's gland, aorta, thyroid, parathyroids and any gross lesions were examined grossly and preserved in 10% neutral buffered formalin. Slides of tissues from all animals in the high dose and half of the male and female controls were prepared. Slides of the liver, thyroid and pituitary of all animals were prepared. Inflammatory and degenerative lesions were graded on a relative scale from 1 to 4 (minimal, mild, moderate or marked) depending on the severity of the lesion. If affected tissues were identified, these tissues were examined from the remaining dose groups.

Hematology samples were evaluated for white and red blood cell counts, hemoglobin, hematocrit, and mean corpuscular volume using a Coulter counter. Serum clinical chemistry parameters measured included glucose, blood urea nitrogen (BUN), creatinine, alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol, lactate dehydrogenase (LDH) and calcium.

Data for males and females were analyzed separately. A one-factor (dose) analysis of variance (ANOVA) was used to analyze normally distributed measures: body weights, organ weights, organ weight ratios, food and water consumption, and hematology and clinical chemistry parameters. When a treatment effect was noted by the F-test ($P <= 0.05$), the difference between the control and treatment groups was analyzed using Tukey's Multiple Comparison Procedure. Hematologic and clinical chemistry data that were not normally distributed were analyzed using the nonparametric Kruskal Wallis test. A Wilcoxon Rank Sum method was then used to analyze differences between means. The data for incidence of histopathological lesions were analyzed by a Fisher Exact test, with a criterion of significance of $P <= 0.05$.

Test substance : GC/MS indicated the purity of the material was > 99.99%.
Reliability : (2) valid with restrictions. The study may not have been evaluated blindly, and some laboratory values reported in tables do not seem accurate.

18.11.2001 (29)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Ames test
System of testing : S. typhimurium strains TA98, TA100, TA1535, TA1537, TA1538
Concentration : 0.02 to 2.56 microliters/plate
Cytotoxic conc. : 1.28 microliters/plate
Metabolic activation : with and without
Result : negative
Method : other
Year : 1983

GLP : no data

Test substance : as prescribed by 1.1 - 1.4

Remark : Thirty different chemicals were tested in this experiment (benzene, 4 fluorobenzenes (mono or di), chlorobenzene, 3 dichlorobenzenes (ortho, meta and para), nitrobenzene, 6 fluoronitrobenzenes (mono or di), 8 chloronitrobenzenes (mono, di or tri), 1 chloro-, fluorobenzene, 3 nitrobenzenes, 1 dinitrofluorobenzene and 1 dinitrochlorobenzene). Fifteen of the compounds containing nitro groups were mutagenic. All compounds without a nitro group showed no mutagenic activity.

This test was given a reliability of 2 because statistical analyses were not performed.

Result : The average number of revertants in the DMSO controls for strains TA98, TA100, TA1535, TA1537 and TA1538 in the absence of S-9 were 28, 181, 32, 8, and 22, respectively. Addition of S-9 to controls did not significantly increase the frequency of mutations. The average number of revertants in all positive control cultures were as follows: ENNG, 1994 in TA100 and 2489 in TA1535; 2-NF, 1798 in TA98 and 1659 in TA1538; 9-AA, 1288 in 1537; and 2-AA, from 132 in TA1537 to 1549 in TA100. The test was valid based on these data.

The number of revertants induced by m-dichlorobenzene was not increased from that of controls at any concentration and tended to decrease with increasing concentration (based on a visual review of the data). Metabolic activation did not appear to significantly increase the number of mutations observed at each concentration. The number of revertants observed in cultures treated with nontoxic concentrations of m-dichlorobenzene (in the presence or absence of S9) ranged from 15-33 in TA98, 135-228 in TA100, 17-38 in TA1535, 6-13 in TA1537, and 17-31 in TA1538. A concentration of 1.28 microliters/plate was toxic to strains TA98 and TA1535 (without S-9), TA1538, and TA1537. A concentration of 2.56 microliters/plate killed all the cells (with the exception of TA100 + S9).

Test condition : All strains of bacteria were supplied by the same supplier (Dr. B. Ames). Test material was dissolved in sterile dimethylsulfoxide. S-9 was prepared from liver homogenate (25% in 0.15M KCl) from male Sprague-Dawley rats (100-200 g) that had been injected with PCB at a dose of 500 mg/kg 5 days before they were killed. S-9 mix contained per ml: S9(0.3 ml), $MgCl_2$ (8 micromoles), KCl (33 micromoles), glucose 6 -phosphate (5 micromoles), NADH (4 micromoles), NADPH (4 micromoles), and sodium phosphate, pH 7.4 (100 micromoles).

Various concentrations (from 0.02 microliters/plate to the concentration that caused toxicity) of test compound (0.1 ml) were added to sterile test tubes containing 3-6 x 10E7 bacterial cells, 0.5 ml of S-9 mix (+ activation) or sodium phosphate buffer (pH 7.4) (- activation). This mixture was preincubated in a shaker water bath at 37 degrees C for 15 min, then added to 2 ml molten top agar (45 degrees C). The contents of each tube were mixed and immediately poured onto the surface of a minimal-agar plate. DMSO (0.05 ml) also was added to plates containing each kind of bacteria (negative control). N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG; 2 or 10 micrograms/plate incubated with strains TA100 and TA 1535 without S-9, 2-nitrofluorene (2-NF; 2 or 5 micrograms/plate incubated with strains TA98 and TA1538 without S-9, 9-aminoacridine (9-AA; 100 micrograms/plate incubated with strain TA1537 without S-9; and 2-aminoanthracene (2-AA;5 micrograms/plate incubated with all strains with S-9 only) were used as positive controls. All tests were performed in duplicate and were repeated at least 3 times separately.

Plates were inverted and incubated at 37 degrees C in the dark for 3 days. Colonies of his+ revertants were counted after incubation. Chemicals inducing more than twice the number of revertant colonies as negative control plates were considered to be mutagenic. Tests without metabolic activation were carried out first. Tests with metabolic activation were only carried out if results of the tests without activation were negative.

Two strains (TA98 and TA100) were checked routinely for the presence of the ampicillin resistance for the R factor. The background bacterial lawn was routinely checked by microscopy for thinning (evidence of toxicity) and contamination.

Test substance : Purity of the test substance was 98%.
Reliability : (2) valid with restrictions. Statistical analyses were not performed.
Flag : Key study for endpoint
18.11.2001 (42)

Type : Ames test
System of testing : S. typhimurium strains TA98, TA100, TA1535, TA1537
Concentration : 1, 3.3, 10, 33, 100 micrograms/plate
Cytotoxic conc. : 100 (in TA100 only)
Metabolic activation : with and without
Result : negative
Method : other
Year : 1983
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Test condition : Two hundred chemicals were tested. Fourteen of the chemicals tested were positive controls. Test material was dissolved in DMSO. DMSO was tested as the negative control. Tests were performed without S-9, or with S-9 from rats and hamsters (separately)
Test substance : Purity was 98%.
Reliability : (2) valid with restrictions.
02.29.2002 (18)

Type : Ames test
System of testing : S. typhimurium strains TA98, TA100, TA1535, TA1537
Concentration : 0.6, 2.4, 12, 60, 180, 600 micrograms/plate (plate incorporation test); 10 mg/plate (spot test)
Cytotoxic conc. : 600 micrograms/plate
Metabolic activation : with and without
Result : negative
Method : other
Year : 1978
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Reliability : (2) valid with restrictions.
02.29.2002 (13)

Type : Ames test
System of testing : S. typhimurium strains TA98, TA100, TA1535, TA1537, TA1538
Concentration : 0.0005 to 1.0 microliters/plate
Cytotoxic conc. : 1.0 microliters/plate
Metabolic activation : with and without
Result : negative
Method : other
Year : 1979

GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Chlorobenzene, o-dichlorobenzene and p- dichlorobenzene also tested negative.

02.27.2002 (43)

Type	:	Ames test
System of testing	:	S. typhimurium TA100, TA98 (repair deficient) and UTH8414, UTH8413 (full repair capacity)
Concentration	:	50, 100, 500, 1000 and 2000 micrograms/plate
Cytotoxic conc.	:	unknown
Metabolic activation	:	with and without
Result	:	negative
Method	:	other
Year	:	1985
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Positive and negative controls were used in study. Ortho and para dichlorobenzene also tested negative.
Reliability	:	(2) valid with restrictions.

18.11.2001 (11)

Type	:	Escherichia coli reverse mutation assay
System of testing	:	E. coli WP2(trp-, uvRA-)
Concentration	:	0.0005 to 1.0 micrograms/l
Cytotoxic conc.	:	1.0 micrograms/l
Metabolic activation	:	with and without
Result	:	negative
Method	:	other
Year	:	1979
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Chlorobenzene, o-dichlorobenzene and p- chlorobenzene also tested negative.
Reliability	:	(2) valid with restrictions. Purity of test material was not given.

19.11.2001 (43)

Type	:	Chromosomal aberration test
System of testing	:	Chinese Hamster Ovary Cells
Concentration	:	125 to 500 micrograms/ml in separate experiments
Cytotoxic conc.	:	500 micrograms/ml
Metabolic activation	:	with and without
Result	:	negative
Method	:	other
Year	:	1985
GLP	:	no data
Test substance	:	other TS
Remark	:	Article is in Japanese
Result	:	There was no test-material related increase in the frequency of aberrant cells in either experiment
Test condition	:	Two different tests were done with the test material. In the first, cells were incubated for 24 or 48 hours with DMSO vehicle, and 125, 250 and 500 micrograms/ml test material. 500 micrograms/ml was toxic at both time points. In the second experiment, cells were incubated with DMSO or 200, 300 or 400 micrograms/ml test material and with DMSO, 300, 400, and 500

micrograms test material in the presence of S9. In this experiment, 500 micrograms/ml was not toxic.

Test substance : Test material was chlorobenzene (CAS No. 108-90-7).
Reliability : (2) valid with restrictions. Reference was not translated.
Flag : Supportive study for clastogenicity endpoint.
27.11.2001 (45)

Type : Chromosomal aberration test
System of testing : Chinese Hamster Ovary Cells
Concentration : 31.3 to 125 micrograms/ml
Cytotoxic conc. : 125 micrograms/ml
Metabolic activation : Without
Result : Negative
Method : other
Year : 1985
GLP : no data
Test substance : other TS
Remark : Article is in Japanese
Result : There was no test-material related increase in the frequency of aberrant cells
Test condition : Cells were incubated for 24 or 48 hours with DMSO vehicle, and 31.3, 62.5 and 125 micrograms/ml test material. 125 micrograms/ml was toxic at both time points. Experiments with S-9 were not performed
Test substance : Test material was 1,2,4- trichlorobenzene (CAS No. 120-82-1).
Reliability : (2) valid with restrictions. Reference was not translated.
Flag : Supportive study for clastogenicity endpoint.
27.11.2001 (45)

Type : Chromosomal aberration test
System of testing : Chinese Hamster Ovary Cells
Concentration : 15.7 to 125 micrograms/ml in separate experiments
Cytotoxic conc. : > 125 micrograms/ml
Metabolic activation : with and without
Result : negative
Method : other
Year : 1985
GLP : no data
Test substance : Other TS
Remark : Article is in Japanese
Result : There was no test-material related increase in the frequency of aberrant cells in either experiment.
Test condition : Two different tests were done with the test material. In the first, cells were incubated for 24 or 48 hours with DMSO vehicle, and 15.7, 31.3 and 62.5 micrograms/ml test material.

In the second experiment, cells were incubated with DMSO or 100 or 125 micrograms/ml test material in the presence and absence of S9.

Test substance : Test material was 1,2,3- trichlorobenzene (CAS No. 87-61-6).
Reliability : (2) valid with restrictions. Reference was not translated.
29.11.2001 (45)

Type : Chromosomal aberration test
System of testing : Chinese Hamster Ovary Cells
Concentration : 25 to 150 micrograms/ml
Cytotoxic conc. : Not stated
Metabolic activation : With and without

Result : Negative
Method : other
Year : 1987
GLP : no data
Test substance : other TS
Result : There was no test-material related increase in the frequency of aberrant cells at doses of 25-150 micrograms/ml. No toxicity or cell cycle delay was found. A precipitate was noted at 150 micrograms/ml. The positive controls induced at least a 7-fold increase in the percentage of cells with aberrations.
Test condition : Doses (50, 100 and 150 micrograms/ml without S-9 and 75 and 25, 50 and 100 micrograms/ml with S-9) were based on a preliminary test of cell survival 24 hours after treatment. Rat liver S-9 mix from Aroclor 1254-induced male Sprague-Dawley rats was used for metabolic activation. In tests without metabolic activation, test chemical was left in culture until colcemid addition (0.1 micrograms/ml). With activation, test chemical was added along with S-9 mix for 2 hr, and then the medium was replaced. The time at which colcemid was added was not stated. Cells were collected 10.5 hours after treatment by mitotic shake-off. Slides were stained with Giemsa and coded.

One hundred cells were scored from each of the dose groups having sufficient metaphases for analysis (metaphases with 19-23 chromosomes) and from positive (0.5 micrograms/ml mitomycin C without S-9 or 25 micrograms/ml cyclophosphamide with S-9) and solvent (DMSO) controls. All types of aberrations were recorded separately, but for analysis were grouped into categories of simple (breaks and terminal deletions), complex (rearrangements and exchanges) and other (included pulverized chromosomes). Gaps and endoreduplications were recorded but were not included in the totals. Aberrations in polyploid cells were not scored. Linear regression of the percentage of cells with aberrations vs. the log-dose was used as the test for trend. A binomial sampling assumption was used, and data were analyzed according to the method of Margolin. P values were adjusted by Dunnett's method to take multiple dose comparisons into account. The criterion for a positive response was that the adjusted p value was < = 0.05. A test was repeated if there was a single elevated point, if the controls were unusually high, or if the range of toxicity was inadequate.

Test substance : Test material was 1,4-dichlorobenzene (CAS No. 106-46-7).
Reliability : (2) valid with restrictions. Purity of test material was not given.
Flag : Supportive study for clastogenicity endpoint.
27.11.2001 (15)

Type : Sister Chromatid Exchange Test
System of testing : Chinese Hamster Ovary Cells
Concentration : 75 to 150 micrograms/ml
Cytotoxic conc. : Not stated
Metabolic activation : With and without
Result : Negative
Method : other
Year : 1987
GLP : no data
Test substance : other TS
Result : There was no test-material related increase in sister chromatid exchanges at doses of 75-150 micrograms/ml in the absence of S-9. A positive result at one concentration (75 micrograms/ml) was obtained in one test with S-9 (10.89 SCE/cell vs. 8.96 SCE/cell in control). The test was repeated and

the results at all three concentrations were similar to control. The overall result was negative. No toxicity or cell cycle delay was found. A precipitate was noted at 150 micrograms/ml. Both positive controls induced approximately a 3-fold increase in SCEs.

Test condition : The material was tested at 75, 100, 125 and 150 micrograms/ml without S-9 and 75, 100 and 125 micrograms/ml in one test with S-9 and 100, 125 and 150 micrograms/ml in another). Positive controls were mitomycin C (0.001 and 0.005 micrograms/ml without S-9) and cyclophosphamide (0.3 micrograms/ml with S-9). The solvent control was DMSO. Rat liver S-9 mix from Aroclor 1254-induced male Sprague-Dawley rats was used for metabolic activation. With activation, test chemical was added along with S-9 mix for 2 hr, and then the medium was replaced. 5-bromodeoxyuridine (1BrDU, 10 micromolar) was added 2 hours after addition of the test chemical (without S-9) or immediately after the S-9 mix with test chemical were removed. The total incubation time in BrDU was 25-26 hours. Colcemid (0.1 micrograms/ml) was present during the final 2-3 hours. Cells were collected by mitotic shake-off. After 1-3 min treatment with hypotonic solution (75 mM KCl), cells were fixed in 3 parts methanol: 1 part glacial acetic acid. Slides were prepared from cells treated with the highest concentration to see if later harvests were necessary.

After staining for 10 min in concentrated Hoechst 33258 and exposure to black light at 55-60 degrees C for 5 minutes, slides were stained in Giemsa. All slides were coded, and 50 cells per concentration were scored (with the exception of 10 for 0.005 micrograms/ml mitomycin C). Linear regression of the number of sister chromosomes per chromosome vs. the log-dose was used as the test for trend. For individual doses, absolute increases in sister chromatid exchanges (SCEs) per chromosome of 20% or more over the positive control were considered significant. A test was repeated if there was a single elevated point, if the controls were unusually high, or if the range of toxicity was inadequate.

Test substance : Test material was 1,4-dichlorobenzene (CAS No. 106-46-7).
Reliability : (2) valid with restrictions. Purity of test material was not given.
Flag : Supportive study for clastogenicity endpoint.
27.11.2001 (15)

Type : Sister chromatid exchange assay
System of testing : Chinese hamster ovary cells
Concentration : 5.9, 19.7 or 59 micrograms/ml (without metabolic activation) and 19.7, 59 or 197 micrograms/ml in one test (with metabolic activation) and 300, 400 or 500 micrograms/ml in another test (with metabolic activation).
Cytotoxic conc. :
Metabolic activation : with and without
Result :
Method : other
Year : 1990
GLP : no data
Test substance : other TS
Remark : It was difficult to read the results in the study, but it looks like the result was just positive and was not dose-dependent.
Result : The test for sister chromatid exchange was negative in the absence of S9 and positive in the presence of S-9 (at concentrations of 59, 197, 200 and 400 micrograms/ml). The test for chromosomal aberrations was negative both in the presence and absence of S-9.
Test condition : Chinese hamster ovary (CHO) cells were obtained at their fifth passage level after cloning. Cells were tested regularly for mycoplasma

contamination. They were not used beyond the fifteenth passage after cloning. Stocks of CHO cells were maintained at 37 degrees C in McCoy's A (modified) medium buffered with 20 mM HEPES and supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 50 IU/ml penicillin and 50 micrograms/ml streptomycin. Test cultures were set up in 75 cm2 flasks 24 hours before treatment at a density of 1.25 x 10E6 cells/flask.

Stock solutions of 1,2 dichlorobenzene were prepared at 500 mg/ml (or the limit of solubility). A series of dilutions were made from the stock solution to achieve 10 test concentrations in a half-log series (covering a range of 5 logs). The highest dose used was that which allowed for a sufficient number of cells to be scored at time of harvest.

Cells were exposed to test material, vehicle, or positive control agent (mitomycin C at a level known to induce an 20-80% increase in sister chromatid exchanges (SCEs) and another to induce a greater than 100% increase in SCEs) in the absence or presence of S-9 for 2 hours before addition of bromodeoxyuridine (BrDU; 10E-5 M). In cells that were incubated with S-9, test medium did not contain serum. Flasks were sealed. Cultures were incubated for 24 additional hours with BrDU. The chemical and BrDU were then removed and cells were rinsed twice with PBS. Fresh medium with BrdU and colcemid were added and the cells were incubated at 37 degrees C for an additional 2-2.5 hr. Cells were then examined for toxicity (% confluence of monolayer). Cells were then harvested, treated for 12 min at 37 degrees C with hypotonic buffer, and resuspended in 3 volumes of fixative. Slides were prepared and examined with fluorescence microscopy to determine the frequency of metaphase cells that had completed one or two cell cycles in BrDU (M1 or M2 cells). Fifty second division M2 cells from each of the top 3 test concentrations were scored for SCEs.

Chromosomal aberrations in CHO cells were determined by incubating cells at 37 degrees C (1.75 x 10E6 cells/75 cm2 flask) with test chemical for 8 hours (without S-9) or incubating cells for 2 hrs in serum free medium (with S-9) and then an additional 8 (without S-9), washing cells, and incubating them with colcemid 2 -2.5 hr before cell harvest. The total durations of the nonactivated and activated experiments were 10 and 12 hrs, respectively. Cells were harvested, stained with Giemsa and scored for aberrations.

All slides (except those from the high dose positive control) were coded and a complete experiment was scored by the same person. A trend test of SCEs per chromosome vs. log of concentration was used to examine data. A 20% increase in sister chromatid exchange at 2 doses was considered positive.

Test substance : Test material was orthodichlorobenzene (CAS No. 95-50-1). It's purity was 99.4%.

Reliability : (1) valid without restriction

Flag : Supportive study for clastogenicity endpoint.

19.11.2001 (27)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Cytogenetic assay

Species : Chinese hamster

Route of admin. : Oral unspecified

Doses	:	1000 mg/kg
Result	:	negative
Method	:	OECD Guide-line 475 "Genetic Toxicology: In vivo mammal bone marrow cytogenetic test- chromosomal analysis"
Year	:	1988
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Source	:	Hoechst AG Frankfurt/Main Clariant GmbH Frankfurt am Main
Remark	:	Since the key study was not available, in vitro (see above) and in vivo (see below) data from other category members and surrogates are used to support it.
Reliability	:	(2) valid with restrictions. OECD Guideline Study. Study was not available for review. Data came from a IUCLID document created by the European Chemicals Bureau, creation date 11-FEB-2000.
Flag	:	Key study for clastogenicity endpoint

18.11.2001 (20)

Type	:	Micronucleus assay
Species	:	mouse
Sex	:	male
Strain	:	NMRI
Route of admin.	:	i.p.
Exposure period	:	48 hrs
Doses	:	175, 350, 525, 700 mg/kg in 2 divided doses 24 hours apart
Result	:	positive
Method	:	other
Year	:	1987
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Chlorobenzene, 1,2-, 1,3- and 1,4- dichlorobenzene, and 1,23- and 1,2,4-trichlorobenzenes also tested positive in this study.
Result	:	The number of micronucleated cells/1000 PCE (mean +/- SD) in control, 175, 350, 525, and 700 mg/kg groups was 1.80 +/- 0.748, 3.40 +/- 0.663, 4.40 +/- 0.916, 6.30 +/- 1.100, and 9.20 +/- 1.248, respectively. The number of micronucleated cells/1000 PCE (mean +/- SD) in animals treated with 264, 528 and 1056 mg/kg (split dose) and 528 mg/kg (in one dose) benzene (positive control) was 4.40 +/- 0.800, 8.10 +/- 0.943, 12.40 +/- 1.356, and 10.83 +/- 1.343, respectively.
Test condition	:	Eight week old male mice (5 per group) were given i.p. doses of test compound (total dose of 175, 350, 525 and 700 mg/kg) such that the highest dose did not exceed 70% of the reported LD50 (1062 mg/kg). Each dose was given in a divided dose 24 hours apart (doses given at each 24 hour injection were 87.5, 175, 262.5 and 350 mg/kg). The control group of 10 mice received corn oil only. Benzene was the positive control. Animals were killed 30 hours after the first injection. The femora were removed and the marrow was suspended in serum. Two smears per femur were prepared and coded. The smears were scored by two different people. One-thousand polychromatic erythrocytes per smear were examined for the presence of micronuclei. T-tests were used to compare data. No further test details are given.
Test substance	:	Purity of test substance (given by manufacturer) was 99.0 %.
Conclusion	:	The test material was positive in the assay
Reliability	:	(4) not assignable. The use of multiple t-tests is an inappropriate means of determining the significance of the data. The study documentation is lacking in sufficient detail as to assess its validity.

18.11.2001 (32)

Type	:	Micronucleus assay
Species	:	mouse
Sex	:	male
Strain	:	B6C3F1
Route of admin.	:	i.p.
Exposure period	:	3 days
Doses	:	chlorobenzene: 128.8, 257.5, 515 mg/kg/day (total dose 386.4, 772.5, 1545 mg/kg); ortho dichlorobenzene: 50, 100, 200 mg/kg/day (total dose 150, 300 and 600 mg/kg)
Result	:	negative
Method	:	other
Year	:	1993
GLP	:	no data
Test substance	:	other TS
Result	:	Chlorobenzene: The % PCE in animals treated with 0, 128.8, 257.5 or 515 mg/kg/day was 57.8, 51.1, 52.8, and 48.0, respectively. The incidences of MN-PCE/1000 (mean +/- SE) in pooled samples from 5-6 animals treated with 0, 128.8, 257.5 or 515 mg/kg/day were 3.70 +/- 0.58, 2.80 +/- 0.64, 2.10 +/- 0.33, and 3.42 +/- 0.57, respectively. The test was negative and was not repeated. Ortho dichlorobenzene: The % PCE in animals treated with 0, 50, 100 or 200 mg/kg/day was 62.5, 56.4, 62.2, and 64.2, respectively. The incidences of MN-PCE/1000 (mean +/- SE) in pooled samples from 5 animals treated with 0, 50, 100 or 200 mg/kg/day were 1.70 +/- 0.49, 1.90 +/- 0.49, 2.40 +/- 0.49, and 2.70 +/- 0.72, respectively. This test was barely positive based on trend analysis (p = 0.049), but no dose group was positive. The test was repeated to 250 mg/kg and was found to be negative by trend analysis (p= 0.358). Because of the relatively small increased in MN-PCE in the initial test and the lack of reproducibility, the overall result was considered negative. Control: Solvent (corn oil) data were scored as 2.12 +/- 0.70 and 2.38 +/- 0.93 MN-PCE/1000 PCE (mean +/- SD) by two separate labs (not significantly different). These values are slightly lower than those reported in the test. The data for the positive control DMBA were 6.93 +/- 2.59 and 7.93 +/- 1.69 MN-PCE/1000 PCE (mean +/- SD) in the two labs. The data for the positive control MMC were 6.82 +/- 1.24 and 6.85 +/- 2.26 MN-PCE/1000 PCE (mean +/- SD) in the two labs (no significant difference).
Test condition	:	Male mice between 9 and 14 weeks of age between 23 and 35 g were used. Test material was mechanically suspended in corn oil and was administered within 30 minutes of preparation. Five mice/group were dosed with 0 (corn oil control), 128.8, 257.5 or 515 mg/kg chlorobenzene, 50, 100 or 200 mg/kg orthodichlorobenzene, or a weakly active dose of the positive control chemicals 7,12-dimethyl benzanthracene (DMBA; 12.5 mg/kg) or mitomycin C (MMC; 0.2 mg/kg) by i.p. injection on three consecutive days (volume 0.4 ml). The doses tested were based on results of toxicity/mortality in a preliminary study. Animals were monitored 2 times/day. Mice were killed 48 hours after the third treatment. Bone marrow and peripheral blood smears (two slides/tissue/mouse) were prepared by a direct technique (Tice et al. 1990. Effect of treatment protocol and sample time on frequencies of micronucleated cells in mouse bone marrow and

peripheral blood. Mutagen.5:313-321). Air-dried smears were fixed using absolute methanol and stained with acridine orange. Smears from each animal were evaluated at 1000 x magnification using epi-illuminated fluoresence microscopy (450-490 nm excitation; 520 nm emission) for the percentage of polychromatic erythrocytes (PCE) among 200 erythrocytes and the number of micronucleated PCE (MN-PCE) among 2000 PCE. Repeat tests were conducted if the results suggested a possible effect or if no toxicity was observed at the highest dose level. Since the result of the experiment with orthodichlorobenzene suggested a possible effect, the experiment was repeated at 0, 150 and 250 mg/kg /day.

The data were analyzed using the Micronucleus Assay Data Management and Statistical software package (version 1.4), which was designed specifically for in vivo micronucleus test data (ILS.1990. Integrated Laboratory Systems, P.O. Box 13501, Research Triangle Park, NC). The level of significance was set at $p < 0.05$. The numbers of MN-PCE at each dose group were pooled and analyzed by a one-tailed trend test. In the software package used, the trend test incorporates a variance inflation factor to account for excess animal variability. In the event that the increase in the dose response curve was nonmonotonic, the software allowed for the data to be analyzed for a significant positive trend after data at the highest dose only had been excluded. In this event, the alpha level was adjusted to $p < 0.01$ to protect against false positives. The %PCE data were analyzed by an analysis of variance (ANOVA) test based on pooled data. Pairwise comparisons between each group and the solvent control were made using an unadjusted one-tailed Pearson chi-squared test which incorporated the calculated variance inflation factor for the study. Solvent (corn oil) and positive control data were analyzed by two separate laboratories.

Test substance : Test materials were chlorobenzene (CAS No. 108-90-7) and ortho dichlorobenzene (CAS No. 95-50-1).
Reliability : (2) valid with restrictions. NTP study. Purity of test material was not noted.
Flag : Supportive study for clastogenicity endpoint.
27.11.2001

(40)(41)

Type : Micronucleus assay
Species : mouse
Strain : NMRI
Route of admin. : oral unspecified
Exposure period : single application
Doses : 100, 330 or 1000 mg/kg bw
Result : negative
Method : Other: OECD Guideline 474 and Directive 84/449/EEC, B.12
Year : 1983
GLP : yes
Test substance : other TS
Test condition : Test material was given in polyethylene glycol 200 vehicle
Test substance : Test material was 1,2,4- trichlorobenzene (CAS No. 120-82-1). Purity of test substance was 99.6 %.
Source : Bayer Leverkusen
Reliability : (2) valid with restrictions. OECD guideline study. The reference was not consulted. Information came from IUCLID file for CAS No. 120-82-1, creation date 11-FEB-2000.
Flag : Supportive study for clastogenicity endpoint.

(10)

Type	:	Micronucleus assay
Species	:	mouse
Strain	:	NMRI
Route of admin.	:	oral gavage, and i.p. (similar to conditions of Mohtashampir et al.)
Doses	:	2500 mg/kg (oral), 2 x 177.5 and 2 x 355 mg/kg (i.p.)
Result	:	negative
Method	:	Other
Year	:	2000
GLP	:	no data
Test substance	:	other TS
Remark	:	The metabolite 2,5, dichlorophenol also tested negative in the oral test. The average number of micronucleated cells/1000 PCE for cyclophosphamide in 87 experiments was used as the positive control in the i.p. test (16.8 +/- 4.2). Because a positive control was not tested in this test, it is not valid.
Result	:	Oral : The % PCE in animals treated with 0 (24 hr) or 2500 mg/kg dichlorobenzene (24, 48 and 72 hrs) was 51.87, 48.59, 38.40 and 34.23 (significantly different from control), respectively. The incidences of MN-PCE/1000 (mean +/- SD) in these same groups was 1.2+/- 0.9, 1.6 +/- 1.1, 1.6 +/- 1.1 and 1.2 +/- 0.6, respectively (no significant difference). The positive control induced 12.0 +/- 5.0 MN-PCE/1000 PCE. i.p.: The % PCE in animals treated with 0 or 2x 177.5 or 2 x 355 mg/kg test material was 48.26, 45.79, and 43.35 (significantly different from control) respectively. The incidences of MN-PCE/1000 (mean +/- SD) in these same groups was 1.6+/- 1.1, 1.3 +/- 1.1, and 2.1 +/- 2.4, respectively. There was no significant difference between treated animals and control
Test condition	:	Oral : Ten NMRI mice (five per sex, 8-12 weeks of age) per group were dosed with vehicle (corn oil), or 2500 mg/kg 1,4-dichlorobenzene, 1500 mg/kg 2,5 –dichlorophenol or 20 mg/kg Cyclophosphamide (positive control, dissolved in water). The materials were administered by stomach tube in a volume of 5 ml/kg (2,5, -dichlorophenol) or 10 ml/kg (1,3-dichlorobenzene). Vehicle control groups received the same volume of corn oil (volume of positive control was not stated). Animals were killed 24, 48 or 72 hours after treatment. i.p.: 1,4-dichlorobenzene in corn oil was administered twice (2 x 177.5 or 2 x 355 m/kg) at an interval of 24 hours. Corn oil vehicle was also given twice. The volume administered was 5 ml/kg. Animals were killed 6hr after the second injection. Femoral bone marrow cells were flushed out with fetal bovine calf serum and the cellular suspension was centrifuged at 100 rpm for 5 min. Smears were prepared from the pellet. 1000 polychromatic erythrocytes (PCEs) per animal were scored for micronuclei. The number of normochromatic erythrocytes per 1000 PCEs was counted. Data were analyzed using Wilcoxon's nonparametric rank sum test. A P value of <= 0.05 was the criterion for significance.
Test substance	:	Test material was 1,4- dichlorobenzene (CAS No. 106-46-7). Purity was > = 99.5%.
Reliability	:	(1) valid without restriction (oral test), invalid (i.p. test). A positive control was not used in the i.p. experiment.
Flag	:	Supportive study for clastogenicity endpoint.

(48)

5.7 CARCINOGENITY

5.8 TOXICITY TO REPRODUCTION

Type	:	other: examination of reproductive organs from 90 day toxicity study
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	90 days
Frequency of treatment	:	daily
Premating exposure period		
Male	:	
Female	:	
Duration of test	:	90 days
Doses	:	9, 37, 147, 588 mg/kg
Control group	:	yes, concurrent vehicle
NOAEL Parental	:	= 147 mg/kg bw
Method	:	other
Year	:	1995
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The NOAEL is based on increased testicular weight in high dose males.
Result	:	High dose males had significantly increased relative testes weights (0.85 +/- 0.14 vs. 0.68 +/- 0.08 in control). This was not observed at lower doses. There were no histological lesions observed at this concentration. There were no effects of treatment on any other reproductive organs (in males or females).
Test condition	:	Rats were 70 days old when received. Males weighed 300-325 g and females weighed 225-250 g. Animals were quarantined for 10 days before treatment. They were allowed free access to food and water. One hundred animals were divided into 5 experimental groups (10/sex/group). Animals were gavaged daily for 90 consecutive days with 0 (corn oil vehicle), 9, 37, 147 or 588 mg/kg. Individual doses were determined weekly from individual body weights. Each rat received a volume of 0.1 ml per 100 g body weight. Dosing solutions were prepared weekly from a stock solution. All rats were observed daily for physiological and behavioral responses and for mortality. Body weights and food and water consumption were recorded weekly throughout the study. Blood samples were collected via cardiac puncture at necropsy for hematological and serum chemistry measurements. Gonads and vital organs were weighed and grossly examined and vital organs, seminal vesicles, prostate, uterus, preputial or clitoral gland were examined grossly and microscopically (as described in Section 5.4). The data for incidence of histopathological lesions was analyzed by a Fisher Exact test, with a criterion of significance of P <= 0.05.
Test substance	:	GC/MS indicated the purity of the material was > 99.99%.

Reliability : (2) valid with restrictions. The effect on mating was not characterized.
Flag : Key study for endpoint.
18.11.2001 (29)

Type : Two generation study
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : inhalation
Exposure period : F0 and F1 generations during mating period, days 0-20 of gestation and days 4-21 of lactation
Frequency of treatment : 6 hrs/day, 7 days/week
Premating exposure period
Male : F0 : 10 weeks, F1:11 weeks
Female : F0 : 10 weeks, F1:11 weeks
Duration of test : until weaning of F2 pups
Doses : 50, 150 or 450 ppm (234, 702 or 2105 mg/m3)
Control group : yes
NOAEL Parental : = 50 ppm
NOAEL F1 Offspr. : = 450 ppm
NOAEL F2 Offspr. : = 450 ppm
Method : other
Year : 1987
GLP : no data
Test substance : other TS
Remark : The NOAELs listed for F1 and F2 offspring are for reproductive indices in F0 and F1 females and males and survival of F1 and F2 fetuses. The NOAEL for toxicity for F0 and F1 parental animals is 50 ppm (based on changes in liver and kidney in males exposed to higher doses).
Result : Cumulative mean (+SD) analytical exposure concentrations were 51 (+/- 5), 151 (+/-8) and 451 (+/- 25) ppm for the F0 generation, and 49 (+/- 4), 150 (+/- 11) and 454 (+/- 21) ppm for the F1 generation.

No mortalities were observed in the adult generations. Mean body weights and food consumption for adult male and females in both generations were comparable for all groups. Mating and fertility indices for males and females were unaffected by treatment. For the F0 generation, the percentage of females that were pregnant in the control, 50, 150 and 450 ppm groups was 90%, 100%, 93.1% and 86.7%, respectively. The percentage of F0 males that successfully impregnated females in the control, 50, 150 and 450 ppm groups was 92.3%, 100%, 93.1% and 89.7%, respectively. For the F1 generation, the percentage of females that were pregnant in the control, 50, 150 and 450 ppm groups was 80%, 100%, 79.3 % and 89.3%, respectively. The percentage of F1 males that successfully impregnated females in the control, 50, 150 and 450 ppm groups was 77.8%, 100%, 79.3% and 88.0%, respectively. The mean (+SD) number of days for F0 males and females to mate in the control, 50, 150 and 450 ppm groups was 4.2 +/- 4.6, 3.1 +/- 2.8, 2.8 +/- 2.0 and 3.1 +/- 2.9, respectively (not significantly different). The mean (+SD) number of days for F1 males and females to mate in the control, 50, 150 and 450 ppm groups was 4.9 +/- 4.2, 3.2 +/- 2.3, 3.1 +/- 2.4 and 4.6 +/- 4.1, respectively. None of these values were significantly different from control.

In the F1 and F2 litters, pup and litter survival for all treated groups was

comparable to controls. The pup viability index at birth for F1 offspring ranged from 96.1 +/- 12.0 in the 50 ppm group to 99.0 +/- 2.4 in the 150 ppm group, and for F2 offspring was 97-98% for all groups. Overall survival indices of F1 litters from F0 rats exposed to 0, 50, 150 or 450 ppm were 100%, 96.7%, 96.3% and 92.3%. Survival indices of F2 litters from F1 rats exposed to the aforementioned concentrations were 91.7%, 96.6%, 91.3% and 88.0%, respectively (not significantly different). In the F2 litters, a slight, nonsignificant decrease in pup survival index (Days 0-4) was seen in offspring from high dose animals. This was not considered to be treatment-related as this was predominantly due to loss of litters from two dams (one dam lost 12/15 pups and another lost all 10).

Significant increases in absolute and relative liver weight were observed in F0 and F1 adults exposed to 150 or 450 ppm. The relative liver weight of F1 males exposed to 50 ppm was also greater than control (3.73 +/- 0.36 vs. 3.47 +/- 0.32). An increase in the incidence of small flaccid testes and dilated renal pelvis was observed in high dose F0 and F1 males. For the 50, 150 and 450 ppm groups the incidence of small flaccid testes was 0, 1, and 3 for F0 males and 0, 1 and 5 for F1 males. For the 0, 50, 150 and 450 ppm males the incidence of dilated renal pelvis was 1, 1, 2 and 5 for F0 males (dose-related) and 1, 4, 6 and 4 for F1 males (not dose-related). In F0 females, the incidence of dilated renal pelvis in treated animals (4-6) was similar to control (5). In the F1 generation, 2 females treated with 150 or 450 ppm had dilated renal pelvis versus 0 in control.

Microscopic changes were observed in the liver and kidneys of treated male rats. Hepatocellular hypertrophy (graded minimal to mild) was noted in 5 mid and 14 high dose F0 males (none in control) and 3 mid and 7 high dose F1 males (2 in control). This lesion was observed in only one female (a high dose F0). The incidence of renal changes in male rats is shown in the Table below:

Table: Incidence of renal changes in male rats inhaling monochlorobenzene for 2 generations

	Group (ppm)							
	F_0 adults				F_1 adults			
	0	50	150	450	0	50	150	450
Total No. of animals	30	30	30	30	30	30	30	30
U/tubular dilation Eosinophilic material	0	3	2	3	4	4	6	6
B/tubular dilation Eosinophilic material	0	1	4	15	4	3	8	16
U/chronic interstitial nephritis	0	0	0	1	1	2	1	0
B/chronic interstitial nephritis	1	2	7	9	0	1	6	11
U/foci of regenerative epithelium	0	0	0	0	0	0	1	1
B/foci of regenerative epithelium	0	1	5	8	1	0	4	10

U/ = unilateral, B/ = bilateral
Lesions were not present in females

Two F0 and 3 F1 males in the 150 ppm group and 6 F1 males in the 450 ppm group exhibited unilateral degeneration and 6 F0 males of the 450 ppm group exhibited bilateral degeneration of varying degrees in the germinal epithelium of the testes. This lesion was observed in 1 male control in each of the generations. In order to determine the effect of these lesions on reproductive performance, the reproductive performance of all males that showed this lesion were reviewed. All controls, all affected F0 and 2/3 F1 males in the 150 ppm group, and 3/6 affected animals in the 2 generations treated with 450 ppm were successful in siring litters.

Test condition : Test article: Test article was administered by the inhalation route with

animals exposed in 6m3 glass and stainless steel chambers. Targeted exposure levels were 50, 150 and 400 ppm; included in the study was a chamber exposed, sham-air control group. The chambers were operated dynamically at an air flow rate of at least 2140 l/min (one air change per 2.8 min). The test material was fed into an atomizing nozzle via an FMI fluid metering pump. The vaporized test material was diluted with preconditioned air prior to entry into the exposure chamber. Test concentrations were monitored hourly during exposures. A daily nominal concentration was determined by dividing the difference in weight of the generation apparatus and test material before and after exposure by the total volume of air delivered during the day.

Test conduct: Animals were acclimated for at least 13 days prior to exposure. They were 6 weeks old at time of exposure. F0 adult animals (30/sex/group) were exposed daily (6 hrs/day, 7 days/week) with 0 (filtered air), 50, 150 or 450 ppm for a 10 week pre-mating treatment period and during mating. Once mated (as evidenced by the presence of a copulatory plug), females were exposed (6 hrs/day) during gestation (Days 0-19) and lactation (Days 5-28) of the FI litters. F0 males continued to be treated daily during the post-mating period until termination. Similarly, F0 females continued to be treated daily post-weaning until terminated after weaning of the last litter.

FI pups (30/sex/group) were exposed to comparable dose levels as the dams one week after weaning to at least 11 weeks prior to mating. Animals chosen to be the F1 parents were selected to maximize representation from the number of available litters. Exposure of F1 animals during the mating, gestation and lactation intervals of the F2 litters was similar to that of the F0 animals.

Animals were given free access to standard laboratory diet and water during all non-exposure periods, and water during lactation. All animals were observed twice daily for toxicity or mortality. Detailed physical examinations were performed weekly. Body weights and food consumption of F0 and F1 adults were generally measured weekly for most animals (with the exception of slightly different intervals for females during gestation and lactation). Litters were examined twice daily for death and general appearance. On day 4 of lactation, all litters with greater than 8 pups were culled to that number. The sex distribution within litters was equalized (if possible). Pup weights, the number of pups in each litter and pup sex distribution were determined on days 0, 4 (pre and post cull), 7, 14, and 21 of lactation. The mating index for males and females, pregnancy rate and fertility index for males were calculated for each of the two matings. Pup survival indices at various intervals during lactation were calculated.

All F0 and F1 adults were killed after all F1 pups and F2 pups were weaned, respectively. All F2 pups were killed at day 21 of lactation. Complete gross postmortem examinations were conducted on all F0 and F1 parents, all F1 weanlings not selected to become parents of the F2 generation, and all F2 weanlings. Liver and brain weights of F0 and F1 adults were recorded. Liver, kidneys, pituitary gland, and reproductive organs (males- epididymes, seminal vesicle and prostate; females- vagina, uterus and ovaries) were examined microscopically for all F0 and F1 adult animals in the control and high dose groups. Liver, kidneys and testes of male rats in the low and mid-dose groups were examined histologically.

Statistical analyses: Mean body weights, food consumption, organ weights, organ to body weight ratios, gestation lengths, and numbers of offspring were evaluated for equal variance using Bartlett's test. Parametric methods (one way analysis of variance followed by a Dunnett's test) were performed on data if variances were equal. A Kruskal-Wallis test or Dunn's summed rank test was used to analyze nonparametric data. The nonparametric test for determining monotonic trend was the Jonckheere's test and standard linear regression was used for parametric data. Pup viability and survival indices were analyzed with the litter as the experimental unit and these data were transformed using arcsine. Incidence data were analyzed using contingency tables. A standard chi-square analysis was performed on these data to determine if the proportion of incidences differed between the groups tested. Next, each treatment group was compared to the control group using a 2 x 2 Fischer exact test. The significance level was corrected using the Bonferroni inequality. An Armitage test for linear trend was performed.

Conclusion : Exposure to male rats of 150 or 450 ppm caused hepatocellular hypertrophy and increased liver weight, degenerative and inflammatory lesions in the kidneys and degenerative testicular changes. The relationship between testicular damage and exposure to monochlorobenzene is unclear because although 3/6 affected high dose males in each generation did not sire litters, the overall incidence of males not siring litters in each generation of the high dose animals (4 F0 and 8 F1) and mid-dose animals (3 F0 and 7 F1) was not different from control (6 F0 and 9 F1).

In the absence of microscopic changes, the increased liver weight in the low dose males and mid and high dose females was not considered to be indicative of an adverse effect. Chlorobenzene was not a reproductive toxicant.

Test substance : Test substance was chlorobenzene (CAS No. 108-90-7). The purity was 99.9%.

Reliability : (1) valid without restriction

Flag : Supportive study for endpoint.

27.11.2001 (33)

Type : Two generation study
Species : rat
Sex : male/female
Strain : other: Charles River CD (Sprague-Dawley derived)
Route of admin. : inhalation
Exposure period :
Frequency of treatment : 6 hr/day, 7 days/week during times specified under test condition
Premating exposure period
Male : 10 weeks
Female : 10 weeks
Duration of test : to weaning of F1 and F2 generations
Doses : 50, 150, 400 ppm (0.305, 0.915, or 2.44 mg/l)
Control group : yes
NOAEL Parental : < 50 ppm
NOAEL F1 Offspr. : = 50 ppm
NOAEL F2 Offspr. : = 150 ppm
Method : other

Year : 1989
GLP : yes
Test substance : other TS
Remark : Study personnel concluded that in the absence of significant mortality in all other litters but one high dose litter during the day 0-4 interval, the decrease in pup survival index for this group (while statistically significant compared to control) was not indicative of a treatment-related effect.

The reduced postnatal weights of pups from high dose animals (both F1 and F2) and mid dose animals (F1) are indicative of slight fetotoxicity.

Result : Mean analytical concentrations for the F0 and FI generation animals were similar to targeted exposure levels. For the F0 generation; the mean analytical concentrations (+ S.D.) for the low-, mid- and high-exposure groups were 50 +/- 3, 150 +/- 5 and 397 +/- 18 ppm, respectively. In the FI, these mean analytical concentrations were 51 +/- 3, 151 +/- 8 and 391 +/- 25 respectively, for these same groups.

Some mortality was seen among the control (1/sex in F0; 1 male in F1) and treated adult animals in each generation (1 mid dose F0 female on day 24 during delivery; 1 high dose male F1, one low dose female F1 during lactation, 1 high dose F1 female killed moribund); however, no adverse effect of treatment was indicated.

The only effect of treatment seen in the low-exposure group was a slight increase in absolute and relative liver weight in both the F0 and FI adult animals. No adverse effect of treatment at the low-exposure level was evident from growth of the adult animals, reproductive performance, fertility, gestation length or litter size data. Pups delivered and weaned to females in this group showed comparable growth and survival rates to weaning as control animals. A slight, but significant increase in mean gestation length was seen in low-dose females; however this was not considered to be relevant as it was not observed at higher dose levels.

In the mid-exposure group, mean weights of adults were lower than control at several weekly intervals early in the pre-mating period of the F0 and throughout this same interval in the F1; however, mean weight gain over the entire pre-mating interval for both generations was comparable to control data. No adverse effect of treatment in the mid-exposure group was evident from reproductive performance or fertility indices, litter size or gestation length or maternal weight gain data during gestation/lactation intervals in either generation. In the F1 litters, mean pup weight at Day 0 (birth) for the mid-exposure group was statistically significantly lower than control; however, pup weight data for the remaining weighing intervals of the FI litters and for all weighing intervals of the F2 litters were comparable to control data. Pup survival indices in the mid-exposure group for each litter interval were comparable to control data. Mean liver weights (absolute and relative to body or brain weights) for the mid-exposure group were higher than control in the F0 while in the FI, only relative liver weights were increased. Additionally, relative kidney weights were increased in the mid-exposure males from both generations.

In the high-exposure group, no adverse effect of treatment was evident from reproductive performance or fertility indices for either generation. Maternal weight gain during gestation/lactation intervals, gestation length, litter size data and pups survival indices were generally comparable to control data for both generations. However, the pup survival index for the day 0-4 lactation interval was lower than control (94.3% vs. 98.1% for

control). This was largely attributed to the loss of all pups within a single litter (one high dose female delivered a litter of 14 live pups and 2 dead pups, but none survived to day 4). Excluding data for this litter, the pup survival index for the day 0-4 interval was 98.4 % (317/322 pups), which was not significantly different from control. The pup surivival for the high dose group over the day 4-21 lactation interval was comparable to control. Mean weekly weights for F0 and FI adults in the high-exposure group during the pre-mating treatment intervals were lower than control, and mean weights continued to be depressed for these animals through to termination.

Mean weight gain over the entire pre-mating intervals were lower than control for males in both generations and for F0 females. Excessive salivation was seen in high dose F0 and F1 males and females at some time points. F0 and F1 adult males and females in the high-exposure group had increased liver weights (absolute and relative) and males (F0, FI) also had increased relative kidney weights. Mean pup weights in the high-exposure group were statistically significantly lower than control on Days 0, 14, 21 and 28 of the FI litters and on Days 14 and 21 of the F2 litters. Growth and food consumption in unselected F1 high dose pups were similar to control.

No adverse effect of treatment was evident from the gross postmortem evaluations of adults and offspring. Treatment-related morphologic abnormalities in the liver and kidneys were seen in the F0 and FI adults.

Liver: Hypertrophy of the central lobular hepatocytes was found in almost all F0 and FI adult males and females in the high-exposure group and numerous males and several females from the mid-exposure group. This effect was not seen in the liver of F0 and F1 males and females from the control group or in the liver of females from the low-exposure group.

Kidneys: Dilated tubular lumens with intraluminal granular casts were seen predominantly at the cortico-medullary junctions in several F0 and FI adult males from the mid- and high-exposure groups. This effect was seen most frequently in the high-exposure group.

Intracytoplasmic granules/droplets in the proximal convoluted tubular epithelium were seen in almost all F0 and FI adult males for which the kidneys were examined microscopically. Based on mean severity this kidney effect was most pronounced in F0 and F1 adult males from the high-exposure group followed by the mid- and low- exposure groups. It was least pronounced in the control group. The granules/droplets were eosinophilic in the hematoxylin and eosin stained sections and they stained positive with the Mallory Heidenhain stain in the specially stained sections.

Other postmortem findings seen in the F0 and FI adults, gross and microscopically, either occurred with comparable incidence and severities in the treated and control animals or they occurred sporadically and were not considered to be related to the test article.

Several F1 generation animals in all groups were noted on week 37 to have findings suggestive of Sialodacryoadenitis viral (SDAV) infection. All males were noted as free of SDAV infection by week 41. Females were generally free of symptoms of SDAV infection at week 41. The presence of SDAV was confirmed by analyses of serum from affected males for

SDAV antibodies. The presence of SDAV did not appear to have adversely affected the study.

Test condition : Test article: Test article was administered by the inhalation route with animals exposed in 6m3 glass and stainless steel chambers. Targeted exposure levels were 50, 150 and 400 ppm; included in the study was a chamber exposed, sham-air control group. Appropriate amounts of the test material were placed into a 2 l erlenmeyer flask connected to a fluid metering pump. The metering pump settings were varied to provide the target exposure levels. The test article was fed from the flask directly into the liquid inlet of an air atomizing nozzle via Teflon tubing. House-supply air was delivered through Teflon tubing from a regulator and flowmeter with a backpressure gauge to the air inlet of the atomizer via tygon tubing to generate the aerosol. The aerosol was directed into the side inlet of the air inlet pipe where it volatized in the chamber airflow stream. This stream entered the exposure chambers. Test concentrations were monitored hourly during exposures.

Test conduct: Each study group consisted of 60 CD rats (30/sex/generation). F0 adult animals (41 days old at treatment) were exposed daily (6 hrs/day) for a 10 week pre-mating treatment period and during mating. Once mated, females were exposed (6 hrs/day) during gestation (Days 0-19) and lactation (Days 5-28) of the FI litters. F0 males continued to be treated daily during the post-mating period until termination. Similarly, F0 females continued to be treated daily post-weaning until terminated after weaning of the last litter. FI pups (2/sex/litter) randomly selected at Day 28 (weaning) became a pool of animals from which the FI adult generation was selected. These animals were exposed at 29 days of age to comparable dose levels as the dams. Once the FI adult generation was chosen, these animals received an 11-week pre-mating treatment period. Exposure of animals during the mating, gestation and lactation intervals of the F2 litters was similar to that for the F0 animals. Animals were given free access to standard laboratory diet during all non-exposure periods, and water during exposure and non-exposure periods. All animals were observed twice daily for toxicity or mortality. Detailed physical examinations were performed for the F0 and F1 adult generation animals and unselected F1 high dose animals (see below). Body weights and food consumption of F0 and F1 adults were generally conducted weekly for most animals (with the exception of slightly different intervals for females during gestation and lactation). Pup weights were determined on days 0, 4 (pre and post cull), 7 (F2 litter only), 14, 21 and 28 of lactation (F1 litters only). They were sexed at each examination.

F0 and FI adult males were terminated as a group three to four weeks post-mating; F0 and FI females were killed as a group after the last litters, FI and F2, respectively, were weaned. Each adult generation animal was given a gross postmortem examination and liver, kidneys, pituitary gland and reproductive tissues were saved in 10% formalin. Liver, kidney, testes and brain weights were recorded at termination and absolute and relative liver, kidney and testes weight data were evaluated. Initially, microscopic evaluations were restricted to tissues for the control and high-dose groups, both generations; however, these evaluations were extended to include the livers of all F0 and F1 adults in the low- and mid-dose groups and the kidneys of all F0 and FI low- and mid-dose males. FI and F2 pups were given a gross external and internal examination and discarded; only abnormal tissues were saved in 10% formalin. FI pups were killed either at weaning (Day 28) or at the time of selection for the FI adult generation. F2 pups were killed at Day 21 of lactation.

If, following weaning of the F1 last litters more than 30 pups/sex were present for a particular group, the excess were culled so that each litter was represented in the parental generation by at least one pup per sex. In the control, low and mid dose groups, the excess pups were culled and given a gross external and internal examination. In the high-exposure group, the excess pups were retained on study. They were removed from the exposure regimen and were maintained on basal diet over an 11-week period that corresponded to the pre-mating treatment period. These animals (unselected F1 high-dose pups) were then killed and given a gross postmortem examination. Only abnormal tissues from these animals were saved.

Data from treated groups were compared to control (methods for statistical tests were not available).

Test substance : The test material was orthodichlorobenzene (CAS No. 95-50-1). The purity of the test material was > 99.2%.

Reliability : (1) valid without restriction

Flag : Supportive study for endpoint.

19.11.2001 (39)

Type : Two generation study
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : inhalation
Exposure period :
Frequency of treatment : 6 hr/day, 7 days/week during times specified under test condition
Premating exposure period
Male : 10 weeks
Female : 10 weeks
Duration of test : to weaning of F1 and F2 generations
Doses : 50, 150, 450 ppm
Control group : yes
NOAEL Parental : < 66.3 ppm (< 50 ppm nominal)
NOAEL F1 Offspr. : 211 ppm (150 ppm nominal)
NOAEL F2 Offspr. : 211 ppm (150 ppm nominal)
Method : other
Year : 1989
GLP : yes
Test substance : other TS
Remark : The fact that the high dose had no effect on any reproductive parameter but caused a significant effect on the number of liveborn and stillborn pups suggests that this dose of material was fetotoxic.
Result : The mean analytical concentrations (+ S.D.) for the low-, mid- and high-exposure groups were 66.3 +/- 8.47, 211 +/- 18.0 and 538 +/- 50.5 ppm, respectively. These were calculated using the charcoal tube values obtained from days 172-282, as these were in better agreement with the values obtained from the direct syringe method than from the syringe/stainless steel tubing sampling method used from Days 1-171. Samples obtained from the syringe/stainless steel method underestimated test concentrations by as mush as 37% (presumably due to condensation problems). The charcoal tube method was successful because the vapor did not contact any surfaces before reaching the charcoal bed. Within

each chamber, vapor concentrations were uniformly distributed.
The maximum amount of test material detected in the control atmosphere was 3 ppm (once on day 194). All other readings in the control atmospheres were at or below the limit of detection (1 ppm). In this summary, the target concentrations will be used when stating results.

All groups: There were no treatment-related gross lesions observed in necropsies of F1 or F2 pups that died during lactation, F1 or F2 weanlings, or F0 or F1 adults. There was no effect of treatment on reproductive parameters (mating or fertility indices of males or females, the gestational index, the 7-, 14-, 21- or 28-day survival index, the lactation index, or the number of copora lutea or implantation sites (including resorptions and live conceptuses)) in either generation. Five adult animals died or were killed in moribund condition during the study (one control F0 male, three F0 females (one each in control, 150 and 450 ppm groups), and one F1 female at 50 ppm). The cause of death of the control F0 male was urinary tract inflammation/obstruction. The cause of death of the other animals was not determined.

450 ppm: F0 and F1 males and F1 females exhibited consistently reduced body weights and weight gains throughout the experiment. F0 females had reduced body weights for the first week of exposure prior to mating, and on gestational day 20. Gestational weight or weight gain of F0 and F1 dams was reduced for gestational days 0-20. Lactational weights of F1 females were reduced for postnatal days 0, 4, and 7. Nonexposed male and female F1 recovery animals exhibited consistently reduced body weights. Food consumption was reduced in F1 males for 5 of the 11 exposure weeks, and was reduced in F0 and F1 females and F0 males during the first week and third week (F0 females only) of exposure. Food consumption was reduced in recovery animals during the first two weeks of the recovery period. Clinical signs observed in F0 and F1 animals included unkempt appearance, tremors, twitches, hypoactivity, ataxia, salivation, and periocular and perioral encrustation. F0 and F1 females had an increased incidence of urogenital wetness. There were no significant clinical observations in recovery animals at 450 ppm. Liver and kidney weights of F0 males and females were increased (as well as relative liver, brain, tested and kidney weights of F0 males and relative liver and kidney weights of F0 females). An increased incidence of hepatocellular hypertrophy was observed in F0 males and females, and increased incidences of hydronephrosis, hyaline droplet nephropathy, tubular proteinosis, granular cast formation, renal tubular cell hyperplasia and interstitial nephritis were observed in F0 males. Exposure-related histologic findings in F1 adults were similar to those of F0 adults.

The mean number of F2 live born pups per litter was significantly reduced in the 450 ppm group. F1 and F2 litter size (but not sex ratio) was reduced on lactational day 4 at 450 ppm. Pups from F1 and F2 litters exhibited reduced body weights per litter during lactation. There was an increase in the incidence of stillborn pups (F2) and postnatal deaths from days 0-4 (both F1 and F2 pups).

150 ppm: Sporadic reductions in body weights and weight gains of F0 and F1 animals were observed at this concentration. F0 and F1 males had increased absolute and/or relative kidney and liver weights, and F0 females had increased relative liver weights. No other effects of treatment were noted. An increased incidence of nephrosis was observed in F0 and F1 males. Histologic findings in the liver were similar to those of males treated

with 450 ppm (with the exception of no renal cell hyperplasia and the additional finding of renal interstitial fibrosis). No histological alterations were noted in the liver. There were no effects of treatment on the pups.

50 ppm: Absolute and/or relative kidney weights of F0 and F1 males and liver weights of F0 males were increased. There was an increased incidence of nephrosis in F0 males, with similar histological findings as in the 150 ppm males. F1 males did not exhibit nephrosis, but had an increased incidence of hyaline droplet formation in the kidneys. No histological alterations were noted in the liver. There were no effects of treatment on the pups.

Test condition : Test article: Test article was administered by the inhalation route with animals exposed in stainless steel chambers approximately 4320 liters in volume. Targeted exposure levels were 50, 150 and 450 ppm; included in the study was a chamber exposed, sham-air control group. Vapor was generated by metering in-house and filtered compressed air at a flow rate of 0.6 – 3.5 L/min (depending on the target concentration) into a heated (130 – 136 degrees C) 12-Liter stainless steel pot containing test material (1.8 – 2.8 kg). The resulting vapor was introduced into the exposure chamber through a heated tube. Chamber airflow was 1000 L/min (at least 14 air changes per hour). Chamber temperature, relative humidity and airflow were monitored. Concentrations of test material in the chambers were measured using gas chromatography with flame ionization detection. Samples were taken with gas-tight syringes up to day 171. Coconut-based charcoal sorbent tubes (placed within the breathing zone) were used for sampling from days 172-282. At least 6 samples were taken daily from each exposure from days 1-171, and 3 were taken from days 172-282. On at least 3 study days, samples were taken with syringes from different positions in the chamber to determine if the concentration of vapor varied throughout the chamber.

Test conduct: Rats (177 virgin females and 140 virgin males) were 28 days old upon receipt (males 75-100 g, females 50-75 g). They were housed 2/same sex for a quarantine period of approximately 2 weeks. Feed was available ad libitum except during exposure periods. Water was available ad libitum in the chambers (except for pre-mating exposure periods).

One hundred twelve healthy rats/sex (F0 rats) were weighed just prior to exposure (avg. Weights of males and females were 199.2 – 200.2 and 139.2 – 140.5 g, respectively) and randomly distributed into 4 treatment groups 28/sex/group). Forty additional females (10/group) were randomly selected for a special mating (satellite) study. Study animals and satellite females were housed individually and exposed to target concentrations of 0, 50, 150 or 450 ppm test material, 6 hrs/day, 7 days/week for 10 consecutive weeks. Animals were examined twice daily for mortality and once daily for clinical signs of toxicity during exposure. Animal weights and food consumption were measured weekly. Clinical observations, body weights or food consumption were not monitored in satellite females.

After the 10-week exposure, animals within each treatment group were mated for a period of 21 days (one male to one female). Exposure continued during mating to gestation day 19. Exposure was discontinued from gestation day 20 through the fifth day postpartum. Beginning on postnatal day 5, mothers were removed from their offspring and exposed to test material (or air) as before, through postnatal day 27. Dams were returned to their offspring after each daily exposure (6 hr/day).

The day a copulation plug or sperm in the vaginal smears was found was considered day 0 of gestation. F0 males that did not show evidence of successful mating within 10 days were removed form the mating cages used in a special mating study with satellite females which was initiated approximately 14 days later. Satellite females and F0 males were exposed throughout this mating period until termination. F0 males were euthanized and necropsied following the satellite mating period. Satellite females were killed on gestation day 15, and examined for evidence of pregnancy.

The corresponding F0 females that did not exhibit evidence of copulation were paired a second time for 11 days with proven males from the same concentration group. For F0 animals that showed no successful mating within 21 days, the last scheduled mating day was designated gestation day 0 for that female and the animals were treated accordingly for subsequent events. Mated study females were weighed on gestation days 0, 4, 7, 14, 21, and 28. Litters were weaned on day 28 postpartum ,and dams were necropsied. When the last F1 litter reached day 28 postpartum, 28 male and female pups per treatment group were randomly selected to produce the F2 generation. Each litter was represented at least once per sex (if possible). Brother/sister matings were avoided. Following selection of the F1 parents, ten female F1 pups were selected as satellite females for special mating, and 20 animals per sex from the control and 450 ppm groups were selected as recovery animals. The remaining F1 pups were euthanized and necropsied.

F1 animals selected to be parents of the F2 generation and F1 satellite females (approximate age of 4-7 weeks) were exposed to test material (or control) for at least 11 weeks as described for F0 animals. The mean weight for F1 males was 192.2-230.6 g, and for F1 females was 143.5 – 172.1 g at the start of exposure. Animals were mated at the end of 11 weeks, and continued to be exposed during mating and gestation day 19 (as described above). F1 males that did not successfully mate within 10 days were mated with F1 satellite females and unsuccessful F1 females were mated with proven F1 males (as described above).

F1 recovery animals were retained for the first 5 weeks of the F1 exposure period. Recovery animals were not exposed but had food and water withheld for 6 hrs/ day during exposure periods of the F1 animals. They were monitored daily for clinical signs of toxicity and weekly for body weights, body weight gains, and food consumption. The animals were euthanized and necropsied after the 5 week period.

All pups from the F1 and F2 generations were sexed and examined on the day of birth. Litters were evaluated twice daily for survival. Each litter was culled to yield 4 pups/sex /litter (if possible0. Survival indices, sex, and weight of the pups were determined at days 0, 4, 7, 14 and 21 days after birth and at weaning (postnatal day 28). All pups were examined for physical abnormalities at birth and throughout the pre-weaning period.

All F0 and F1 parental animals in all groups and F1 recovery animals were euthanized and subjected to a gross necropsy. Tissues from the control and 450 ppm groups (pituitary, liver, kidneys (2), vagina, uterus, ovaries, testes, epididymes, seminal vesicles, prostate and any tissues with gross lesions) were examined histopathologically. Kidney sections from all groups of F) and F1 animals were examined for the presence of alpha2μ globulin protein droplets. The number of copora lutea, implantation sites (including

resorptions and live conceptuses) in F0 and F1 satellite females were recorded. Any apparently nonpregnant uteri were stained with 10% ammonium sulfide for confirmation of pregnancy status. Satellite females were not subjected to gross necropsies. A complete necropsy and histopathologic examination was conducted on any parental animals dying on test. Absolute organ weights for testes, ovaries, brain, liver and kidneys were recorded and organ to body and brain weight ratios were determined for all animals scheduled for termination. A gross internal examination was performed on any pup appearing abnormal or that died on test, and all remaining F1 and F2 pups.

Statistical Analyses: The unit of comparison was the male, female, or litter. Body weight, food consumption and organ weight data were tested for homogeneity using Levene's test for equal variances. When variances were homogeneous, data were compared using analysis of variance and pooled t-tests (for pairwise comparisons). An analysis of variance for unequal variances, followed by the separate variance t-test for pairwise comparisons was used when data were not homogenous. The significance levels for the t-test comparisons were corrected by the Bonferroni method.

Nonparametric data were evaluated using the Kruskal-Wallis test, followed by the Mann-Whitney U test for pairwise comparisons (when appropriate). Frequency data were compared using the Fisher's exact test. For all tests, the fiducial limit of 0.05 (two-tailed) was used as the criterion for statistical significance.

Test substance : The test material was para dichlorobenzene (CAS No. 106-46-7). The purity of the test material was approximately 100%.

Reliability : (1) valid without restriction

Flag : Supportive study for endpoint.

19.11.2001 (49)

Type : Two generation study
Species : rat
Sex : female
Strain : no data
Route of admin. : drinking water
Exposure period : from birth of F0 generation through weaning of F2 generation
Frequency of treatment : continuously
Premating exposure period
Male : F0: 90 days; F1:90 days
Female : F0: 90 days, F1:90 days
Duration of test : until day 32 of F2 generation
Doses : 25, 100 or 400 ppm
Control group : other: two controls (no treatment and vehicle treatment)
NOAEL Parental : = 100 ppm
NOAEL F1 Offspr. : = 400 ppm
Method : other
Year : 1981
GLP : no data
Test substance : other TS
Remark : Results of an additional study with i.p. dosing confirmed the effect of test material on adrenal weight and also showed that the test material had no effect on ovarian weight and decreased (rather than increased) uterine

weight. This showed that the test compound did not have any estrogenic activity.

Result : The fertility of the F0 and F1 animals was not affected by treatment. The number of females delivering/number of females bred ranged from 17/24 to 22/24 in treated F0 and F1 females and 19/24 to 22/24 in Tween treated controls. Litter size was slightly lower in the F2 than the F1 generation in all treatment groups (ranged from 9.8 to 12.8 in F2 vs. 12.8 to 14.5 in F1), but did not differ between treatment groups. Percent mortality to day 12 was lower in the F2 generation than the F1 generation (ranged from 3.7 to 9.3 in F2 vs. 13.1 to 21.8 in F1), but was unaffected by treatment. Percent mortality from d12 to weaning was low in all groups (ranged from 1.0 to 4.3 in F1 and F2) and was not affected by treatment. The treated F2 females also did not differ from controls in the time of vaginal opening.

An increase in food intake was noted in F0 high dose males at 29 days and a decrease in water intake of high dose males and females at 83 days of age. There were no changes in food or water consumption of F1 animals treated with test material. There was no effect of treatment on locomotor activity.

Slight increases in the weight of the left adrenal gland (the only one weighed) of males and females treated with 400 ppm test material for 90 days were observed. The values obtained in high dose F0 and F1 males were 31.8 +/- 2.43 mg and 29.6 +/- 1.66 mg vs. 28.6 +/- 1.09 mg and 28.0 +/- 1.57 mg in their respective Tween- treated controls. The values obtained in high dose F0 and F1 females were 41.5 +/- 1.99 mg and 38.5 +/- 1.89 mg vs. 36.8 +/- 1.14 mg and 37.0 +/- 1.36 mg in their respective Tween- treated controls. No histological damage was found in livers or kidneys of F1 generation animals examined at 95 days. No dose-related changes were found in blood chemistries.

Test condition : One hundred 90 day old, timed pregnant animals received regular tap water and food ad libitum until birth of the F0 generation. At birth, the litters (17-23) were randomly reduced to 4 males and 4 females per litter. These F0 animals were randomly distributed into 5 treatment groups (25, 100 or 400 ppm test material plus 0.125% Tween 20 as a solubilizer in drinking water, 0.125% Tween 20 in drinking water, or normal drinking water). No test material precipitated from these preparations over at least a 1 week period. Animals were given freshly prepared solutions at least twice weekly and were allowed to drink ad libitum.

After 90 days on treatment, 12 breeding cages were established, each containing 2 F0 females and 1 F0 male (within treatment nonsiblings). After 2 weeks of cohabitation, males were euthanized and the females were housed individually. Litters were weaned at 25 days of age and were housed as unisexual littermates until breeding or euthanization. F1 litters (reduced to 3 males and 4 females at weaning) received similar treatment as their F0 parents and were similarly bred. The experiment was terminated when the F2 animals were 32 days old, at which time the females were examined for vaginal opening.

Maternal weights, litter size, neonate sex and weights and 24 hour food and water intake of test animals were recorded on days 1, 8, 15, and 22 for the F0 generation and days 1 and 12 for the F1 and F2 generations. Body weights and 24 hour food and water intake were measured on days 29, 35, 43, 50 and 83 for the F0 generation and on days 25, 53 and 80 for the F1 generation. Individual locomotor activity was measured for 12-20 animals per treatment group on days 16, 27, 48, and 90 for the F0

generation, days 31 and 90 in the F1 generation and at 26 days in the F2 generation by placing animals in a residential maze. Blood and organs were obtained from 10 males and females per treatment group (approximately 1 animal per litter was used) at 37 and 95 days of age for the F0 generation and 95 days only for the F1 generation. The liver, lungs, kidneys, adrenals, gonads and seminal vesicles were removed and weighed. Blood samples were collected by cardiac puncture. Serum was analyzed for glucose, blood urea nitrogen, creatinine, sodium, potassium, chloride, uric acid, calcium, phosphorus, cholesterol, triglyceride, bilirubin, alkaline phosphatase, glutamic-pyruvic transaminase, glutamic oxaloacetic transaminase, creatinine phosphokinase, lactic dehydrogenase, total protein, albumin, and globulin. The livers and kidneys from Tween 20 and 400 ppm test material treated animals in the F1 generation were preserved in neutral buffered formalin and examined histologically.

Since the vehicle was found to have a significant effect on kidney weight, the Tween 20 control group was used as the 0 dosage group in all statistical comparisons. Data were analyzed by analysis of variance, and when results were significant, by linear regression. When a significant dose response effect was found, Dunn's procedure was used to determine the significance of pairwise comparisons. The organ weight and blood chemistry data in the F0 and F1 generations at 95 days were analyzed by regression procedures as a 2 x 2 x 4 (sex, generation, dose) factorial design.

The calculated mean dosages for F0 females administered 25, 100 or 400 ppm test material (based on water intake) were 8.3, 28.0 and 133 mg/kg at 29 days of age and 3.7, 14.8 and 53.6 mg/kg at 83 days of age. The calculated mean dosages for F0 males administered 25, 100 or 400 ppm test material (based on water intake) were 8.5, 27.6 and 133.6 mg/kg at 29 days of age and 2.5, 8.9 and 33.0 mg/kg at 83 days of age.

Conclusion : At the concentrations tested, the test material did not affect fertility, growth, viability, locomotor activity or blood chemical analyses of two generations of rats

Test substance : Test material was 1,2,4-trichlorobenzene (CAS No. 120-82-1).

Reliability : (2) valid with restrictions. Purity of test material was not stated. The test material was a related substance.

Flag : Supportive study for endpoint.

18.11.2001 (37)

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : rat
Sex : female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : Days 6 to 15 of gestation
Frequency of treatment :
Duration of test :
Doses : 50, 100, 200 mg/kg
Control group : no data specified
NOAEL Maternalt. : = 200 mg/kg bw
NOAEL Teratogen : = 200 mg/kg bw
Method : other

Year	:	1983
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Ortho and para dichlorobenzene also tested negative in this study.
Result	:	Treatment was not associated with any teratological effect. Maternal toxicity was not mentioned.
Test condition	:	Test material was given by gavage at 50, 100 and 200 mg/kg to pregnant rats (number not stated) on days 6 - 15 of gestation. Maternal weight gain, changes in microscopic examination and 15 biochemical parameters (types not stated) were used to evaluate maternal toxicity. Changes in litter size, fetal weight, deciduoma, skeletal and visceral examination, residue analysis and microscopic pathology were used to evaluate fetal toxicity.
Reliability	:	(4) not assignable. There is not enough information given to determine the validity of the study or the NOAEL for fetotoxicity.
Flag	:	Supportive study for endpoint.

18.11.2001 (38)

Species	:	rat
Sex	:	female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	days 6 to 15 of gestation
Frequency of treatment	:	daily
Duration of test	:	to day 22 of gestation
Doses	:	150, 300 and 600 mg/kg
Control group	:	yes, concurrent vehicle
NOAEL Maternalt.	:	= 150 mg/kg bw
NOAEL Teratogen	:	= 600 mg/kg bw
Method	:	other
Year	:	1988
GLP	:	no data
Test substance	:	other TS
Remark	:	The NOAEL listed for teratogenicity is also the NOAEL for fetoxicity. Teratogenicity of 75, 150 and 300 mg/kg 1,2,4-trichlorobenzene and 150, 300 and 600 mg/kg 1,3,5- trichlorobenzene also were tested in this study. High doses of these materials induced similar changes in dams as 1,2,3-trichlorobenzene. The results suggested that neither of these trichlorobenzenes was embryotoxic, fetotoxic or teratogenic.
Result	:	One control animal and one treated with 300 mg/kg test material died over the course of the study. None of the tested animals displayed clinical signs of toxicity. A significant increase in relative liver weight was observed in rats treated with 600 mg/kg (5.8 +/-0.26%). APDM activity was significantly increased in high dose animals (27.03 +/- 0.9 nanomoles formaldehyde/hr/mg protein vs. 22.8 +/- 0.6 in control). Hemoglobin concentrations were slightly decreased in animals dosed with 300 or 600 mg/kg test material (11.4 +/- 0.2 and 11.3 +/- 0.2 g/dl vs. 12.0 +/- 0.3 g/dl in control, respectively). Mild changes in the thyroid were noted in animals dosed with 300 and 600 mg/kg. The changes consisted of a reduction of follicle size which was often accompanied by angular collapse. Test material residue was only found in fat tissue of the dams treated with 300 and 600 mg/kg (0.02 and 0.4 ppm, respectively, with a detection limit of 0.01 ppm). No test material residue was found in the fetus.

There was no effect of treatment on pregnancy rate (ranged from 10-11/13 treated vs. 12/14 in control), number of resorptions + dead fetuses (ranged from 0.4 +/- 0.1 to 0.5 +/- 0.2 in treated vs. 0.7 +/- 0.2 in control), litter size (ranged from 11.5 +/-1.1 to 13.3 +/- 0.6 in treated vs. 11.5 +/- 1.2 in control), or fetal weight (ranged from 5.1 +/- 0.2 to 5.4 +/- 0.1 in treated vs. 5.4 +/- 0.1 in control).

No visceral or skeletal abnormalities were found in the 27-37 pups/group examined for visceral changes and the 49-67 pups/group examined for skeletal changes. The incidence of sternal anomalies was 11 in 3 litters of control animals, 1 each in the 150 and 300 mg/kg groups, and 13 in 3 litters in the 600 mg/kg group. Wavy ribs were found in one fetus/group (except the 150 mg/kg group), and a short 13th rib was found in one animal in the 150 mg/kg group. Based on these data, the test material was not concluded to be fetotoxic or teratogenic.

Test condition : Rats (122-175 g) were acclimatized one week prior to mating. Two females were placed in a cage overnight with a male. Vaginal swabs were checked for evidence of mating. The day on which sperm was detected was designated as day 1 of pregnancy. Test material was dissolved in corn oil and was administered to groups of animals (N=13/group) at 150, 300 and 600 mg/kg by gavage from days 6 to 15 of gestation. Controls (N =14) received an equal volume of corn oil. Dams were weighed on day 22 of gestation and killed. The uterus was transected and removed with the ovaries. Dams were reweighed and the liver, kidney, spleen, heart and brain were removed and weighed. The heart, brain, pituitary, eye, thyroid, parathyroid, trachea, bronchi, lung, thymus, stomach, small and large intestine, pancreas, liver, kidney, spleen, adrenal, skeletal muscle, peripheral nerve, skin, bone marrow, ovary, uterus and bladder were examined histologically.

Hemoglobin concentration, hematocrit, erythrocyte count, total and differential leukocyte count, mean corpuscular volume, and mean corpuscular hemoglobin concentration of maternal blood were determined. Serum from each dam was analyzed for sodium, potassium, inorganic phosphorus, total bilirubin, alkaline phosphatase, glutamic oxaloacetic transaminase, total protein, calcium, cholesterol, glucose, uric acid and lactate dehydrogenase. Liver homogenate from each dam was analyzed for aniline hydroxylase, aminopyrene-N-demethylase (ADPM) and protein. Portions of kidney, brain, spleen, heart, liver and perirenal fat were analyzed for trichlorobenzene residue analysis. The concentration of trichlorobenzene in hexane extracts of tissues was measured using gas chromatography.

The fetuses were removed and weighed individually. Live fetuses were examined grossly at necropsy for birth defects. Two thirds of each litter were examined for skeletal abnormalities by examining the cleared and stained skeletons stereoscopically. The remainder were fixed for visceral examination (using dissection and razor sectioning). The amount of residual test material in one fetus/litter and the liver and brain of a litter mate was determined as described above.

The data from organ weight, body weights, hematology and biochemical parameters were analyzed using a one way analysis of variance. Duncan's multiple range test was used to compare means.

Test substance : Test material was 1,2,3- trichlorobenzene (CAS No. 87-61-6). Purity was 99.5%.

Reliability : (1) valid without restriction

Flag : Supportive study for endpoint.
18.11.2001 (5)

Species : rat
Sex : female
Strain : Fischer 344
Route of admin. : inhalation
Exposure period : day 6-15 of gestation
Frequency of treatment : 6 hours/day
Duration of test : to day 21 of gestation
Doses : 75, 210 or 590 ppm (351, 982 or 2760 mg/m3)
Control group : yes
NOAEL Maternalt. : = 210 ppm
NOAEL Teratogen : = 590 ppm
Method : other
Year : 1984
GLP : no data
Test substance : other TS
Remark : The NOAEL for fetotoxicity is 210 ppm, as evidenced by increased incidences of some skeletal variations at 590 ppm.
Result : Maternal: No maternal deaths or abnormal clinical signs were noted in treated rats. High dose animals lost weight (2 grams) during the first three days of exposure. Other groups gained an average of 2-4 g. Absolute and relative liver weights of high dose rats were slightly, but significantly greater than control at necropsy, indicating slight maternal toxicity at the high dose.

The pregnancy rate was not altered by exposure to any concentration of test material (data not shown). No adverse effects were noted in mean litter size or incidence of implantations that underwent resorption.

Fetal: Fetal body measurements for exposed animals were similar to controls. The incidence of malformations was not altered by treatment (N = 4 in control, N= 1 at 76 ppm, N = 2 in two litters at 210 ppm and N = 3 in 3 litters at 590 ppm). With the exception of a cleft palate in one animal in the 75 ppm group, the malformations observed in litters from treated rats were similar to the study control and were at historical incidences for controls. Decreases in the incidence of focal necrosis in the liver were seen in litters from animals exposed to 210 (22 vs. 30 in control) and 590 ppm (19 vs. 30 in control). This was not considered to be of toxicological importance.

There was an increased incidence of some minor skeletal variants in fetuses from treated animals. A higher incidence of delayed ossification of centra of the cervical veterbrae was noted in offspring from rats exposed to 75 ppm (92 in 27 litters vs. 59 in 23 litters in controls) and 590 ppm (103 in 27 litters vs. 59 in 23 litters in controls), but not 210 ppm (73 in 23 litters). A higher incidence of bilobed centra of the thoracic vertebrae (12 in 11 litters vs. 8 in 5 litters in controls) and a lower incidence of cervical spurs (13 in 11 litters vs. 25 in 17 control litters) was noted in offspring from high dose animals. The skeletal changes observed in offspring from high dose animals were concluded to be indicative of a slight delay in skeletal development. None of the variants described were considered to be indicative of a specific teratogenic response.
Test condition : Test vapor generation: Temperature and relative humidity were controlled at 21 degrees C and 50%, respectively. Vapor was generated by metering

the liquid test material at controlled rates into vaporization tubes. Vapors from the tubes were swept into the chamber inlet ducts with preheated compressed air (120 to 135 degrees C) where they were mixed and diluted with incoming air by turbulence. Concentrations of test material were determined 1-2 times per hour throughout the exposure by infrared spectrometry. The actual concentration in the exposure chamber was determined by interpolation from standard curves derived from vapor standards of known concentrations. At least one standard was run before each daily exposure. The nominal concentration (ratio of amount of test material vaporized/total amount of air through the chamber) was calculated for each chamber for each exposure day. The mean daily time weighted average analytical concentrations for each chamber were within 7-8 % of intended chamber concentrations. Mean daily nominal concentrations were in close agreement with the time weighted analytical concentrations indicating that test material losses were negligible.

Study conduct: After a 2 week acclimation period, females (175 to 225 g) were bred to adult males of the same strain (one female to one male). The day sperm were found in a vaginal smear was considered to be Day 0 of gestation. Groups of 30-32 bred rats were exposed to filtered room air (control), or 75, 210 or 590 ppm test material for 6 hr/day on Days 6 through 15 of gestation. Exposure levels were based on results of preliminary studies that showed that 1000 ppm produced maternal toxicity. Inhalation exposures were conducted in 4.3 m3 stainless steel and glass chambers under dynamic airflow conditions (800 liters air/min). Food and water were available ad libitum except during exposures. Treated and control animals were held in separate rooms.

Animals were observed daily throughout the experimental period for toxicity. Body weights were recorded on gestation days 6, 9, 12, 16 and 21. Food and water consumption was recorded at 3-day intervals beginning on Day 6 of gestation. Animals were killed on Day 21 of gestation. Maternal liver and kidney weights were recorded. The uterine horns were exteriorized and the following data were recorded: number and position of fetuses in utero, number of live and dead fetuses, number and position of resorption sites, number of corpora lutea, the sex, body weight and crown -rump length of each fetus, and any gross external alterations. The uteri of apparently nonpregnant animals were stained with a 10% solution of sodium sulfide and examined for implantation sites. These data were not used to calculate the incidence of resorptions. One-half of each litter was randomly selected for immediate examination under a dissection microscope for soft tissue abnormalities. The heads of these fetuses were removed, placed in Bouin's fixative and examined by serial sectioning. All fetuses were preserved in alcohol, eviscerated, stained with alizarin red-S and examined for skeletal alterations.

Statistical evaluations: Statistical evaluations of the frequencies of alterations and resorptions among litters and the fetal population were conducted by the Wilcoxon test as modified by Haseman and Hoel (J Statis. Comput. Simul. 3:117-135, 1974). Statistical analyses of the percentage of pregnancy and other incidence data were conducted by the Fisher exact probability test. Analyses of other data were made by parametric or nonparametric analysis of variance followed by the Dunnett's test (for parametric data) or the Wilcoxon test (for nonparametric data). The reported level of statistical significance was $p < 0.05$. Statistical outliers in feed and water consumption data were identified by a sequential outlier test and were not used when calculating mean values.

Test substance	:	Test material was chlorobenzene (CAS No. 108-90-7). It was 99.982 % pure. Impurities (% by weight) were benzene(< 0.005%), bromobenzene (0.018%) and water (0.0077%).
Conclusion	:	Test material was not embryotoxic or teratogenic. Fetal effects (slight delays in skeletal development) were only noted at a concentration that produced maternal toxicity.
Reliability	:	(1) valid without restriction
Flag	:	Supportive study for endpoint.

27.11.2001 (26)

Species	:	rabbit
Sex	:	female
Strain	:	New Zealand white
Route of admin.	:	inhalation
Exposure period	:	days 6 - 18 of gestation
Frequency of treatment	:	6 hours/day
Duration of test	:	to day 29 of gestation
Doses	:	75, 210, 590 ppm (first study), and 10, 30, 75 and 590 ppm (second study)
Control group	:	yes
NOAEL Maternalt.	:	= 75 ppm
NOAEL Teratogen	:	= 590 ppm
Method	:	other
Year	:	1984
GLP	:	no data
Test substance	:	other TS
Remark	:	Since the 61% resorption rate in high dose animals in the second study was within the historical range (19-67%) from 21 studies conducted in the laboratory and was not observed in the first study (where 63% of the control litters had resorptions) it was not interpreted by the authors to be indicative of an embryotoxic effect. The increased incidence of extra ribs observed at the high dose in the first study also was not confirmed by the second study. Therefore, the NOAELs for embryotoxicity, teratogenicity and fetotoxicity are 590 ppm.
Result	:	In the first study, there were increased absolute and relative liver weights in rabbits exposed to 210 or 590 ppm. There was no effect of treatment on pregnancy rate, mean litter size or the incidence of resorption. However, exposure to 0.75, 210 or 590 ppm test material resulted in a variety of malformations in all groups at incidences slightly higher than historical controls. The incidence of malformations at these doses was 11 (in 6 litters), 8 (in 7 litters), 6 (in 5 liters) and 8 (in 7 litters). There was no effect of treatment on the incidence of any malformation or variation, except for an increased incidence of extra ribs (a variation) in the high dose animals (113 in 26 litters vs. 79 in 24 control litters). Head/facial abnormalities were present in one fetus from the 75 ppm group and another from the 590 ppm group, and heart defects were seen in 1 fetus from the 210 ppm group and 2 fetuses (in 2 litters) in the 590 ppm group. To determine if these malformations were true effects of treatment, the study was repeated. In the second group, exposure to 590 ppm produced an increase in liver weight of maternal animals. A significant increase in the percentage of implantations undergoing resorption was observed in the 590 ppm group (61% vs. 41% in controls). There was no effect of treatment on the number of bred females, % pregnant, number of litters, implantation sites/dam, number of fetuses/litter, % of implantations resorbed, fetal body weight or fetal crown-rump length. The incidence of malformations in fetuses from animals treated with 0, 10, 30, 75 or 590 ppm was 14 (in 11 litters), 3 (in 3

liters), 14 (in 8 litters), 7 (in 5 litters) and 14 (in 5 litters). Fetuses with external, soft tissue and skeletal malformations were observed among all groups (including controls). There were seven fetuses in one litter with ablepharia (missing eyelid) in the 590 ppm group. This anomaly was not observed in the first study. Heart abnormalities were observed at similar incidences in controls and treated animals (2 in controls and 0-2 in treated) and there was no dose-dependent effect. No head or facial abnormalities were observed in any group. Skeletal examinations revealed a significant increase in the incidence of extra ribs in the 10 ppm group (94 in 23 litters vs. 72 in 21 control litters). This alteration was considered a skeletal variant.

Test condition : Test vapor generation: Temperature and relative humidity were controlled at 21 degrees C and 50%, respectively. Vapor was generated by metering the liquid test material at controlled rates into vaporization tubes. Vapors from the tubes were swept into the chamber inlet ducts with preheated compressed air (120 to 135 degrees C) where they were mixed and diluted with incoming air by turbulence. Concentrations of test material were determined 1-2 times per hour throughout the exposure by infrared spectrometry. The actual concentration in the exposure chamber was determined by interpolation from standard curves derived form vapor standards of known concentrations. At least one standard was run before each daily exposure. The nominal concentration (ratio of amount of test material vaporized /total amount of air through the chamber) was calculated for each chamber for each exposure day. The mean daily time weighted average analytical concentrations for each chamber were within 7-8 % of intended chamber concentrations. Mean daily nominal concentrations were in close agreement with the time weighted analytical concentrations indicating that test material losses were negligible.

Study conduct: After a 2 week acclimation period, rabbits (3.5 to 4.5 kg) were artificially inseminated. The day of insemination was considered gestation day 0. Groups of 30 inseminated rabbits were exposed to filtered room air (control), or 75, 210 or 590 ppm test material for 6 hr/day on Days 6 through 18 of gestation. Exposure levels were based on results of preliminary studies that showed that 1000 ppm produced maternal toxicity. Inhalation exposures were conducted in 4.3 m3 stainless steel and glass chambers under dynamic airflow conditions (800 liters air/min). Food and water were available ad libitum except during exposures. Treated and control animals were held in separate rooms.

Animals were observed daily throughout the experimental period for toxicity. Body weights were recorded on gestation days 6, 9, 12, 16 and 21. Food and water consumption was recorded at 3-day intervals beginning on Day 6 of gestation. Animals were killed on Day 21 of gestation. Maternal liver and kidney weights were recorded. The uterine horns were exteriorized and the following data were recorded: number and position of fetuses in utero, number of live and dead fetuses, number and position of resorption sites, number of corpora lutea, the sex, body weight and crown -rump length of each fetus, and any gross external alterations. The uteri of apparently nonpregnant animals were stained with a 10% solution of sodium sulfide and examined for implantation sites. These data were not used to calculate the incidence of resorptions. One-half of each litter was randomly selected for immediate examination under a dissection microscope for soft tissue abnormalities. The heads of these fetuses were removed, placed in Bouin's fixative and examined by serial

sectioning. All fetuses were preserved in alcohol, eviscerated, stained with alizarin red-S and examined for skeletal alterations.

Statistical evaluations: Statistical evaluations of the frequencies of alterations and resorptions among litters and the fetal population were conducted by the Wilcoxon test as modified by Haseman and Hoel (J Statis. Comput. Simul. 3:117-135, 1974). Statistical analyses of the percentage of pregnancy and other incidence data were conducted by the Fisher exact probability test. Analyses of other data were made by parametric or nonparametric analysis of variance followed by the Dunnett's test (for parametric data) or the Wilcoxon test (for nonparametric data). The reported level of statistical significance was $p < 0.05$. Statistical outliers in feed and water consumption data were identified by a sequential outlier test and were not used when calculating mean values.

Repeat Study: Due to the finding of a variety of external and visceral malformations in the exposed animals, a second study was initiated. This study was initiated prior to the completion of the skeletal examinations in the first study to ascertain if the low incidences of head and facial anomalies and heart defects in the first study were true effects of treatment. In this study, groups of 30-32 inseminated rabbits were exposed to filtered room air (control), or 10, 30, 75 or 590 ppm test material for 6 hr/day on Days 6 through 18 of gestation. A control group of 22 rabbits was exposed to filtered room air.

Conclusion : Maternal toxicity (as evidence by increased liver weight) was observed in rabbits exposed to 210 or 590 ppm. Inhalation of up to 590 ppm test material was not teratogenic in rabbits. Malformations were evenly distributed among groups (including controls) and were not dose-related. The increase in incidence of resorptions at 590 ppm test material in the second study, although significantly increased from control, was within the historic range of 19-67% in controls from 21 teratology studies performed by the laboratory. Since an increase in resorptions did not occur in the first experiment the increase in the second experiment was not interpreted to be indicative of an embryotoxic effect.

Test substance : Test material was chlorobenzene (CAS No. 108-90-7). It was 99.982 % pure. Impurities (% by weight) were benzene(< 0.005%), bromobenzene (0.018%) and water (0.0077%).

Reliability : (1) valid without restriction

Flag : Supportive study for endpoint.

27.11.2001 (26)

Species : rat
Sex :
Strain : Fischer 344
Route of admin. : inhalation
Exposure period : days 6 through 15 of gestation
Frequency of treatment : 6 hr/day
Duration of test : Day 21 of gestation
Doses : 100, 200, 400 ppm (0.6, 1.2, 2.4 mg/l)
Control group : other: filtered room air
NOAEL Maternalt. : < 100 ppm
NOAEL Teratogen : = 400 ppm
Method : other
Year : 1985
GLP : no data

Test substance : other TS

Remark : The NOAEL for fetotoxicity is 200 ppm, based on an increased occurrence of delayed ossification of cervical vertebral centra at 400 ppm.

Result : Maternal observations: A slight to moderate degree of urine soaking of the perineal area was observed in 8/32 rats in the high dose group. Mean body weights of high dose females were reduced from gestation days 6 though 30, and mean body weight gains of all groups were depressed from days 6-8, 12-15, and 6-20 of gestation. Food consumption was slightly depressed during the first three exposure days (data not shown). A significant increase in both absolute (10.25 +/- 0.69 g versus 9.71 +/- 0.61 g in control) and relative liver weight (3.98 +/- 0.23 vs. 3.62 +/- 0.19 in control) was seen in rats exposed to 400 ppm and relative liver weight in rats exposed to 100 ppm (3.79 +/- 0.24 vs. 3.62 +/- 0.19 in control). There were no significant differences in the % pregnant, number of litters, number of corpora lutea/dam, number of implantation sites/dam, number of fetuses/litter, % of implantation sites resorbed, % litters with resorptions, litters totally resorbed, the ratio of resorptions/liters with resorptions, sex ratio, fetal body weight or fetal crown-rump length between groups.

Fetal alterations: The incidence of major malformations, when considered individually or collectively, was not significantly increased in treated groups. The incidence of major malformations in the control, 100, 200 and 400 ppm treatment groups was 5 in 2 litters, 3 in three litters, 1 in 1 litter and 5 in three litters, respectively.

Four fetuses in one control litter were missing one pair of ribs and a single thoracic vertebra; two of these fetuses had cervical ribs. Three fetuses in three litters in the 100 ppm group had cervical ribs. One fetus in the 200 ppm group had a cervical rib. In the 400 ppm group, the malformations included single cases of coarcted and retroesophageal aortic arch, unilateral testicular agenesis, polydactyly, cervical ribs and microphthalmia. Skeletal evaluation of the fetuses revealed a significant increase in the occurrence of spurs on the first lumbar vertebra (6 in 6 litters vs. 1 in 1 control litter) and delayed ossification of sternebrae in the 200 ppm group (84 in 27 litters vs. 66 in 22 litters in control). These variations were not considered to be related to treatment, as their incidence was not increased in the 400 ppm group. The occurrence of delayed ossification of cervical vertebral centra was significantly increased with respect to controls in the 400 ppm group (79 in 25 litters vs. 60 in 22 litters). This variation was not considered to be a major malformation.

Test condition : Test animals: Male and female F344 rats were acclimated for at least 2 weeks prior to breeding. Adult virgin females (175-220 g at breeding) were bred to adult males (one female to one male). The day sperm were found in a vaginal smear was considered Day 0 of gestation. Animals were randomly assigned to test groups according to their day 0 of gestation. Food and water were available ad libitum except during test material exposure.

Test vapor generation: Temperature and relative humidity were controlled at 21 degrees C and 50%, respectively. Vapor was generated by metering the liquid test material at controlled rates into vaporization tubes. Vapors from the tubes were swept into the chamber inlet ducts with preheated compressed air (120 to 135 degrees C) where they were mixed and diluted with incoming air by turbulence. Concentrations of test material were determined 1-2 times per hour throughout the exposure by infrared spectrometry. The actual concentration in the exposure chamber was determined by interpolation from standard curves derived form vapor

standards of known concentrations. At least one standard was run before each daily exposure. The nominal concentration (ratio of amount of test material vaporized /total amount of air through the chamber) was calculated for each chamber for each exposure day. The mean daily time weighted average analytical concentrations for each chamber were within 1% of intended chamber concentrations. Mean daily nominal concentrations were in close agreement with the time weighted analytical concentrations indicating that test material losses were negligible.

Study conduct: Groups of 30-32 bred rats were exposed to filtered room air (control), or 100, 200 or 400 ppm test material for 6 hr/day on Days 6 through 15 of gestation. Exposure levels were based on results of preliminary studies that showed that 400 ppm produced maternal toxicity. Inhalation exposures were conducted in 4.3 m3 stainless steel and glass chambers under dynamic airflow conditions (800 liters air/min). Animals were observed daily throughout the experimental period for toxicity. Body weights were recorded on gestation days 6, 9, 12, 16 and 21. Food and water consumption was recorded at 3-day intervals beginning on Day 6 of gestation. Animals were killed on Day 21 of gestation. Maternal liver and kidney weights were recorded. The uterine horns were exteriorized and the following data were recorded: number and position of fetuses in utero, number of live and dead fetuses, number and position of resorption sites, number of corpora lutea, the sex, body weight and crown -rump length of each fetus, and any gross external alterations. The uteri of apparently nonpregnant animals were stained with a 10% solution of sodium sulfide and examined for implantation sites. These data were not used to calculate the incidence of resorptions. One-half of each litter was randomly selected for immediate examination under a dissection microscope for soft tissue abnormalities. The heads of these fetuses were removed, placed in Bouin's fixative and examined by serial sectioning. All fetuses were preserved in alcohol, eviscerated, stained with alizarin red-S and examined for skeletal alterations.

Statistical evaluations: Body weights and absolute and relative organ weights were evaluated by Bartlett's test for equality of variance. A parametric or nonparametric analysis of variance (ANOVA) was then conducted (as needed) followed by analysis with Dunnett's test (parametric data) or the Wilcoxon rank-sum test with Bonferroni's correction (nonparametric data) if the ANOVA was significant. Statistical outliers for food and water consumption data were identified using a sequential outlier test and excluded from analysis. Frequency of fetal alterations and resorption data among litters and the fetal population were analyzed using a sensored Wilcoxon test. Other incidence data were analyzed with the Fisher exact probability test. The litter was considered the basic unit of analysis. The final interpretation of numerical data considered statistical analyses along with other factors such as dose-response relationships and whether the results were significant in the light of other biologic and pathological findings.

Test substance : Test material was orthodichlorobenzene (CAS No. 95-50-1). It was analyzed by gas chromatography and found to be 98.81 % pure.

Conclusion : The only developmental treatment-related effect (small increase in occurrence of delayed ossification of cervical vertebral centra) occurred at a dose (400 ppm) that clearly produced maternal toxicity. 1,2, dichlorobenzene was not teratogenic or embryotoxic.

Reliability : (1) valid without restriction

Flag : Supportive study for endpoint.

19.11.2001 (19)

Species	:	rabbit
Sex	:	female
Strain	:	New Zealand white
Route of admin.	:	inhalation
Exposure period	:	days 6 through 18 of gestation
Frequency of treatment	:	6 hr/day
Duration of test	:	to day 29 of gestation
Doses	:	100, 200, 400 ppm (0.6, 1.2, 2.4 mg/l)
Control group	:	other: filtered room air
NOAEL Maternalt.	:	< 100 ppm
NOAEL Teratogen	:	= 400 ppm
Method	:	other
Year	:	1985
GLP	:	no data
Test substance	:	other TS
Remark	:	The majority of malformations observed in the study have occurred historically among rabbits in the laboratory and have been reported by others to occur spontaneously. The NOAEL listed above for teratogenicity (400 ppm) is also the NOAEL for fetotoxicity.
Result	:	Maternal: Rabbits exposed to all concentrations of test material lost weight during the first 3 days of exposure. Average body weight gains of controls, 100, 200 and 400 ppm groups on gestation days 6-8 were 21 +/- 62 g, -54 +/ 67 g, -44 +/- 50 g, and -62 +/- 54 g. Total weight gain over days 6-28 was less in treated animals than controls (but only significant for the 100 ppm group). There were no effects of treatment on absolute or relative liver or kidney weights. There were no significant differences in the % pregnant, number of litters, number of corpora lutea/dam, number of implantation sites/dam, number of fetuses/litter, % of implantation sites resorbed, % litters with resorptions, litters totally resorbed, the ratio of resorptions/liters with resorptions, fetal body weight or fetal crown-rump length between groups. The ratio of male/female offspring was significantly different from a 50:50 distribution in the 200 ppm group (61 males:39 females). It was not significantly altered in the 400 ppm group. Fetal alterations: The incidence of major malformations, when considered individually or collectively, was not significantly increased in treated groups. The incidence of major malformations in the control, 100, 200 and 400 ppm treatment groups was 4 in 4 litters, 4 in 4 litters, 2 in 2 litters and 8 in 7 litters, respectively. One fetus from the control group exhibited anencephaly (failure of formation of the brain), aprosopia (missing facial structures), and forelimb flexure. Examination of the cephalic tissue indicated that they also exhibited agnathia (missing jaw) and epitheliogenesis imperfecta (focal lack of formation of skin and adnexa). Another fetus from the control group had forelimb flexure, another had calloused ribs, and an additional control fetus had multiple malformations. In the 100-ppm group, a single fetus had a dilated renal pelvis, single fetuses from 2 litters had forked ribs, and one fetus from another litter had multiple malformations. In the 200 ppm group, one fetus had coarctation of the aortic arch and a fetus from a second litter was missing a vertebra. In the 400 ppm group, one fetus exhibited anencephaly, one had forelimb flexure and a rotated hind limb, and another had a rotated hind limb. One fetus from another litter had multiple malformations. A second fetus from this litter had a missing vertebra and fused ribs. Other skeletal malformations in the 400 ppm group included single cases of fused ribs, missing vertebra and hemivertebra. None of the malformations in treated

animals were at a higher statistical frequency than controls.

Test condition : Test animals: Rabbits (3.5 to 4.5 kg) were artificially inseminated. The day of insemination was considered day 0 of gestation. Animals were randomly assigned to test groups. Food and water were available ad libitum except during test material exposure.

Test vapor generation: Temperature and relative humidity were controlled at 21 degrees C and 50%, respectively. Vapor was generated by metering the liquid test material at controlled rates into vaporization tubes. Vapors from the tubes were swept into the chamber inlet ducts with preheated compressed air (120 to 135 degrees C) where they were mixed and diluted with incoming air by turbulence. Concentrations of test material were determined 1-2 times per hour throughout the exposure by infrared spectrometry. The actual concentration in the exposure chamber was determined by interpolation from standard curves derived form vapor standards of known concentrations. At least one standard was run before each daily exposure. The nominal concentration (ratio of amount of test material vaporized/total amount of air through the chamber) was calculated for each chamber for each exposure day. The mean daily time weighted average analytical concentrations for each chamber were within 1% of intended chamber concentrations. Mean daily nominal concentrations were in close agreement with the time weighted analytical concentrations indicating that test material losses were negligible.

Study conduct: Groups of 28-30 inseminated rabbits were exposed to filtered room air (control), or 100, 200 or 400 ppm test material for 6 hr/day on Days 6 through 18 of gestation. Exposure levels were based on results of preliminary studies that showed that 500 ppm produced maternal toxicity. Inhalation exposures were conducted in 4.3 m3 stainless steel and glass chambers under dynamic airflow conditions (800 liters air/min). Animals were observed daily throughout the experimental period for toxicity. Body weights were recorded on gestation days 6, 9, 12, 15, 19 and 29. Animals were killed on Day 29 of gestation. Maternal liver and kidney weights were recorded. The uterine horns were exteriorized and the following data were recorded: number and position of fetuses in utero, number of live and dead fetuses, number and position of resorption sites, number of corpora lutea, the sex, body weight and crown -rump length of each fetus, and any gross external alterations. The uteri of apparently nonpregnant animals were stained with a 10% solution of sodium sulfide and examined for implantation sites. These data were not used to calculate the incidence of resorptions. One-half of each litter was randomly selected for immediate examination under a dissection microscope for soft tissue abnormalities. The heads of these fetuses were removed and examined using a free-hand sectioning technique. All fetuses were preserved in alcohol, eviscerated, stained with alizarin red-S and examined for skeletal alterations.

Statistical evaluations: Body weights and absolute and relative organ weights were evaluated by Bartlett's test for equality of variance. A parametric or nonparametric analysis of variance (ANOVA) was then conducted (as needed) followed by analysis with Dunnett's test (parametric data) or the Wilcoxon rank-sum test with Bonferroni's correction (nonparametric data) if the ANOVA was significant. Statistical outliers for food and water consumption data were identified using a sequential outlier test and excluded from analysis. Frequency of fetal alterations and resorption data among litters and the fetal population were analyzed using a sensored Wilcoxon test. Other incidence data were analyzed with the

Fisher exact probability test. The litter was considered the basic unit of analysis. The final interpretation of numerical data considered statistical analyses along with other factors such as dose-response relationships and whether the results were significant in the light of other biologic and pathological findings.

Test substance : Test material was orthodichlorobenzene (CAS No. 95-50-1). It was analyzed by gas chromatography and found to be 98.81 % pure.

Conclusion : Test material was not embryotoxic or teratogenic at the doses used.

Reliability : (1) valid without restriction

Flag : Supportive study for endpoint.

19.11.2001 (19)

Species : rabbit
Sex : female
Strain : New Zealand white
Route of admin. : inhalation
Exposure period : days 6 through 18 of gestation
Frequency of treatment : 6 hr/day
Duration of test : to day 29 of gestation
Doses : 100, 300, 800 ppm (0.59, 1.77, 4.72 mg/l)
Control group : other: filtered room air
NOAEL Maternalt. : 300 ppm
NOAEL Teratogen : 800 ppm
Method : other
Year : 1985
GLP : no data
Test substance : other TS

Remark : The percentage of litters with resorptions for the 300 ppm group was within the range of historical controls in the laboratory (range 0-70%). Therefore, the effect at the 300 ppm level was not considered to be indicative of an embryolethal effect of the test material.

The increase in the incidence of retroesophageal right subclavian artery in the 800 ppm group was not considered by the authors to be indicative of a teratogenic response, as it is a minor variation in the circulatory system and had been previously observed in control rabbits (2%) in the laboratory. The NOAEL for fetotocixity is 300 ppm based on the increased incidence of this variation.

Result : Maternal: A single pregnant rabbit in the 300 ppm group was found dead on day 10 of gestation. This death was not considered to be treatment-related. Rabbits exposed to 800 ppm gained significantly less weight than controls on days 6 through 8 of gestation. There were no significant effects on liver or kidney weights in any of the exposed groups compared to controls. There was a significant increase in the percentage of implantations undergoing resorption (16 vs 7 in control) and the percentage of litters with resorptions (63% vs. 29% in control) in the 300 ppm, but not the 100 or 800 ppm groups. The number of litters, corpora lutea/dam, implantation sites/dam, fetuses/litter, resorptions/litter, litters totally resorbed and resorptions/litters with resorptions was not significantly different from control in any of the treated groups.

Fetal: Sex ratio, fetal weight and crown-rump length of treated animals were not significantly different from control. The overall incidence of major malformations in the 100 (6 in 4 litters), 300 (3 in 3 litters) and 800 ppm groups (11 in 7 litters) was not significantly different from control (8 in 7

litters). A significant increase in the incidence of retroesophageal right subclavian artery (not considered to be a major malformation) was observed in the 800-ppm group (N=5) compared to control (N=1). The incidences of other malformations were similar to controls.

Test condition : Test animals: Rabbits (3.5 to 4.5 kg) were artificially inseminated. The day of insemination was considered day 0 of gestation. Animals were randomly assigned to test groups. Food and water were available ad libitum except during test material exposure.

Test vapor generation: Temperature and relative humidity were controlled at 21 degrees C and 50%, respectively. Vapor was generated by metering the liquid test material at controlled rates into vaporization tubes. Vapors from the tubes were swept into the chamber inlet ducts with preheated compressed air (120 to 135 degrees C) where they were mixed and diluted with incoming air by turbulence. Concentrations of test material were determined 1-2 times per hour throughout the exposure by infrared spectrometry. The actual concentration in the exposure chamber was determined by interpolation from standard curves derived form vapor standards of known concentrations. At least one standard was run before each daily exposure. The nominal concentration (ratio of amount of test material vaporized/total amount of air through the chamber) was calculated for each chamber for each exposure day. The mean daily time weighted average analytical concentrations for each chamber were within 1% of intended chamber concentrations. Mean daily nominal concentrations were in close agreement with the time weighted analytical concentrations indicating that test material losses were negligible.

Study conduct: Groups of 28-30 inseminated rabbits were exposed to filtered room air (control), or 100, 300 or 800 ppm test material for 6 hr/day on Days 6 through 18 of gestation. Exposure levels were based on results of preliminary studies that showed that 1000 ppm produced maternal toxicity. Inhalation exposures were conducted in 4.3 m3 stainless steel and glass chambers under dynamic airflow conditions (800 liters air/min). Animals were observed daily throughout the experimental period for toxicity. Body weights were recorded on gestation days 6, 9, 12, 15, 19 and 29. Animals were killed on Day 29 of gestation. Maternal liver and kidney weights were recorded. The uterine horns were exteriorized and the following data were recorded: number and position of fetuses in utero, number of live and dead fetuses, number and position of resorption sites, number of corpora lutea, the sex, body weight and crown -rump length of each fetus, and any gross external alterations. The uteri of apparently nonpregnant animals were stained with a 10% solution of sodium sulfide and examined for implantation sites. These data were not used to calculate the incidence of resorptions. One-half of each litter was randomly selected for immediate examination under a dissection microscope for soft tissue abnormalities. The heads of these fetuses were removed and examined using a free-hand sectioning technique. All fetuses were preserved in alcohol, eviscerated, stained with alizarin red-S and examined for skeletal alterations.

Statistical evaluations: Body weights and absolute and relative organ weights were evaluated by Bartlett's test for equality of variance. A parametric or nonparametric analysis of variance (ANOVA) was then conducted (as needed) followed by analysis with Dunnett's test (parametric data) or the Wilcoxon rank-sum test with Bonferroni's correction (nonparametric data) if the ANOVA was significant. Statistical outliers for

food and water consumption data were identified using a sequential outlier test and excluded from analysis. Frequency of fetal alterations and resorption data among litters and the fetal population were analyzed using a sensored Wilcoxon test. Other incidence data were analyzed with the Fisher exact probability test. The litter was considered the basic unit of analysis. The final interpretation of numerical data considered statistical analyses along with other factors such as dose-response relationships and whether the results were significant in the light of other biologic and pathological findings.

Test substance : Test material was para dichlorobenzene (CAS No. 106-46-7). It was analyzed by gas chromatography and found to be 99.9 % pure.

Conclusion : The authors concluded that the test material was not embryotoxic or teratogenic at the doses used.

Reliability : (1) valid without restriction

Flag : Supportive study for endpoint.

19.11.2001 (19)

5.10 OTHER RELEVANT INFORMATION

5.11 EXPERIENCE WITH HUMAN EXPOSURE

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7.1 END POINT SUMMARY

7.2 HAZARD SUMMARY

7.3 RISK ASSESSMENT