I U C L I D

Data Set

Existing Chemical	:	ID: 70024-85-0
CAS No.	:	70024-85-0
Generic name	:	Methyl chloropyridine derivatives
Producer related part		
Company	:	The Dow Chemical Company
Creation date	:	27.09.2004
Substance related part		
Company	:	The Dow Chemical Company
Creation date	:	27.09.2004
Status	:	
Memo	:	
Printing date	:	27.09.2004
Revision date	:	
Date of last update	:	27.09.2004
Number of pages	:	16
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

Type	:	manufacturer
Name	:	Dow Agrosciences PLC
Contact person	:	
Date	:	
Street	:	9330 Zionsville Road
Town	:	46268 Indianapolis, IN
Country	:	United States
Phone	:	(+1) 317-337-3890
Telefax	:	(+1) 317-337-4444
Telex	:	
Cedex	:	
Email	:	
Homepage	:	
Source	:	The Dow Chemical Company, Midland, MI.
Reliability	:	(1) valid without restriction

27.09.2004

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

Remark	:	Methyl chloropyridine derivatives (CAS No. 70024-85-0) is a stream containing members of a group of chemicals known as chloropyridines, used in the production of chlorinated pesticides. The composition of the stream varies in relative proportion, but not identity of components. A major component of this stream is pentachloropyridine (CAS No. 2176-62-7).
Source	:	The Dow Chemical Company, Midland, MI.
Reliability	:	(1) valid without restriction

27.09.2004

1.1.1 GENERAL SUBSTANCE INFORMATION

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

1.3 IMPURITIES

1.4 ADDITIVES

1.5 TOTAL QUANTITY

1.6.1 LABELLING

1.6.2 CLASSIFICATION

1.6.3 PACKAGING

1.7 USE PATTERN

1.7.1 DETAILED USE PATTERN

1.7.2 METHODS OF MANUFACTURE

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

1.8.4 MAJOR ACCIDENT HAZARDS

1.8.5 AIR POLLUTION

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

1.13 REVIEWS

2.1 MELTING POINT

2.2 BOILING POINT

2.3 DENSITY

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

2.5 PARTITION COEFFICIENT

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

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 DISSOCIATION CONSTANT

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

Memo : Methyl chloropyridine derivatives is a stream containing chloropyridines. This stream varies by proportion of, but not nature of, component. Thus, determination of consistent physical properties is not possible for this stream.

Source : The Dow Chemical Company, Midland, MI.
Reliability : (1) valid without restriction
27.09.2004

3.1.1 PHOTODEGRADATION

3.1.2 STABILITY IN WATER

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

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 SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

5.1.1 ACUTE ORAL TOXICITY

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

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

5.5 GENETIC TOXICITY ‘IN VITRO‘

5.6 GENETIC TOXICITY ‘IN VIVO‘

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

6.1 ANALYTICAL METHODS

6.2 DETECTION AND IDENTIFICATION

7.1 FUNCTION

7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED

7.3 ORGANISMS TO BE PROTECTED

7.4 USER

7.5 RESISTANCE

8.1 METHODS HANDLING AND STORING

8.2 FIRE GUIDANCE

8.3 EMERGENCY MEASURES

8.4 POSSIB. OF RENDERING SUBST. HARMLESS

8.5 WASTE MANAGEMENT

8.6 SIDE-EFFECTS DETECTION

8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER

8.8 REACTIVITY TOWARDS CONTAINER MATERIAL

10.1 END POINT SUMMARY

10.2 HAZARD SUMMARY

Chapter : Toxicity

Remark : In an effort to reduce animal testing and to leverage existing data, published and unpublished data for 2,3,4,5,6-pentachloropyridine (CAS No. 2176-62-7), as detailed in the Robust Summaries, will be used as a surrogate to satisfy the requirements of all required mammalian testing. This material is component of the methyl chloropyridine derivatives stream, and the safe handling procedures detailed for pentachloropyridine are conservatively estimated to provide adequate protection from the derivatives stream. Additional testing would be unlikely to change safe handling recommendations for the derivatives stream. Thus, the Robust Summaries provide adequate data to characterize the human health effects endpoints under the Program.

Source : The Dow Chemical Company, Midland, MI.

Reliability : (2) valid with restrictions
The comparison of this stream with pentachloropyridine and the use of pentachloropyridine data as surrogate data for this stream is in keeping with current hazard assessment methods and practices.

27.09.2004

Chapter : Environmental Fate and Pathways

Remark : In an effort to reduce testing and to leverage existing data, published and unpublished data for 2,3,4,5,6-pentachloropyridine (CAS No. 2176-62-7), as detailed in the Robust Summaries, will be used as a surrogate to satisfy the requirements of all required environmental fate testing. This material is component of the methyl chloropyridine derivatives stream, and the safe handling procedures detailed for pentachloropyridine are conservatively estimated to provide adequate protection from the derivatives stream. Additional testing would be unlikely to change safe handling recommendations for the derivatives stream. In addition, current practice in environmental fate assessment recognizes that components of a mixture characteristically behave as individual components in the environment. Thus, the Robust Summaries provide adequate data to characterize environmental fate endpoints under the Program.

Source : The Dow Chemical Company, Midland, MI.

Reliability : (2) valid with restrictions
The comparison of this stream with pentachloropyridine and the use of pentachloropyridine data as surrogate data for this stream is in keeping with current hazard assessment methods and practices.

27.09.2004

Chapter : Ecotoxicity

Remark : In an effort to reduce testing and to leverage existing data, published and unpublished data for 2,3,4,5,6-pentachloropyridine (CAS No. 2176-62-7), as detailed in the attached Robust Summaries, will be used as a surrogate to satisfy the requirements of all required ecotoxicity testing. This material is component of the methyl chloropyridine derivatives stream, and the safe handling procedures detailed for pentachloropyridine are conservatively estimated to provide adequate protection from the derivatives stream. Additional testing would be unlikely to change safe handling recommendations for the derivatives stream. Thus, the Robust Summaries

provide adequate data to characterize the ecotoxicity endpoints under the Program.

Source : The Dow Chemical Company, Midland, MI.

Reliability : (2) valid with restrictions
The comparison of this stream with pentachloropyridine and the use of pentachloropyridine data as surrogate data for this stream is in keeping with current hazard assessment methods and practices.

27.09.2004

10.3 RISK ASSESSMENT

IUCLID

Data Set

Existing Chemical	:	ID: 2176-62-7
CAS No.	:	2176-62-7
Common name	:	2,3,4,5,6-Pentachloropyridine
Producer related part		
Company	:	The Dow Chemical Company
Creation date	:	20.05.2002
Substance related part		
Company	:	The Dow Chemical Company
Creation date	:	20.05.2002
Status	:	
Memo	:	
Printing date	:	22.09.2004
Revision date	:	
Date of last update	:	22.09.2004
Number of pages	:	64
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

Type	:	
Name	:	Dow AgroSciences
Contact person	:	
Date	:	
Street	:	9330 Zionsville Road
Town	:	Indianapolis, IN 46268-1189
Country	:	United States
Phone	:	
Telefax	:	
Telex	:	
Cedex	:	
Email	:	
Homepage	:	

04.06.2002

Type	:	
Name	:	The Dow Chemical Company
Contact person	:	
Date	:	
Street	:	2020 Dow Center
Town	:	48674 Midland, Michigan
Country	:	United States
Phone	:	
Telefax	:	
Telex	:	
Cedex	:	
Email	:	
Homepage	:	

20.05.2002

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

Type	:	
Name of plant	:	
Street	:	
Town	:	Freeport, TX
Country	:	United States
Phone	:	
Telefax	:	
Telex	:	
Cedex	:	
Email	:	
Homepage	:	

04.06.2002

Type	:	
Name of plant	:	
Street	:	
Town	:	Pittsburg, CA
Country	:	United States
Phone	:	
Telefax	:	

Telex	:	
Cedex	:	
Email	:	
Homepage	:	

04.06.2002

1.0.3 IDENTITY OF RECIPIENTS

Name of recipient	:	The Dow Chemical Company
Street	:	
Town	:	Freeport, TX
Country	:	United States
Phone	:	
Telefax	:	
Telex	:	
Cedex	:	
Email	:	
Homepage	:	

04.06.2002

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type	:	
Substance type	:	inorganic
Physical status	:	solid
Purity	:	> 99 % w/w
Colour	:	
Odour	:	

Test substance	:	Molecular formula = C5Cl5N Molecular weight = 251.3 Substance Type = organic Physical status = white solid Odor = sharp pyridine-like

04.06.2002

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

:Pentachloropyridine

20.05.2002

PCP

04.06.2002

1.3 IMPURITIES

Purity :
CAS-No :
EC-No :
EINECS-Name : 2,5,6-trichloro-3-pyridinecarboxylic acid
Molecular formula :
Value :

04.06.2002

Purity :
CAS-No : 2808-86-8
EC-No :
EINECS-Name : Tetrachloropyridine
Molecular formula :
Value : = .4 % w/w

04.06.2002

1.4 ADDITIVES

1.5 TOTAL QUANTITY

Quantity : 10 - 50 tonnes produced in

04.06.2002

1.6.1 LABELLING

1.6.2 CLASSIFICATION

1.6.3 PACKAGING

1.7 USE PATTERN

Type of use : type
Category : Non dispersive use

Remark : 1) 75 % used in the manufacturing of Symtet
2) 24.9 % sent to Freeport, Texas
3) 0.1% sent to external customers

04.06.2002

Type of use : type
Category : Use in closed system

04.06.2002

Type of use : industrial
Category : Agricultural industry

04.06.2002

Type of use : industrial
Category : other: pharmaceutical industry

04.06.2002

Type of use : use
Category : Intermediates

04.06.2002

1.7.1 DETAILED USE PATTERN

1.7.2 METHODS OF MANUFACTURE

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

Type of limit : other: Dow AgroSciences Industrial Hygiene Guide
Limit value : 7 mg/m3

04.06.2002

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

1.8.4 MAJOR ACCIDENT HAZARDS

1.8.5 AIR POLLUTION

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

Remark : Sampling conducted using Proper Protective Equipment per the MSDS recommendation.
Sources of Exposure
This chemical is produced in Pittsburg, California and is shipped to Freeport, Texas. Therefore, chemical is present at two sites. The chemical known as PCP is an intermediate in the production of Symtet and Starane Herbicide. Chlorine and Picolines are reacted in a vapor phase reactor followed by a series of liquid phase reactors. This material is then distilled with the PCP product stored in a tank prior to loading into a rail car. The unreacted material is recycled back to the reactors and reprocessed. The system is fully contained with no atmospheric vents. Vents are collected and sent to a vent condenser followed by thermal incineration or caustic scrubber. The scrubber effluent is sent to a Chlorinolysis facility for treatment and disposal. We have in process flow meters that perform material balances to ensure and track that PCP volumes do not escape into the environment. PCP is present in the Symtet intermediate at the 0.1 - 0.6 wt% level. PCP is not present in the end-use products of Garlon (Triclopyr) or Chlorpyrifos. PCP is also present in N-Serve 24 at the 0.2 - 0.44 wt% levels. This is an end use product.

04.06.2002

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

1.13 REVIEWS

2.1 MELTING POINT

Value	:	= 125 - 126 °C
Sublimation	:	
Method	:	
Year	:	1982
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Measured value

04.06.2002 (1)

2.2 BOILING POINT

Value	:	= 273 °C at
Decomposition	:	
Method	:	other: calculated
Year	:	2002
GLP	:	
Test substance	:	

04.06.2002 (2)

2.3 DENSITY

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Decomposition	:	
Method	:	other (measured)
Year	:	1967
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	0.014 mm Hg at 25 0C

04.06.2002 (3)

2.5 PARTITION COEFFICIENT

Partition coefficient	:	
Log pow	:	= 3.53 at °C
pH value	:	
Method	:	other (measured)
Year	:	1967
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4

04.06.2002 (3)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	
Value	:	= 8.5 mg/l at 25 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	slightly soluble (0.1-100 mg/L)
Stable	:	
Deg. product	:	
Method	:	other: measured
Year	:	1982
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Dissociation Constant: Not applicable. Does not ionize within environmentally relevant pH ranges.

04.06.2002 (4)

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 DISSOCIATION CONSTANT

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

INDIRECT PHOTOLYSIS

Sensitizer	:	OH
Conc. of sensitizer	:	1500000 molecule/cm³
Rate constant	:	= .000000000000011 cm³/(molecule*sec)
Degradation	:	ca. 50 % after 974 day(s)
Source	:	The Dow Chemical Company, Midland, MI.

05.06.2002 (5)

3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH4	:	at °C
t1/2 pH7	:	at °C
t1/2 pH9	:	at °C
Deg. product	:	
Method	:	other (calculated)
Year	:	2002
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Because the test material does not ionize at environmentally relevant pH ranges, no rate constants could be calculated for stability in water.
Reliability	:	(1) valid without restriction

12.09.2003 (6)

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	:	fugacity model level III
Media	:	other
Air	:	73.55 % (Fugacity Model Level I)
Water	:	6.5 % (Fugacity Model Level I)
Soil	:	19.5 % (Fugacity Model Level I)
Biota	:	% (Fugacity Model Level II/III)
Soil	:	% (Fugacity Model Level II/III)
Method	:	other
Year	:	2003
Method	:	Test: Environmental Distribution and Transport between Environmental Compartments Method: Level I and Level III Fugacity Models. Year: 2003 Remarks: Level I model version 2.11, Level III model version 2.70.

Obtained from the Canadian Environmental Modeling Centre, Trent University, Peterborough, Ontario, Canada

Input Parameters for Level I Model:

Property	Value	Source
Data Temperature (°C)	25	
Chemical Type	1	Type 1 indicates chemical can partition into all environmental compartments
Molecular Mass (g/mol)	251.33	Calculated from molecular structure
Water Solubility (g/m3)	8.5	Measured value
Vapor Pressure @ 25°C (Pa)	1.9	Measured value
Melting Point (°C)	125	Measured value
Log Kow Octanol-Water Partition Coefficient	3.53	Measured value
Amount of Chemical input (kg)	100,000	Level I Default Value

Input Parameters for Level III Model:

Property	Value	Source
Data Temperature (°C)	25	
Chemical Type	1	Type 1 indicates chemical can partition into all environmental compartments
Molecular Mass (g/mol)	251.33	Calculated from molecular structure
Water Solubility (g/m3)	8.5	Measured value
Vapor Pressure @ 25°C (Pa)	1.9	Measured value
Melting Point (°C)	125	Measured value
Estimated Henry's Law Constant (H) (Pa m3/mol)	56.1	Calculated by Level I Fugacity Model
Log Kow Octanol-Water Partition Coefficient	3.53	Measured value
Amount of Chemical input (kg/hr)	1,000 per compartment	Level III Default Values
Reaction Half-lives (hr.) Input to Level III Model		
Air (vapor phase)	11700	Estimated value
Water(no susp. solids)	*1.0 x 1011	
Soil	*1.0 x 1011	
Sediment	*1.0 x 1011	
Suspended Sediment	*1.0 x 1011	
Fish	*1.0 x 1011	
Aerosol	*1.0 x 1011	

*Default value used in Level III model when reaction is expected to be negligible in this compartment

Result **:** Fugacity Level I: Distribution among air, water, soil, and sediments

Emission Scenario	Percentage and amount distributed to Air	Water	Soil	Sediment
100,000 kg total emissions	73.55 % 73550 kg	6.50% 6499 kg	19.50 % 19502 kg	0.43 % 433 kg

Fugacity Level III: Distribution among air, water, soil, and sediments

Emission Scenario	Percentage and amount distributed to Air	Water	Soil	Sediment
1,000 kg/hr to Air	81.90 %	1.10 %	17.0 %	0.05 %
	2000 kg	26.9 kg	415 kg	1.21 kg
1,000 kg/hr to Water	0.33 %	95.3 %	0.07 %	4.28 %
	1266 kg	3.66	262 kg	16452 kg
		E+5 kg		
1,000 kg/hr to Soil	0.03 %	0.55 %	99.4 %	0.02 %
	1927 kg	36328 kg	6.58	1631 kg
			E+6 kg	
1,000 kg/hr simultaneously to Air, Water, and Soil	0.07 %	5.75 %	93.9 %	0.26 %
	5193 kg	4.03	6.58	18084 kg
		E+5 kg	E+6 kg	

Residence Time (days)
[without advection in brackets]

0.10
[859]
16
[2.14E+5]
276
[2.42E+6]
97.3
[9.49 x 105]

Source : The Dow Chemical Company, Midland, MI.

Conclusion : Pentachloropyridine has a moderate potential to volatilize from aqueous solution, based on the estimated Henry's Law constant (56.1 Pa m3/mol). The compound has a moderate potential to bioaccumulate in aquatic organisms based on the log Kow value (3.53).
Assuming an equal input of pentachloropyridine into air, water and soil, the Level III fugacity model predicts that most of the compound (94%) will move to the soil compartment.

Reliability : (1) valid without restriction

21.09.2004 (7)

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

3.6 BOD5, COD OR BOD5/COD RATIO

COD

Method	:	other: ThOD
Year	:	1975
COD	:	= .64 mg/g substance
GLP	:	no

Method	:	The theoretical oxygen demand is computed by assuming all carbon is oxidized to CO2 and the hydrogen to H2O. TODs are values obtained using the Dow Total Oxygen Demand Analyzer (Clifford, 1968). The oxygen demand is obtained by comparing peak heights of the sample to those of a known standard solution (standard potassium acid phthalate). TOD values are usually very close to the theoretical oxygen demand of the material.

12.09.2003 (8) (7)

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
NOEC	:	= .28 measured/nominal
LC50	:	= .47 calculated
LC100	:	= .66 measured/nominal
Limit test	:	
Analytical monitoring	:	yes
Method	:	other
Year	:	1985
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4

Method : Test Chemical

Test chemical was supplied by Aldrich Chemical Co., with a purity of 98% as determined by gas-liquid chromatography/mass spectrometry (GC/MS).

Analytical Technique

Gas-liquid chromatography was used to analyze toxicants in water samples from the fish exposure tanks. All compound analyses included one spike and one duplicate sample for every 6-12 water samples. Calibration curves were established by linear regression analysis of from 3-5 standards. Peak areas were used.

All test chambers were sampled at approximately mid-depth at 0, 24, 48, 72, and 96 hours in all exposure chambers. All samples were analyzed immediately or adequately preserved for later analysis.

Water Quality

Five water quality parameters were routinely measured. They were: water temperature, dissolved oxygen, total hardness, total alkalinity, and pH.

Water temperature was determined using a partial immersion thermometer. Measurements were made in each exposure chamber daily. The desired test temperature was 25 +/- 1 degree C.

Dissolved oxygen was determined in high, medium, low, and control exposure chambers at least once during the test. Daily measurements were taken in five treatments and the control exposure chambers during a 96-hour test if surviving fish existed in those chambers. Determinations were made with an oxygen-sensitive electrode (Yellow Springs Instrument, Yellow Springs, OH, Model 54 polarograph) which was calibrated weekly using the azide modification of the Winkler method.

Total hardness and total alkalinity measurements were made on the control (~45 mg/L as CaCO3) and low, medium and high chambers were sampled once during the exposure duration.

pH was measured daily in the control and five treatment chambers. Measurements were made with a meter, calibrated prior to each test.

The test was conducted at the USEPA Environmental Research Laboratory-Duluth, using Lake Superior water which was filtered through

sand and a cotton fiber filter.

Test Fish

Fathead minnows used in the test were cultured at the USEPA Environmental Research Laboratory-Duluth. Adults were held at 25 degrees C. in flowing water with a 16-hour light-controlled photoperiod and fed frozen adult brine shrimp (Artemia sp.) They were provided with asbestos pipes (cut in half longitudinally) as spawning substrates. The naturally spawned and fertilized embryos became attached to the underside of the spawning substrates. The substrates, with intact embryos, were removed daily and placed in another 25 degree C. bath where hatching occurred.

Fish were reared in flow-through tanks in the lab's culture units using water from the same source as that used in the test. Larvae were fed 40-48 hour old brine shrimp nauplii in excess two times daily (once on weekend days).

Fish approximately 26 to 37 days old were used in the toxicity test. Only groups of fish having a healthy appearance and no history of unusual thermal exposure or abnormally high mortality rate were used for toxicant exposure. Test fish were not fed 24 hours before or during a test.

Fish were randomly assorted to treatment chambers from a pooled group. Dose levels tested were 0, 0.28, 0.43, 0.66, 1.02, and 1.57 mg/L.

Death was the major test endpoint. The number of dead fish were noted every 24 hours after the beginning of a test, at which time they were also removed. Observations of fish behavior and toxic signs were made at 2-8, 24, 48, 72, and 96 hours. Unique behavior was also recorded using a color video camera and 0.5" tape recorder.

Individual control fish were weighed (wet weight) and measured (standard length). Four surviving fish each from the control, the lowest concentration and the concentration nearest the LC50 were preserved in 10% buffered formalin for histological examination.

Result : The 96 hour LC50 was approximately 0.47 mg/L, with confidence limits of 0.44-0.50. Affected fish lost schooling behavior and swam near the tank surface. They were hypoactive and underreactive to external stimuli, had increased respiration, were hemmorhagic and deformed, had rigid musculature, and lost equilibrium prior to death.

Source : The Dow Chemical Company, Midland, MI.

Reliability : (1) valid without restriction

19.09.2003 (9)

Type : static
Species : Notropis atherinoides
Exposure period : 72 hour(s)
Unit : mg/l
LC0 : = 1 measured/nominal
LC50 : = 1.23 calculated
LC100 : = 2 measured/nominal
Limit test :
Analytical monitoring : no
Method : other
Year : 1972
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Method : Lake Emerald shiners were exposed to 1.0, 1.5, or 2.0 mg/L PCP for 72 hours in dechlorinated Lake Huron water at 50 deg. F. under static conditions.

04.06.2002 (10)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	static
Species	:	Crangon septemspinosa (Crustacea)
Exposure period	:	43 hour(s)
Unit	:	mg/l
EC50	:	= 1.8
Analytical monitoring	:	yes
Method	:	other
Year	:	1979
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4

Method : Shrimp, collected locally in St. Andrews, New Brunswick, Canada, were held in running sea water at 10 degrees C and 30 ppt salinity for at least a week before tests. They were fed brine shrimp and clams at 2-day intervals. They ranged in length from 6.4 to 8.3 cm (2.4 to 4.5 g).

A lethality test of 96 hours duration was carried out on three shrimp in 4 liters of aerated sea water at 10 degrees C, with the solution changed at 48 hours. A stock solution was prepared in either ethanol or dimethyl sulfoxide. From the stock solution, 5 dilutions were prepared such that 1 ml added to 4 L sea water produced the required test concentration. The control test contained 1 ml of ethanol or dimethyl sulfoxide in 4 liters of sea water, as appropriate.

Concentration of the test material was measured by UV spectrophotometry at the beginning and immediately after the solution change at 48 hours. In addition, the concentration of one solution of intermediate nominal concentration was measured at 2, 4, 6, 12, 24, and 48 hours.

The time to 50% mortality (LT50) at a particular concentration of a chemical was read from a plot of percentage mortality against time to death (logarithmic scales). Lethality lines were drawn from plots of LT50 against test concentration (logarithmic scales). The 96 hour threshold was taken as the geometric mean of the highest concentration with no deaths and the next higher concentration (step by a factor of 2) at which all three shrimp died.

Result : The measured concentration of the test material remained practically constant throughout the 48 hours. The highest dose level tested was 6 mg/l. The LC50 was calculated as 1.8 mg/l at 43 hours.

Source : The Dow Chemical Company, Midland, MI

Reliability : (1) valid without restriction

26.09.2003 (11)

Type	:	static
Species	:	other aquatic mollusc: soft-shelled clam (Mya arenaria)
Exposure period	:	96 hour(s)
Unit	:	mg/l
NOEC	:	= 6 measured/nominal
EC50	:	> 6 measured/nominal
Analytical monitoring	:	yes
Method	:	other
Year	:	1979
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4

Method : Clams, collected locally in St. Andrews, New Brunswick, Canada, were

held in running sea water at 4 degrees C and 30 ppt salinity for at least a week before tests. They were uniform in size, measuring about 5 cm in length (20 g).

A lethality test of 96 hours duration was carried out on three clams in 4 liters of aerated sea water at 10 degrees C, with the solution changed at 48 hours. A stock solution was prepared in either ethanol or dimethyl sulfoxide. From the stock solution, 5 dilutions were prepared such that 1 ml added to 4 L sea water produced the required test concentration. The control test contained 1 ml of ethanol or dimethyl sulfoxide in 4 liters of sea water, as appropriate.

Concentration of the test material was measured by UV spectrophotometry at the beginning and immediately after the solution change at 48 hours. In addition, the concentration of one solution of intermediate nominal concentration was measured at 2, 4, 6, 12, 24, and 48 hours.

The time to 50% mortality (LT50) at a particular concentration of a chemical was read from a plot of percentage mortality against time to death (logarithmic scales). Lethality lines were drawn from plots of LT50 against test concentration (logarithmic scales). The 96 hour threshold was taken as the geometric mean of the highest concentration with no deaths and the next higher concentration (step by a factor of 2) at which all three clams died.

Result : The measured concentration of the test material remained practically constant throughout the 48 hours. The highest dose level tested was 6 mg/l. No mortality was observed throughout the 96 hour test period, so the LC50 was greater than 6 mg/l.
Source : The Dow Chemical Company, Midland, MI
Reliability : (1) valid without restriction
26.09.2003 (11)

Type : static
Species : other: ciliate protozoan, Tetrahymena pyriformis
Exposure period :
Unit :
Method :
Year : 1989
GLP :
Test substance :

04.06.2002 (12)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Selenastrum capricornutum (Algae)
Endpoint : biomass
Exposure period : 96 hour(s)
Unit : mg/l
NOEC : = 1 measured/nominal
EC50 : = 2.03 measured/nominal
Limit test :
Analytical monitoring : yes
Method : OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year : 2004
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Method : Testing Facility
The testing was conducted by the Toxicology & Environmental Research

and Consulting Laboratory, The Dow Chemical Company, Midland, Michigan.

Test Substance
The sample of pentachloropyridine was supplied by Dow AgroSciences LLC, Indianapolis, Indiana. Information on the test material is summarized below [7,8].

Test Substance Number: TSN103958 [7]
Chemical Name: 2,3,4,5,6-Pentachloropyridine [7]
Common Name: Pentachloropyridine, K-039636, LSN061122 [7]
Synonyms: Pentachloropyridine
Lot Number: T-171C [7]
Physical State: Solid [7]
Purity: > 99% [8]
Reference Substance - Analytical Standard
Same as test material listed above

Culture and Test Medium
The culture conditions are listed in Table 1. The growth and test medium used was that designed for the EPA Algal Assay Bottle Test [9]. A complete description of the algal
assay medium (AAM) is provided in Appendix B.

Test Organism
Axenic samples of Pseudokirchneriella subcapitata (formerly known as Selenastrum capricornutum) were received on 13 February 2003 from the University of Toronto Culture Collection at the University of Toronto, Toronto, Ontario, Canada. Stock cultures of this organism were maintained aseptically by weekly transfer into sterile AAM.

Test Vessels
Test vessels were sterilized 250-mL borosilicate Erlenmeyer flasks with Shimadzu closures, each containing 100 mL test medium. Each flask was labeled with a unique number for identification purposes.

Algal Inoculum
The algal inoculum was prepared from a 3-day old stock culture of Pseudokirchneriella subcapitata. A Coulter Multisizer 3 was used to determine the algal density of the stock culture. This evaluation determined the aliquot of the culture required so that each test vessel would contain approximately 10,000 cells/mL (0.790 mL).

Dose Level Selection
The dose levels selected for evaluating the effects of pentachloropyridine on the growth of Pseudokirchneriella subcapitata were based on the results of a probe test and the preliminary solubility work of the compound in AAM. The solubility of pentachloropyridine in AAM with an acetone carrier (using = 0.1 mL acetone carrier/L media) was evaluated and determined to be approximately 1.0 to 5.0 mg
pentachloropyridine/L AAM. The probe test was conducted between 16 October and 20 October 2003 using four nominal pentachloropyridine concentrations of 0.008, 0.04, 0.2,
and 1.0 mg/L, plus a medium and solvent control. Percent inhibition compared to controls was -7, -3, -2, and 27% for the 0.008, 0.04, 0.2, and 1.0 mg/L test levels, respectively (negative percent inhibition values indicate stimulation of growth).

The definitive test levels were set at target concentrations of 0 (control), 0 (solvent control), 0.125, 0.250, 0.500, 1.00, 2.00, and 4.00 mg pentachloropyridine/L AAM. The 2.00 and 4.00 test concentrations were within the approximate range of solubility of pentachloropyridine in AAM.

The probe determined a response of 27% inhibition at the target concentration of 1.00 mg/L. Based on this, the addition of the 2.00 and 4.00 mg/L dose concentrations would possibly allow the determination of an EC50 value.

Test Solutions
Since the test material had low inherent solubility, an organic solvent (acetone) was used to aid in dissolution of the test material in AAM. Stock solutions were prepared at the following concentrations in acetone: 40, 20, 10, 5.0, 2.5, and 1.25 mg/mL. Pentachloropyridine was weighed out directly for the three highest concentration stock solutions. To prepare the 40, 20, and 10 mg/mL stock solutions, 80.15, 40.87, and 20.58 mg of pentachloropyridine, respectively, were dissolved into 2 mL of acetone. To prepare the 5.0, 2.5, and 1.25 mg/mL stock solutions, 1.0 mL of the next highest stock solution was diluted with 1.0 mL of acetone, respectively. The 4.00, 2.00, 1.00, 0.500, 0.250, and 0.125 mg pentachloropyridine/L test solutions were prepared by addition of 50 µL of the 40, 20, 10, 5.0, 2.5, and 1.25 mg/mL stock solutions, respectively, into approximately 400 mL of AAM in a 500-mL volumetric flask. The flasks were stoppered, mixed thoroughly, unstoppered, filled to the mark (500 mL) with AAM, restoppered, and mixed again. Following addition of the stock solution to the AAM during preparation of the 4.00 mg/L test solution, a powdery film was observed on the surface of the water. This solution was sonicated for approximately three minutes, which appeared to dissolve the precipitate into the media. Test solutions
were then dispensed to the appropriate test vessels.

Sample Collection and Analysis
The bulk dose solutions were sampled for analytical confirmation on day 0 of the study immediately following preparation. On day 4, the three test solutions containing algae at each dose level were pooled to provide one composite algae-containing sample per dose level for analytical confirmation. The test solutions at each dose level containing no algae were sampled separately. A 4-mL aliquot was collected from each test solution and
centrifuged for 10 minutes at 2000 rpm. The day 0 samples were centrifuged in order to maintain consistency with the day 4 sample preparation, although there was no algae in
the day 0 bulk dose solutions. A 1-mL aliquot of the supernatant was transferred to 4-dram vials and extracted with 10-mL iso-octane by shaking on a flat-bed shaker (low
speed) for 30 minutes followed by 10 minutes of centrifuging at 2000 rpm. For the control, solvent (acetone) control, 0.125, 0.250, and 0.500 mg/L solutions, a 100-µL aliquot of the extract was transferred to autosampler vials containing 0.9-mL iso-octane. For the 1.00, 2.00, and 4.00 mg/L solutions, a 10- µL aliquot of the extract was transferred to autosampler vials containing 1 mL of iso-octane. The samples were mixed with vortexing and analyzed using gas chromatography with an electron capture detector (GC/ECD).

Method Precision and Homogeneity
To assess analytical method precision and solution homogeneity, three additional samples were taken on day 0 from bulk dose solutions at nominal concentrations of 0.125 and 4.00 mg/L. These additional samples were prepared for analysis as described above and analyzed along with the other day 0 samples.

Extraction Efficiency of Pentachloropyridine from Algal Assay Media
The recovery (extraction efficiency) of pentachloropyridine from AAM by solventpartitioning with iso-octane was determined by fortifying AAM with pentachloropyridine
at concentrations representative of the expected range of dose solutions

and then conducting the extraction as described in Sample Collection/Extraction. Triplicate
spikes at of pentachloropyridine in AAM were prepared at target concentrations of ~ 0.1, 0.5, and 4 mg/L AAM. This assessment of extraction efficiency yielded average
recovery values of 95.4% for day 0 and 103% for day 4, which were used to adjust the analyzed concentrations of the test solutions for method recovery on each analysis day as needed.

Standard Preparation/Detector Calibration
Primary stock solutions of pentachloropyridine were prepared in acetone at nominal concentrations of ~ 200 mg pentachloropyridine/L for each analysis. The stock solutions were further diluted and used to prepare analytical standards in iso-octane over concentration ranges extending from ~ 0.1 to 6 µg pentachloropyridine/L, which encompassed the expected sample concentration range (after accounting for dilution during the sample preparation). Standards were analyzed with each set of samples to define the detector response.

Response factors calculated for each of the individual analytical standards were averaged to yield an overall mean response factor and standard deviation for each daily set of analyses. The measured concentrations (mg pentachloropyridine/L AAM) of the test solutions were then calculated as follows:
Response Factor (RF) = Standard Pentachloropyridine Concentration/Peak Area
Sample Concentration (mg/L) = (RF * Peak Area * Dilution Factor)(Spike Recovery)
To provide some measure of the quality (applicability) of the daily calibration factors (mean response factors), a relative standard deviation was calculated for each daily set of analyses by dividing the standard deviation of the individual response factors by the mean response factor. The relative standard deviation values derived from the two calibrations did not exceed 7% for pentachloropyridine.

Instrument and Conditions for GC Analysis
GC: Agilent 6890N (EGC-0719) SN#: US10211081
Autosampler: Agilent 7683 SN#: US84603523 (tower)
Detector: Electron Capture Detector (ECD)
Capillary Column: 30 m x 0.32 mm, 0.25 mm film J&W Scientific HP-5 SN#: US2108471H
Temperatures:
Column Oven: 150oC isothermal for 5 minutes
Injection Port: 250oC
Detector: 275oC
Gases:
Carrier Gas: Nitrogen @ 20 psig headpressure
Make-up Gas: Nitrogen @ 20 mL/minute
Injection: 1-mL splitless; purge on at 0.25 minute
Data System: PerkinElmer's TurboChromÔ System Perkins- Elmer, Inc., Wellesley, Massachusetts

Exposure Phase
The definitive test was conducted from 27 October to 31 October 2003. Four replicate test vessels were prepared per test concentration and control, each containing 100 mL of
test solution. Three replicates at each test concentration and the control group were inoculated with approximately 10,000 cells/mL. Inoculations were made after all the
replicate test vessels at each test concentration were poured. The fourth replicate at each test concentration and control group was not inoculated with algae to serve as a counting blank. These blanks were used to correct

the daily counts for the interference of the test material and to monitor pH and concentration of the test material without the algal biomass. The exposure phase was carried out aseptically under static conditions for four days (approximately 96 hours). The replicate test flasks were placed in a walk-in environmental chamber (Lab-Line Environmental Chamber, Lab-Line Inc., Melrose,
Illinois) according to a computer-generated randomization scheme. The replicate test flasks were randomized daily after sampling for cell counts. The incubator was thermostated at 24 ± 2°C with continuous light at approximately 8000 ± 1600 lux. Test conditions are presented in Table 2.

Physical Analysis
At test initiation, a pH measurement was taken from a sample of the bulk preparation of each test concentration and the control. At test termination, a final pH measurement was taken from a pooled sample of the replicates with algae at each test concentration and the
control and from each blank replicate. The incubator temperature was continuously monitored with a Fischer minimum/maximum thermometer probe placed in a
representative vessel within the incubator. The light intensity was monitored daily at positions corresponding to the test flasks in the incubator with a VWR Dual Display
Light Meter.

Density Determinations/Observations
Algal cell densities of the initial inoculum and test cultures were determined by electron particle counting using a Coulter Multisizer 3. Total cell counts were determined at approximately 24, 48, 72, and 96 hours. Cells were cumulatively counted at a lower threshold equivalent spherical diameter of approximately 2.6 µm to a higher threshold equivalent spherical diameter of approximately 8.7 µm. Three separate cell count readings were made per replicate. The readings for the blank replicates were used to correct for background in daily calculations. Since there was no test material effect on the blanks, the mean of all the blanks was calculated and used to adjust the cell counts. The adjusted cell counts were converted to cells x 10000/mL (cell density) for statistical analysis and reporting.

In addition, at test termination morphological observations were done on a composited sample of the three inoculated replicates at each test concentration and control. The cells were observed under a microscope (Olympus® BHB-DO System Microscope; 20x or 40x objective lens; WF10x eyepiece; 1.25x Dual Observation Deck) using a Bright Line Hemacytometer Counting Chamber.

Statistical Analysis
The results (study endpoints) of the study were evaluated based on the nominal pentachloropyridine concentrations and are expressed in terms of algal growth (cells x 10,000/mL). The endpoints analyzed were cell density, growth rate (day-1), and biomass (area under the growth curve).
The control and the acetone control groups were compared using a t-test (a = 0.25) to determine whether the control groups could be pooled. The EC25 and EC50 values for cell density (those concentrations that limited cell density
to 25% and 50% of the test population, respectively, when compared to the control population) were determined by a least squares linear regression of cell density against the concentration at 72 and 96 hours for test concentrations where a clear dose relationship was observed.
The ErC50 value (the concentration that inhibited the growth rate to 50% of the test population, when compared to the control population) was empirically determined since
reduction in growth rate at both time points was less than 50%. The following formula was used to calculate growth rate:

$$mui\text{-}j = \frac{lnNj - lnNi}{tj - ti}$$

Where: mu = mean specific growth rate from moment i to j (days-1)
ln = natural logarithm
Ni = initial cell density at time i (cells/ml x 104)
Nj = cell density at time j
ti = the moment time for the start of the period
tj = the moment time for the end of the period

The EbC50 value (the concentration that inhibited biomass to 50% of the test population, when compared to the control population) was calculated by regression of the differences
in area under the growth curves for each dose group compared to the control against the log of the concentrations for 72 and 96 hours where a clear dose-response relationship was observed. Area under the growth curve was calculated using the following formula:

$$A = \frac{N1-N0}{2} \times t1 + \frac{N1+N2-2N0}{2} \times (t2-t1) + \frac{Nn-1+Nn-2N0}{2} \times (tn-tn-1)$$

Where: A = area under the growth curve
N0 = nominal number of cells/mL (x 104) at t0
N1 = measured number of cells/mL (x 104) at t1
Nn = measured number of cells/mL (x 104) at tn
t1 = time of first measurement after beginning of test
tn = time of nth measurement after beginning of test

Prior to evaluation of the no-observed-effect concentrations (NOECs), the data were tested for normality using the Shapiro-Wilk's Test and for homogeneity of variance using the Bartlett's Test. The 72- and 96- hour endpoints met the assumptions of homogeneity and normality, so the untransformed data for these endpoints were
evaluated using the Dunnett's test. Based on this, the 72- and 96- hour data for cell density, growth rate, and biomass (area under the growth curve) were analyzed using the analysis of variance and Dunnett's test (a = 0.05) to determine NOEC values.

Quality Assurance
The study conduct and data generated were reviewed according to the procedures of the Quality Assurance Unit of Toxicology & Environmental Research and Consulting, The
Dow Chemical Company, Midland, Michigan. Permanent records of all data generated during the course of this study, the protocol, any changes/revisions to the protocol, and a
copy of the final report were available for inspection by the Quality Assurance Unit.

Archival Statement
All data generated are archived at Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan.

Result **:** Chemical Analysis
The results obtained from the analyses of test solutions for pentachloropyridine are presented in Table 3. Results from the day 0

analysis yielded percent recovery of target
values for the bulk dose solutions ranging from 29.5 to 118%. The data exhibited a general trend in which the percent of recovery of target values decreased as the target concentrations increased, which is likely due to the limited solubility of pentachloropyridine in AAM. The measured pentachloropyridine concentrations were similar for the 0.500, 1.00, and 2.00 mg/L bulk dose solutions with values ranging from 0.437 to 0.613 mg/L, suggesting that a concentration plateau was achieved. Pentachloropyridine was not present at quantifiable concentrations in any of the day 4 test solutions (with or without algae). Mean measured concentrations for the exposure period were calculated for all dose levels by averaging the day 0 bulk dose solution
concentrations and the day 4 exposure solution concentrations. However, since the day 4 measured concentrations were all less than the lowest levels quantified (LLQ) of 0.0100 mg pentachloropyridine/L AAM for the controls, 0.125, 0.250, and 0.500 mg/L solutions and 0.101 mg pentachloropyridine/L AAM for the 1.00, 2.00, and 4.00 mg/L solutions, values of 0.005 and 0.05 mg/L (equivalent to ½ LLQ, respectively) were used in the calculations as a conservative estimate of day 4 exposure concentrations. The resulting overall mean measured concentrations ranged from 16.0 to 60.4 percent of target dose levels.

None of the analyses of the AAM or solvent controls exhibited a peak eluting at the retention time of pentachloropyridine at concentrations exceeding the lowest level quantified of 0.0100 mg/L AAM.

Method Precision and Solution Homogeneity
The variability associated with the analytical method, as well as solution homogeneity, was assessed on day 0 of the study. Four replicate samples were collected from day 0
bulk dose solutions at nominal concentrations of 0.125 and 4.00 mg pentachloropyridine/L AAM. Four repeated measurements (4 samples x 1 injection/sample) resulted in percent relative standard deviation (RSD) values of 2.84 and 7.99% in the low and high samples, respectively (data not shown).

Linearity
The GC/ECD instrumentation exhibited a linear response over the concentration range extending from ~0.1 to 6 µg pentachloropyridine/L iso-octane.

Lowest Level Quantified (LLQ)
Different LLQ values were used for the low concentration dose levels (controls, 0.125, 0.250, and 0.500 mg/L) and the high concentration dose levels (1.00, 2.00, and 4.00
mg/L) to account for the difference in sample dilution during preparation for analysis. The low concentration samples were diluted 100- fold resulting in an LLQ value of
0.0100 mg pentachloropyridine/L AAM while the high concentration samples were diluted 1010-fold to provide an LLQ value of 0.101 mg pentachloropyridine/L AAM.
The LLQ values were based upon the concentration of the lowest standard analyzed times the dilution factor.

Test Conditions
Temperature (°C), light intensity (lux), and pH data ranges observed during the four-dayexposure phase are summarized in Table 5. Temperatures during the exposure period
ranged from 24.4 - 24.6°C. The mean (± standard deviation) light intensity was 7346 ± 445 lux, with a range of 6410 - 8200 lux. The pH values ranged 6.8 to 7.0 at test
initiation, from 8.9 to 9.6 in pooled replicates with algae at test termination,

and from 6.6 to 7.1 in blank replicates without algae at test termination.

Biological Data
All biological results are expressed in terms of nominal concentrations of pentachloropyridine. There was a clear dose-related inhibition in cell density over all test
levels. However, analytical recoveries from the Day 0 test solutions indicated no dose related increase in residues above the 0.500 mg/L nominal test level. The samples taken
for residue determinations were centrifuged prior to extraction, which most likely removed undissolved test material from the test media (see Test Material section). Thisindicates that the solubility of pentachloropyridine in AAM was approximately 0.500 mg/L. Since there was a dose related biological response, it was decided to
statistically analyze the data using the nominal concentrations. This is the only way a meaningful dose-response endpoint (i.e., EC50) could be calculated.

Mean cell densities at 72 hours were 201.0, 152.6, 174.1, 166.2, 140.1, 138.4, 76.76, and 23.99 x 104 cells/mL for the control, acetone control, 0.125, 0.250, 0.500, 1.00, 2.00, and 4.00 mg/L test levels, respectively. The t-test analysis indicated that the control data should not be pooled ($p < 0.25$), so the statistical comparisons for this endpoint were made to the acetone control. Response relative to the acetone controls ranged from 14% stimulation to 84% inhibition of growth. The 72-hour calculated EC25 and EC50 values (95% confidence
intervals) for cell density were 1.26 (< 0.125-3.15) and 2.39 (0.474-> 4.00) mg/L, respectively. Based on the Dunnett's test, the 72-hour cell density was significantly less than the acetone controls at the 4.00 mg/L test level; therefore, the 72-hour NOEC value for cell density was determined to be 2.00 mg/L. Mean cell densities at 96 hours were 457.5, 409.1, 449.9, 425.7, 379.5, 372.0, 241.4, and
108.6 x 104 cells/mL for the control, acetone control, 0.125, 0.250, 0.500, 1.00, 2.00, and 4.00 mg/L test levels, respectively. The t-test analysis indicated that the control data should not be pooled ($p < 0.25$), so the statistical comparisons for this endpoint were made
to the acetone control. Response relative to the acetone controls ranged from 10% stimulation to 73% inhibition of growth. The 96-hour calculated EC25 and EC50 values
(95% confidence intervals) for cell density were 1.44 (0.107-2.77) and 2.74 (1.38-> 4.00) mg/L, respectively. Based on the Dunnett's test, the 96-hour cell density was significantly less than the controls at test levels >= 2.00 mg/L; therefore, the 96-hour NOEC value for cell
density was determined to be 1.00 mg/L.

Mean specific growth rates at 72 hours were 1.765, 1.672, 1.710, 1.691, 1.627, 1.640, 1.445, and 1.033 day-1 for the control, acetone control, 0.125, 0.250, 0.500, 1.00, 2.00, and 4.00 mg/L test levels, respectively. The t-test analysis indicated that the control data should not be pooled ($p < 0.25$), so the statistical comparisons for this endpoint were made to the acetone control. Response relative to the acetone controls ranged from 2% stimulation to 38% inhibition of growth rate. The empirically determined 72-hour ErC50 value for specific growth rate was > 4.00 mg/L, the highest level tested. Based on the Dunnett's test, the 72-hour specific growth rate was significantly less than the controls attest levels >= 2.00 mg/L; therefore, the 72-hour NOEC value for specific growth rate was determined to be 1.00 mg/L.

Mean specific growth rates at 96 hours were 1.531, 1.502, 1.526, 1.513, 1.483, 1.480,1.367, and 1.149 day-1 for the control, acetone control, 0.125, 0.250, 0.500, 1.00, 2.00,
and 4.00 mg/L test levels, respectively. The t-test analysis indicated that

the control data should not be pooled ($p < 0.25$), so the statistical comparisons for this endpoint were made to the acetone control. Response relative to the acetone controls ranged from 2% stimulation to 24% inhibition of growth rate. The empirically determined 96-hour ErC50 value for specific growth rate was > 4.00 mg/L, the highest level tested. Based on the
Dunnett's test, the 96-hour specific growth rate was significantly less than the controls at test levels >= 2.00 mg/L; therefore, the 96-hour NOEC value for specific growth rate was determined to be 1.00 mg/L.

Mean biomass area values at 72 hours were 3656, 2948, 3212, 3078, 2537, 2342, 1320, and 383 day-1 for the control, acetone control, 0.125, 0.250, 0.500, 1.00, 2.00, and 4.00 mg/L test levels, respectively. The t-test analysis indicated that the control data should not be pooled ($p < 0.25$), so the statistical comparisons for this endpoint were made to the acetone control. Response relative to the acetone controls ranged from 9% stimulation to 87%
inhibition of biomass. The 72-hour calculated EbC50 value (95% confidence intervals) for biomass was 1.66 (0.871-3.15) mg/L. Based on the Dunnett's test, the 72-hour biomass was significantly less than the controls at test levels >= 2.00 mg/L; therefore, the 72-hour
NOEC value for biomass was determined to be 1.00 mg/L.
Mean biomass area values at 96 hours were 11533, 9665, 10676, 10157, 8749, 8444, 5113, and 1951 day-1 for the control, acetone control, 0.125, 0.250, 0.500, 1.00, 2.00, and 4.00 mg/L test levels, respectively. The t-test analysis indicated that the control data should not be pooled ($p < 0.25$), so the statistical comparisons for this endpoint were made to the acetone control. Response relative to the acetone controls ranged from 10% stimulation to 80% inhibition of biomass. The 96-hour calculated EbC50 value (95% confidence intervals) for biomass was 2.03 (0.937-> 4.00) mg/L. Based on the Dunnett's test, the 96-hour biomass was significantly less than the controls at test levels >= 2.00 mg/L;
therefore, the 96-hour NOEC value for biomass was determined to be 1.00 mg/L.

Microscopic evaluation of cells at each test concentration and the control revealed no abnormal observations at any test level.

Table 1. Typical Culturing Conditions
Organism: Pseudokirchneriella subcapitata (formerly know as Selenastrum capricornutum), a freshwater green alga
Temperature: 24 ± 2°C
Light (lux): 4300 ± 650
Photoperiod: Continuous
Medium: Algal assay medium (AAM) designated for the EPA algal assay bottle test
pH: Range: approximately 7.0-7.5
Culture Conditions: Axenic
Culture Volume: 200 mL
Culture Vessel: 500-mL Erlenmeyer flask
Culture Vessel Cap: Shimadzu closure

Table 2. Typical Testing Conditions
Habitat: Environmental Growth Chamber
Temperature °C: 24 ± 2°C
Light (lux): 8000 ± 1600
Photoperiod: Continuous
Agitation: Continuous (100 rpm)
Medium: Algal Assay Medium
Test Conditions: Axenic

Test Vessel 250-mL Erlenmeyer flask
Test Volume 100 mL
Replicates/Concentration 4 (3 with algae, 1 without algae)
Replicates/Control 4 (3 with algae, 1 without algae)
Length of Exposure 4 days (approximately 96 hours)
Initial Cell Density:
Exposure Phase ~ 10,000 cells/mL
Measurements:
Lights Daily
Temperature Continuous
pH Initiation and Termination
(with and without algae)
Observations:
Hours 24, 48, 72, and 96 Total cell counts/mL (microscopic evaluation of cell morphology at 96 hours)
Endpoints: Cell Density (cells/mL), Growth Rate, and Biomass (area under the growth curve)

Source **:** The Dow Chemical Company, Midland, MI.

Conclusion **:** The purpose of this study was to assess the effects of pentachloropyridine on the growth of Pseudokirchneriella subcapitata (formerly known as Selenastrum capricornutum), a freshwater green alga. The freshwater alga was exposed to six nominal test concentrations of 0.125, 0.250, 0.500, 1.00, 2.00, and 4.00 mg/L mg pentachloropyridine/L of algal assay medium, plus a medium control and an acetone control, over a 96-hour period. Recoveries on day 0 ranged from 29.5 to 118% of nominal. The wide range in recoveries was most
likely due to decreasing solubility as nominal concentrations increased, combined with centrifugation of the analytical samples prior to extraction. Measured concentrations did not increase in a dose dependent fashion at nominal test levels above 0.500 mg/L. Based on
these recoveries, the solubility of pentachloropyridine in algal assay medium was determined to be approximately 0.500 mg/L. Analytical residues at test termination (day 4)
were less than the LLQ at all test concentrations. Even though measured test levels did not increase in dose dependent fashion above 0.500 mg/L (nominal), there was a clear dose response in biological effect. This indicated that the measured concentrations at the higher test levels were not reflective of the actual exposure (i.e., undissolved test material was present that was not measured due to centrifugation). Based on this, statistical analysis of the biological data was conducted using the nominal test concentrations.
The 72-hour results, based on nominal pentachloropyridine concentrations, were as follows:
· The 72-hour EC25, EC50, and NOEC values for cell density were 1.26, 2.39, and
2.00 mg/L, respectively.
· The 72-hour ErC50 and NOEC values for growth rate (day-1) were > 4.00 and 1.00
mg/L, respectively.
· The 72-hour EbC50 and NOEC values for biomass (area under the growth curve)
were 1.66 and 1.00 mg/L, respectively.
The 96-hour results, based on nominal pentachloropyridine concentrations, were as
follows:
· The 96-hour EC25, EC50, and NOEC values for cell density were 1.44, 2.74, and
1.00 mg/L, respectively.
· The 96-hour ErC50 and NOEC values for growth rate (day-1) were > 4.00 and 1.00
mg/L, respectively.
· The 96-hour EbC50 and NOEC values for biomass (area under the

growth curve)
were 2.03 and 1.00 mg/L, respectively.

Reliability : (1) valid without restriction
21.09.2004 (13)

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 SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	= 435 mg/kg bw
Species	:	rat
Strain	:	Fischer 344
Sex	:	male
Number of animals	:	12
Vehicle	:	other: corn oil
Doses	:	
Method	:	other
Year	:	1987
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Method : Young adult male rats were fasted overnight. They were administered the material as a solution in corn oil at a dose volume of 10 ml/kg bw at dose levels of 100, 250, 500, or 750 mg/kg bw. Animals were observed closely for two weeks, then submitted for pathological examination. All animals which died prior to scheduled necropsy were also submitted for pathological examination. Body weights were recorded on the day of treatment (Study Day 0), and Study Days 1, 8, and 15.

Result : Acute oral toxicity was characterized as moderate. The acute oral LD50 for male rats was approximately 435 mg/kg, when calculated using the moving average method.

Dose (mg/kg)	Number Treated	Number Dead
100	3	0
250	3	0
500	3	2
750	3	3

In-life signs of toxicity were observed only in rats receiving 500 or 750 mg/kg, and included lethargy, tremors/muscle spasms, lacrimation, palpebral closure, and death on the day of treatment. No clinical evidence of treatment-related effects were seen at 100 or 250 mg/kg. All surviving rats gained weight over the 2-week observation period.

Source : The Dow Chemical Company, Midland, MI.

Reliability : (1) valid without restriction
Study conducted in accordance with generally accepted scientific principles.
GLP not compulsory at time study was performed.

05.06.2002 (14)

Type	:	LD50
Value	:	= 126 - 1000 mg/kg bw
Species	:	rat
Strain	:	no data
Sex	:	female
Number of animals	:	3
Vehicle	:	other: rodent chow
Doses	:	
Method	:	other
Year	:	1963
GLP	:	no

Test substance : as prescribed by 1.1 - 1.4

Source : The Dow Chemical Company, Midland, MI.
Reliability : (2) valid with restrictions
05.06.2002 (15)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

Species : rabbit
Concentration : undiluted
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 1
Vehicle :
PDII :
Result : moderately irritating
Classification :
Method : other
Year : 1965
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Method : Neat Material: A male rabbit was prepared by shaving the hair from the entire abdomen with a straight razor and barber soap. The animal was then rested for several days to allow any abrasions to heal completely and to be sure skin was suitable for use. Two sites on the abdomen were used for applications: one intact, the other cross-hatched with a sharp hypodermic needle to penetrate the stratum corneum but not to produce more than a trace of bleeding. Ten applications were made to the intact abdominal site over a period of 14 days. Three consecutive daily applications were made to the abraded site. Both abdominal sites were covered with 1X1 cotton pads and held place with a single cotton cloth taped to remaining body hair. Applications were discontinued upon production of a substantial skin burn, or if the animal died.

10% Dilution in Dowanol* DPM: A male rabbit was prepared by shaving the hair from the entire abdomen with a straight razor and barber soap. The animal was then rested for several days to allow any abrasions to heal completely and to be sure skin was suitable for use. Ten applications (unoccluded) were made to the ear over a period of 14 days. Two sites on the abdomen were used for applications: one intact, the other cross-hatched with a sharp hypodermic needle to penetrate the stratum corneum but not to produce more than a trace of bleeding. Ten applications were made to the intact abdominal site over a period of 14 days. Three consecutive daily applications were made to the abraded site. Both abdominal sites were covered with 1X1 cotton pads and held place with a single cotton cloth taped to remaining body hair. Applications were discontinued upon production of a substantial skin burn, or if the animal died.

Result	:	Neat Material: At the intact abdominal site, slight to moderate hyperemia and slight edema was observed during the first week of application. Slight necrosis appeared after the 5th application. All signs of irritation resolved within 21 days. Similar results were seen at the abraded abdominal site, with the exception that necrosis was first observed after the 4th application. 10% Dilution in Dowanol* DPM: The site at the rabbit ear had no signs of irritation. Both the intact and abraded abdominal sites had slight to moderate hyperemia and edema appear within the first week. All signs of irritation resolved within 21 days.
Source	:	The Dow Chemical Company, Midland, MI.

05.06.2002 (16)

5.2.2 EYE IRRITATION

Species	:	rabbit
Concentration	:	undiluted
Dose	:	.1 ml
Exposure time	:	24 hour(s)
Comment	:	
Number of animals	:	1
Vehicle	:	
Result	:	not irritating
Classification	:	not irritating
Method	:	other
Year	:	1965
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Both eyes of a white rabbit were stained with 5% fluorescein dye and examined for evidence of injury or alterations. The rabbit was then allowed to rest for 24 hours before test. Two drops of the material were introduced into the right eye. The eye was washed within 30 seconds for 2 minutes in a flowing stream of tepid water. Two drops of material were introduced in a similar fashion to the left eye, but this eye was left unwashed. Immmediately after instillation into each eye, the rabbit was examined for signs of discomfort. Within 2-3 minutes after the unwashed eye was treated, each eye was observed for conjunctival and corneal response. Similar observations were made on both eyes at 1 hour, 24 hours, 48 hours, and 6-8 days post-treatment. Examinations were conducted both with and without fluorescein dye.
Result	:	In both washed and unwashed eyes, the material caused very slight discomfort and very slight conjunctival irritation which resolved within 1 hour.
Source	:	The Dow Chemical Company, Midland, MI.

05.06.2002 (16)

5.3 SENSITIZATION

Type	:	Split adjuvant test
Species	:	guinea pig
Concentration	:	1st: Induction 5 % intracutaneous 2nd: Challenge 5 % open epicutaneous 3rd:
Number of animals	:	8

Vehicle	:	other: Dowanol* DPM/Tween* 80, 9/1
Result	:	sensitizing
Classification	:	
Method	:	other
Year	:	1965
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Source	:	The Dow Chemical Company, Midland, MI.

05.06.2002 (16)

5.4 REPEATED DOSE TOXICITY

Type	:	
Species	:	rat
Sex	:	male/female
Strain	:	no data
Route of admin.	:	oral feed
Exposure period	:	90 days
Frequency of treatm.	:	continuous
Post exposure period	:	none
Doses	:	0, 0.3, 1, 3, 10, 30 mg/kg/day
Control group	:	yes, concurrent vehicle
NOAEL	:	= 10 mg/kg bw
LOAEL	:	= 30 mg/kg bw
Method	:	other
Year	:	1968
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Method :

Groups of 10-15 45-day old rats/sex/dose group were treated with 0, 0.3, 1, 3, 10, or 30 mg/kg/day via diet. Rats were randomly assigned to treatment groups. Vehicle for the test material and feed for the controls was Purina ground rodent chow.

Diets designed to deliver the nominal dose were mixed weekly on the basis of rat body weight and feed consumption. Body weights and feed consumption were collected once/week for the duration of the study. All animals were observed frequently for clinical signs of toxicity.

Blood samples were collected from 5 rats/sex/dose from the 0, 10, and 30 mg/kg/day levels via orbital sinus puncture during weeks 3 and 12, and at termination. Hematological parameters examined included Hgb, crit, RBC, WBC, and differential counts. Blood urea nitrogen determinations were run on 10 rats/sex/dose at termination, and SGPT determinations were run for 5 rats/sex/dose at 0 and 30 mg/kg/day levels on days 1, 3, 7, 14, 30, and termination (10 rats/sex/dose).

A complete necropsy examination, including both gross pathological and histopathological examinations, was conducted on a standard set of tissues, including reproductive organs. Weights were collected for lungs, heart, liver, kidneys, spleen, testes, and brain.

In an effort to clarify testicular findings among dosed rats, additional studies were undertaken.

Repeated intubation: Groups of 10 male rats/dose were given 0, 62.5, 125, or 250 mg/kg/day via gavage 5 days/week for 2 weeks. Rats were necropsied 3 and 18 days after the last dose Body weights and testicular weights were recorded, and testes, prostate, seminal vesicles, coagulating

gland, and epididymis were examined for microscopic lesions. SGPT determinations were conducted at necropsy.

Dietary: Groups of 30 male rats were given diets at dose levels of 0, 62.5, 125, or 250 mg/kg/day. 5 rats/dose were necropsied on test days 49, 119, 175, and 242. Body weights and testicular weights were recorded, and testes, prostate, seminal vesicles, coagulating gland, and epididymis were examined for microscopic lesions. Livers were also examined on rats killed on days 175 and 242. SGPT determinations were conducted at necropsy.

Result : There were no treatment-related morphological changes observed at any level in females.

Male rats given 30 mg/kg/day had increased relative liver and kidney weights and mild focal hyaline droplet degeneration of the convoluted tubules of the renal cortex. No histological changes were observed in livers.

Testicular tubal atrophy of varying degrees was observed at all dose levels in the male rats. Not all animals within a dose level were affected, and severity was not dose-related.

In the follow-up studies, no treatment-related differences were observed for final body weight, testicular weight, gross pathology and histopathology. There was a marked degeneration of SGPT values at all dose levels. In the repeated intubation experiment, values were moderately depressed 3 days after final dosing, but returned to normal by the 18 day kill. In the dietary experiment, SGPT values were severely depressed at 49 and 119 days. Values at 175 and 242 days improved, but were still markedly lower than controls. Testicular effects observed in the earlier study could not be replicated, even at these much higher dose levels.

Histopathology Peer Review of Two Pentachloropyridine 90-Day Dietary Feeding Studies in Rats:

In the first study ten adult rats per sex per dose level were provided dose concentrations of 0 (controls), 0.3, 1, 3, 10 or 30 mg pentachloropyridine (PCP) per kilogram body weight per day in the feed for 90 days. The histopathologic peer review of this study consisted of microscopic evaluation of both testes from all male rats at all dose levels. The peer review was conducted by a Diplomate of the American College of Veterinary Pathologists. Results of the peer review histopathologic evaluation showed that there were no treatment-related testicular effects. This was in agreement with the final conclusions of the original pathologist. There were comparable numbers of rats at all dose levels, including the control group, with very slight or slight degeneration of testicular seminiferous tubules. The quality of the microscopic slides from this study was less than optimal, with artifacts of poor fixation or processing methods, and evidence of rough physical handling of some testicular specimens. Some of the histopathologic diagnoses made by the original pathologist were determined to be reflective of artifactual changes, based on examination by the peer review pathologist. The diagnoses that were attributed to poor fixation or rough tissue handling consisted of interstitial edema, vacuoles in seminiferous tubules, and the presence of primary or secondary spermatocytes in the lumens of seminiferous tubules.

In the second study groups of 30 male rats per dose level were provided dose concentrations of 0 (controls) 62.5, 125 or 250 mg PCP per kilogram body weight per day in the feed. Five rats per dose group were necropsied after 49, 119, 175 and 242 days on the diet. The histopathologic peer review of this study consisted of microscopic evaluation of both testes from all male rats at all dose levels. The peer review was conducted by a Diplomate of the American College of Veterinary Pathologists. Results of

the peer review histopathologic evaluation showed that there were no treatment-related testicular effects. This was in agreement with the original pathologist. As with the previous 90-day study, there were comparable numbers of rats at all dose levels, including the control group, with very slight or slight degeneration of testicular seminiferous tubules. The quality of microscopic slides in the second study was optimal, with no significant artifacts related to fixation, processing, or tissue handling.

Source : The Dow Chemical Company, Midland, MI.
Reliability : (2) valid with restrictions
26.09.2003 (17)

Type :
Species : rat
Sex : no data
Strain : other: Alderly Park
Route of admin. : inhalation
Exposure period : 6 hours
Frequency of treatm. : 16 exposures
Post exposure period : none
Doses : saturated vapor; ~1 ppm (0.01 mg/L)
Control group : no data specified
NOAEL : = 1 ppm
Method : other
Year : 1970
GLP : no
Test substance : no data

Result : No rats died, no toxic signs were observed, and no organs were affected at necropsy.
Source : The Dow Chemical Company, Midland, MI.
Reliability : (2) valid with restrictions
05.06.2002 (18)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Bacterial reverse mutation assay
System of testing : Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and Echerichia coli strain WP2uvrA
Test concentration : 0.33-3333 (activation) and 3.3-3333 microg/plate (no activation)
Cycotoxic concentr. : 1000 microg/plate
Metabolic activation : with and without
Result : negative
Method : OECD Guide-line 471
Year : 2003
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Method : The Salmonella-E. coli mammalian-microsome bacterial reverse mutation assay is used to evaluate the mutagenic potential of test agents in bacteria with and without
mammalian-microsome activation (Ames et al., 1975; Maron and Ames, 1983; Green and Muriel, 1976). The strains of Salmonella typhimurium used in this assay (TA98, TA100,
TA1535, TA1537) are histidine auxotrophs while E. coli WP2uvrA is a tryptophan auxotroph, by virtue of conditionally lethal mutations in the appropriate operons. When these histidine (his-) or tryptophan (trp-) dependent cells are exposed to the test article and grown under selective conditions (minimal media with a trace amount of histidine or tryptophan), only those cells that revert to histidine (his+) or tryptophan (trp+) independence are able to form colonies. The trace amount of histidine or

tryptophan in the media allows all the plated bacteria to undergo a few cell divisions that are essential for mutagenesis to be fully expressed. The (his+) or (trp+) revertants are readily discernable as colonies against the limited background growth of the his- or trp- cells.

In addition to a mutation in the histidine operon, the Salmonella typhimurium tester strains contain additional mutations that enhance their sensitivity to some mutagenic compounds. A mutation of the uvrB gene results in a deficient DNA excision repair system that greatly enhances the sensitivity of these strains to some mutagens. Since the uvrB deletion extends through the bio gene, the tester strains also require the vitamin biotin for growth.

These Salmonella typhimurium tester strains also contain the rfa wall mutation that results in the loss of one of the enzymes responsible for the synthesis of part of the
lipopolysaccharide (LPS) barrier that forms the surface of the bacterial cell wall. The resulting cell wall deficiency increases permeability to certain classes of chemicals such as those containing large ring systems (e.g., benzo[a]pyrene) that would otherwise be excluded by a normal intact cell wall. Strain TA98 and TA100 also contain the pKM101 plasmid, which further increases the sensitivity of this strain to some mutagens. The mechanism by which this plasmid increases sensitivity to mutagens has been suggested to be a consequence of its modification of an existing bacterial DNA-repair polymerase complex involved in the mismatch-repair process. The tester strains TA98 and TA1537 are reverted from histidine dependence (auxotrophy) to histidine independence (prototrophy) by frameshift mutagens, while TA100 and TA1535 and the E. coli strain WP2uvrA are reverted by base-pair substitution. The utilization of a mammalian microsomal enzyme preparation
(S-9 mix) also allows for detection of potentially mutagenic metabolites of the test material.

The specific genotypes of the strains are shown in Text Table 1.
TEXT TABLE 1. TESTER STRAIN GENOTYPES

	Mutation Type				
	his/trp Mutation	Additional Mutations	Repair	LPS	Plasmid pKM101	Detection
Tester Strain						
TA98	hisD3052	uvrB	rfa	+		Frameshift
TA100	hisG46	uvrB	rfa	+		Base-pair Substitution
TA1535	hisG46	uvrB	rfa	-		Base-pair Substitution
TA1537	hisC3076	uvrB	rfa	-		Frameshift
WP2uvrA	trp	uvrA	-	-		Base-pair Substitution

Bacterial Tester Strains
The Salmonella typhimurium and E. coli tester strains used in this study (TA98, TA100, TA1535, TA1537 and E.coli WP2uvrA) were acquired from a vendor (Moltox Inc., Boone, North Carolina), who prepared them from master cultures originally obtained from the laboratory of Dr. Bruce N. Ames (Maron and Ames, 1983) and the National Collections of Industrial and Marine Bacteria (Green and Muriel, 1976). Their characteristics are detailed in the Text Table 1 above. Frozen permanent stocks were prepared by growing fresh overnight cultures, adding DMSO (0.09 mL/mL of culture) and freezing appropriately vialed aliquots. Frozen permanent stocks of the tester strains were stored in liquid nitrogen vapor or at least at

£ -70°C.

Tester Strain Media
All tester strain media and experimental reagents were acquired from (Moltox Inc., Boone, North Carolina). The broth used to grow overnight cultures of the tester strains
was Vogel-Bonner salt solution (Vogel and Bonner, 1956) supplemented with 2.5% (w/v) Oxoid Nutrient Broth #2 (dry powder). Bottom agar (25 mL per 15 x 100 mm petri dish)
was Vogel-Bonner minimal medium E (Vogel and Bonner, 1956) supplemented with 1.5% (w/v) agar and 0.2% (w/v) glucose. Top (overlay) agar was prepared with 0.7% agar (w/v) and 0.5% NaCl (w/v) supplemented with 10 mL of 0.5 mM histidine/biotin solution per 100 mL agar for selection of histidine revertants or 0.5 mM tryptophan solution per 100 mL agar for selection of tryptophan revertants. For an agar overlay, 2.0 mL of the supplemented top agar was used.

Chemicals and S-9
Glucose-6-phosphate, nicotine adenine dinucleotide phosphate (NADP), dimethylsulfoxide (DMSO), 2-nitrofluorene, sodium azide, ICR-191, 4-nitroquinolone-Noxide, benzo(a)pyrene and 2-aminoanthracene were obtained from Sigma Chemical
Company, St. Louis, Missouri. The S-9 activation system consisted of an NADPH regeneration system plus the S-9 fraction of rat liver homogenate. The S-9 fraction of rat
liver homogenates prepared from Aroclor®-1254-induced male Sprague-Dawley rats (500 mg/kg, i.p.) was purchased from Moltox Inc. and stored at –100oC or below. Immediately prior to use, the S-9 was thawed and mixed with a cofactor pool to contain 10% S-9 homogenate, 5 mM glucose-6-phosphate, 4 mM b-nicotinamide-adenine
dinucleotide phosphate, 8 mM MgCl2 and 33 mM KCl in a 200 mM phosphate buffer at pH 7.4. This mixture is referred to as S-9 mix. In the absence of S-9, the non-activation mix was 200 mM phosphate buffer at pH 7.4. All other constituents were reagent grade or better.

Assay Control Chemicals
Vehicle controls were plated for all strains in the absence and presence of S-9 mix as appropriate and constituted the solvent (0.2 M phosphate buffer) used for the test material.

TEXT TABLE 2. POSITIVE CONTROLS FOR THE BACTERIAL MUTAGENICITY ASSAY

Strain	S-9 Mix	Positive Control	Concentration Per Plate
TA98	-	2-NITROFLUORENE	5 microg
TA100	-	SODIUM AZIDE	2 microg
TA1535	-	SODIUM AZIDE	2 microg
TA1537	-	ICR-191	2 microg
WP2uvrA (E. coli)	-	4-NITROQUINOLINE -N-OXIDE	0.4 microg
TA98	+	BENZO(A)PYRENE	2.5 microg
TA100	+	2-AMINOANTHRACENE	2.5 microg
TA1535	+	2-AMINOANTHRACENE	2.5 microg
TA1537	+	2-AMINOANTHRACENE	2.5 microg
WP2uvrA (E. coli)	+	2-AMINOANTHRACENE	25 microg

The most concentrated test article dilution and the buffers and S-9 mixes were checked for sterility by being mixed with top agar, poured onto

nutrient agar and incubated along with the other treated test plates. Text Table 2 above outlines the positive control chemicals used in the study. The positive controls were of reagent grade or better. The concentrations of positive controls were based on concentration response data from this laboratory. The
selected concentrations of the test material in the treatment solutions used for the main assay were verified by the Analytical Chemistry Laboratory, Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan using high performance liquid chromatography with ultraviolet detection (HPLC/UV).

Confirmation of Tester Strain Genotype
Tester strain cultures were checked for the following genetic markers concurrent with their use in the assay. The presence of the rfa wall mutation was confirmed by
demonstration of the sensitivity of the cultures to crystal violet. The presence of the pKM101 plasmid was confirmed for cultures of tester strain TA98 and TA100 by
demonstration of resistance to ampicillin.

Culturing and Harvest
Overnight cultures for each strain, for use in all testing procedures, were inoculated by transferring a colony from the appropriate master plate to a flask containing 20 ml of
culture medium. Inoculated flasks were placed in a shaker-incubator at 100 RPM 37 ± 2°C overnight and harvested once a predetermined density was reached according to spectrophotometric turbidity measurements at 650 nm. The cultures were adjusted to a density of at least 0.3 x 109 cells per mL and held at 5 ± 3°C until used in the assay.

Preliminary Mutagenicity Assay
Selection of dose levels for the main mutagenicity assay was based upon the toxicity and precipitation profile of the test article assessed in a preliminary assay. The tester strains were exposed to the test article via the pre-incubation modification of the Ames test described by Maron and Ames (1983). In the pre-incubation methodology, the tester strain and the test article were pre-incubated on an orbital shaker at 37oC for approximately 20 minutes prior to the addition of molten agar. The test material was
evaluated up to a concentration of 5000 mg/plate. The concentrations selected under these conditions were 5000, 3333, 1000, 333, 100, 33, 10, 3.3, and 1 mg/plate in the
presence and absence of S-9.

For the assay with activation, the bacteria (0.1 ml), test article (0.05 ml of the appropriately diluted test material or solvent), and the S-9 mix (0.5 ml) were placed into
sterile tubes, pre-incubated on an gyratory shaker (100 rpm) at 37°C for approximately 20 minutes prior to the addition of 2 ml of molten top agar (supplemented with trace amounts of histidine and biotin or tryptophan). The mixture was then poured onto minimal glucose agar plates. For the non-activation assay, S-9 mix was omitted and
replaced by 0.5 ml of 0.2M phosphate buffer, pH 7.4. The plates were then incubated for approximately 52 ± 4 hour in an incubator at 37°C. This preliminary mutagenicity assay
was conducted by exposing all strains to negative controls (three plates) and positive controls (two plates) and to nine concentrations of test article (two plates/dose), in both the presence and absence of S-9 activation.

Mutagenicity Assay
In selecting concentration levels for the mutagenicity assay, the following guidelines were employed. Concentrations were selected based on the

preliminary mutagenicity assay such that precipitate did not interfere with the counting of colonies. The highest concentration for the mutagenicity assay was also selected to give some indication of toxicity without exceeding 5 mg/plate.

Pentachloropyridine was tested at 6-8 concentration levels along with appropriate negative and positive controls with all tester strains (TA98, TA100, TA1535, TA1537, and WP2uvrA), with and without S-9 activation. All concentration levels of test article, negative controls, and positive controls were plated in triplicate.

Plate Evaluation
Prior to scoring the assay plates, all tester strain cultures were checked for the appropriate genetic markers. The condition of the bacterial background lawn was evaluated for evidence of cytotoxicity and test article precipitate. Evidence of cytotoxicity was scored relative to the vehicle control and recorded along with the revertant counts for that concentration. Revertant colonies were counted either by an Artek automated colony counter or by hand when revertant colony numbers were low. Plates that were not evaluated immediately following the incubation period were held at 5 ± 3°C until such time that colony counting and bacterial background lawn evaluation took place, but not more than two weeks.

Evaluation Criteria
To demonstrate the presence of the rfa mutation, all Salmonella typhimurium tester strain cultures must exhibit sensitivity to crystal violet. To demonstrate the presence of the pKM101 plasmid R-factor, the tester strain culture of TA98 and TA100 must exhibit resistance to ampicillin as described above.

Positive and negative controls were run concurrently with the test chemical, and appropriate responses for these controls were prerequisite for evaluating the response of
the bacteria to the test chemical.

A minimum of three non-toxic dose levels was required to evaluate assay data. For a test article to be judged positive for TA98, TA1535, TA1537, and E. coli WP2uvrA, a
concentration-related increase in mean revertants must be equal to or greater than 3.0-times the mean negative control value (vehicle). Similarly, for strain TA100, data
sets were judged positive if the concentration-related increase in mean revertants is equal to or greater than 2.0-times the mean negative control value (vehicle).

Result **:** The mutagenicity of pentachloropyridine was evaluated using Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and Echerichia coli strain WP2uvrA in the presence and absence of a metabolic activation system (S-9).

Preliminary Assay
A preliminary assay (A1, Tables 1 and 2) was conducted on all tester strains (two plates per treatment) in both the presence and absence of metabolic activation (S-9 mix) at
concentrations ranging from 1-5000 mg/plate. Precipitate was observed at 3333 and 5000 mg both in the presence and in the absence of S-9 in all strains. Furthermore,
precipitation and cytotoxicity was also observed at 1000 mg/plate and above for strains TA98, TA100, and TA1537 in the absence of S-9 only. No evidence of increases in
mutant counts required to satisfy the conditions for a positive response were observed in any of the tester strains.

Mutagenicity Assay
The assay was conducted in all five tester strains, with 6-8 different treatments of pentachloropyridine in both the presence and absence of S-9 metabolic activation at
concentrations ranging from 0.33-3333 and 3.3-3333 mg/plate in the presence and absence of S-9 metabolic activation, respectively.

This mutagenicity assay (Assay B1) was repeated for strains TA100 and TA1537 under non-activation conditions because of a failure of the positive control for TA100, and a greater than expected cytotoxicity for TA1537, which did not allow for enough non-cytotoxic concentration levels to be evaluated for this strain. The remaining strain and activation conditions did not exhibit increases in revertants/plate required to fulfill the criteria for a positive response. The concentrations of test material in the dosing solutions were analytically confirmed to be between 77-101% of those targeted.

The mutagenicity assay (Assay B1) was repeated for strains TA100 and TA1537 under non-activation conditions. The repeat mutagenicity assay (Assay B2) for these strains (TA100 and TA1537) were performed at nine concentrations
from 0.033-3333 mg/plate in the absence of S-9. While evidence of cytotoxicity and precipitation was observed at the three highest concentrations, there were no positive
increases in mutant colonies in either strain. All criteria for a valid assay were met. The concentrations of test material in the dosing solutions were analytically confirmed to be between 102-124% with the exception of the two lower concentrations near the limit of detection, which were 135 and 159% of the target.

Source : The Dow Chemical Company, Midland, MI.

Conclusion : Results of this study indicate that pentachloropyridine did not induce an increase in revertants/plate over the negative control in any tester strain either in the presence and absence of S-9, required to satisfy the criteria for a positive response. Hence, pentachloropyridine is concluded to be negative in the bacterial reverse mutation assay under the conditions used in this study.

Reliability : (1) valid without restriction
22.09.2004 (7)

Type : Chromosomal aberration test
System of testing : Rat lymphocytes
Test concentration : 3.8, 15 and 30 (no activation); 7.5, 30 and 60 (activation) microg/ml
Cycotoxic concentr. : 60 µg/ml
Metabolic activation : with and without
Result : negative
Method : OECD Guide-line 473
Year : 2003
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Method : Animal Husbandry
Blood samples were collected from male Sprague-Dawley rats (outbred Crl: CD BR strain purchased from Charles River, Kingston, New York), aged approximately 10 weeks. Upon arrival at the laboratory1, each animal was evaluated by a laboratory veterinarian to determine general health status and acceptability for study purposes. The rats were entered into an animal log, given unique numbers, and ear tagged with their numbers. The rats were allowed to acclimate for at least seven days prior to the start of the study.

Housing

Animals were housed one per cage in suspended stainless steel cages in rooms designed to maintain adequate conditions (temperature, humidity, and photocycle). The relative humidity was maintained within a range of 40-70%. A 12-hour light/dark photocycle was maintained for all animal rooms with lights on at 6:00 a.m. and off at 6:00 p.m. Room air was exchanged at 12-15 times/hour and the room temperature was maintained at 22 ± 1 (with a maximum range of ± 3 °C). Cages had wire-mesh floors and were
suspended above catch pans. Cages contained a stainless steel feeder and a pressure activated nipple-type watering system.

Identification
Animals were identified with each animal receiving an unique alphanumeric metal ear tag.

Feed and Water
Animals were provided LabDietâ Certified Rodent Diet #5002 (PMI Nutrition International, St. Louis, Missouri) in pelleted form. Feed and municipal water was provided ad libitum. Analysis of the feed was performed by PMI Nutrition International to confirm the diet provided adequate nutrition and to quantify the levels of selected contaminants. Drinking water obtained from the municipal water source was periodically analyzed for chemical parameters and biological contaminants by the municipal water department. In addition, specific analyses for chemical contaminants were conducted at periodic intervals by an independent testing facility. Copies of these analyses are maintained at Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Results of the feed and water analysis were
judged to be acceptable.

Animal Welfare
In response to the Final Rules amending the U.S. Animal Welfare Act promulgated by the U.S. Department of Agriculture effective October 30, 1989, the Animal Care and Use Activities (ACUA) required for the conduct of this study were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). The IACUC has determined that the proposed Activities are in full accordance with these Final Rules. The IACUC assigned File No. Genetic Tox 02 and Animal ID 01 to these Animal Care and Use Activities.

Lymphocyte Cultures
Blood samples were collected by cardiac puncture, following euthanasia with carbon dioxide, from four rats. In this assay, blood samples from individual rats were pooled
and whole blood cultures were set up in RPMI 1640 medium (with 25 mM HEPES, GIBCO, Grand Island, New York) supplemented with 10% heat-inactivated dialyzed fetal bovine serum (GIBCO), antibiotics and antimycotics (Fungizone 0.25 µg/ml; penicillin G, 100 µ/ml; and streptomycin sulfate, 0.1 mg/ml; GIBCO), 30 µg/ml PHA
(HA16, Murex Diagnostics Ltd., Dartford, England), and an additional 2 mM L-glutamine (GIBCO). Cultures were initiated by inoculating approximately 0.5 ml of whole blood/5 ml of culture medium. Cultures were set up in duplicate at each dose level in T-25 plastic tissue culture flasks and incubated at 37°C.

Controls
The solvent selected for dissolving the test material was used as the negative control treatment. Mitomycin C (MMC, Sigma, St. Louis, Missouri, CAS No. 50-07-7) was used
as the positive control chemical for the non-activation assay at a concentration of 0.5 µg/ml (4 h treatment) or 0.05 and 0.075 µg/ml (24 h

treatment), while cyclophosphamide monohydrate (CP, Sigma, CAS No. 6055-19-2) was the positive control for the activation assay at final concentrations of 4 and 6 µg/ml.

In Vitro Metabolic Activation System
S-9 liver homogenate prepared from Aroclor 1254 treated (500 mg/kg) male Sprague-Dawley rats was purchased from Molecular Toxicology, Inc., Boone, North Carolina, and
stored at -100°C or below. Thawed S-9 was reconstituted at a final concentration of 10% (v/v) in a "mix" (O'Neill et al., 1982). The mix consisted of 10 mM $MgCl_2 \cdot 6H_2O$ (Sigma), 5 mM glucose-6-phosphate (Sigma), 4mM nicotinamide adenine dinucleotide phosphate (Sigma), 10mM $CaCl_2$ (Fisher, Fair Lawn, New Jersey), 30 mM KCl (Sigma), and 50 mM sodium phosphate (pH 8.0, Sigma and Fisher). The reconstituted mix was added to the culture medium to obtain the desired final concentration of S-9 in the culture, i.e., 2% v/v. Hence, the final concentration of the co-factors in the culture
medium was 1/5 of the concentrations stated above.

Preparation of the Treatment Solution
The test material was first dissolved in dimethyl sulfoxide (DMSO, Sigma) and further diluted (1:100) with the treatment medium to obtain the desired concentrations. All
prepared stock solutions were submitted to the Analytical Chemistry Laboratory of the testing facility for the verification of test material concentrations. MMC and CP were dissolved directly in treatment medium. The treatment medium was RPMI 1640 with HEPES and antibiotics, without the serum and the PHA. The pH of treatment medium containing approximately 120 µg/ml the test material (above the limit of solubility in culture medium) and medium containing 1% DMSO was determined using a Denver
Basic pH meter (Denver Instrument Co., Arvada, Colorado) and an Osmette A freezing point osmometer (Precision Systems, Inc., Natick, Massachusetts), respectively. There
was no appreciable change in either the pH or osmolality at this concentration as compared to the culture medium with solvent alone (culture medium with the test material, pH = 7.41, osmolality = 421 mOsm/kg H_2O; culture medium with 1% DMSO, pH = 7.35, osmolality = 429 mOsm/kg H_2O).

Analytical Verification of Dosing Solutions
The selected concentrations of the test material in the stock solutions used for treatment were verified by the Analytical Chemistry Laboratory, Toxicology & Environmental
Research and Consulting, The Dow Chemical Company, Midland, Michigan. Samples were diluted in an appropriate solvent and analyzed by high performance liquid chromatography (HPLC) with ultra violet (UV) detection.

Identification of the Test System
All test cultures were identified using self adhesive labels containing a code system that identified the test material, experiment number, treatment, and replicate.

Treatment Procedure without Metabolic Activation
Approximately forty-eight hours after initiation of the cultures, the cell suspension was dispensed into 15 ml sterile centrifuge tubes (approximately 5.5 ml/tube, two cultures per dose level). The cells were sedimented by centrifugation and the culture medium removed and saved. The cells were exposed to medium (RPMI 1640, HEPES, and antibiotics) containing the test or positive or negative control treatments for approximately 4 hr at 37°C and the exposure was terminated by washing

the cells with
culture medium. The cells were then placed in individual sterile disposable tissue culture flasks (T-25) along with approximately 4.5 ml of the original culture medium until the time of harvest. The cultures were harvested at approximately 24 hr after treatment initiation (i.e., approximately 20 hr after treatment termination).

A second set of cultures was treated with the test material continuously for 24 hr (approximately 1.5 normal cell cycle length). Stock solutions of the treatments were added directly to the culture flasks at 48 hr after initiation of the cultures and these cultures were harvested 24 hr later.

Treatment Procedure using Metabolic Activation
Approximately 48 hr after initiation of the cultures, the cell suspension was dispensed into sterile disposable centrifuge tubes. The cells were sedimented by centrifugation and the culture medium removed and saved. The cells were exposed to medium (RPMI 1640, HEPES, antibiotics, and S-9) containing the test and positive and negative control treatments for 4 hr at 37°C and the exposure was terminated by washing the cells with culture medium (without serum and PHA).

The cells were then placed in individual sterile disposable tissue culture flasks (T-25) along with 4.5 ml of the original culture media until the time of harvest. The cultures were harvested approximately 24 hr after treatment initiation (i.e., 20 hr after treatment termination).

Harvesting of Cultures and Slide Evaluation
Colcemid was added approximately 3 hr prior to harvest at a final concentration of 0.2 µg/ml. The cells were swollen by hypotonic treatment (0.075 M KCl), fixed with methanol:acetic acid (3:1), dropped on microscope slides, and stained in Giemsa. All slides were coded prior to evaluation. Mitotic indices were determined as the number of cells in metaphase among 1000 cells/replicate and expressed as percentages. One hundred metaphases/replicate were examined, where possible, from coded slides at each
selected concentration of the test chemical and the negative controls (a total of 200 cells/treatment) for structural abnormalities (Buckton and Evans, 1973; Sinha et al., 1984; Gollapudi et al., 1986). In the positive control cultures, 50-75 metaphases/replicate (a total of 100-150 cells/treatment) were examined for abnormalities unless otherwise indicated. The microscopic coordinates of those metaphases containing aberrations were recorded. Only those metaphases that contained 42 + 2 centromeres were scored with the exception of cells with multiple aberrations, in which case accurate counts of the chromosomes were not always possible. Structural chromosomal abnormalities that were counted included chromatid and chromosome gaps, chromatid breaks and exchanges, chromosome breaks and exchanges, and miscellaneous (chromosomal disintegration, chromosomal pulverization, etc.). Those cells having five
or more aberrations/cell were classified as cells with multiple aberrations. Gaps were not included in calculations of total cytogenetic aberrations. In addition, one hundred metaphases/replicate were examined for the incidence of polyploidy. The data was used to calculate the following parameters:

% Cells with aberrations : Aberrant cells (excluding cells with gaps only)/# metaphases evaluated x 100

Aberrations/100 cells : Total aberration (excluding gaps, miscellaneous and severely damaged)/# metaphases evaluated

Statistics

The proportion of cells with aberrations (excluding gaps) was compared by the following statistical methods. At each dose level, data from the replicates were pooled. A two-way
contingency table was constructed to analyze the frequencies of aberrant cells. An overall Chi-square statistic, based on the table, was partitioned into components of interest. Specifically, statistics were generated to test the two global hypotheses of (1) no
differences in average number of cells with aberrations among the dose groups, and (2) no linear trend of increasing number of cells with aberrations with increasing dose (Armitage, 1971). An ordinal metric (0, 1, 2, etc.) was used for the doses in the statistical evaluation. If either statistic was found to be significant at a = 0.05 versus a one-sided increasing alternative, pairwise tests (i.e., control vs. treatment) were performed at each
dose level and evaluated at a = 0.05 again versus a one-sided alternative.

Polyploid cells were analyzed by the Fisher Exact probability test (Siegel, 1956). The number of polyploid cells was pooled across replicates for the analysis and evaluated at a = 0.05. The data was analyzed separately based on the presence or absence of S-9 and based on the exposure time.

For a test to be acceptable, the chromosomal aberration frequency in the positive control cultures should be significantly higher than the negative controls. The aberration frequency in the negative control should be within reasonable limits of the laboratory historical values. A test chemical is considered positive in this assay if it induces a significant dose-related and reproducible increase in the frequency of cells with
aberrations.

1 Fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International)

Result **:** The analytically detected concentrations of the test material in the stock solutions varied from 91.5 to 97.5% of the target.

4 Hr Treatment Assay
In the 4 hour treatment, cultures were treated with the test material in the absence and presence of S-9 activation at concentrations of 1.9, 3.8, 7.5, 15, 30, 60, and 120 µg/ml. The highest concentration evaluated was based upon solubility limitations. Without metabolic activation, the 60 and 120 µg/ml levels induced excessive toxicity as assessed by reductions in mitotic indices of 70 and 72%, respectively. Cultures treated with 15 and 30 µg/ml had reductions in mitotic indices of 40 and 55%, respectively. The remaining cultures showed little toxicity. Based upon these results, cultures treated with 3.8, 15, and 30 mg/ml were chosen for the determination of chromosomal aberration frequencies and incidence of polyploidy in the absence of S-9 activation.

In the presence of S-9 activation, the highest concentration evaluated (120 mg/ml) induced a 54% reduction in mitotic index. Cells treated with concentrations of 30 and 60 µg/ml had reductions in mitotic indices of 34 and 61%, respectively, while the remaining cultures showed little toxicity. Based upon these results, cultures
treated with 7.5, 30, and 60 µg/ml were selected for evaluating aberrations in the presence of S-9 activation.
Among the cultures treated with the positive control chemicals, 0.5 mg/ml of MMC and 4 mg/ml of CP were selected for evaluation of aberrations in the absence and presence of S-9, respectively.

There were no significant increases in the incidence of polyploid cells in the test material treated cultures as compared to the negative control values.

In the non-activation assay, the frequency of cells with aberrations in the

negative control was 2.0% and the corresponding values at treatment levels 3.8, 15, and
30 µg/ml were 2.5, 6.0, and 2.5%, respectively. In the activation assay, cultures treated with the test material at concentrations of 7.5, 30, and 60 µg/ml had aberrant cell frequencies of 3.5, 3.5, and 2.0%, respectively as compared to the negative control value of 4.0%. Statistical analyses of these data did not identify significant
differences between the negative control and any of the treated cultures either with or without S-9 activation. The frequencies of aberrant cells in the test material treated
cultures were within the laboratory historical background range.

Significant increases in the frequency of cells with aberrations were observed in cultures treated with the positive control chemicals. Aberrant cell frequencies in MMC (without S-9) and CP (with S-9) treated cultures were 14.3% and 29%, respectively.

A second assay with treatment of cultures in the presence of S-9 was not considered necessary in this assay since the results of the initial test yielded clearly negative results.

24 Hr Treatment Assay (without S-9)
Rat lymphocyte cultures were treated continuously for 24 hr with 0.9, 1.9, 3.8, 7.5, 15, 30, 60, and 120 µg/ml of the test material in the absence of S-9 activation. Cultures were harvested 24 hr after treatment initiation.

The higher concentrations tested, (i.e., 60 and 120 µg/ml) showed excessive toxicity as evidenced by mitotic index reductions of 74 and 88%, respectively. Cultures treated with 15 and 30 µg/ml had reductions of 43 and 69%, respectively. The remaining cultures had reductions in mitotic index of 7 to 10% (Table 2C). Based upon these results, cultures treated with 3.8, 15, and 30 µg/ml were selected for determining the chromosomal aberration frequencies.

Among the cultures treated with the positive control chemical, 0.05 µg/ml of was selected for the evaluation of aberrations.

There were no significant increases in the incidence of polyploid cells in test material treated cultures as compared to the negative control values.

In the 24 hr treatment assay, the frequency of cells with aberrations in the negative control was 1.5% and the corresponding values at treatment levels 3.8, 15, and
30 µg/ml were 0, 2.5, and 3.0%, respectively. Statistical analyses of these data did not identify significant differences between the negative control and any of the treated cultures either with or without S-9 activation. The frequencies of aberrant cells in the test material treated cultures were within the laboratory historical background range.

A significant increase in the frequency of cells with aberrations was observed in cultures treated with the positive control chemical (MMC). The aberrant cell frequency was 14%.

Source : The Dow Chemical Company, Midland, MI.

Conclusion : The test material, pentachloropyridine, did not induce a significant increase in the frequency of cells with chromosomal abnormalities at any of the concentrations
evaluated. Hence, it was concluded that under the experimental conditions used, pentachloropyridine was not genotoxic in this in vitro chromosomal aberration test.

Reliability : (1) valid without restriction
22.09.2004

(7)

Type	:	Cytogenetic assay
System of testing	:	Mouse bone marrow cells
Test concentration	:	11.75, 100 mg/kg
Cycotoxic concentr.	:	Not indicated
Metabolic activation	:	no data
Result	:	negative
Method	:	other
Year	:	1993
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4

Method : Ten male CFLP mice weighing approximately 30 g were used in each experimental group. The animals were given single oral doses of 11.75 mg/kg (1/20 of the i.p. LD50 in mouse) and 100 mg/kg PCP in pharmaceutically pure sunflower oil (Oleum helianthi); parallel to these experiments the solvent (0.1 ml Oleum helianthi per mouse), the positive control (100 mg/kg cyclophosphamide), and the untreated control group were studied. The bone marrow preparation was carried out 24 and 48 hours after treatment (cyclophosphamide: 24 hours after treatment). Following band technique staining, 20 mitoses in metaphase per mouse were evaluated using the technique of Datta et al. (1970). Significance calculations were made by the Fisher probe.

Result : No significant increase in the number of cells showing alterations as well as in the frequency of numerical and structural chromosome aberrations could be observed, neither 24 nor 48 hours after treatment with PCP. When the chromosomes of cyclophosphamide-treated animals were examined 24 hours after treatment, total aberrations in bone marrow cells were 78.5% ($p<0.001$). Thus, PCP cannot be regarded as a mutagen in the chosen test system.

Source : The Dow Chemical Company, Midland, MI.

Reliability : (2) valid with restrictions
Study does not satisfy the requirements of SIDS-level endpoints.

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5.6 GENETIC TOXICITY 'IN VIVO'

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rat
Sex	:	female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	Days 6-20 of gestation
Frequency of treatm.	:	Daily
Duration of test	:	Until gestation day 21
Doses	:	50, 100, 200, or 400 mg/kg BW/day
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	= 50 mg/kg bw
NOAEL teratogen.	:	= 200
Method	:	EPA OPPTS 870.3700
Year	:	2003

GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Method : TEST MATERIAL INFORMATION

Test Material Name
Pentachloropyridine

Chemical Name
2,3,4,5,6-Pentachloropyridine

Synonyms
PCP, Perchloropyridine

Supplier, City, State (lot, reference number)
Dow AgroSciences LLC, Indianapolis, Indiana (T-171C, TSN103958)

Purity/Characterization (method of analysis and reference)
The purity of PCP, lot T-171C, was determined to be > 99% by gas chromatography and liquid chromatography. Compound structure was confirmed by infrared mass spectrometry (IR/MS) and proton and carbon nuclear magnetic resonance (CNMR) analysis.

Characteristics

Appearance (physical state, color)
Gray crystals

Molecular Formula
C5Cl5N

Molecular Weight
251.33

TEST SPECIES AND HUSBANDRY

Species and Sex
Rats, time-mated female

Strain and Justification
CD (Crl:CD(SD)IGS BR) rats were selected because of their general acceptance and suitability for toxicity testing, availability of historical background data and the reliability of the commercial supplier.

Supplier and Location
Charles River Laboratories Inc. (Portage, Michigan)

Age and Weight at Study Start
Sexually mature adult, 10-11 weeks of age and weighing approximately 200-250 grams.

Physical and Acclimation
Each animal was evaluated by a laboratory veterinarian or a trained animal/toxicology technician, under the direct supervision of a lab veterinarian to determine their general health status and acceptability for study purposes upon arrival at the laboratory1. The animals were housed one per cage and allowed to acclimate to the laboratory conditions for five days prior to the start of dosing.

Housing
After assignment to study, animals were housed one per cage in stainless steel cages. The relative humidity and temperature were maintained within

a range of 40-70% and
22 ± 1°C with a maximal allowable excursion range of ± 3°C. These values were within the laboratory recommended range for rats. A 12-hour light/dark photocycle was maintained with lights on at 6:00 a.m. and off at 6:00 p.m. Room air was exchanged approximately 12-15 times/hour. Cages had wire-mesh floors and were suspended above catch pans. Cages contained a glass feed crock and a pressure activated nipple-type watering system.

Randomization and Identification
Animals were stratified by gestation day 0 body weight and then randomly assigned to treatment groups using a computer program designed to increase the probability of uniform group mean weights and standard deviations at the start of the study. Rats placed on study were uniquely identified via subcutaneously implanted transponders (BioMedic Data Systems, Seaford, Delaware) which were correlated to unique alphanumeric identification numbers.

Feed and Water
Animals were provided LabDiets Certified Rodent Diet #5002 (PMI Nutrition International, St. Louis, Missouri) in meal form. Feed and municipal water were provided ad libitum. Analyses of the feed were performed by PMI Nutrition International to confirm the diet provided adequate nutrition and to quantify the levels of selected
contaminants. Drinking water obtained from the municipal water source was periodically analyzed for chemical parameters and biological contaminants by the municipal water department. In addition, specific analyses for
chemical contaminants were conducted at periodic intervals by an independent testing facility. Copies of these analyses are maintained at Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland,
Michigan. The results of the feed and water analyses indicated that there were no contaminants present at levels that would interfere with the conduct of the study or
interpretation of the results.

Animal Welfare
In response to the Final Rules amending the U.S. Animal Welfare Act promulgated by the U.S. Department of Agriculture effective October 30, 1989, the Animal Care and Use Activities (ACUA) required for the conduct of this study were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). The IACUC determined
that the proposed Activities were in full accordance with these Final Rules.

Breeding Procedures
Sexually mature virgin females were naturally mated with males of the same strain (one male:one female) at the supplier's facility. Females were checked for in situ copulation plugs the following morning and those found with such a plug were removed from the males' cages. The day on which a vaginal plug was detected was considered day 0 of
gestation. Gestation day 0 body weights were provided by the supplier, and maintained in the study record. Rats arrived in our laboratory on gestation day 1.

STUDY DESIGN

Experimental Design and Critical Dates
Groups of eight time-mated female CD rats were administered pentachloropyridine by gavage at dose levels of 0, 50, 100, 200, or 400 mg/kg/day on days 6-20 of gestation. This dosing schedule was based on the Health Effects Test Guideline of the United States Environmental

Protection Agency (OPPTS 870.3700 Prenatal Developmental Toxicity Study). Test material administration began on April 8, 2003 and the animals were euthanized on April 23, 2003.

Text Table 1. Study Parameters

Study Parameters	Day(s) of Gestation
Clinical Observations	Daily
Body Weights	0, 6-20, 21 (terminal)
Dosing	6-20
Feed Consumption	3-6, 6-9, 9-12, 12-15, 15-18, 18-21
Maternal Necropsy	21
Organ Weights	21

Reproductive Parameters:

Number of Corpora Lutea	21
Number of Implantation Sites	21
Number of Viable Fetuses	21
Number of Resorptions	21

Route, Method of Administration, Frequency, Duration and Justification
Pentachloropyridine was administered by oral gavage once daily for seven days per week on days 6-20 of gestation. Gavage administration is the preferred route of exposure
specified in the relevant test guideline and will be the route used in the subsequent developmental toxicity study.

Dose Levels and Justification
These dose levels were selected to provide adequate data to establish a maximum tolerated dose and to provide dose-response data for any toxicity observed. The top dose
level of 400 mg/kg/day was based on prior toxicity data (discussed previously) indicating very little toxicity at doses of approximately 250 mg/kg/day, yet lethality at
500 mg/kg/day. In light of this steep dose response, it was considered necessary to set the high dose between 250 and 500 mg/kg/day in order to accurately estimate the
maximum tolerated dose.

Dose Preparation and Analysis
Pentachloropyridine was administered as a suspension in corn oil such that a dose volume of 4 ml/kg body weight yielded the targeted dose. Corn oil was selected as the vehicle based on preliminary method development work. Dose volumes were adjusted daily based on individual body weights.

Analysis

Homogeneity
The low- and high-dose suspensions were analyzed concurrent with the study to verify homogeneous distribution of the test material in vehicle.

Stability
Stability of the 50 and 200 mg/kg/day dose group suspensions were determined concurrent with the study using HPLC with ultraviolet detection and external standards.

Concentration Verification
Concentrations of all dose suspensions were verified in conjunction with the stability and homogeneity analyses.

Retainer Samples
Reference samples were not retained, as this study was less than four

weeks in duration.

In-Life Observations
Clinical examinations were conducted daily throughout the study period. This examination included careful, hand-held evaluations of the skin, fur, mucous membranes, respiration, nervous system function (including tremors and convulsions), swelling, masses and animal behavior at the time of dosing. At the expected time of peak effects,
animals were observed for general behavior and appearance, respiration, nervous system function (including tremors and convulsions) and any other signs of clinical toxicity. In
addition, a cage-side examination was conducted and to the extent possible, the following were evaluated: skin, fur, mucous membranes, respiration, nervous system function
(including tremors and convulsions), animal behavior, moribundity, mortality, and the availability of feed and water. Any animals found dead were necropsied on that day.

Body Weights
Body weights were recorded on day 0 by the supplier, daily during the dosing period, and on day 21 of gestation. Statistical analyses of body weights and body weight gains were performed using data collected on gestations days 0, 6, 9, 12, 15, 18, and 21.

Feed Consumption
Feed consumption was recorded for all animals beginning on day 3 of gestation by weighing feed containers at the start and end of a measurement cycle and consumption was calculated using the following equation:

Feed consumption (g/day) = (initial weight of feed container - final weight of feed container)/
(# of days in measurement cycle)

Anatomic Pathology

Necropsy
On day 21 of gestation, all animals that survived were submitted for a complete necropsy by a team of trained individuals under the direct supervision of a veterinary
pathologist. The animals were weighed, anesthetized with CO_2, the tracheas exposed and clamped and the animals decapitated. The eyes were examined in situ by visual
inspection using a moistened glass slide technique. Weights of the liver and kidneys were recorded, and the organ-to-body weight ratios calculated. Sections of liver,
kidneys and any gross lesions were preserved in neutral, phosphate-buffered10% formalin. Histopathologic evaluation of preserved tissues was not performed.

A detailed examination of the uterus for the number of implantations and resorptions, and the ovaries for the number of corpora lutea was performed. The position and
number of early and/or late resorptions and normally developing fetuses were recorded. As the objective of this study was limited to the evaluation of maternal and
developmental toxicity potential, a detailed external examination of individual fetuses was not performed. The fetuses were euthanized via sublingual deposition of sodium
pentobarbital, and discarded. Corpora lutea were not counted for non-pregnant females. The uteri of animals
lacking visible implantations were stained with a 10% aqueous solution of

sodium sulfide (Kopf et al., 1964) and examined for evidence of early resorptions in order to
verify pregnancy status. Any animal that died was submitted for a complete necropsy examination by a veterinary pathologist. This necropsy was performed as described above with the following exceptions. Terminal body weight was not recorded. Representative sections of the liver, kidneys, and gross lesions were preserved in neutral, phosphate-buffered 10% formalin, however, liver and kidney weights were not recorded. During the ovarian and uterine exam, the number of corpora lutea was not recorded.
The degree to which implantation site(s) had developed was determined to the extent possible by external examination (as appropriate for gestational age). Following
external examination, these implantation sites were discarded. Near term fetuses were euthanized as described above.

STATISTICS
Maternal body weights, maternal body weight gains, organ weights (absolute and relative) and feed consumption were evaluated by Bartlett's test for equality of variances.
Based on the outcome of Bartlett's test, a parametric (Steel and Torrie, 1960) or nonparametric (Hollander and Wolfe, 1973) analysis of variance (ANOVA) was performed. If the ANOVA was significant at a = 0.05, analysis by Dunnett's test (a = 0.05; Winer, 1971) or the Wilcoxon Rank-Sum test (a = 0.05; Hollander and Wolfe, 1973) with Bonferroni's correction (Miller, 1966) was performed, respectively. Frequency of pre- and post-implantation loss was analyzed using a Censored Wilcoxon test with Bonferroni's correction (Haseman and Hoel, 1974). The number of corpora lutea, implantations, resorptions per litter and litter size were evaluated using a
nonparametric ANOVA (a = 0.05) followed by the Wilcoxon Rank-Sum test (a = 0.05) with Bonferroni's correction. Pregnancy rates were analyzed using the Fisher exact
probability test (a = 0.05; Siegel, 1956) with Bonferroni's correction. Non-pregnant females, females with resorptions only, or females found to be pregnant after staining of
their uteri were excluded from the appropriate analyses. Statistical outliers were identified, using a sequential method (a = 0.02; Grubbs, 1969), and excluded if justified
by sound scientific reasons. Both Dunnett's test and Bonferroni's correction correct for multiple comparisons to the control group to keep the experiment-wise a at 0.05. Both were reported at the experiment-wise a level.
Because numerous measurements were statistically compared in the same group of animals, the overall false positive rate (Type I errors) was greater than the nominal
a levels. Therefore, the final interpretation of the data considered statistical analyses along with other factors, such as dose-response relationships and whether the results were consistent with other biological and pathological findings and historical control values.

Calculation of Pre- and Post-implantation Loss
· Pre-implantation loss* = (No. corpora lutea-implantations)/No. corpora lutea x 100
· Post-implantation loss* = (No. implantations-viable fetuses)/No. implantations x 100

* Note: Percent pre- and post-implantation loss was determined for each litter, followed by calculation of the mean of these litter values.

1 Fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

Result : Analytical Results

Analyses of all dosing suspensions from the first mix indicated mean concentrations of PCP ranging from 98.8 to 110% of the targeted concentration. A re-mix of the high dose (100 mg/g) suspension was necessary due to an apparent homogeneity problem (see below). The concentration of this second mix of the high-dose suspension assayed at 120% of the targeted concentration. Stability analyses indicated that the test material atconcentrations as low as 1 mg/g was stable in the vehicle for at least 24 days. Analysis of the low-dose suspension indicated that the PCP was homogeneously distributed in the vehicle. As mentioned above, the initial mix of the high-dose suspension indicated apparent heterogeneity, and therefore was not used for dosing. A new mix of the high-dose suspension was prepared and analysis of multiple aliquots at different levels of the storage vessel revealed that PCP was homogeneously distributed. This second mix was used for dosing.

In-Life Observations
Animals 1327 and 1330 from the 400 mg/kg/day dose group were found dead on test day 9 and 8, respectively. Prior to the death of animal 1327, soiling of the perioral (salivation), perineal and perinasal areas was seen on gd 7, 8 and/or 9, with extensive whole body soiling noted on gd 9. Aside from perioral soiling (salivation) on gd 6, animal 1330 did not exhibit any clear treatment-related signs of toxicity prior to death. At necropsy, both of these animals were found to have watery contents in the gastrointestinal tract, liver congestion, and a white, pasty material (presumably test material) in the small intestine. Animal 1327 also had soiling on the skin consistent with its clinical signs, whereas Animal 1330 also exhibited congestion of the adrenals and a cyst in the nonglandular mucosa of the stomach. Animal 1327 was not pregnant (confirmed by sodium sulfide staining), while the uterus of Animal 1330 contained 12 implantations. Due to the excessive degree of toxicity at 400 mg/kg/day, as indicated by these treatment-related deaths, all remaining animals in this group were euthanized on gestation day 9 with no further collection of data.

In the remaining dose groups, increased incidences of perioral soiling (salivation) were apparent at all dose levels. This salivation occurred at the time of dosing and was transient, suggesting that it was a local response to the test material and not a sign of toxicity. Red perinasal soiling was seen at 100 and 200 mg/kg/day and was considered to be treatment-related. Increases in the incidence of perineal soiling and red/dark urine were seen at 200 mg/kg/day only and were also considered to be treatment-related. All other clinical findings were minor in nature and/or occurred on isolated days, and did not appear to be of toxicological significance.

Body Weights
There were no statistically identified differences in the body weights of any treated groups when compared to their respective controls. However, in dams given 200 mg/kg/day, body weight gains during the first few days of dosing (gd 6-9) were significantly decreased. This difference was attributed to PCP exposure.

Feed Consumption
PCP at a dose level of 200 mg/kg/day produced a statistically significant decrease in feed consumption during gd 6-9 relative to their respective controls. This correlated with decreased body weight gain for the same time period. There were, however, no significant differences in the amount of feed consumed for any other time period in the 200 mg/kg/day group, nor at any time in the other treated groups.

Anatomic Pathology

Organ Weights
Dose levels of 100 and 200 mg/kg/day produced statistically significant increases in relative kidney weights of 16% and 20%, respectively. Increases in absolute kidney weight of 16% and 14%, respectively, also were recorded. While not statistically identified, these increases were considered treatment-related. PCP at a dose level of 200 mg/kg/day also produced a statistically significant increase in relative liver weight (26% increase) and although not statistically identified, a 20% increase in absolute liver weight. The 100 mg/kg/day dose level produced a 19% increase in absolute and relative liver weights. Although these increases were not statistically significant, they were considered to be treatment-related. There were no significant effects on absolute or relative weights of the liver or kidney at 50 mg/kg/day.

Gross Pathology
There were no treatment-related gross pathologic observations in animals surviving to the scheduled necropsy. All gross pathologic observations were considered to be spontaneous alterations, unassociated with exposure to PCP.

Reproductive Parameters
Pregnancy rate was low across all groups, including controls, and was likely due to a supplier problem with the
detection of mating. There were no significant treatment related-effects on pregnancy rates, number of corpora lutea, implantations, mean percent preimplantation loss,
number of resorptions per litter, resorptions per litters with resorptions, mean percent postimplantation loss, viable fetuses per litter, or litter size in animals given PCP.

Source : The Dow Chemical Company, Midland, MI.

Conclusion : Oral administration of 400 mg PCP/kg/day to time-mated CD rats resulted in excessive toxicity as evidenced by the spontaneous deaths of two of eight animals. Therefore, the
remaining animals in the 400 mg/kg/day dose group were terminated on day 9 of gestation with no further collection of data. Perioral soiling was seen at all dose levels.
In the remaining animals, treatment-related clinical signs of toxicity were limited to increased incidences of perineal soiling and red/dark urine observed only at
200 mg/kg/day. Maternal body weight was not significantly altered at any dose level, although decreases in body weight gains and food consumption from gestation day 6-9
were statistically identified in the 200 mg/kg/day dose level group. Absolute and relative weights of the liver and kidneys were increased at 100 and 200 mg/kg/day. There were
no treatment-related gross pathological observations, nor were there any effects on reproductive parameters.

Thus, the no-observed-adverse-effect level (NOAEL) for maternal toxicity was 50 mg/kg/day while 200 mg/kg/day was considered a no-observed-effect level (NOEL) for embryo/fetal lethality.

Reliability : (1) valid without restriction
21.09.2004 (7)

Species : rat
Sex : female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : Gestation Days 6-20
Frequency of treatm. : Daily
Duration of test : Until Gestation Day 21
Doses : 10, 50, 200 mg/kg BW/day

Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	= 10 mg/kg bw
NOAEL teratogen.	:	= 200 mg/kg bw
NOAEL Fetotoxicity	:	= 50 - mg/kg bw
Result	:	Fetotoxic at maternally toxic dose levels
Method	:	OECD Guide-line 414 "Teratogenicity"
Year	:	2004
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Method :

Species and Sex
Rats, time-mated females

Strain and Justification
CD rats (Crl:CD(SD)IGS BR) were selected because of their general acceptance and suitability for toxicity testing, the availability of historical background data, and the
reliability of the commercial supplier.

Supplier and Location
Charles River Laboratories Inc. (Portage, Michigan)

Age and Weight at Study Start
Sexually mature adult, 10-11 weeks of age and weighing approximately 200-250 g

Physical and Acclimation
Each animal was evaluated by a laboratory veterinarian or a trained animal/toxicology technician, under the direct supervision of a lab veterinarian to determine their general health status and acceptability for study purposes upon arrival at the laboratory1. The animals were placed in their cages (one per cage) and allowed to acclimate to the
laboratory conditions for approximately four days prior to the start of dosing. The animal rooms of the facility are designed to maintain adequate environmental conditions
(temperature, humidity, and photocycle).

Housing
Animals were housed, one per cage, in stainless-steel cages in rooms designed to maintain adequate conditions (temperature, humidity, and photocycle). Room temperature was recorded daily. The relative humidity was maintained within a range of 40-70%. The room temperature was maintained at 22 ± 1°C (with a maximum permissible excursion range of ± 3°C). These values were within the laboratory recommended range for rats. A 12-hour light/dark photocycle was maintained in all animal rooms with lights on at 6:00 a.m. and off at 6:00 p.m. Room air was exchanged approximately 12-15 times/hour. Cages had wire-mesh floors and were suspended above catch pans. Cages contained feed containers and pressure activated, nipple-type watering
systems.

Randomization and Identification
Animals were stratified based upon GD 0 body weights and then randomly assigned to treatment groups using a computer program designed to increase the probability of
uniform group mean weights and standard deviations at the start of dosing. Animals placed on study were uniquely identified via subcutaneously implanted transponders
(BioMedic Data Systems, Seaford, Delaware) which were correlated to unique alphanumeric identification numbers. If a transponder stopped functioning or was lost, it was replaced with a new transponder that correlated with the unique animal number.

Feed and Water
Animals were provided LabDiet Certified Rodent Diet #5002 (PMI Nutrition International, St. Louis, Missouri) in meal form. Feed and municipal water were provided ad libitum. Analyses of the feed were performed by PMI Nutrition International to confirm the diet provided adequate nutrition and to quantify the levels of selected
contaminants. Drinking water obtained from the municipal water source was periodically analyzed for chemical parameters and biological contaminants by the municipal water department. In addition, specific analyses for
chemical contaminants were conducted at periodic intervals by an independent testing facility. Copies of these analyses are maintained at Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland,
Michigan. The results of the feed and water analysis indicated that there were no contaminants present at levels that would interfere with the conduct of the study or
interpretation of the results.

Animal Welfare
In response to the Final Rules amending the U.S. Animal Welfare Act promulgated by the U.S. Department of Agriculture effective October 30, 1989, the Animal Care and Use Activities (ACUA) required for the conduct of this study were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). The IACUC determined
that the proposed Activities were in full accordance with these Final Rules. The IACUC-approved Animal Care and Use Activities to be used for this study are DART 02 and
Animal ID 01.

Breeding Procedures
Sexually mature, adult virgin females were naturally mated with males of the same strain at the supplier's facility. Females were checked for in situ copulation plugs the following morning and those found with such a plug were removed from the males' cages. The day on which a vaginal plug was detected was considered GD 0. GD 0 body weights were provided by the supplier, and maintained in the study record. Rats arrived in our laboratory on GD 1 or 2.

STUDY DESIGN

Experimental Design and Critical Dates
Groups of 25 time-mated female CD rats were administered PCP by oral gavage at dose levels of 0, 10, 50, or 200 mg/kg/day on GD 6-20.

The following study parameters were evaluated at the designated gestational ages:

Text Table 1. Study Parameters

Study Parameters	Gestation Day
Clinical Observations	Daily
Body Weights	0, 6-20, 21 (terminal)
Dosing	6-20
Feed Consumption	3-6, 6-9, 9-12, 12-15, 15-18, 18-21
Maternal Necropsy	21
Organ Weights (kidneys, liver, gravid uterus)	21

Reproductive Parameters:

Number of Corpora Lutea	21
Number of Implantation Sites	21
Number of Viable Fetuses	21
Number of Resorptions	21

Fetal Data:

Fetal Sex	21
Fetal Body Weights	21
External Examination	21
Visceral Examination	21
Craniofacial Examination	Post-necropsy (after fixation in Bouin's solution
Skeletal Examination	Post-necropsy (after skeletal staining)

Test material administration began for the first group of rats on 02 November 2003 and the last group of animals was necropsied on 02 December 2003.

Route, Method of Administration, Frequency, Duration and Justification
Test material was administered daily by oral gavage from GD 6-20 as recommended by the applicable guideline.

Dose Levels and Justification

Text Table 2. Dose Levels

Dose Levels (mg/kg/day)	No. of Rats/Dose Level
0	25
10	25
50	25
200	25
TOTAL	100

These dose levels were selected based on the preliminary results of the probe study (see study summarized as ordinate 1). The high-dose of 200 mg/kg was expected to
induce overt signs of maternal toxicity. The lower dose levels were selected to provide dose response data for any toxicity observed in high-dose group rats.

Dose Preparation and Analysis
Dose suspensions were prepared in corn oil at concentrations of 0, 2.5, 12.5, and 50 mg/ml and administered a dose volume of 4 ml/kg body weight in order to achieve the targeted dose levels. Dose volumes were adjusted daily based on individual body weights.

Analysis

Homogeneity
Dosing suspensions were analyzed prior to the start of dosing to verify homogeneous distribution of the test material in the vehicle.

Stability
PCP was found to be stable in corn oil at concentrations ranging from 1 mg/g up to 50 mg/g for at least 24 days.

Concentration Verification
Analysis of all dosing suspensions were initiated prior to the start of dosing using high performance liquid chromatography (HPLC) with ultraviolet detection and
external standards to determine concentrations.

Retainer Samples
Reference samples (one/dose/mix) were retained and stored at ambient temperature in sealed vials in a manner consistent with the sample retention policy of the laboratory.

STUDY SPECIFIC PARAMETERS

In-Life Observations
Clinical examinations were conducted daily throughout the study period. This examination included a careful, hand-held evaluation of the skin, fur, mucous membranes, respiration, nervous system function (including tremors and convulsions), unusual swelling or masses, and animal behavior. In addition, at least once each day a
cage-side examination was conducted and to the extent possible, the following were evaluated: skin, fur, mucous membranes, respiration, nervous system function (including
tremors and convulsions), animal behavior, moribundity, mortality, and the availability of feed and water.

Moribund animals that were not expected to survive until the next observation period, and any animals found dead, were necropsied that day.

Body Weights
Body weights were recorded on GD 0 by the supplier, daily during the dosing period, and at necropsy (GD 21). Statistical analysis of body weights was performed using data collected on GD 0, 6, 9, 12, 15, 18, and 21. Statistical analysis of body weight gains was conducted for the following intervals: GD 0-6, 6-9, 9-12, 12-15, 15-18, 18-21, 6-21, and 0-21.

Feed Consumption
Feed consumption was recorded for all animals on GD 3-6, 6-9, 9-12, 12-15, 15-18, and 18-21 by weighing feed containers at the start and end of a measurement cycle. Feed consumption was calculated using the following equation:

Feed consumption (g/day) = (initial weight of feed container - final weight of feed container)/
(# of days in measurement cycle)

Anatomic Pathology

Necropsy
On GD 21, all surviving females (not fasted) were euthanized by CO_2 inhalation and a limited gross pathologic examination (necropsy) was performed. The sequence of
the maternal necropsies was counterbalanced across groups (e.g., control, high, middle, low) to control for potential confounding influences of timing on fetal growth
and skeletal ossification.

The maternal necropsy included an examination of the external tissues and all orifices. The skin was reflected from the carcass, the thoracic and abdominal cavities
were opened, and the viscera was examined. The stomach, liver, and kidneys were dissected from the carcass and were incised. Any obvious

gross pathologic alterations were recorded, and the weight of the liver, kidneys, and gravid uterus was recorded. The ratios of liver and kidney weights to terminal body weight were calculated. Representative sections of liver, kidneys, and gross lesions were
preserved in neutral, phosphate-buffered 10% formalin. Transponders were removed and placed in jars with the tissues. A detailed examination of the reproductive tract was performed and the number and position of implantations, viable fetuses, dead fetuses, and resorptions was recorded. Resorptions were classified as either "early" or "late" based on the presence (late resorption) or absence (early resorption) of grossly recognizable embryonic/fetal form, while a "dead fetus" indicated a very recent death as evidenced by a lack external degenerative changes. For females with one or more viable fetuses, the number of ovarian corpora lutea was counted. The uteri of females lacking visible implantations was stained with a 10% aqueous solutio n of sodium sulfide (Kopf et al., 1964) and examined for evidence of early resorptions in order to verify pregnancy status.

The sex of all fetuses was recorded and the body weight of all viable fetuses determined. All fetuses were given an external examination that included observations on body proportions, the head and face (including closure of the palate), abdomen, spine, extremities, genitalia, rectum, and tail. All viable fetuses were euthanized by sublingual administration of a sodium pentobarbital solution. At least
one-half of all the fetuses in each litter was chosen randomly using a computer program and a visceral examination was conducted by dissection under a low power
stereomicroscope for evidence of visceral alterations (Staples, 1974; Stuckhardt and Poppe, 1984). The visceral examination included observation of the thymus, trachea,
esophagus, lungs, great vessels, heart (external and internal), liver, gastrointestinal tract, pancreas, spleen, kidney (sectioned), adrenal glands, ureters, bladder, and
reproductive organs. The heads of these fetuses were removed, placed in Bouin’s fixative and serially sectioned to allow for inspection of the eyes, brain, nasal passages, and tongue (Wilson, 1965). Remaining fetuses not selected for visceral examination were then skinned, eviscerated, preserved in ethyl alcohol (95%), and double stained with Alcian Blue and Alizarin Red S for cartilage and bone
respectively, according to methods based on Trueman et al. (1999). After staining, skeletons were macerated and cleared. A thorough evaluation of the fetal skeleton
was conducted on the remaining fetuses not selected for visceral examination. However, a fetus may have been intentionally changed from one selected for visceral examination to one processed for skeletal examination (and vice versa) if it was deemed that such examination would provide more meaningful data about a
suspected abnormality.

All fetal alterations were classified as variations or malformations. A variation was defined as a divergence beyond the normal range of structural constitution that may
not have adversely affected survival or health. A malformation was defined as a permanent structural change that may adversely affected survival, development or
function and/or which occurred at a relatively low incidence in the specific species/strain. Maternal necropsy and fetal examinations were conducted such that
investigators were blind to treatment. During routine working hours, any animals found dead were necropsied on that day. This necropsy was performed as described above with the following exceptions: 1) animals submitted alive for necropsy were euthanized by the inhalation of CO2 and

subsequent decapitation, 2) the head was removed, the cranial cavity opened and the brain, pituitary, and adjacent cervical tissues were examined, 3) all viscera were dissected from the carcass and re-examined, 4) terminal body weight, liver, kidney, and gravid uterine weights were not recorded, 5) the number of corpora lutea and the sex and body weight of fetuses from these animals was not recorded), and 6) evaluation of the implantations was limited to a general assessment of viability and external development (to the extent possible).

STATISTICS AND CALCULATIONS
Maternal body weights, maternal body weight gains, organ weights (absolute and relative), fetal body weights, and feed consumption were evaluated by Bartlett's test (alpha = 0.01; Winer, 1971) for equality of variances. Based on the outcome of Bartlett's test, a parametric (Steel and Torrie, 1960) or nonparametric (Hollander and Wolfe, 1973) analysis of variance (ANOVA) was performed. If the ANOVA was significant at alpha = 0.05, analysis by Dunnett's test (alpha = 0.05; Winer, 1971) or the Wilcoxon Rank-Sum test (alpha = 0.05; Hollander and Wolfe, 1973) with Bonferroni's correction (Miller, 1966) was performed, respectively. Feed consumption values were excluded from analysis if the feed was spilled or scratched.

Frequency of pre- and post- implantation loss (calculations shown below), and fetal alterations were analyzed using a censored Wilcoxon test (Haseman and Hoel, 1974) with Bonferroni's correction. The number of corpora lutea, implantations, and litter size was evaluated using a nonparametric ANOVA (alpha = 0.05) followed by the Wilcoxon Rank-Sum test (alpha = 0.05) with Bonferroni's correction. Pregnancy rates were analyzed using the Fisher exact probability test (alpha = 0.05; Siegel, 1956) with Bonferroni's correction. Fetal sex ratios were analyzed using a binomial distribution test. Females lacking visible implantations at the scheduled necropsy were excluded from the appropriate analyses. Statistical outliers were identified using a sequential method (alpha = 0.02; Grubbs, 1969), but were not excluded unless justified by sound scientific reasons unrelated to treatment. Both Dunnett's test and Bonferroni's correction corrected for multiple comparisons to the control groups and kept the experiment-wise alpha at 0.05. Both were reported at the experiment-wise alpha level.

Because numerous measurements were statistically compared in the same group of animals, the overall false positive rate (Type I errors) was greater than the nominal alpha levels. Therefore, the final interpretation of the data considered statistical analyses along with other factors, such as dose-response relationships and whether the results were consistent with other biological and pathological findings and historical control values.

Calculation of Pre- and Post-Implantation Loss
· Pre-implantation loss* = (No. corpora lutea - implantations) / No. corpora lutea X 100
· Post-implantation loss* = (No. implantations - viable fetuses) / No. implantations X 100

* Note: Percent pre- and post- implantation loss were determined for each litter, followed by calculation of the mean of these litter values.

1 Fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

Result : Analytical
Analysis of all dosing suspensions from the first of two mixes revealed mean concentrations of PCP ranging from 94.0% to 101% of targeted

concentrations. PCP was homogeneously distributed throughout the dosing suspensions as verified by analysis of multiple aliquots at different levels in the storage vessels.

In-Life Observations
Examinations performed on all animals prior to the start of dosing (GD 0-6) revealed incidental abrasions, which were apparently inflicted during breeding at the supplier's facility. One middle-dose rat (# 4734) appeared thin upon arrival (GD 1-4), but was otherwise healthy. By GD 5, this animal appeared normal. One animal (# 4736) from the middle-dose group was found dead on GD 8. A gross necropsy examination was performed. This examination revealed general cyanosis, and visceral congestion, but the cause of death was not determined. This animal was pregnant.

Oral administration of PCP produced an increase in transient, post-dosing salivation (recorded as clear, perioral soiling) in 2 out of 25 animals in the mid-dose group and 24 out of 25 animals in the high-dose group on various days during the dosing period. In one high-dose animal, the soiling was extensive and was observed beyond the perioral region. The incidence of this soiling among affected animals was transient and sporadic across test days and in only one case, appeared prior to dosing (on GD 13). No low-dose animals showed signs of pre- or post-dosing salivation. The mechanism of this salivation effect is unknown, but is likely related to a localized reaction to the test material.

Additionally, 7 out of 25 high-dose animals (all of which showed signs of perioral soiling), had urine soiling at least once during dosing. These observations, though treatment related, were not associated with any persistent toxic effect.

Body Weights
The body weight of animals administered 200 mg/kg/day was significantly decreased relative to control on GD 12, 15, 18, and 21. Also, decreases in weight gain were statistically significant in the high-dose group on GD 6-9, 9-12, 18-21, 6-21, and 0-21 compared to control.

Feed Consumption
There were no significant differences in feed consumption between the mid- and low-dose groups and controls in any of the intervals examined. In the high-dose group, differences in feed consumption were statistically significant compared to control only during the GD 6-9 and 9-12 intervals.

Anatomic Pathology

Organ Weights
Administration of PCP at dose levels of 50 and 200 mg/kg/day produced statistically-significant increases, relative to control, in absolute and relative kidney weights (increased by 6.8% and 17.3%, and 8.6% and 27.1%, respectively). PCP also produced treatment-related increases in absolute and relative liver weight (by 8.2% and 17.1%, respectively) in high-dose animals and these increases were statistically significant. In mid-dose rats, absolute and relative liver weights were also increased (by 6.2 and 7.6% compared to controls, respectively), but only the increase in relative liver weight was statistically significant. Overall, increases in kidney and liver weights were considered

treatment related. There were no significant effects on absolute or relative liver or kidney
weights at 10 mg/kg/day.

Gross Pathology
In one high-dose animal (#4759), the size of both kidneys was increased and each had a pale cortex. Because of increases in relative and absolute kidney weights in high-dose animals, these effects were likely related to treatment, despite its low incidence. The kidneys of all other high-dose animals were unremarkable.

All observations were considered to be spontaneous alterations, and unassociated with exposure to PCP.

Reproductive Parameters
There were no treatment-related effects on pregnancy rates, resorption rates, litter size, numbers of corpora lutea or
implantations, percent preimplantation loss, percent postimplantation loss, fetal sex ratios, or gravid uterine weights at any dose level.

At the highest dose level, fetal body weights of male fetuses, female fetuses, and both sexes combined were less than controls and these differences were statistically
significant. This effect on fetal body weight was correlated with decreased body weight in high-dose dams and was likely subsequent to maternal toxicity.

Fetal Alterations
The incidence of irregular pattern of ossification of the sternebrae in the low- and high-dose groups was
significantly different than control. However, because the incidences were less than control, this result was dismissed as due to random variation across all groups. There were no statistically- significant differences in the incidence of any other fetal alteration in any of the treated groups compared to controls. The small number of
alterations observed in fetuses from dams administered PCP either occurred at low frequencies and/or were not dose related.

Text Table 3. Summary of Fetal Malformations

Dose Group	Dam ID#	Fetus ID#	Malformation
Control	4678	3	Irregular cartilage in a thoracic centrum.
			Extra thoracic rib and vertebrae.
			Fused thoracic ribs
		5	Extra thoracic rib, vertebrae and centra
	4696	15	13th thoracic rib missing
	4701	9	Sternoschisis
10 mg/kg/day	N/A1	N/A	N/A
50 mg/kg/day	4742	15	Macrophthalmia
	4748	5	Extra thoracic rib, vertebrae and centra
200 mg/kg/day	4756	13	Anophthalmia
			Domed skull

1N/A No fetal malformations were observed in the 10 mg/kg/day group.

Source	:	The Dow Chemical Company, Midland, MI.
Conclusion	:	Oral gavage administration of PCP at a dose level of 200 mg/kg/day caused maternal toxicity as evidenced by decreases in body weight, body weight gain, feed consumption, and increases in kidney and liver weight. Treatment-related clinical observations included clear, perioral soiling in the high and middle-dose group, and urine soiling in the high-dose group. Developmental effects were limited to decreased fetal body weight at 200 mg/kg/day. There were no other effects on fetal development at any dose level. Therefore, under the conditions of this study, the no-observed-effect level (NOEL) for maternal toxicity was 10 mg/kg/day and the NOEL for developmental toxicity was 50 mg/kg/day.
Reliability	:	(1) valid without restriction

22.09.2004 (7)

Species	:	mouse
Sex	:	female
Strain	:	DBA
Route of admin.	:	gavage
Exposure period	:	Days 6-15 of gestation
Frequency of treatm.	:	Daily during treatment period
Duration of test	:	Until gestation day 18
Doses	:	100 mg/kg
Control group	:	no
Method	:	other
Year	:	1993
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Embryotoxic effects were studied following oral administration of 100 mg/kg PCP in sunflower oil (Oleum helianthi) daily to pregnant Halle:DBA and Halle:AB mice on days 6-15 of gestation. On day 18 of gestation the mice were killed and the reproductive status was determined (number of corpora lutea and dead and live fetuses; the latter were examined for gross malformations). The data were analyzed statistically using the Chi quadrate test.
Result	:	There were no significant changes in the number of fetal deaths, the weight of live embryos and the rate of malformations after PCP treatment.
Source	:	The Dow Chemical Company, Midland, MI
Reliability	:	(2) valid with restrictions No examinations for visceral or skeletal malformations were conducted.

22.09.2004 (20)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

6.1 ANALYTICAL METHODS

6.2 DETECTION AND IDENTIFICATION

7.1 FUNCTION

7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED

7.3 ORGANISMS TO BE PROTECTED

7.4 USER

7.5 RESISTANCE

8.1 METHODS HANDLING AND STORING

8.2 FIRE GUIDANCE

8.3 EMERGENCY MEASURES

8.4 POSSIB. OF RENDERING SUBST. HARMLESS

8.5 WASTE MANAGEMENT

8.6 SIDE-EFFECTS DETECTION

8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER

8.8 REACTIVITY TOWARDS CONTAINER MATERIAL

(1) Dictionary of Organic Compounds. 5th ed. Vol. 4. Chapman and Hall. 1982.

(2) MPBPWIN v1.40,) 2000 U.S. EPA

(3) Gehring J. et. al., Toxicol Applied Pharmacol 11: 361-71. 1967

(4) Lyman WJ, et. al. Handbook of Chemical Property Estimation Methods. NY, McGraw-Hill. p2 - 14. 1982.

(5) U.S. EPA, 2000. - AOPWin, v1.90, Atmospheric half-life estimating software & experimental value database.

(6) EPIWIN. 1999. Estimation Program Interface for Windows, version 3.02. Syracuse Research Corporation, Syracuse, NY, USA.

(7) Unpublished data, The Dow Chemical Company.

(8) Clifford, Dennis A. (1968). Automatic measurement of total oxygen demand: a new theoretical method. Presented at Purdue University, Lafayette, IN.

(9) Vol. III, "Acute Toxicities of Organic Chemicals to Fathead Minnow", Univ. of WI, 1985.

(10) Unpublished data, The Dow Chemical Company, 1972.

(11) McLeese, D.W., et al. SAR for Phenols, Anilines, and Other Aromatic Compounds in Shrimp and Clams. Chemosphere 8(2): 53-57, 1985.

(12) Schultz, T.W., et al. Comparative Toxicity of Selected N-containing Aromatic Compounds in Two Test Systems. Chemosphere 18(11/12): 2283-2291, 1989.

(13) Unpublished data, The Dow chemical Company.

(14) Unpublished data, The Dow Chemical Company, 1987.

(15) Unpublished data, The Dow Chemical Company, 1963.

(16) Unpublished data, The Dow Chemical Company, 1965.

(17) Unpublished data, The Dow Chemical Company, 1968.

(18) Gage, J.C. (1970). Brit. J. Ind. Med. 27: 1-18.

(19) Datta, P.K., Frigger, H., and Schleiermacher, E. (1970). The effect of chemical mutagens on the mitotic chromosomes of the mouse in vivo. In: Chemical Mutagenesis in Mammals and Man. F. Vogel and G. Rohrborn, eds., pp. 194-213. Berlin: Springer-Verlag.

(20) Nehez, M., Fischer, G.W., Scheufler, H., Schmidt, R., and Desi, I. (1993). Investigations on the cytogenetic and embryotoxic activity of pentachloropyridine.

10.1 END POINT SUMMARY

10.2 HAZARD SUMMARY

10.3 RISK ASSESSMENT