201-14886B2

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Data Set

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Existing Chemical	:	ID: 102-06-7
CAS No.	:	102-06-7
EINECS Name	:	1,3-diphenylguanidine
EC No.	:	203-002-1
TSCA Name	:	Guanidine, N,N'-diphenyl-
Molecular Formula	:	C13H13N3

Producer related part

Company	:	Atofina
Creation date	:	06.11.2000

Substance related part

Company	:	Atofina
Creation date	:	06.11.2000
Status	:	
Memo	:	
Printing date	:	14.11.2001
Revision date	:	
Date of last update	:	14.11.2001
Number of pages	:	146
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	
Flags (profile)	:	

1.0.1 APPLICANT AND COMPANY INFORMATION

Type	:	lead organisation
Name	:	M.L.P.C.
Contact person	:	
Date	:	
Street	:	BP 2
Town	:	40370 RION DES LANDES
Country	:	France
Phone	:	
Telefax	:	
Telex	:	
Cedex	:	
Email	:	
Homepage	:	
Source	:	ECB - Existing Chemicals Ispra (VA)
Flag	:	non confidential

07.11.2000

Type	:	cooperating company
Name	:	Akzo Chemicals b.v.
Contact person	:	
Date	:	
Street	:	Stationsplein 4, PO Box 247
Town	:	3800AE Amersfoort
Country	:	Netherlands
Phone	:	
Telefax	:	
Telex	:	
Cedex	:	
Email	:	
Homepage	:	
Source	:	ECB - Existing Chemicals Ispra (VA)
Flag	:	non confidential

07.11.2000

Type	:	cooperating company
Name	:	Monsanto plc
Contact person	:	
Date	:	
Street	:	
Town	:	RG24 OUL Basingstoke
Country	:	United Kingdom
Phone	:	
Telefax	:	
Telex	:	
Cedex	:	
Email	:	
Homepage	:	
Source	:	ECB - Existing Chemicals Ispra (VA)
Flag	:	non confidential

07.11.2000

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type :
Substance type : organic
Physical status : solid
Purity : ca. 97.5 % w/w
Colour :
Odour :

Flag : non confidential
12.09.2001 (1)

Purity type :
Substance type :
Physical status : solid
Purity : > 96 % w/w
Colour :
Odour :

Remark : 96% is the minimum acceptable purity of the batch. In practice analysis shows 97-98.5%
12.09.2001 (2)

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

12.09.2001 (3)

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

1,2-DIPHENYLGUANIDINE

23.10.1995

1,3-DIPHENYLGUANIDINE

23.10.1995

DENAX

23.10.1995

DFG

23.10.1995

DPG

23.10.1995

GUANIDINE, 1,3-DIPHENYL-

05.09.2001

GUANIDINE, N,N'-DIPHENYL-

23.10.1995

MELANILINE

23.10.1995

N,N'-DIPHENYLGUANIDIN

06.09.2001

N,N'-diphenylguanidine

23.10.1995

SYM-DIPHENYLGUANIDINE

23.10.1995

Vulkacit D

23.10.1995

VULKAZIT

06.09.2001

Source : M.L.P.C. RION DES LANDES
ECB - Existing Chemicals Ispra (VA)

05.09.2001

Source : M.L.P.C. RION DES LANDES
ECB - Existing Chemicals Ispra (VA)

05.09.2001

Source : Monsanto plc Basingstoke
ECB - Existing Chemicals Ispra (VA)

05.09.2001

Source : Monsanto plc Basingstoke

ECB - Existing Chemicals Ispra (VA)

05.09.2001

1.3 IMPURITIES

Purity	:	
CAS-No	:	
EC-No	:	
EINECS-Name	:	Others and unknown
Molecular formula	:	
Value	:	ca. .7 % w/w

06.09.2001

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

06.09.2001

Purity	:	
CAS-No	:	62-53-3
EC-No	:	200-539-3
EINECS-Name	:	aniline
Molecular formula	:	
Value	:	< .04 % w/w

06.09.2001

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

Remark : No occupational exposure limit has been set.
06.09.2001

Remark : No data available on Occupational Exposure Limit Values on the referred chemical.
Source : M.L.P.C. RION DES LANDES
ECB - Existing Chemicals Ispra (VA)
23.10.1995

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 : Batch process. The powder in suspension is extracted by a centrifugal dryer. The final product is obtained after flash dryer and cyclone.
Effluents containing powder in suspension are puirfied in a waster tip treatment. Wet wastes are burning in an incinerator.
In the atmospher, dust only appears on the area of the process unit.
If dust on soil, recuperation and incineration.
06.09.2001

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

1.13 REVIEWS

2.1 MELTING POINT

Value : 142 °C
Sublimation :
Method :
Year :
GLP :
Test substance : other TS: DPG no indication of purity

Remark : No further information available
Reliability : (2) valid with restrictions
06.09.2001 (4)

Value : 145 - 147 °C
Sublimation :
Method :
Year :
GLP :
Test substance : other TS: DPG no indication of purity

06.09.2001 (5)

Value : = 147 - 150 °C
Decomposition : no, at °C
Sublimation : no
Method : other: Differential Scanning Calorimetry
Year : 2001
GLP : no
Test substance : other TS: commercial grade

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
05.09.2001 (6)

Value : 147 °C

Reliability : (2) valid with restrictions
06.09.2001 (7)

Value : 148 - 148.5 °C
Sublimation :
Method :
Year : 1926
GLP :
Test substance :

Reliability : (2) valid with restrictions
06.09.2001 (8)

Value : 149 - 150 °C
Sublimation :
Method :
Year : 1992
GLP :
Test substance :

Reliability : (2) valid with restrictions
06.09.2001 (9)

Value : 150 °C
Sublimation :
Method :
Year : 1985
GLP :
Test substance :

Reliability : (2) valid with restrictions
06.09.2001 (10) (11)

Value : 151.6 °C
Sublimation :
Method :
Year : 1989
GLP :
Test substance :

06.09.2001 (12)

2.2 BOILING POINT

Value : > 170 °C at 1013 hPa
Decomposition : yes
Method :
Year : 1985
GLP :
Test substance : other TS: DPG no indication of purity

Result : No further information
Reliability : (2) valid with restrictions
06.09.2001 (10)

Value : > 200 °C at 1013 hPa
Decomposition : yes
Method : other: Differential Scanning Calorimetry
Year : 2001
GLP : no
Test substance : other TS: DPG no indication of purity

Remark : The exact boiling point temperature is not well determined because the DSC graph does not show a clearly defined threshold. We just notice that the curve goes up instead of being straight (see attached graph). The analysis equipment used does not allow to go up to 300°C.
Attached document : BP chromatogram dpg.doc
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
05.09.2001 (6)

2.3 DENSITY

Type : density
Value : 1.13 g/cm³ at 20 °C
Method :
Year : 1985
GLP :
Test substance : other TS: DPG no indication of purity

Reliability : (2) valid with restrictions
06.09.2001 (10) (13)

Type : density
Value : 1.19 g/cm³ at 20 °C
Method :
Year :
GLP :
Test substance : as prescribed by 1.1 - 1.4

Reliability : (2) valid with restrictions
06.09.2001 (4)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value : .0000000174 hPa at 20 °C
Decomposition :
Method : other (calculated)
Year :
GLP :
Test substance : as prescribed by 1.1 - 1.4

Remark : data extrapolated from measurements at 87-128 degree C
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
06.09.2001 (4)

Value : .0000000409 hPa at 25 °C
Decomposition :
Method : other (calculated)
Year : 1988
GLP :
Test substance : as prescribed by 1.1 - 1.4

Remark : data extrapolated from measurements at 87-128 degree C
Reliability : (2) valid with restrictions
06.09.2001 (14)

2.5 PARTITION COEFFICIENT

Partition coefficient :
Log pow : 1.69 at °C
pH value :
Method : other (measured)
Year : 1992
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : 4 measurements of log Pow were made:
1.76
1.65
1.81
1.54

However, as no pH was reported it is not possible to

determine the state of ionisation of DPG during this experiment.

The values can only be used as an indication of log Pow.

Test condition : 1-4 mg test substance dissolved in 2 ml n-octanol by addition of 20 ml water; HPLC-analysis

Reliability : (3) invalid

06.09.2001 (15)

Partition coefficient :
Log pow : 2.9 at °C
pH value :
Method : other (calculated)
Year : 2001
GLP :
Test substance : other TS: modeled data

Result : The result is presumed to be an indication of the log Pow of DPG in an unionised state

Reliability : (2) valid with restrictions

06.09.2001 (16)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in :
Value : .217 g/l at 30 °C
pH value : 10
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method :
Year : 1989
GLP :
Test substance : as prescribed by 1.1 - 1.4

Reliability : (2) valid with restrictions

06.09.2001 (17)

Solubility in :
Value : < 1 g/l at 21 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method :
Year : 1985
GLP : no data
Test substance : other TS: DPG no indication of purity

06.09.2001 (10)

Solubility in :

Value : 1.5 g/l at 20 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method :
Year : 1974
GLP : no
Test substance : other TS: DPG no indication of purity

Reliability : (2) valid with restrictions
06.09.2001 (18)

Solubility in :
Value : 1 g/l at 25 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method :
Year : 1992
GLP : no data
Test substance : other TS: no indication of purity

Reliability : (2) valid with restrictions
06.09.2001 (9)

Solubility in :
Value : 22 g/l at 20 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method :
Year : 1974
GLP : no
Test substance : other TS: DPG no indication of purity

Test substance : DPG x H3PO4
Reliability : (2) valid with restrictions
06.09.2001 (18)

Solubility in :
Value : 43.28 g/l at 20 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :

Stable :
Deg. product :
Method :
Year : 1974
GLP : no
Test substance : other TS: DPG no indication of purity

Test condition : Strongly acid conditions increasing water solubility
Test substance : (DPG)2 x H2SO4
Reliability : (2) valid with restrictions
06.09.2001 (18)

Solubility in :
Value : 519 g/l at 20 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method :
Year : 1974
GLP : no
Test substance : other TS: DPG no indication of purity

Test condition : strongly acid conditions increasing water solubility
Test substance : DPG x HCl
Reliability : (2) valid with restrictions
06.09.2001 (18)

Solubility in :
Value : = 860 mg/l at 25 °C
pH value : = 7
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method :
Year : 1980
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Method : measurements made at pH 5, 7 and 9 using Campbell method. Substance was allowed to equilibrate for 7 or 8 days at 25°C.

Saturated solution was exracted with methylene chloride (4 x 2 ml) and extract measured by HPLC.

Result : At pH 5 the substance was observed to have completely decomposed and no data was obtained. Decomposition was proposed as a brown residue was formed while "DPG is light grey" and th e fact that DPG kept disslving no matter how much was added.

Solubilities at pH 7 and 9 were based on two measurements so wide standard errors were obtained:

pH7 860 +/ - 110

Reliability : (2) valid with restrictions
06.09.2001 (19)

Solubility in :
Value : = 1470 mg/l at 25 °C
pH value : = 9
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method :
Year : 1980
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Method : measurements made at pH 5, 7 and 9 using Campbell method. Substance was allowed to equilibrate for 7 or 8 days at 25°C.

Saturated solution was exracted with methylene chloride (4 x 2 ml) and extract measured by HPLC.

Result : At pH 5 the substance was observed to have completely decomposed and no data was obtained. Decomposition was proposed as a brown residue was formed while "DPG is light grey" and the fact that DPG kept disslving no matter how much was added.

Solubilities at pH 7 and 9 were based on two measurements so wide standard errors were obtained:

pH9 1470 +/- 380

Reliability : (2) valid with restrictions
06.09.2001 (19)

Solubility in :
Value : = 475 mg/l at 20 °C
pH value : = 7
concentration : 1015 mg/l at 60 °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description : other:DPG is slightly soluble in water at pH 7 and basic pH (11). At acid pH DPG is transformed into DPG chlorhydrate which is very soluble in water.
Stable :
Deg. product :
Method : other: comparable to OECD guideline n°. 105 (NF T 20-046 AFNOR 1985)
Year : 2001
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Result : Solubility

Temp (°C)	pH 7	pH11
10	422	422
20	475	485
30	537	541
40	680	701
60	1015	1009
90		

Test condition : The NF T 20-046 method was used, instead of NF T 20-045 method, although 1,3-diphenylguanidine is slightly soluble.
Attached document : solubility curve dpg.doc
Conclusion : The results of this method are good and the results are used in industrial production.
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
06.09.2001 (20)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

Value : 170 °C
Type : closed cup
Method : other: DIN 51578
Year :
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
06.09.2001 (4)

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

Remark : dissociation constant (pKa): 10.12 at 25 degree C for first protonation

The pKa at which the second protonation occurs is still unknown but will be inferior or equal to the first.
11.09.2001 (21)

3.1.1 PHOTODEGRADATION

Type : water
Light source :
Light spectrum : nm
Relative intensity : based on intensity of sunlight

Result : No photolysis was found due to exposure to sunlight for 7 d.
No loss was found in dark controls.

Test condition : 1 mg/l solutions of DPG in water were prepared in milli-Q water with 1% acetonitrile. The solutions were placed in test tubes at a 60° angle from horizontal and exposed to sunlight for 7 d.

Dark controls were mintained at 23°C.

After the test some samples were analysed immediately while others were refrigerated until analysis.

Analysis
Extraction with methylene chloride and analysis by HPLC

Conclusion : Study valid with restrictions as light intensity was not measured and no subjective indication of weather conditions provided. No information is given on the pH of solutions used during the test.

The results are supported by the low extinction coefficient of DPG in the sunlight region (E300 nm <100).

Reliability : (2) valid with restrictions
26.09.2001

INDIRECT PHOTOLYSIS
Sensitizer : OH
Conc. of sensitizer : 1000000 molecule/cm^3
Rate constant : = .0000000000085 cm^3/(molecule*sec)
Degradation : = 50 % after 2.3 hour(s)

Remark : Calculated from Atmospheric Oxidation Programme V.1.89. Syracuse Corp.

Flag : Risk Assessment
13.07.2001

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
Year : 1984
GLP : no data
Test substance : other TS: no indication of purity

Remark : Hydrolysis of test substance (0.3 g/l resp. 0.03 % of weight in water) at various pH values at 80 degree C: no hydrolysis at pH 3.5 after 500 h; 18.1 % at pH 7.0 after 1000 h; t1/2

at pH 10.5 ca. 168 h; hydrolysis products:
N,N'-diphenyl -urea and aniline (IR- and UV-spectroscopy)

Reliability : (2) valid with restrictions
26.09.2001 (22)

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

Type of measurement : background concentration
Media : biota
Concentration :
Method :

Remark : Japanese EPA investigated 42 water and sediment samples in Japan in 1978. Samples not directly contaminated byindustrila emissions. No DPG determined at a LOD of 2 to 5 µg/l for water and 100 to 500 µg/kg in sediment. reported in BUA report no. 96: N,N'-diphenylguanidine

Reliability : (2) valid with restrictions
06.09.2001 (23)

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

Type : aerobic
Inoculum : activated sludge
Concentration : 100 mg/l related to Test substance
related to
Contact time :
Degradation : = 0 (±) % after 14 day(s)
Result : other: not readily biodegradable
Deg. product :
Method : OECD Guide-line 301 C "Ready Biodegradability: Modified MITI Test (I)"
Year : 1981
GLP :
Test substance : other TS: no indication of purity

Method : Test type: MITI test
Sludge concentration: 30 mg/l unadapted activated sludge
Substance concnetration: 100 mg/l

Reliability : (1) valid without restriction
06.09.2001 (24)

Type : aerobic
Inoculum : aerobic microorganisms
Concentration : 20 mg/l related to Test substance
related to
Contact time :
Degradation : 18 (±) % after 3 day(s)
Result :
Deg. product :
Method : other
Year : 1988
GLP : no data
Test substance : other TS: no indication of purity

Remark : Screening test with microorganisms of river water from not environmental polluted regions (COD < 3 ppm); 5 ml of a 0.2 % peptone solution (pH 7.0) was mixed with 4.9 ml river water and 0.1 ml aqueous solution of test substance (dissolved in water, acetone or DMSO; final concentration: 20 mg/l); measurement of turbidity at 610 nm of optical density
Test condition : 30 degree C
Reliability : (4) not assignable
11.09.2001 (25)

Type : aerobic
Inoculum : activated sludge, domestic, adapted
Contact time : 28 day(s)
Degradation : ca. 75 (±) % after 28 day(s)
Result : inherently biodegradable
Deg. product : not measured
Method : other: equivalent to OECD 301 D
Year : 1988
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Experiment 1

Closed bottle test
Unadapted sludge
0.8 2.4 8.0 24 mg/l DPG solutions

Measured after 5, 10 and 20 d

Experiment 2

Closed bottle test
Adapted sludge (aerated for 14 d in contact with DPG)
0.8 2.4 8.0 24 mg/l DPG solutions

Measured after 5, 10 and 20 d
Result : Expt 1
No degradation observed within 20 d at any concentration

Expt 2
Results based on % degradation after X d

Conc (mg/l)	T (d) 5	10	20
0.8	0	62	74
2.4	0	66	76
8.0	16	>LOQ	>LOQ
24	>LOQ	>LOQ	>LOQ

>LOQ = all available oxygen used. No measurement possible

Conclusion : Rapid mineralisation of DPG by adapted micro-organisms
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
06.09.2001 (26)

Type : aerobic
Inoculum : activated sludge, adapted
Concentration : 20 mg/l related to Test substance
related to
Contact time :
Degradation : = 55 - 71 (±) % after 28 day(s)
Result : other: data suggest that this material is intrinsically biodegradable
Deg. product :
Method : other: see test conditions
Year : 1979
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : Flasks with DPG:
21 mg 71% of ThOD
20 mg 68% of ThOD
20 mg 55% of ThOD

Flasks with DPG plus HgCl2:
21 mg 5% of ThOD

Test condition : Draft method Nr 2 for the proposed standard for the determination of the ultimate biodegradability of organic chemicals, August 1979, ASTM Committe E35.24.

Inoculum: Aclimated SCAS supernatant

Method similar to sturm test OECD 301B except that the flasks were shaken.

Preparation
100 ml of acclimated bcterial inoculum mixed with 900 ml of mineral salt medium in 2l Ehlenmeyer flask.

The solution was aerated and 20 to 21 mg of DPG added to test flasks. No substance added to control. One flask contained 21 mg DPG and 50 mg/l HgCl2.

CO2 produced was trapped by Ba(OH)2 suspended within the flasks. No aeration is provided during the study.
Periodically (e.g. 3, 7, 14, 21, 28 and 35 d) the flasks were unstoppered and the Ba(OH)2 analysed for CO2. Fresh barium solution was replaced at each sample time.

The duration of the DPG test was consideed to be 28 d but no exact study length is provided in the report.

Conclusion : Considered as valid with restrictions as method followed a defined norm but no study duration was provided in the report. However, the duration is expected to be either 28 or 35 d.
Reliability : (2) valid with restrictions
06.09.2001 (27)

Type : aerobic
Inoculum : other: screen filtered river water
Concentration : 1 mg/l related to Test substance

related to

Contact time : 14 day(s)
Degradation : (±) % after
Result : other: primarily degradable
Kinetic of testsubst. : 0 day(s) = 0 %
3 day(s) ca. 3 %
7 day(s) = 78 %
14 day(s) = 100 %
%
Control substance : other: quinoline
Kinetic : %
%
Deg. product : not measured
Method :
Year : 1980
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Closed 4 l bottle half filled with 2 l of screen filled river water containing 20 ml of (1 g/l) potassium phosphate buffer (pH 7.5) and 50 µl/litre of DPG stock solution (containing 40 mg/ml DMSO).

Final concentration DPG = 1 mg/l
Final concentration DMSO = 25 µl/l

Steril control with autoclaved river water.

Positive control of 4 ml of 2 mg/ml quinoline into buffered river water.

Incubation temperature: 21-25°C.

Analysis
DPG analysed at each sample time using HPLC. Sample adjusted to pH>10 with NaOH and extracted with CH2Cl2 (2 x 5 ml).

Quantified using 1.5% ethanoic acid/48.5% ethanol/50% hexane through a silica 100 µl loop column.
Flow rate: 2 ml/min
detector: 254 nm UV

Result : DPG residues were measured:

time	sterile control	DPG
0	0.82	0.82
3	0.84	0.81
7	0.96	0.21
14	0.81	0

Conclusion : Primary degradation complete within 14 days at a concentration of 1 mg/l DPG in filtered river water.

No mineralisation was determined in this study.

Reliability : (2) valid with restrictions
27.06.2001 (28)

3.6 BOD5, COD OR BOD5/COD RATIO

BOD5
Method :
Year : 1960

Concentration : related to
BOD5 : mg/l
GLP : no

Remark : BOD5: 2.3 % (referred to TOD); no further information available
Reliability : (2) valid with restrictions
06.09.2001 (29)

3.7 BIOACCUMULATION

Species : Cyprinus carpio (Fish, fresh water)
Exposure period : 42 day(s) at 25 °C
Concentration : .1 mg/l
BCF : < 2
Elimination : no data
Method : OECD Guide-line 305 C "Bioaccumulation: Test for the Degree of Bioconcentration in Fish"
Year : 1992
GLP : no data
Test substance : other TS: no indication of purity

Remark : Exposure of test organisms for 6 weeks in a flow-through system (0.2 -0.8 l/min); no solvent; BCF determined as below the limit of detection; no further information available
Test condition : 6-8 mg O2/l; test concentration analyzed twice a week
Reliability : (1) valid without restriction
06.09.2001 (9)

Species : Cyprinus carpio (Fish, fresh water)
Exposure period : 42 day(s) at 25 °C
Concentration : .01 mg/l
BCF : < 20
Elimination : no data
Method : OECD Guide-line 305 C "Bioaccumulation: Test for the Degree of Bioconcentration in Fish"
Year : 1992
GLP : no data
Test substance : other TS: no indication of purity

Remark : Exposure of test organisms for 6 weeks in a flow-through system (0.2 -0.8 l/min); no solvent; BCF determined as below the limit of detection; no further information available
Test condition : 6-8 mg O2/l; test concentration analyzed twice a week
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
06.09.2001 (9)

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	flow through
Species	:	Cyprinus carpio (Fish, fresh water)
Exposure period	:	
Unit	:	
Limit test	:	
Analytical monitoring	:	no data
Method	:	other
Year	:	1963
GLP	:	no
Test substance	:	other TS: no indication of purity
Remark	:	effect after a single oral application of test substance in gelatine capsules; 3.2; 6.0 and 8.7 mg/kg bw: no effects within 114 h; 9.5 and 17 mg/kg bw: not specified symptoms after < 120 h, recovery after < 312 h; 5.6 mg/kg bw: mortality after 71 h; 9.5 and 70 mg/kg bw: not specified symptoms after >= 22 h, mortality after 125 h no further information available
Test condition	:	18 degree C
Conclusion	:	gavage of fish cannot be considered as relevant for aquatic ecotoxicological hazard assessment
Reliability	:	(3) invalid

06.09.2001 (30)

Type	:	other: static or semistatic test
Species	:	Oryzias latipes (Fish, fresh water)
Exposure period	:	48 hour(s)
Unit	:	mg/l
LC50	:	10
Limit test	:	
Analytical monitoring	:	no data
Method	:	other: Japanese Industrial Standard (JIS K 0102-1986-71) "Testingmethods for industrial waste water"
Year	:	1992
GLP	:	no data
Test substance	:	other TS: no indication of purity
Remark	:	LC50 referred to nomimal concentration; no further information available
Test condition	:	solvent not specified
Conclusion	:	not considered valid for hazard assessment only because of short length of study (48 h); unacceptable test methodology.
Reliability	:	(3) invalid

06.09.2001 (9)

Type	:	static
Species	:	Lepomis macrochirus (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
NOEC	:	< 7.5 measured/nominal
LC50	:	= 9.6 calculated
LC100	:	= 14 measured/nominal
Limit test	:	
Analytical monitoring	:	no
Method	:	other: static method : US EPA Ecological Research series 660/3-75009

Year : 1979
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Remark : 24h LC50 = 18 mg/l
48h LC50 = 17 mg/l

Result : Nominal T.S. concentrations:
0, 7.5, 14, 24, 42, 75, mg/l

Nominal concentrations	Mortality 24	48	72	96
0	0	0	0	0
7.5	0	0	0	2
14	0	0	9	10
24	10	10	10	10
42	10	10	10	10
75	10	10	10	10

Concentration of solvent at 75 mg/l DPG = 1000 mg/l. Only lowest test solution contained <100 mg/l of solvent.
Solvent control was not included
This is not thought to have had a major impact upon the test results as 20 % mortality was noted at 96 h at the lowest concentration.

Sub-lethal effects were noted as "loss of equilibrium" from 48 h at 14 mg/l and from 72 h in all surviving groups

Reference substance test included (antimycin A). The 96 h LC50 (0.029 µg/l) was reported as being within limits quoted in literature

Test condition : species/Supplier: Bluegill sunfis h from Ossage Catfisheries Inc, Missouri, USA
Statistical method: probit

Test fish (control at termination):
length 29.9 mm S.D. 3.54 mm
weight 0.72 g S.D. 0.29 g

Diluion water: well water with hardness 255 mg/l as CaCO3; alkalinity 368 mg/l as CaCO3; conductivity 50 µOhms/cm; TOC no information; TSS not measured; pH = 8.2; Chlorine not measured.

Stock solution: prepared at 150 g/l in acetone. Diluted directly into the final test solutions
Exposure vessels: 40 l aquaria containing 30 l test solution
no. of replicates: 1 rep per concentration and 10 fish per vessel

Test conditions:

Nominal conc.	0 hours T°C	DO	pH	48 hours T°C	DO	pH	96 hours T°C	DO	pH
O	22	7.9	8.2	22	7.3	8.2	22	3.8	8.3
7.5	22	8.3	8.2	22	7.8	8.3	22	4.3	8.3
14				22	7.6	8.3			
24	22	8.3	8.3						

Conclusion : Three negative points compromise the validity of the study:
1) No analytical information
2) static test system

3) The oxygen concentration at 96 h
but due to the stability of the substance (abiotically as well as biotically) and the low log Pow of this substance the nominal concentrations were likely to have been maintained over the test period.

The oxygen concentration in the only surviving test group at the end of the study was below the levels considered acceptable for fish ecotoxicity testing (4.3 mg/l). However, the control oxygen concentration was even lower (3.8 mg/l) and no mortality was observed in this group.

The results of this study are therefore considered valid with restrictions.

Reliability : (2) valid with restrictions
06.09.2001 (31)

Type : static
Species : Leuciscus idus (Fish, fresh water)
Exposure period : 48 hour(s)
Unit : mg/l
LC100 : 10
Limit test :
Analytical monitoring : no
Method : other: Bestimmung der akuten Wirkung von Stoffen auf Fische.Arbeitskreis "Fischtest" im Hauptausschuss "Detergentien" (15.10.73)
Year : 1975
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : direct weight; range finding test
Conclusion : Endpoint calculated from a full range finding study. This endpoint is not considered valid for hazard assessment - refer to 96 h endpoint.
Reliability : (3) invalid
06.09.2001 (4)

Type : static
Species : Leuciscus idus (Fish, fresh water)
Exposure period : 72 hour(s)
Unit : mg/l
LC0 : 1
Limit test :
Analytical monitoring : no
Method : other: Bestimmung der akuten Wirkung von Stoffen auf Fische.Arbeitskreis "Fischtest" im Hauptausschuss "Detergentien" (15.10.73)
Year : 1975
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : direct weight
Conclusion : Endpoint calculated from a full range finding study. This endpoint is not considered valid for hazard assessment - refer to 96 h endpoint.
Reliability : (3) invalid
06.09.2001 (4)

Type : static
Species : Oncorhynchus mykiss (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
NOEC : = 5.6 measured/nominal

LC50 : = 11 calculated
LC100 : = 18 measured/nominal
Limit test :
Analytical monitoring : no
Method : other: Static method : US EPA Ecological Research series 660/3-75009
Year : 1979
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Remark : 24 and 48h LC50 = 18 mg/l
Result : Nominal T.S. concentrations:
0, 3.2, 5.6, 10, 18, 32 mg/l

Nominal concentrations	Mortality 24	48	72	96
0	0	0	0	0
3.2	0	0	0	0
5.6	0	0	0	0
10	2	3	3	4
18	3	3	6	10
32	10	10	10	10

Concentration of solvent in highest test solution = 213 mg/l
All other solutions contained <120 mg/l of solvent
Solvent control was not included
This is not thought to have had an impact upon the test results.

Sub-lethal effects were noted as "loss of equilibrium" from 48 h at 10 and 18 mg/l.

Reference substance test included (antimycin A). The 96 h LC50 (0.029 µg/l) was reported as being within limits quoted in literature

Test condition : species/Supplier: rainbow trout from Spring Creek Hatchery, Lewistown, Montana, USA
Statistical method: probit

Test fish (control at termination):
length 29.3 mm S.D. 2.26 mm
weight 0.26 g S.D. 0.07 g

Diluion water: well water with hardness 255 mg/l as CaCO3; alkalinity 368 mg/l as CaCO3; conductivity 50 µOhms/cm; TOC no information; TSS not measured; pH = 8.2; Chlorine not measured.

Stock solution: prepared at 150 g/l in acetone. Diluted directly into the final test solutions
Exposure vessels: 15 l
no. of replicates: 1 rep per concentration and 10 fish per vessel

Test conditions:

Nominal conc.	0 hours T°C	DO	pH	48 hours T°C	DO	pH	96 hours T°C	DO	pH
O	12	8.8	8.0	12	8.7	8.2	12	8.9	8.2
3.2	12	9.1	8.4	12	8.9	8.3	12	9.2	8.4
5.6									
10							12	8.3	8.4

32 12 9.5 8.2 12 9.0 8.1

Conclusion : Two negative points compromise the validity of the study:
1) No analytical information
2) static test system
but due to the stability of the substance (abiotically as well as biotically) and the low log Pow of this substance the nominal concentrations were likely to have been maintained over the test period.

The results of this study are considered valid with restrictions.

Reliability : (2) valid with restrictions
06.09.2001 (32)

Type : static
Species : Pimephales promelas (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
NOEC : = 3.2 measured/nominal
LC50 : = 4.2 calculated
LC100 : = 5.6 measured/nominal
Limit test :
Analytical monitoring : no
Method : other: static method : US EPA Ecological Research series 660/3-75009
Year : 1979
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Remark : 24h LC50 = 7.2 mg/l
48h LC50 = 6.4 mg/l

Result : Nominal T.S. concentrations:
0, 1.0, 1.8, 3.2, 5.6, 10 mg/l

Nominal concentrations	Mortality 24	48	72	96
0	0	0	0	0
1.0	0	0	0	0
1.8	0	0	0	0
3.2	0	0	0	0
5.6	1	2	8	10
10	9	10	10	10

Concentration of solvent in highest test solution = 66 mg/l
Solvent control was not included

Sub-lethal effects were noted as "loss of equilibrium" from 24 h at 5.6 and 10 mg/l and from 48 h at 3.2, 5.6 mg/l.

Reference substance test included (antimycin A). The 96 h LC50 (0.028 µg/l) was reported as being within limits quoted in literature

Test condition : species/Supplier: Fathead minnows from Fattig Fish Hatchery, Brady, Nebraska, USA
Statistical method: probit

Test fish (mean control at termination):
length 23.3 mm
weight 0.2 g

Diluion water: well water with hardness 255 mg/l as $CaCO_3$; alkalinity 368 mg/l as $CaCO_3$; conductivity 50 µOhms/cm; TOC

no information; TSS not measured; pH = 8.2; Chlorine not measured.

Stock solution: prepared at 150 g/l in acetone. Diluted directly into the final test solutions
Exposure vessels: contained 15 l test solution
no. of replicates: 1 rep per concentration and 10 fish per vessel

Water chemistry:

Nominal conc.	0 hours T°C	DO	pH	48 hours T°C	DO	pH	96 hours T°C	DO	pH
O	22	9.4	8.2	22	7.3	8.2	22	6.8	8.1
1.0	22	9.6	8.2	22	7.2	8.2	22	6.9	8.3
5.6				22	6.5	8.2	22	6.8	8.2
10	22	9.6	8.3						

Conclusion : Two negative points compromise the validity of the study:
1) No analytical information
2) static test system
but due to the stability of the substance (abiotically as well as biotically) and the low log Pow of this substance the nominal concentrations were likely to have been maintained over the test period.

The results of this study are considered valid with restrictions.

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
06.09.2001 (33)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 24 hour(s)
Unit : mg/l
NOEC : 22 measured/nominal
EC0 : 3.9 calculated
EC50 : 73.6 calculated
EC100 : 177 calculated
Analytical monitoring : no
Method : other: UBA-Verfahrensvorschlag "Bestimmung der Schwimmunfaehigkeit beimWasserfloh "Daphnia magna" (EC0, EC50, EC100; statisches System) (Mai,1984)
Year : 1990
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Remark : EC50 as geometric mean of nominal concentrations; 24 h EC50 of organisms to reference substance
(potassium dichromate) was 3 mg/l as opposed to the average 24 h EC50 reported in the EC directive of 1.5 mg/l.

Given the relatively high stability and water solubility of the test substance and the normal variation between intra- and inter-laboratory test results, the study is not considered invalid by the reviewer.

Result : Nominal T.S. concentrations:
0, 1.4, 2.8, 5.5, 11, 22, 44, 88, 177 mg/l

Nominal concentrations	Mortality 24 h No.	%
0	0	0
1.4	0	0
2.8	0	0
5.5	0	0
11	0	0
22	0	0
44	2	10
88	13	65
177	20	100

Results are provided in the report which do not correspond.

Therefore, the EC0 is taken as 3.9 mg/l calculated
the EC100 as 125 mg/l calculated
and the EC50 as 73.6 mg/l with 95% confidence limits of 61.4-88.4 mg/l) recalculated using pooled data provided in the report and using the probit method

The concentration at which no immobility was seen was 22 mg/l although at this level only 10% immobilisation was observed which is within the limits accepted for the control without invalidating the study. If this is taken into account the concentration at which no unacceptable immobility was observed was 44 mg/l.

The concentration at which 100% immobility was observed was 177 mg/l.

Test condition : species/Supplier: Daphnia magna STRAUS. No strain reported
Supplier: Bundesgesundheitsamtes, Berlin, Germany
Statistical method: probit

Culture methods: Daphnids between 6 and 24 h old used for testing.

Diluion water: Elendt synthetic medium

Test conditions:
pH 7.8-8.3
T°C 20.5 -20.9

Stock solution: prepared at 200 mg/l, heated to 50°C for one hour and allowed to cool for one hour while stirring.
Diluted directly into the final test solutions
Exposure vessels: no vessels
no. of replicates: 2 reps per concentration and 10 daphnids per vessel

Test substance : purity: 73.8 %

Conclusion : The lack of analytical information compromises the validity of the study but due to the stability of the substance (abiotically as well as biotically) and the low log Pow of this substance the nominal concentrations were likely to have been maintained over the test period.

The results of this study are considered valid with restrictions.

Reliability : (2) valid with restrictions

06.09.2001 (34)

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
NOEC : = 5.6 measured/nominal
EC50 : = 17 calculated
EC100 : = 32 measured/nominal
Analytical monitoring : no
Method : other: static method : APHA 1975 US EPA Ecological Research series 660/3-75009
Year : 1979
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Remark : 24h EC50 = 33 mg/l
Result : Nominal T.S. concentrations:
0, 3.2, 5.6, 10, 18, 32 mg/l

Nominal concentrations	Mortality 24 h	48 h
0	0 0	0 0
0 + acetone	0 0	0 0
3.2	0 0	0 0
5.6	0 0	0 0
10	0 0	1 2
18	0 0	6 5
32	5 4	10 10

Concentration of solvent at 32 mg/l DPG = 1600 mg/l. Only lowest test solution contained <100 mg/l of solvent.
Solvent control at 1600 mg/l included
This is not thought to have had a major impact upon the test results.

No reference substance test included.

Test condition : species/Supplier: Daphnia magna cultured at ABC fa cilities.
No strain reported
Statistical method: probit

Culture methods: adult daphnids fed on trout chow and alfalfa (PR-11) daily until 24 h prior to testing. Daphnids less than 24 h old used for testing.

Photoperiod: 16h daylight: 8 h dark

Diluion water: well water with hardness 255 mg/l as CaCO3; alkalinity 368 mg/l as CaCO3; conductivity 50 µOhms/cm; TOC no information; TSS not measured; pH = 8.2; Chlorine not measured.

Stock solution: prepared at 150 g/l in acetone. Diluted directly into the final test solutions
Exposure vessels: 250 ml glass beakers containing 200 ml test solution
no. of replicates: 2 reps per concentration and 10 daphnids per vessel

Water chemistry (at end of test):
D.O 8.8 mg/l

pH 7.9
T°C reported as maintained at 20°C (+/-1°C) but not confirmed by measurement

Effect measured: immobilisation

Conclusion : The lack of analytical information compromises the validity of the study but due to the stability of the substance (abiotically as well as biotically) and the low log Pow of this substance the nominal concentrations were likely to have been maintained over the test period.

The results of this study are considered valid with restrictions.

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
06.09.2001 (35)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Scenedesmus subspicatus (Algae)
Endpoint : biomass
Exposure period : 72 hour(s)
Unit : mg/l
EC10 : = .013 calculated
EC50 : = 2.6 calculated
Limit test :
Analytical monitoring : no
Method : other: cell multiplication inhibition test according to DIN 38412, part 9
Year : 1990
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Method : Following DIN norm 38 412 Teil 9
Result : Cell count

Nominal concs	24h	48h	72h	% biomass
0.01	58889	267778	845556	8.7
0.032	57778	230000	800000	16.5
0.1	58889	234444	775556	17.3
0.32	55556	210000	727778	23.8
1.0	51111	190000	744445	25.8
3.2	53333	111111	432222	55.2
10.0	21111	42222	51111	91.9
32.0	14444	14444	13333	98.7
100.0	10000	10000	8889	100.1

Control response satisfactory (>factor of 16)

No reference substance test included.

Test condition : species/Supplier: No strain reported
origin: Pflazenphysiologisches Institut der Universität, 3400 Göttingen
Statistical method: probit

Culture methods: constant temperature (23+/- 2°C) and continuous illumination (120 µE/m2s). Algal culture maintained in suspension by magnetic stirrer.

Diluion water: millipore deionised water used to prepare culture water.

Stock solution: Agitated for two hours at 50°C. No further information
Exposure vessels: 300 ml glass beakers containing 100 ml test solution
no. of replicates: no information

Nominal T.S. concentrations:
0, 0.01, 0.032, 0.1, 0.32, 1.0, 3.2, 10, 32, 100 mg/l

Test conditions:
T°C 23°C

	pH values	
	T0	T72
0	7.6	8.9
0.01	7.6	8.8
0.032	7.6	8.9
0.1	7.6	8.7
0.32	7.6	8.3
1.0	7.7	8.0
3.2	7.7	8.0
10	7.9	7.9
32	8.2	8.2
100	8.4	8.3

Effect measured: biomass

Attached document : 102067alg.doc
Reliability : (2) valid with restrictions
06.11.2001 (36)

Species : Scenedesmus subspicatus (Algae)
Endpoint : growth rate
Exposure period : 72 hour(s)
Unit : mg/l
EC10 : = 2.1 calculated
EC50 : = 7.5 calculated
Limit test :
Analytical monitoring : no
Method : other: cell multiplication inhibition test according to DIN 38412, part 9
Year : 1990
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Method : Following DIN norm 38 412 Teil 9
Result : Cell count

Nominal concs	24h	48h	72h	% growth rate
0.01	58889	267778	845556	1.17
0.032	57778	230000	800000	2.40
0.1	58889	234444	775556	3.09
0.32	55556	210000	727778	4.51
1.0	51111	190000	744445	4.01
3.2	53333	111111	432222	16.11
10.0	21111	42222	51111	63.66
32.0	14444	14444	13333	93.59

100.0	10000	10000	8889	102.62

Control response satisfactory (>factor of 16)

Test condition : No reference substance test included.
species/Supplier: No strain reported
origin: Pflazenphysiologisches Institut der Universität, 3400 Göttingen
Statistical method: probit

Culture methods: constant temperature (23+/- 2°C) and continuous illumination (120 µE/m2s). Algal culture maintained in suspension by magnetic stirrer.

Diluion water: millipore deionised water used to prepare culture water.

Stock solution: Agitated for two hours at 50°C. No further information
Exposure vessels: 300 ml glass beakers containing 100 ml test solution
no. of replicates: no information

Nominal T.S. concentrations:
0, 0.01, 0.032, 0.1, 0.32, 1.0, 3.2, 10, 32, 100 mg/l

Test conditions:
T°C 23°C

	pH values	
	T0	T72
0	7.6	8.9
0.01	7.6	8.8
0.032	7.6	8.9
0.1	7.6	8.7
0.32	7.6	8.3
1.0	7.7	8.0
3.2	7.7	8.0
10	7.9	7.9
32	8.2	8.2
100	8.4	8.3

Effect measured: growth rate

Reliability : (2) valid with restrictions
06.09.2001 (37)

Species : Selenastrum capricornutum (Algae)
Endpoint : other: growth (no. of cells or chlorophyll a)
Exposure period : 96 hour(s)
Unit : mg/l
NOEC : = .3 measured/nominal
EC50 : = 1.4 - 1.7 calculated
Limit test :
Analytical monitoring : no
Method : other: Static method US EPA, 1971, Algae assay procedure : bottle test
Year : 1979
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Remark : Determination of cell multiplication by cell counter.
In vivo chlorophyll EC50 24h > 5.6 mg/l
48h = 3.5 mg/l
72h = 2.0 mg/l
96h = 1.4 mg/l
Init Inoc. = 20 000 cells/ml; 2800 lux; Carrier: DMF; 24°C

Result : Chlorophyll a determination

	Fluorimeter reading (window setting)			
	24h	48h	72h	96h
	(30)	(10)	(3)	(1)
Nominal concs	1 2 3	1 2 3	1 2 3	1 2 3
0	47 49 54	60 58 68	99 92 98	45 44 51
0 + dmf	53 50 53	64 68 60	99 98 91	49 51 46
0.3	51 52 55	58 60 60	88 84 85	43 42 42
0.6	53 52 52	47 45 47	68 64 67	32 27 30
1.0	50 52 52	43 45 41	51 54 51	20 22 19
3.2	48 46 48	34 31 35	42 40 45	19 16 20
5.6	45 46 45	28 31 26	30 32 29	9 10 7

Cell growth determination

	cell counts (in no. of haemocytometer squares) 96h	
Nominal concs	1 2 3	mean (x10 000)
0	74 73 84 (in 2)	38.8
0 + dmf	79 82 75 (in 2)	39.3
0.3	75 73 76 (in 2)	37.3
0.6	57 51 53 (in 2)	26.8
1.0	35 34 30 (in 2)	16.5
3.2	55 51 58 (in 4)	13.7
5.6	37 40 38 in 4	9.6

EC50s based on chlorophyl a determination:
24h >5.6 mg/l
48h 3.5 mg/l (0.2-56)
72h 2.0 mg/l (0.2-17)
96h 1.4 mg/l (0.2-7.4)

96h EC50 based on cell number = 1.7 mg/l (0.4-7.4)

NOEC not calculated but based on available data can be taken as 0.3 mg/l at both 72 and 96h (for chlorophyl a and growth rate data at 96h).

Concentration of solvent at 5.6 mg/l DPG = 1000 mg/l.
Solvent control at 1000 mg/l included
This is not thought to have had a major impact upon the test results.

Control response satisfactory (initial cell count (20 000): final cell count (mean 388 000) = 194 divisions).

No reference substance test included.
No growth curves provided.

Test condition : species/Supplier: Selenastrum capricornutum obtained from US EPA Environmental Research Laboratory, Oregan, USA and maintained in stock at BMRL. No strain reported
Statistical method: probit

Culture methods: As recommended by EPA (1971).

Diluion water: no information

Stock solution: prepared in dimethylformamide. Secondary stock solutions prepared by serial dilution. Secondary stock used to prepare the test solutions.
Exposure vessels: 125 ml glass beakers containing 50 ml test solution
Initial cell density: 20 000 cells/ml
Illumination approximately 3800 lux
no. of replicates: 3 reps per concentration

Nominal T.S. concentrations:
0, 0.3, 0.6, 1.0, 3.2, 5.6 mg/l

Test conditions: (at beginning and end of test):
pH 7.3-7.6
T°C reported as 24 +/-1°C (no measurements)

Effect measured: inhibition of growth measured as chlorophyll a concentration or cell numbers at each concentration.

Conclusion : The lack of analytical information compromises the validity of the study but due to the stability of the substance (abiotically as well as biotically) and the low log Pow of this substance the nominal concentrations were likely to have been maintained over the test period.

The results of this study are considered valid with restrictions.

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
06.09.2001 (38)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type : aquatic
Species : activated sludge, industrial
Exposure period : 3 hour(s)
Unit : mg/l
EC50 : 147
Analytical monitoring : no
Method : OECD Guide-line 209 "Activated Sludge, Respiration Inhibition Test"
Year : 1989
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : Not adapted inoculum (content: 6 g dry substance/l) from a laboratory treatment plant
Result : Conc respiration (% inibition based on mean control)

Test sub
100 40
180 53.3
320 73.3
560 80
1000 90

Ref sub
1 20
20 73.3

Test condition : Nominal concentrations:
0, 1, 3.2, 5.6, 10, 18, 32, 56, 100, 180, 320, 560, 1000, 1800, 3200, 5600 and 10000 mg/l

One control measurement at begining and one at endof test

Tested using 6 g/l activated sludge in aerobic conditions

Reference substance: 3,5-dichlorophenol tested at 1 and 20 mg/l

Stock concentration and preparation: no information

Test duration: 3 hours

Water chemistry:

	Temperature	pH
0	20.4	7.8
100	21.3	8.1
180	21.2	8.3
320	21.3	8.3
560	21.2	8.3
1000	21.1	8.3
1 (ref)	20.3	8.0
25 (ref)	20.6	8.1
0	21.3	8.2

Conclusion : No information on test solution preparation but accepted as validity 1 as report indicates that OECD guidelines were followed
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
06.09.2001 (39)

Type : aquatic
Species : Escherichia coli (Bacteria)
Exposure period :
Unit : mg/l
EC50 : 202.8
Analytical monitoring : no data
Method : other
Year : 1975
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : Inhibiton of specific steps of protein-biosynthesis as a result of a non-competitive inhibition of phenylalanyl-tRNA-synthetase; in vitro-test with homogenized cells; 50 % inhibition at a molar ratio of inhibitor/amino acid of 3.2 (L-phenylalanyl concentration 49.5 mg/l); no further information available
Conclusion : Not considered valid for use in hazard assessment - inapropriate methodology
Reliability : (3) invalid
06.09.2001 (40)

Type : aquatic
Species : other bacteria: Pre-cleaned activated sludge in particle-free communal wastewater (BOD5: 250 mg/l; NH4-N/l: 50-80 mg)
Exposure period : 4 hour(s)
Unit : mg/l
EC75 : > 50
Analytical monitoring : yes

Method	:	other: Quantitative determination of the nitrification rate,colorimetric measurement of the NO2/NO3 concentration; static test system
Year	:	1966
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	EC75 related to the effective concentration which caused a decrease in the 1st step of the nitrification rate (NH4 to NO2)
Test condition	:	25 degree C; pH 7.6-7.8
Reliability	:	(2) valid with restrictions

26.09.2001 (41)

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Species	:	Daphnia magna (Crustacea)
Endpoint	:	reproduction rate
Exposure period	:	21 day(s)
Unit	:	mg/l
NOEC	:	= .6
LCEC	:	= 1.9
EC50	:	> 1.9 - 6
Analytical monitoring	:	yes
Method	:	OECD Guide-line 202, part 2 "Daphnia sp., Reproduction Test"
Year	:	1990
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	EC50 of immobilization/mortality or reproduction rate resp.: > 1.9 - < 6.0 mg/l; at 0.6 - 1.9 mg/l: decreasing of reproduction rate by 4.1 or 19.8 % resp.; at 6.0 - 60 mg/l: 100 % mortality after 5 d; HPLC-analysis
Result	:	No adult mortality up to 1.9 mg/l. All adults died within 7 days from 6 mg/l onwards.

Control

Day	Number of neonates per replicate							
	1	2	3	4	5	6	7	8
9	46	47	45	31	46	57	43	45
10								
11								
12	72	81	76	80	79	90	84	66
13								
14	19	8	25	37	263	3	18	19
15								
16	117	126	131	95	106	162	120	113
17								
18								
19	148	165	134	175	161	186	121	127
20								

21 125 41 3 74 53 38 46

mean 105.4 93.6 82.8 98.4 94.2 99.6 84.8 83.2

0.6 mg/l

Day	Number of neonates per replicate							
	1	2	3	4	5	6	7	8
9	33	44	16	22	48	32	10	21
10								
11								
12	77	75	78	68	78	62	70	58
13								
14	34	17	52	33	3	40	62	65
15								
16	119	100	62	102	108	116	111	86
17								
18								
19	112	121	88	144	141	112	107	106
20								
21	106	103	100	68	41	72	110	128

mean 96.2 92 79.2 87.4 83.8 86.8 94 92.8

1.9 mg/l

Day	Number of neonates per replicate							
	1	2	3	4	5	6	7	8
9	3							
10								
11								
12	51	56	37	37	60	38	36	40
13								
14	45	54	70	68	63	54	42	61
15								
16	93	125	106	102	126	63	100	38
17								
18								
19	119	96	63	55	77	110	78	145
20								
21		63	102	99	90	112	103	95

mean 62.2 78.8 75.6 72.2 83.2 75.4 71.8 75.8

EC50 immobilisation = 1.9 -6.0 mg/l
EC50 reproduction = 1.9-6.0 mg/l

Based on the results of a Dunnetts test the lowest concentration at which an effect was observed was 1.9 mg/l
Therefore the NOEC = 0.6 mg/l

Test condition : Daphnia magna STRAUS in parthenogenetically reproducing condition

Origin: Budesgesundheitsamtes, Berlin, Germany

Age at start of test: 6-24 h

test type: semistatic

19.7-21.8 degree C;

pH 7.7-8.8
O2 concentration: 8.2-10.5 mg/l

Test containers: glass beakers containing 250 ml of test solution. Not aerated.

Test medium: Elendt synthetic daphnid medium

Stock concentration: 300 mg/l

Lighting conditions: 16h:8h light:dark ratio

Light intensity: 2700 lux

No. of daphnids per group: 5

No. of reps per group: 8

Room temperature: 20-22°C

Feeding conditions: Concentrated algae (Scenedesmus subspicatus) cultures added to daphnid cultures at a specific number of cells per day (up to 6 700 000)

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
06.09.2001 (42)

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

Species : Lactuca sativa (Dicotyledon)
Endpoint : emergence
Exposure period : 3 day(s)
Unit : mg/l
Method : other
Year : 1965
GLP : no data
Test substance : other TS: no indication of purity

Result : No effect compared to negative control at 0.21 mg/l.
At 2.1 and 21.1 mg/l germination induction of 57 or 104 % respectively occurred compared to positive control.

In a test carried out in parallel using tobacco cells no influence of test concentrations on cell multiplication could determined;

Test condition : Effect of different test concentrations on germination of seedlings compared to a negative (1 ml water; 20 % germination rate) and a positive control (1 ml germ-inducing kinetin solution (5 * 10E-5 mol/l);

Temperature: 25 degree C

static test on prepared filter paper in the dark (preincubation for 24 h; afterwards illumination with red light; incubation for another 48 h)

Light intensity set to induce negative control germination rate of 20%
Positive control gave a corresponding 85-95 % germination at the same light intensity.

Conclusion : Induction of germination cannot be considered a valid endpoint for hazard assessment

Reliability : (3) invalid

06.09.2001 (43)

Species : Avena sativa (Monocotyledon)
Endpoint : growth
Exposure period : 16 day(s)
Unit : mg/kg soil dw
NOEC : = 316 measured/nominal
EC50 : = 1169 calculated
Method : other: BBA Guideline "Phtytotoxicity test to a monocotyledonous plant species (Avena sativa L.) and a dicotyledenous plant species (Brassica rapa ssp. rapa [DC.] Metzg.) " adopted March 1984
Year : 1995
GLP : no
Test substance : other TS: DPG purity 97%

Remark : Emergence not determined

Result :

All seedlings places in spiked or control soil emerged by day 9 of the test. All control plants emerged by day 3 of the test.

EC50 = 1169 mg/kg based on plant fresh weight
LOEC = 1000 mg/kg based on plant fresh weight
NOEC = 316 mg/kg based on plant fresh weight

LOEC based on observed toxic effects = 31.6 mg/kg - brown leaf tips observed in 12 out of 20 plants, however, at 10 mg/kg this symptom was noted in 3 out of 20 plants, while 1 out of 20 was observed to have yellow leaves.

LC50 cannot be calculated as no plant mortality was found at any concentration.

The only toxic effects noted at any of the concentrations in any of the plants were sedlings smaller than controls, brown leaf tips, yellowing of leaves (one plant at one concentration) and height of seedlings about 1 cm (significantly lower than control).

The reference substance (Trichloraoacetic acid)
NOEC 10 mg/kg dw
LOEC 100 mg/kg
Effect on mortality LC50 31.6 mg/kg
Based on observations on effcts the LOEC for TCA was 100 mg/kg

Validity criteria were respected (fresh wt of controls >800 mg and control produced 100% healthy seedling

Test condition : Administration method:
Seeds places on moist filter paper and placed in closed stainless steel vessels at room temperature in the dark for 53 h prior to test

Germinated seeds were used for the test

5 germinated seeds per vessel, 4 replicates per concentration

Concentrations:
Test substance
0 1, 3.16, 10, 31.6, 100, 316, 1000 mg/kg soil (dry wt)

Reference substance (Trichloroacetic acid)
0.1, 1, 10, 100, 1000 mg/kg soil

The concentrations were applied once at test initiation.

Total test exposure time was 16 days equivalent to 14 days after emergence of 50% of the control

Light/dark cycle: 16/8 h
intensity:
280-290 µE/sec.m2 (400-700 nm) first 6 d
210-220 µE/sec.m2 (400-700 nm) T7-10 d
160-170 µE/sec.m2 (400-700 nm) T10-16

Temperature: mean 23.1°C max. 30.2°C min 15.2°C (recorded every 30 min)

Containers:
Plastic rectangular beakers 7X10X10

Soil: standard unsterilised
OC content 2.32+/-0.38%
Particle size <0.02 mm) 12.1 +/-2.3%
pH value 5.6+/ - 0.2
Total N 0.23+/ -0.03% dw
max water content 48+/ -7 g/100g dw

Water loss was compensated by daily addition of demineralised water

Satistics: ANOVA with Bonferroni multiple range test for growth test results for NOEC

Probit analysis using Finey's method for EC50s

Conclusion : No analysis of test concentrations and no conclusions provided on the results of the reference substance

However, given the stability of the substance and the details provided in the study report, the test is considered valid.

Reliability : (1) valid without restriction
Flag : Critical s tudy for SIDS endpoint
06.09.2001 (44)

Species : Brassica rapa (Dicotyledon)
Endpoint : growth
Exposure period : 16 day(s)
Unit : mg/kg soil dw
NOEC : = 100 measured/nominal
EC50 : = 358 calculated
Method : other: BBA Guideline "Phtytotoxicity test to a monocotyledonous plant species (Avena sativa L.) and a dicotyledenous plant species (Brassica rapa ssp. rapa [DC.] Metzg.) " adopted March 1984
Year : 1995
GLP : no

Test substance : as prescribed by 1.1 - 1.4

Remark : Emergence not determined

Result : All seedlings places in spiked or control soil emerged by day 9 of the test. All control plants emerged by day 3 of the test.

EC50 = 358 mg/kg based on plant fresh weight
LOEC = 316 mg/kg based on plant fresh weight
NOEC = 100 mg/kg based on plant fresh weight

LOEC based on observed toxic effects = 100 mg/kg - dry leaf edges observed in 12 out of 20 plants, however, at 31.6 mg/kg this symptom was noted in 1 out of 20 plants.

LC50 cannot be calculated as no plant mortality was found at any concentration.

The toxic effects noted at any of the concentrations in any of the plants were seedlings smaller than controls, yellow leaf edges, yellowing of leaves (1 out of 20 was observed to have yellow leaves at one time point at one concentration) dry leaf tips or leaves, and at the highest concentration only, height of seedlings about 1 cm (significantly lower than control) and chlorosis of the leaves.

The reference substance (Trichloraoacetic acid)
NOEC 10 mg/kg dw
LOEC 100 mg/kg
Effect on mortality LC50 31.6 mg/kg
Based on observations on effcts the LOEC for TCA was 100 mg/kg

Validity criteria were respected (fresh wt of controls >800 mg and control produced >80% healthy seedlings

Test condition : Administration method:
Seeds places on moist filter paper and placed in closed stainless steel vessels at room temperature in the dark for 53 h prior to test

Germinated seeds were used for the test

5 germinated seeds per vessel, 4 replicates per concentration

Concentrations:
Test substance
0 1, 3.16, 10, 31.6, 100, 316, 1000 mg/kg soil (dry wt)

Reference substance (Trichloroacetic acid)
0.1, 1, 10, 100, 1000 mg/kg soil

The concentrations were applied once at test initiation.

Total test exposure time was 16 days equivalent to 14 days after emergence of 50% of the control

Light/dark cycle: 16/8 h
intensity:
280-290 µE/sec.m2 (400-700 nm) first 6 d
210-220 µE/sec.m2 (400-700 nm) T7-10 d
160-170 µE/sec.m2 (400-700 nm) T10-16

Temperature: mean 23.1°C max. 30.2°C min 15.2°C (recorded every 30 min)

Containers:
Plastic rectangular beakers 7X10X10

Soil: standard unsterilised
OC content 2.32+/-0.38%
Particle size <0.02 mm) 12.1 +/-2.3%
pH value 5.6+/ - 0.2
Total N 0.23+/-0.03% dw
max water content 48+/ -7 g/100g dw

Water loss was compensated by daily addition of demineralised water

Satistics: ANOVA with Bonferroni multiple range test for growth test results for NOEC

Probit analysis using Finey's method for EC50s

Conclusion : No analysis of test concentrations and no conclusions provided on the results of the reference substance

However, given the stability of the substance and the details provided in the study report, the test is considered valid without restriction.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
07.09.2001 (44)

Species : other terrestrial plant: Vicia faba
Endpoint : other
Exposure period :
Unit : mg/l
Method : other
Year : 1972
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : Influence of 1-10 mg/l test substance in vitro on mitosis-cycle (G1-,
S- and G2-phase) in root cells of the field bean (Vicia faba).

Cells were pretreated for 3 h in 0.02 % colchicine, and transferred to aerated water for 5, 10 or 16 h to allow the cells to reach different stages of DNA sythesis at the time of treatment.
The cells were exposed to 0.1, 1.0, 5.0 or 10 mg/l for 30 mins under intensive aeration; after
washing the cells were transferred into aerated water again for another 5-29 h.
The nmitosis index was calculated

Result : A reduced mitosis index was observed.
0.43-9.48 with concentration dependence;
control: 13.94
aberrations of chromosomes at 5-55 % depending on mitotic phase of cells was also noted

Conclusion : in vitro study not suitable for use in hazard assessment - inappropriate methodology

Reliability : (3) invalid

06.09.2001 (45)

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

Type : artificial soil
Species : soil dwelling microorganisms
Endpoint : other
Exposure period :
Unit :
Method : other
Year : 1984
GLP : no data
Test substance : other TS: no indication of purity

Remark : Effect of test substance (concentration not specified) on growth rate of soil microorganisms both applied on a polycarbonate membrane or embeded in a epoxyde resin (Araldit) on aluminium foil resp.; indirect (by membrane pores) or direct contact with a not specified soil (moistured John Innes No. 1 soil) resp.; no colony formation within the test period of 3 months; no further information available
Conclusion : Not valid for hazard assessment - inappropriate methodology
Reliability : (3) invalid
06.09.2001 (46)

Type : other
Species : soil dwelling microorganisms
Endpoint : mortality
Exposure period : 4 day(s)
Unit : other
LC50 : <= .1
Method : other
Year : 1984
GLP : no data
Test substance : other TS: no indication of purity

Remark : Inoculation of aqueous soil extract (3.0 * 10E+7 ind./ml) in nutrient agar containing the test substance (concentration not specified but probably O.1%); incubation for 4 and 14 d; LD50: < or = 0.1 % referred to growth rate of control; no further information available
Test condition : 25 degree C
Conclusion : Single effect concentration providing response of LD50 > or = 1 g/l.
Reliability : (2) valid with restrictions
06.09.2001 (46)

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

Species : other avian: see remarks
Endpoint : mortality
Exposure period :
Unit : mg/kg bw
LC50 : > 100
Method : other
Year : 1983
GLP : no data

Test substance : other TS: no indication of purity

Remark : No acute effects at max. applied dose (100 mg/kg bw by gavage) on
three species of songbirds: red winged blackbirds (Agelaius phoeniceus), Starlings (Sturnus
vulgaris) in 1971 and Passer domesticus in 1983; single oral application of
test substance (dissolved in propylene glycol) after a settling in-phase of 2-6 weeks;
no further information available

Reliability : (2) valid with restrictions
06.09.2001 (47) (48)

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 : = 290 - 420 mg/kg bw
Species : rat
Strain :
Sex : male/female
Number of animals : 30
Vehicle : other: 5.0% in corn oil
Doses :
Method : other: Younger Laboratory standard protocol
Year : 1977
GLP : no
Test substance : no data

Result : LD50 = 350 mg/kg.

Signs of intoxication: reduced appetite and activity (one to three days in survivors), increasing weakness, collapse, and death.
Gross autopsy of decedents: hemorrhagic areas of the lungs, liver discoloration and gastrointestinal inflammation.
Source : MLPC, Rion-des-Landes, France
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
06.09.2001 (49)

Type : LD50
Value : = 320 - 662 mg/kg bw
Species : rat
Strain :
Sex : male
Number of animals : 100
Vehicle : other: corn oil
Doses :
Method : other
Year : 1977
GLP : no
Test substance : no data

Result : LD50 = 460 mg/kg

Observed toxic symptoms;
100 mg/kg: slight ataxia;
130 mg/kg: at 20-30 min. p.a. decrease of spontaneous motor activity, irregular respiration and hind limb ataxia (symptoms disappeared within 1 day);
>=170 mg/kg: in addition to above toxic symptoms, dyspnea and ataxia were observed, death occurred mostly 1-4 h p.a., toxic symptoms in surviving animals disappeared within 2-3 days.
Source : MLPC, Rion-des-Landes, France
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
06.09.2001 (50)

Type : LD50
Value : = 309 - 477 mg/kg bw
Species : rat
Strain :
Sex : female
Number of animals : 100
Vehicle : other: corn oil
Doses :
Method : other
Year : 1977
GLP : no
Test substance : no data

Result : LD50 = 384 mg/kg.

Observed toxic symptoms;
100 mg/kg: slight ataxia;
130 mg/kg: at 20-30 min. p.a. decrease of spontaneous motor activity, irregular respiration and hind limb ataxia (symptoms disappeared within 1 day);
>=170 mg/kg: in addition to above toxic symptoms, dyspnea and ataxia were observed, death occurred mostly 1-4 h p.a., toxic symptoms in surviving animals disappeared within 2-3 days.

Source : MLPC, Rion-des-Landes, France
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
06.09.2001 (50)

Type : LD50
Value : = 850 mg/kg bw
Species : rat
Strain :
Sex : male/female
Number of animals : 21
Vehicle : CMC
Doses :
Method : other
Year :
GLP : no
Test substance : no data

Result : Survival time was 2 to 24 hours. In some cases, animals were prostrated in 15 minutes and remained in a comatose condition for several hours although death did not always result. A short period of convulsions preceded most death. At autopsy the mucosa of the stomach and intestinal tract were found to be severely irritated and the liver and spleen were exceptionally dark.

Source : MLPC, Rion-des-Landes, France
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
06.09.2001 (51)

Type : LD50
Value : = 375 mg/kg bw
Species : rat
Strain :
Sex : no data
Number of animals :
Vehicle : no data

Doses :
Method : other
Year :
GLP : no
Test substance : no data

Remark : Symptoms of toxicity: increased muscle tension, spasms of the limbs, liver damage (no further information available)
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (52) (53)

Type : LD50
Value : ca. 500 mg/kg bw
Species : rat
Strain :
Sex : no data
Number of animals : 7
Vehicle : other: propylene glycol
Doses :
Method : other
Year : 1947
GLP : no
Test substance : no data

Source : MLPC, Rion-des-Landes, France
Reliability : (3) invalid
06.09.2001 (54)

Type : other: minimum lethal dose
Value :
Species : rat
Strain :
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Method : other
Year : 1971
GLP : no
Test substance : no data

Remark : 1 % emulsion was given to adult rats and to 2-week-old rats; symptoms of toxicity appeared rapidely (20-30 min.) and included unsteady walk and flabbiness, followed by spasmodic jerking of the limbs and sharp increases of pain sensitivity, the body muscles were tensed; death occurred during the first and second day of the experiment; young rats were more sensitive than adult animals (no further information available).
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (55)

Type : other: maximum tolerable dose
Value : = 250 mg/kg bw
Species : rat
Strain :
Sex : no data
Number of animals :
Vehicle : no data
Doses :

Method : other
Year :
GLP : no
Test substance : no data

Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (52) (56)

Type : LDLo
Value : = 500 mg/kg bw
Species : rat
Strain :
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Method : other
Year :
GLP : no
Test substance : no data

Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (52)

Type : LD50
Value : = 323 mg/kg bw
Species : rat
Strain :
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Method : other
Year :
GLP : no
Test substance : no data

Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (57)

Type : LD50
Value : = 290 mg/kg bw
Species : mouse
Strain :
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Method : other
Year :
GLP : no
Test substance : no data

Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (53)

Type : LD100
Value : = 450 mg/kg bw

Species : mouse
Strain :
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Method : other
Year :
GLP : no
Test substance : no data

Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
26.06.2001 (53)

Type : other: maximum tolerated dose
Value : = 250 mg/kg bw
Species : mouse
Strain :
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Method : other
Year :
GLP : no
Test substance : no data

Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (52) (56)

Type : LD50
Value : = 258 mg/kg bw
Species : mouse
Strain :
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Method : other
Year :
GLP : no data
Test substance : no data

Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
26.06.2001 (57)

Type : LD50
Value : = 520 mg/kg bw
Species : mouse
Strain :
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Method : other
Year :
GLP : no data
Test substance : no data

Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (58)

Type : LD50
Value :
Species : mouse
Strain :
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Method : other
Year :
GLP : no
Test substance : no data

Remark : LD50 (m): 150 (120-188) mg/kg bw
LD50 (f): 211 (176-253) mg/kg bw
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (59)

Type : LD50
Value : = 246 mg/kg bw
Species : rabbit
Strain :
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Method : other
Year :
GLP : no
Test substance : no data

Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (57)

Type : other: minimal lethal dose
Value : = 250 mg/kg bw
Species : rabbit
Strain :
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Method : other
Year :
GLP : no
Test substance : no data

Source : MLPC, Rion-des-Landes, France
Reliability : (3) invalid
27.12.2000 (60)

Type : LD50
Value : = 250 mg/kg bw
Species : rabbit
Strain :
Sex : no data

Number of animals :
Vehicle : no data
Doses :
Method : other
Year :
GLP : no
Test substance : no data

Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (61)

Type : LD50
Value : = 250 mg/kg bw
Species : guinea pig
Strain :
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Method : other
Year :
GLP : no
Test substance : no data

Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (61)

Type : other: minimal lethal dose
Value : = 250 mg/kg bw
Species : guinea pig
Strain :
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Method : other
Year :
GLP : no
Test substance : no data

Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (60)

Type : other: emetic dose
Value : = 10 mg/kg bw
Species : dog
Strain :
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Method : other
Year :
GLP : no
Test substance : no data

Remark : DPG is a powerful emetic in single oral doses as little as 10 mg/kg
Source : MLPC, Rion-des-Landes, France

Reliability : (4) not assignable
27.12.2000 (58)

5.1.2 ACUTE INHALATION TOXICITY

Type : LC0
Value : = .5 mg/l
Species : other: not specified
Strain :
Sex : no data
Number of animals :
Vehicle :
Doses :
Exposure time : 30 minute(s)
Method : other: no detail available
Year : 1949
GLP : no
Test substance : no data

Remark : Slight hypnotic effect (no further information available)
Source : MLPC, Rion-des-Landes, France
Reliability : (3) invalid
06.09.2001 (62)

5.1.3 ACUTE DERMAL TOXICITY

Type : LD0
Value : > 2000 mg/kg bw
Species : rabbit
Strain :
Sex : male/female
Number of animals : 10
Vehicle : other: none
Doses :
Method : other: EEC N°L251/103 part B3, Sept 1984
Year : 1991
GLP : yes
Test substance : no data

Result : No mortality occured during the study. The most notable clinical signs were generally limited to transient dermal irritation at the site of test article application. Body weight gain was exhibited by all animals during the study. At necropsy on day 15, the pancreas or pancreatic lymph nodes of 4/10 animals were noted to have an abnormal red discoloration. The cause of this finding could not be determined.
Source : MLPC, Rion-des-Landes, France
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
06.09.2001 (63)

Type : LD50
Value : > 794 mg/kg bw
Species : rabbit
Strain :
Sex : male/female
Number of animals : 6

Vehicle	:	other: 10% in corn oil
Doses	:	
Method	:	other
Year	:	1977
GLP	:	no
Test substance	:	no data
Result	:	Signs of intoxication: reduced appetite and activity (2 to 3 days in survivors), increasing weakness, paralysis, collapse and death. Gross autopsy in decedents: liver and spleen discoloration, enlarged gall blader and slight gastrointestinal inflammation.
Source	:	MLPC, Rion-des-Landes, France
Reliability	:	(3) invalid

06.09.2001 (64)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Type	:	LD50
Value	:	ca. 25 - 50 mg/kg bw
Species	:	mouse
Strain	:	
Sex	:	no data
Number of animals	:	
Vehicle	:	no data
Doses	:	
Route of admin.	:	i.p.
Exposure time	:	
Method	:	no data
Year	:	
GLP	:	no
Test substance	:	no data
Source	:	MLPC, Rion-des-Landes, France
Reliability	:	(4) not assignable

27.12.2000 (65)

Type	:	LD50
Value	:	= 75 mg/kg bw
Species	:	mouse
Strain	:	
Sex	:	no data
Number of animals	:	
Vehicle	:	no data
Doses	:	
Route of admin.	:	i.p.
Exposure time	:	
Method	:	no data
Year	:	
GLP	:	no
Test substance	:	no data
Source	:	MLPC, Rion-des-Landes, France
Reliability	:	(4) not assignable

27.12.2000 (66)

Type	:	LD100
Value	:	= 50 mg/kg bw
Species	:	mouse
Strain	:	
Sex	:	no data

Number of animals :
Vehicle : no data
Doses :
Route of admin. : s.c.
Exposure time :
Method : no data
Year :
GLP : no
Test substance : no data
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (66)

Type : LDLo
Value : = 50 mg/kg bw
Species : rat
Strain :
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Route of admin. : s.c.
Exposure time :
Method : no data
Year :
GLP : no
Test substance : no data
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (67)

Type : LDLo
Value : = 200 mg/kg bw
Species : guinea pig
Strain :
Sex : no data
Number of animals :
Vehicle : other: propylene glycol
Doses :
Route of admin. : s.c.
Exposure time :
Method : no detail available
Year : 1949
GLP : no
Test substance : no data
Source : MLPC, Rion-des-Landes, France
Reliability : (3) invalid
06.09.2001 (62)

Type : other
Value :
Species : rabbit
Strain :
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Route of admin. : s.c.
Exposure time :
Method : no data
Year :
GLP : no

Test substance : no data
Remark : 10 mg/kg: no symptoms of toxicity
20 mg/kg: convulsions, dyspnoea, rapid breathing 1 h p.a.
50 mg/kg: convulsions, dyspnoea, rapid breathing, prostration 20 min p.a.
DPG-HCl-solution was applied; 1 animal/dose was treated
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (67)

Type : other
Value :
Species : rabbit
Strain :
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Route of admin. : s.c.
Exposure time :
Method : no data
Year :
GLP : no
Test substance : no data
Remark : Influence on blood sugar levels was investigated;
100 mg/kg: transient slight hypoglycemia, followed by a more intense hyperglycemia
50 mg/kg: hypoglycemia developed more slowly but lasted longer
20 mg/kg: hypoglycemia appeared less constantly
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (62)

Type : LDLo
Value : = 25 mg/kg bw
Species : dog
Strain :
Sex : no data
Number of animals :
Vehicle : other: propylene glycol
Doses :
Route of admin. : i.v.
Exposure time :
Method : no detail available
Year : 1949
GLP : no
Test substance : no data
Source : MLPC, Rion-des-Landes, France
Reliability : (3) invalid
06.09.2001 (62)

Type : other
Value : = 1 mg/kg bw
Species : rabbit
Strain :
Sex : female
Number of animals :
Vehicle : no data
Doses :
Route of admin. : i.v.
Exposure time :
Method : no data

Year	:	
GLP	:	no
Test substance	:	no data
Remark	:	2 females were treated. Symptoms of toxicity (circulatory and respiratory effects): fall in blood pressure, decrease in heart rate, the amplitude of respiration was slightly affected.
Source	:	MLPC, Rion-des-Landes, France
Reliability	:	(4) not assignable

27.12.2000 (67)

5.2.1 SKIN IRRITATION

Species	:	rabbit
Concentration	:	undiluted
Exposure	:	Occlusive
Exposure time	:	24 hour(s)
Number of animals	:	6
Vehicle	:	
PDII	:	0
Result	:	not irritating
Classification	:	not irritating
Method	:	Draize Test
Year	:	
GLP	:	no
Test substance	:	no data
Method	:	0.5 g was applied as finely ground sample moistened with water
Remark	:	Primary irritation index = 0.0/8.0
Source	:	MLPC, Rion-des-Landes, France
Reliability	:	(2) valid with restrictions

06.09.2001 (68)

Species	:	rabbit
Concentration	:	
Exposure	:	no data
Exposure time	:	
Number of animals	:	
Vehicle	:	
PDII	:	
Result	:	slightly irritating
Classification	:	
Method	:	Draize Test
Year	:	
GLP	:	no
Test substance	:	no data
Remark	:	no further information available
Source	:	MLPC, Rion-des-Landes, France
Reliability	:	(4) not assignable

26.06.2001 (66)

5.2.2 EYE IRRITATION

Species	:	rabbit
Concentration	:	undiluted
Dose	:	20 other: mg

Exposure time	:	24 hour(s)
Comment	:	not rinsed
Number of animals	:	6
Vehicle	:	
Result	:	sli ghtly irritating
Classification	:	not irritating
Method	:	Draize Test
Year	:	
GLP	:	no
Test substance	:	no data
Remark	:	Primary irritation indice : 20.2/110
Source	:	MLPC, Rion-des-Landes, France
Reliability	:	(2) valid with restrictions

06.09.2001 (69)

Species	:	rabbit
Concentration	:	undiluted
Dose	:	100 other: mg
Exposure time	:	24 hour(s)
Comment	:	not rinsed
Number of animals	:	6
Vehicle	:	
Result	:	irritating
Classification	:	irritating
Method	:	Draize Test
Year	:	
GLP	:	no
Test substance	:	no data
Result	:	Primary irritation indice : 47.6/110
Source	:	MLPC, Rion-des-Landes, France
Reliability	:	(2) valid with restrictions

06.09.2001 (70)

Species	:	rabbit
Concentration	:	
Dose	:	
Exposure time	:	
Comment	:	
Number of animals	:	
Vehicle	:	
Result	:	irritating
Classification	:	
Method	:	Draize Test
Year	:	
GLP	:	no
Test substance	:	no data
Remark	:	Effects were reversible (48 h); no further information available
Source	:	MLPC, Rion-des-Landes, France
Reliability	:	(4) not assignable

26.06.2001 (66)

5.3 SENSITIZATION

Type	:	Guinea pig maximization test
Species	:	guinea pig
Concentration	:	1st: Induction 1 % intracutaneous

2nd: Induction 25 % occlusive epicutaneous
3rd: Challenge 25 % occlusive epicutaneous

Number of animals : 10
Vehicle : other: paraffin oil
Result : not sensitizing
Classification : not sensitizing
Method : OECD Guide-line 406 "Skin Sensitization"
Year : 1992
GLP : yes
Test substance : other TS: purity 99.9%

Method : Fifteen guinea-pigs were allocated to 2 groups: a control group 1 of 5 females and a treated group 2 of 10 females. The sensitization potential of the test substance was evaluated after a 10-day induction period during which time the animals were treated with paraffin oil (control group) or the test substance (treated group). On day 1, in presence of Freund's complete adjuvant, 0.1 ml of the test substance at a concentration of 1 % (w/w) in the vehicle was administered by intradermal route. On day 8, 0.5 ml of the test substance at a concentration of 25% (w/w) in the vehicle was applied by cutaneous route during 48 hours by means of an occlusive dressing. After a period of 12 days without treatment, a challenge cutaneous application of 0.5 ml of the vehicle (left flank) and 0.5 ml of the test substance at a concentration of 25% (w/w) in the vehicle (right flank) were administered to all animals. The test substance and the vehicle were prepared on a dry gauze pad then were applied to the skin and held in place for 24 hours by means of an occlusive dressing. Cutaneous reactions on the challenge application sites were then evaluated 24 and 48 hours after removal of the dressing.

After the final scoring period, the animals were killed. Due to the absence of cutaneous reactions, no skin samples were taken from the challenge application sites from all the animals.

The sensitivity of the guinea-pigs in C.I.T. experimental conditions were checked in recent studies with a positive sensitizer: Dinitro-2,4-Chlorobenzene.

Result : No clinical signs and no deaths were noted during the study. After 24 and 48 hours following removal of the dressing of the cutaneous challenge application of the test substance, no cutaneous reactions were recorded.
The guinea-pigs which were used in recent studies showed a satisfactory sensitization response
Source : MLPC, Rion-des-Landes, France
Conclusion : According to the maximization method established by Magnusson and Kligman, no cutaneous reactions attributable to the sensitization potential of 1,3-DIPHENYLGUANIDINE (DPG), at the concentration of 25% (w/) were observed in guinea-pigs.
Reliability : (1) valid without restriction
27.12.2000 (71)

5.4 REPEATED DOSE TOXICITY

Type :
Species : rat

Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	oral feed
Exposure period	:	14 days
Frequency of treatm.	:	continously in diet
Post exposure period	:	none
Doses	:	300, 500, 800, 1500 and 3000 ppm (approx. 36, 56, 73, 119 or 200 mg/kg/day)
Control group	:	yes, concurrent no treatment
LOAEL	:	= 300 ppm
Method	:	other: range finding study
Year	:	1980
GLP	:	yes
Test substance	:	no data

Method : Groups of 5 male and 5 female CD rats were doses with DPG orally via diet for 2 weeks at constant dose levels of 0, 300, 500, 800, 1500 and 3000 ppm. At the end of this period all the surviving rats were killed and subjected to a macroscopic post-mortem examination.

Result : Mortality
There were 5 premature decedents (2M and 3F)during the second week of dosing. Three rats were killed in extremis and 2 brats were found dead in their cages. All the premature decedents were in the top dose group receiving 3000 ppm DPG.

Clinical Signs
No abnormalities were detected in the gr oups receiving 0, 300 and 500 ppm DPG. Animals receiving 800 ppm appeared slightly emaciated during week 2 of dosing. A reduction in body tone, piloerection and emaciation was observed in animals receiving 1500 ppm.
The animals receiving 3000 ppm DPG showed an initial reduction in body tone leading to extreme emaciation by the end of the 2 week dosing period. Ataxia, piloerection, hunched posture and subdued appearance were observed in some animals. Hair loss was observed on the abdomens of 2 animals. One animal was observed having uncontrollable muscular spasms and another one having convulsive fits on day 11 of dosing. Both animals were killed in extremis. Hypersensitivity to audio stimuli was observed in the surviving animals during the last 3 days of treatment.

Body Weight
Clear dose related reductions in body weight gain were observed. Animals receiving 3000 ppm DPG showed an actual reduction in body weight over the course of the 2 week dosing period.

Food Consumption
Dose related reductions in food consumption were observed.

Water Consumption
No differences were detected between control animals and those treated with DPG.

Terminal Studies
No significant gross lesions were present in DPG treated animals.
At 3000 ppm, post mortem examinations revealed reduced spleen size in 2 males and 3 females. A reduction in the

size of the thymus was also observed in one male and one female. The bladder of one female was distended with dark red urine.
Hydronephrosis was observed in the left kidney of one female rat at 800 ppm. Small seminal vesicles were observed for one male at 3000 ppm.

Organ Weights
Males : slight reductions were observed for the absolute weight of the heart and kidneys (p<0.01) and for the liver and spleen (p<0.05) in animals receiving 500 ppm DPG. Statistically significant reductions (p<0.001) for the absolute weights of the heart, liver, kidneys and spleen were observed in animals receiving 800, 1500 and 3000 ppm. Animals receiving 1500 and 3000 ppm also showed significant reductions in absolute lung (p<0.01) and brain (p<0.05) weights.
Relative organ weight analysis showed significant increases in brain weight in all groups dosed with DPG. The relative weights of the ardrenals in animals receiving 1500 ppm were also increased.

Females : absolute organ weight analysis showed slight reductions (p<0.05) for the brain, liver and lungs in animals receiving 800 ppm and for the liver only in animals receiving 500 ppm. Significant reductions for heart and liver (p<0.001) and slight reductions for adrenals, kidneys, lungs and spleen were observed in animals receiving 1500 ppm. Absolute weights for the brain, heart and spleen were significantly reduced (p<0.001) whilst kidneys and lungs were slightly reduced (p<0.05) in animals receiving 3000 ppm DPG.
Relative organ weight analysis showed a statistically significant increase in brain weights (p<0.001) for animals receiving 500 and 1500 ppm DPG.

The differences observed in absolute and relative organ weight profiles between control animals and those treated with DPG are attributed to the reduction in body weight gain shown by the DPG treated animals.

Source : MLPC, Rion-des-Landes, France

Conclusion : Dosing rats with DPG caused reduced body tone and emaciation at dose levels of 800 ppm and above. Ataxia, piloerection, hunched posture and subdued appearance were also observed in animals receiving 3000 ppm. There were 5 premature decedents in the 3000 ppm dose group. Dose related reductions in food consumption with corresponding decreases in body weight gain were observed in animals receiving DPG. The significant differences observed in organ weight profiles, between control and DPG treated animals, are attributed to the above mentioned reduction in body weight gain.
There is little doubt that the above effects were the direct result of the administration of DPG, it can, however, be speculated that poor palatability of the DPG/diet mixture may have been a contributory factory, the degree of such a contribution being unascertained.
Gross pathological examination did not reveal any gross lesions in the DPG treated animals.

Reliability : (1) valid without restriction
29.10.2001 (72)

Type :

Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	oral feed
Exposure period	:	90 days
Frequency of treatm.	:	continously in diet
Post exposure period	:	none
Doses	:	50, 150 and 500 ppm (approx. 4, 11 or 37 mg/kg/day)
Control group	:	yes, concurrent no treatment
NOAEL	:	= 150 ppm
Method	:	OECD Guide-line 408 "Subchronic Oral Toxicity - Rodent: 90-day Study"
Year	:	1981
GLP	:	yes
Test substance	:	other TS: Monsanto, batch LLN/AS/811920, 97.7%

Method : Groups of 15 male and 15 female rats were administered 0, 50, 150 or 500 ppm 1,3-diphenylguanidine in feed that was available ad libitum forn 13 weeks.

Rats were housed 3 per cage. Animal rooms were maintained at a target temperature of 20°C and a target relative humidity of 50%, with 12 hours of fluorescent light per day and approximately 14 air changes per hour. Feed and water were available ad libitum.

Hematology, clinical chemistry and urinalysis evaluations were performed on 10 rats per sex and exposure level at weeks 6 and 13. Hematology parameters evaluated included: hemoglobin, erythrocytes, packed cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, platelets and leukocyte count and differential. Clinical chemistry parameters evaluated included: urea nitrogen, total protein, albumin, cholesterol, glucose, sodium, potassium, calcium, chloride, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and lactate dehydogenase. Urinalysis parameters evaluated included: glucose, protein, ketones, urobilinogen, volume/colour/appearence, specific gravity pH, blood pigment and microscopic examination.

Complete necropsies were performed on all animals. The heart, kidneys, liver, lungs, ovaries, prostate gland, spleen, testis, epididymides, thymus, pituitary, adrenals, and thymus were removed and weighed. Organs and tissues were examined for gross lesions and fixed in 10% neutral buffered formalin. Tissues to be examined microscopically were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Complete histopathologic examinations were performed on all rats in the 0 and 500 ppm groups. Gross lesions and selected tissues were examined in the lower exposure groups. The following tissues were examined: adrenal glands, aortic arch, bladder, brain (three sections), eyes, gross lesions, heart, intestines (caecum, colon, duodenum, jejunum, ileum), kidneys, liver, lung/mainstem bronchi, lymph nodes, skin, spleen, spinal cord/sciatic nerve, stomach (forestomach and glandular stomach), testes (with epididymis and seminal vesicle), thymus, thyroid glands, tongue, trachea and uterus.

Result : OBSERVATION
Mortality
There were 2 unscheduled deaths during the course of the

study: 1 male receiving 500 ppm DPG (in Week 4) and 1 female receiving untreated diet (in Week 13). Neither death could be attributed to dosing with the test compound.

Clinical Signs
There were no clinical signs observed that could be attributed to dosing with the test compound.

Body Weights
Males : Rats receiving 500 ppm DPG showed a lower group mean body weight than the control rats from Week 1 of dosing, whereas the other dose groups tended to perform in a similar fashion to the controls. The reduction in the top dose animals was roughly 15% (P<0.001) for the majority of the dosing period. The exceptions were Week 9 (2%, P<0.001), Week 10 (19%, P<0.001) and Week 13 (21%, P<0.001).
On the first occasion (Week 9) all groups receiving DPG showed a dose related deviation from the trends showed prior to and after this week. Apart from a depression in food consumption in all groups during Week 9 there appeared to be no obvious cause of this effect. A similar effect was seen in rats receiving 500 ppm DPG only in Week 13; again no cause was evident.

Females :Rats receiving 50 or 150 ppm DPG tended to perform in a similar fashion to the control rats.
The rats receiving 500 ppm DPG showed a significant difference from the controls from Week 1 of dosing (9% reduction, P<0.001). The effect tended to increase as the study went on, reaching a maximum in Week 9 (18% reduction, P<0.001). After this the effect tended to stabilise at approximately 16% (P<0.001).
In Week 9, all groups (including the controls) showed a body weight reduction, followed by a recovery in Week 10. As in the males, there was an accompanying food consumption decrease, but no obvious cause was discovered.

Food Consumption
Male and female rats receiving 500 ppm DPG showed a reduced food consumption compared with the controls over the dosing period whereas rats receiving 50 or 150 ppm DPG tended to consume comparable quantities to the controls.
In Weeks 9 and 13 all groups showed a reduced food consumption when compared to other week's data, no reason for this change could be discovered.

Water Consumption
No intergroup differences were observed.

LABORATORY INVESTIGATIONS
Haematology
Males:
Week 6 - There were slight dose related reductions in both white blood cell count and platelets (11% and 8% respectively when comparing rats receiving 500 ppm DPG and controls; neither being statistically significant). These effects are considered to be of little biological significance due to the large degree of variation between the individuals in any one group.
Week 13 - The slight effects seen at Week 6 were not evident. A doubling of the monocyte count (P<0.05) in rats

receiving 500 ppm DPG when compared to the controls, is thought to be of little biological significance.

Females:
Week 6 - There was an increase in white blood cell count (13% in rats receiving 500 ppm DPG when compared to controls), but the large degree of intergroup variation suggests that it is of little biological significance.
Week 13 - Again rats receiving 500 ppm DPG showed an increased white blood cell count when compared to controls, but again there was a large intergroup variation.

Clinical Chemistry
Males:
Week 6 - Rats receiving 500 ppm DPG showed higher BUN levels than controls (13%, P<0.01). Alanine aminotransferase levels showed a slight dose related increase (16%, P<0.05 in rats receiving 500 ppm DPG when compared to controls).
Differences were also seen in alkaline phosphatase (P<0.01) and sodium levels (P<0.05) in rats receiving 500 ppm DPG when compared to controls.
All the results were within expected levels and are thought to be of no biological significance.
Week 13 - The effects seen at Week 6 were not evident.
Differences were seen in aspartate aminotransaminase (P<0.01) and potassium levels (P<0.01) in rats receiving 150 ppm DPG when compared to controls, but the effects are considered of little biological significance. All results were within expected ranges.

Females:
Week 6 - Alkaline phosphatase was increased (P<0.05) in rats receiving 500 ppm DPG, when compared to controls. Reductions were evident in rats receiving 500 ppm DPG, when compared to controls in chloride (P<0.05), total protein (P<0.05), albumin (P<0.01) and calcium levels (P<0.05). Most of the results obtained were within expected ranges and are thought to be of no biological significance.
Rats receiving 50 ppm DPG showed reduced BUN levels (P<0.05) when compared to controls, but this is considered not to be of biological significance.
Week 13 - The effects seen at Week 6 were again evident in chloride (P<0.05), total protein (P<0.05) and albumin levels (P<0.01) in rats receiving DPG, but the reductions were so small as to be of little biological significance. In addition, potassium levels (P<0.05) were increased in rats receiving 500 ppm DPG and calcium levels (P<0.05) decreased in rats receiving 50 ppm DPG, when compared to controls. In both cases the changes were of no biological significance.

Urinalysis
Males:
Week 6 - There was a higher incidence of glucose found in the urine of rats receiving DPG than in controls. The incidence of ketones in the urine was also increased. There was a slight increase in specific gravity and a reduction in volume of urine produced by rats receiving 500 ppm DPG when compared to controls.
All tests for faecal occult blood were negative.
Week 13 - The dose related occurrence of glucose and ketones evident in the urine at Week 6 were not seen again.
The increase in specific gravity and decrease in volume were

present again in rats receiving 500 ppm DPG.
There was a slight dose related decrease in pH noted.

Females:
Week 6 - No significant intergroup differences were observed. All tests for faecal occult blood were negative.
Week 13 - There was a slight increase in specific gravity in rats receiving 500 ppm DPG when compared to controls. Slight dose related decreases in pH and volume were also seen.

TERMINAL STUDIES
Organ Weights
Males: The absolute organ weights of rats receiving 500 ppm DPG tended to be reduced when compared to controls. In the case of heart (17%), kidney (14%) , liver (23%) and spleen (25%) the reductions were highly statistically significant (P<0.001). Absolute lung weight was also decr eased (14%, P<0.01). The absolute brain weight of rats receiving 50 ppm DPG was increased (3%, P<0.05) when compared to controls, but this is thought not to be biologically significant.
After the body weight effects were taken into account the relative organ weights of rats receiving 500 ppm DPG tended to be increased when compared to controls. Brain (27%) and testes (25%) were found to be highly statistically significant (P<0.001). Heart (7%, P<0.01), kidneys (10%, P<0.01), lungs (9%, P<0.01), adrenals (17%, P<0.05) and pituitary (33%, P<0.05) also showed statistical significance.

Females: The absolute organ weights of rats receiving 500 ppm DPG tended to be reduced compared to control rats. High statistical significance was found in heart (18%, P<0.001), liver (17%, P<0.001) and brain (4%, P<0.01). Less significant effects were seen in adrenals (17%, P<0.05) and lungs (8%, P<0.05). Kidneys were also reduced in weight (12%) when compared with the controls, but did not show statistical significance.
On taking account of body weight effects, there was an increase in relative organ weight when compared to controls in brain (19%, P<0.001), lungs (13%, P<0.001), uterus (34%, P<0.01) and spleen (32%, P<0.05).

Pathology Findings
No macroscopic intergroup differences of any significance were observed at necropsy and histopathological examination showed no specific lesion that could be attributed to dosing with Diphenylguanidine.
There was a small range of background lesions in all groups. They included
i) a mild interstitial pneumonitis,
ii) slight mammary hyperplasia (males only),
iii) small inflammatory lesions in prostate and pancreas,
iv) renal pelvic dilatations,
v) small foci of renal tubular calcification (females only).
One animal receiving 500 ppm DPG showed multiple mononuclear foci in the liver; the aetiology of these is unknown, but they are not considered to be treatment related. Several animals had ocular inflammatory foci, probably associated with blood sampling; these lesions were mild in nature, with the exception of one animal receiving 500 ppm DPG.

Source : MLPC, Rion-des-Landes, France

Conclusion : Dosing rats with Diphenylguanidine in the diet at a

concentration of 500 ppm produced a marked reduction in growth rate of both males and females, with respect to controls. The food consumption of these animals was also reduced, when compared to the controls. The effects were most pronounced over the first few weeks of dosing, suggesting that the cause may partly be due to the unpalatability of the test substance.

Dose levels of DPG up to 500 ppm did not cause an increase in mortality or the appearance of any abnormal clinical signs.

Laboratory investigations revealed small differences between rats receiving DPG and the controls in both haematological and clinical chemical parameters, but as the same effects were not present at both Weeks 6 and 13 they are considered to be of little or no significance. Male rats receiving 500 ppm DPG tended to produce more concentrated urine than the controls at Weeks 6 and 13, with a slightly reduced pH on the latter occasion. Female rats at the same dose level also showed a slight aciduria, but at Week 13 only. These effects were only slight and could not be attributed to any obvious structural change to the kidney as seen on histopathological examination. The changes seen lacked a dose response and were of marginal magnitude, thereby confirming their insignificance.

The terminal studies revealed no dose related lesions; those lesions found were spread across the dose groups with roughly equal incidence and are considered to be common findings in rats of this age and strain.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
29.10.2001 (73)

Type :
Species : rat
Sex : male/female
Strain : Fischer 344
Route of admin. : oral feed
Exposure period : 2 weeks
Frequency of treatm. : ad libitum
Post exposure period : none
Doses : 250, 500, 750, 1500 and 3000 ppm (22, 45, 64, 121 and 200 mg/kg/d in males and 23, 44, 65, 127 and 166 mg/kg in females)
Control group : yes, concurrent no treatment
Method : other: range-finding toxicity study, see comment
Year : 1995
GLP : yes
Test substance : other TS: purity 98.9% +/- 0.6%

Method : In the 2-week studies, groups of five male and five female rats and mice were administered 0, 250, 500, 750, 1,500. or 3,000 ppm 1,3-diphenylguanidine in feed that was available ad libitum.

Rats were housed five per cage. Animal rooms were maintained at 69° to 75° F and 35% to 65% relative humidity, with 12 hours of fluorescent light per day and approximately 10 air changes per hour. Feed and water were available ad libitum. Because of the limited stability of the 1,3-diphenylguanidine feed mixtures, feeders were changed

daily, 7 days per week.

The rats were observed twice daily for mortality/morbidity and clinical signs of toxicity. Clinical observations and indvidual body. Complete necropsies were performed on all animals. The heart, right kidney, liver, lungs, ovaries, prostate gland, seminal vesicles, spleen, right testis, and thymus were removed and weighed. Organs and tissues were examined for gross lesions and fixed in 10% neutral buffered formalin. Tissues to be examined microscopically were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Histopathologic examinations were performed on all tissues with gross lesions.

Result : All rats survived to the end of the 2-week study. The final mean body weights and body weight gains of males and females exposed to 1,500 or 3,000 ppm 1,3-diphenylguanidine were notably less than those of the control groups. During the second week of the study, clinical signs of toxicity were observed in males and females in the 3,000 ppm groups and included ruffled fur and thin appearance.

During the first week of the study rats exposed to 3,000 ppm consumed 35% less feed than controls and the final mean body weights of these groups remained the same or decreased slightly from their initial values. During the second study week, feed consumption by the 3,000 ppm groups increased relative to controls and body weight gains increased but the final mean body weights remained lower than controls. Feed consumption and final mean body weights of groups receiving 750 or 1,500 ppm were also lower than the controls during both study weeks; however, animals in these groups gained weight continuously during the study.

The pattern of organ weight changes observed during the 2-week study was not indicative of chemical-related toxicity. Absolute organ weights of male rats that received 3,000 ppm were uniformly lower than controls due to the markedly reduced final mean body weights of this group. Ovarian weights of females that received 750 or 1,500 ppm were lower than controls, however final mean body weights of both groups were also lower than the controls. Relative liver weights of males that received 500 or 1,500 ppm and relative kidney weight of females that received 750 ppm were greater than those of the controls but the influences were small in magnitude, not exposure related, and not considered biologically meaningful.

No gross lesions associated with exposure to 1,3-diphenylguanidine were observed in male or female rats. No microscopic examination was conducted.

Source : MLPC, Rion-des-Landes, France
Reliability : (2) valid with restrictions
29.10.2001 (74)

Type :
Species : rat
Sex : male/female
Strain : Fischer 344
Route of admin. : oral feed
Exposure period : 13 weeks
Frequency of treatm. : ad libitum
Post exposure period : none

Doses	:	250, 500, 750, 1500 and 3000 ppm (17, 32, 50, 100, 181 mg/kg/d in males and 17, 32, 49, 95, 184 mg/kg/d in females)
Control group	:	yes, concurrent no treatment
NOAEL	:	= 500 ppm
LOAEL	:	= 750 ppm
Method	:	other: equivalent to OECD guide-line 408
Year	:	1995
GLP	:	yes
Test substance	:	other TS: purity 98.9% +/- 0.6%

Method : Groups of 10 male and 10 female rats were administered 0, 250, 500, 750, 1,500, or 3,000 ppm 1,3-diphenylguanidine in feed that was available ad libitum. Additional rats (10 males and 10 females per exposure group) were used in a supplemental clinical pathology study.

Rats were housed five per cage. Animal rooms were maintained at 69° to 75° F and 35% to 65% relative humidity, with 12 hours of fluorescent light per day and approximately 10 air changes per hour. Feed and water were available ad libitum. Because of the limited stability of the 1,3-diphenylguanidine feed mixtures, feeders were changed daily, 7 days per week.

The rats were observed twice daily for mortality/morbidity and clinical signs of toxicity. Clinical observations were recorded weekly. Individual body weights were recorded at the start of the study, weekly thereafter, and at the end of the study. Feed consumption was recorded daily for 5 consecutive days per week for 13 weeks.

Hematology and clinical chemistry evaluatlons were performed on 10 male and 10 female supplemental rats per group at Days 5 and 21 and at study termination (Week 13). For these evaluations, rats were anesthetized with CO2, and blood samples were collected from the retroorbital sinus. Samples for hematology analysis were placed in tubes containing potassium EDTA, and samples for clinical chemistry evaluations were placed in similar tubes devoid of anticoagulant. The latter samples were allowed to clot at room temperature; the samples were then centrifuged and serum was removed.

Complete necropsies were performed on all animals. The heart, right kidney, liver, lungs, ovaries, prostate gland, seminal vesicles, spleen, right testis, and thymus were removed and weighed. Organs and tissues were examined for gross lesions and fixed in 10% neutral buffered formalin. Tissues to be examined microscopically were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Complete histopathologic examinations were performed on all rats in the 0 and 3,000 ppm groups, on all rats in the 1,500 ppm groups, and on all animals that died early. Gross lesions and selected tissues were examined in the lower exposure groups. The following tissues were examined: adrenal glands, brain (three sections), esophagus, femur with marrow, gross lesions, heart, intestines (large: cecum, colon, rectum; small: duodenum, jejunum, ileum), kidneys, liver, lunglmainstem bronchi, lymph nodes (mandibular, mesenteric), mammary gland with adjacent skin, nasal cavity

and turbinates (three sections), ovaries, pancreas, parathyroid glands, pituitary gland, preputial or clitoral glands, prostate gland, salivary glands, skin, spleen, spinal cord/sciatic nerve, stomach (forestomach and glandular stomach), testes (with epididymis and seminal vesicle), thigh muscle, thymus, thyroid glands, trachea, urinary bladder, uterus, and vagina (females in vaginal cytology studies only). The uterus and prostate gland were examined in the lower exposure groups.

At the end of the 13-week studies, vaginal cytology and sperm motility evaluations were performed on all base-study rats in the 0, 500, 750, and 1,500 ppm groups. Methods were those outlined in the National Toxicology Program's Technical Protocol for Sperm Morphology and Vaginal Cytology Evaluation in Toxicity Testing for Rats and Mice (NTP, 1987). Beginning 12 days prior to sacrifice, the vaginal vaults of 10 females from each exposure group were lavaged, and the aspirated lavage fluid and cells were stained with toluidine blue. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (Le., diestrus, proestrus, estrus, or metestrus).

Sperm motility was evaluated at necropsy in the following manner. The left testis and epididymis were weighed. The tail of the epididymis (cauda epididymis) was then removed from the corpus epididymis and weighed. Test yolk (rats) or Tyrode's buffer (mice) was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers.
Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced and swirled, and the tissue was incubated and then heat fixed. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in 10% dimethyl sulfoxide in phosphate-buffered saline. Homogenization-resistant spermatid nuclei were counted using a hemacytometer.

Result : Six males and all females in the 3,000 ppm groups died or were killed moribund before the end of the 13-week study; all rats in the lower exposure groups survived to the end of the study. Mean body weights of male and female rats that were exposed to 1,500 or 3,000 ppm were markedly lower than those of controls throughout the 13-week study. Mean body weights of the 3,000 ppm groups decreased during the first week of the study for males and the first 2 weeks of the study for females before starting to increase. No final mean body weight or body weight gain was determined for female rats administered 3,000 ppm 1,3-diphenylguanidine due to 100% mortality in this exposure group.

Clinical signs of toxicity were noted primarily in rats in the 1,500 and 3,000 ppm groups beginning at Week 2. The majority of rats in these groups appeared thin and had ruilled fur, with discolorations of the tail, ears, and scrotum or vaginal area. Salivation, hypoactivity, and

convulsions and seizures were also observed in some male and female rats in these groups, and abnormal posture (staggering) was noted in most males and females. Other clinical signs observed in these groups included hyperactivity, hunched posture, ptosis, ataxia, dyspnea, and bristly hair.

Average feed consumption decreased as exposure concentrations increased above 500 ppm with feed consumption 34% to 40% less than the controls during the 13-week study period in males and females that received 3,000 ppm. During the first week of the study feed consumption by groups receiving 3,000 ppm were 57% and 63% lower than control for males and females respectively, indicating poor palatability at this exposure concentration.

Organ weights for groups receiving 750 ppm or greater were significantly lower than those of the controls and were the result of low body weights and low feed consumption by these groups rather than a specific toxic response to 1,3-diphenylguanidine.

In general, changes in hematology parameters were limited to rats receiving 1,500 and 3,000 ppm. A mild polycythemia occurred at Day 5 in the 3,000 ppm male and female rats, and to a lesser extent in the 1,500 ppm females. This was indicated by greater erythrocyte counts, hematocrit values, and hemoglobin concentrations than controls and would be consistent with a relative polycythemia related to dehydration and hemoconcentration. There were slightly lower reticulocyte counts at Day 5 in 3,000 ppm male and female rats and 1,500 ppm females. Other changes in hematology parameters were minor, sporadic, and did not suggest a treatment effect.

Changes in clinical chemistry parameters occurred primarily in the 1,500 and 3,000 ppm groups, although some minor changes were observed in other groups. Greater alkaline phosphatase activity and bile acid concentration than controls occurred in an exposure-related manner in male and female rats. Males exhibited greater increases in activity and at earlier time periods. By Week 13, alkaline phosphatase activity and bile acid concentration were greater than the controls in all groups of exposed rats; these changes are consistent with cholestasis. The lack of an increase of alkaline phosphatase activity in groups that received 3,000 ppm was probably related to inanition and a decreased contribution of the intestinal fraction of alkaline phosphatase to the total serum activity. Total protein, creatinine, cholesterol, and triglyceride concentrations in the 1,500 and 3,000 ppm groups were lower than the controls and these differences are consistent with inanition.

Gross necropsy observations related to 1,3-diphenylguanidine treatment were limited to thinness of the carcass in higher exposure rats. Microscopic changes associated with chemical administration were observed in the bone marrow, thymus, uterus, testes, prostate gland/seminal vesicle, and salivary glands. All of the gross and microscopic changes occurred in the two highest exposure groups and were attributed to the lower feed intake, reduced weight gains, and poor body

condition of these animals.

In the thymus, lymphoid depletion and necrosis were present in several 3,000 ppm females which were found dead or were killed in moribund condition. Depletion of hematopoietic cells in the femoral bone marrow was also variably present in the 3,000 ppm females which died early. Both of these lesions are common in moribund animals and are not considered to be direct toxic effects of chemical administration.

An exposure-related effect in the uterus of females was characterized by an overall reduction in size and was diagnosed as hypoplasia. This finding occurred with greater incidence and severity in the three highest exposure groups. In general, this change was attributed to poor body condition and delayed development due to lower feed consumption; the younger age of those females which died or were killed during the study may have been a reason for the smaller size of the uterus.

Several lesions were noted sporadically in the reproductive organs of 3,000 ppm males. In two of ten 3,000 ppm males, lower numbers of mature spermatozoa were present in the seminiferous tubules than in the controls; lower numbers of spermatozoa were also noted in the epididymal tubules than in the controls. Secretory depletion of the prostate gland and seminal vesicles was observed in several 3,000 ppm males; this difference was characterized by alveolar size smaller than controls and smaller amounts of secretory material within the lumen. Decreased spermatogenesis and secretory depletion of the accessory sex glands were considered secondary to poor body condition. In the salivary glands of several 3,000 ppm males and females, a change diagnosed as cytologic alteration was observed, characterized by smaller size and increased basophilia of the secretory acini. This change was interpreted to be a reflection of physiological atrophy due to reduced feed intake. No specific cause of death could be determined for the early death animals from the 3,000 ppm groups.

Evaluation of male reproductive tissues in groups that received 500, 750, or 1,500 ppm revealed a significant reduction in sperm motility in 1,500 ppm males. Among 750 and 1,500 ppm group females the length of the estrous cycle was greater than the controls.

Source : MLPC, Rion-des-Landes, France
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
29.10.2001 (74)

Type :
Species : rat
Sex : no data
Strain : no data
Route of admin. : oral feed
Exposure period : 28 days
Frequency of treatm. : continously in diet
Post exposure period : no data
Doses : 100 or 1000 ppm (approx. 7 or 75 mg/kg/day)
Control group : no data specified
NOAEL : = 100 ppm

Method : other
Year :
GLP : no data
Test substance : no data

Remark : no further information available
Result : 1000 ppm: reduced food consumption, reduced body weight gain (no details reported)
100 ppm: no substance related effects
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
29.10.2001 (75)

Type :
Species : rat
Sex : no data
Strain : no data
Route of admin. : oral unspecified
Exposure period : 4 months
Frequency of treatm. : no data
Post exposure period :
Doses : 32 mg/kg
Control group : yes
Method : other
Year :
GLP : no data
Test substance : no data

Remark : Frequency of application not reported; no further information available.
Result : Symptoms of toxicity: increased mortality, anemia, reticulocytosis, eosinophilia, increased bilirubin concentration; inhibition of the iron containing enzymes catalase and peroxidase; reduced thresholds of nerve and muscle excitability (no details reported).
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
29.10.2001 (76)

Type :
Species : mouse
Sex : male/female
Strain : B6C3F1
Route of admin. : oral feed
Exposure period : 2 weeks
Frequency of treatm. : ad libitum
Post exposure period : none
Doses : 250, 500, 750, 1500 and 3000 ppm (48, 92, 133, 266, 573 mg/kg/d in males and 53, 112, 150, 303, 691 mg/kg/d in females)
Control group : yes, concurrent no treatment
Method : other: range-finding toxicity study, see method section
Year : 1995
GLP : yes
Test substance : other TS: purity 98.9% +/- 0.6%

Method : Groups of five male and five female mice were administered 0, 250, 500, 750, 1,500. or 3,000 ppm 1,3-diphenylguanidine in feed that was available ad libitum.

Mice were housed individually. Animal rooms were maintained at 69° to 75° F and 35% to 65% relative humidity, with 12 hours of fluorescent light per day and approximately 10 air

changes per hour. Feed and water were available ad libitum. Because of the limited stability of the 1,3-diphenylguanidine feed mixtures, feeders were changed daily, 7 days per week, throughout the 2-week and 13-week studies.

Mice were observed twice daily for mortality/morbidity and clinical signs of toxicity. Clinical observations and individual body weights were recorded on Days 1 and 8 and at the end of the studies. Feed consumption was recorded 5 consecutive days per week for 2 weeks.

Complete necropsies were performed on all animals. The heart, right kidney, liver, lungs, ovaries, prostate gland, seminal vesicles, spleen, right testis, and thymus were removed and weighed. Organs and tissues were examined for gross lesions and fixed in 10% neutral buffered formalin. Tissues to be examined microscopically were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Histopathologic examinations were performed on all tissues with gross lesions.

Result : All mice survived to the end of the 2-week study. The final mean body weight of female mice in the 3,000 ppm group was 6% lower than the controls; final mean body weights of other exposed groups were similar to controls. Clinical signs of toxicity were observed in a few female mice during the latter part of the study; one female in the 1,500 ppm group appeared thin, and one female each in the 750 and 3,000 ppm groups had hunched posture and appeared thin. The average amounts of feed consumed by females in the 750 and 1,500 ppm groups were slightly lower than the control value; the average amounts of feed consumed by all other exposed groups were similar to control values.

Only a few significant organ weight changes were observed (data on file at NIEHS). Absolute and relative liver weights of males and females in the 1,500 and 3,000 ppm groups were lower than those of the control groups, and the relative heart weight of females in the 500 ppm group was greater than that of the control group.

No gross or microscopic lesions related to 1,3-diphenylguanidine exposure were observed in male or female mice.

Source : MLPC, Rion-des-Landes, France
Reliability : (2) valid with restrictions
06.09.2001 (74)

Type :
Species : mouse
Sex : male/female
Strain : B6C3F1
Route of admin. : oral feed
Exposure period : 13 weeks
Frequency of treatm. : ad libitum
Post exposure period : none
Doses : 250, 500, 750, 1500 and 3000 ppm (38, 75, 114, 231, 457 mg/kg/d in males and 46, 93, 141, 285, 577 mg/kg/d in females)
Control group : yes, concurrent no treatment
NOAEL : = 500 ppm
LOAEL : = 750 ppm
Method : other: NTP protocol, see method section

Year : 1995
GLP : yes
Test substance : other TS: purity 98.9% +/- 0.6%

Method : Groups of 10 male and 10 female mice were administered 0, 250, 500, 750, 1,500, or 3,000 ppm 1,3-diphenylguanidine in feed that was available ad libitum.

Mice were housed individually. Animal rooms were maintained at 69° to 75° F and 35% to 65% relative humidity, with 12 hours of fluorescent light per day and approximately 10 air changes per hour. Feed and water were available ad libitum. Because of the limited stability of the 1,3-diphenylguanidine feed mixtures, feeders were changed daily, 7 days per week, throughout the 2-week and 13-week studies.

Mive were observed twice daily for mortality/morbidity and clinical signs of toxicity. Clinical observations were recorded weekly. Individual body weights were recorded at the start of the study, weekly thereafter, and at the end of the study. Feed consumption was recorded daily for 5 consecutive days per week for 13 weeks.

Complete necropsies were performed on all animals. The heart, right kidney, liver, lungs, ovaries, prostate gland, seminal vesicles, spleen, right testis, and thymus were removed and weighed. Organs and tissues were examined for gross lesions and fixed in 10% neutral buffered formalin. Tissues to be examined microscopically were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Complete histopathologic examinations were performed on all mice in the 0 and 3,000 ppm groups, and on all animals that died early. Gross lesions and selected tissues were examined in the lower exposure groups. The following tissues were examined: adrenal glands, brain (three sections), esophagus, femur with marrow, gallbladder, gross lesions, heart, intestines (large: cecum, colon, rectum; small: duodenum, jejunum, ileum), kidneys, liver, lung/mainstem bronchi, lymph nodes (mandibular, mesenteric), mammary gland with adjacent skin, nasal cavity and turbinates (three sections), ovaries, pancreas, parathyroid glands, pituitary gland, preputial or clitoral glands, prostate gland, salivary glands, skin, spleen, stomach (forestomach and glandular stomach), testes (with epididymis and seminal vesicle), thigh muscle (rats only), thymus, thyroid glands, trachea, urinary bladder, uterus, and vagina (females in vaginal cytology studies only). No tissues were designated for examination in the lower exposure groups.

At the end of the 13-week studies, vaginal cytology and sperm motility evaluations were performed all mice in the 0, 250, 750, and 3,000 ppm groups. Methods were those outlined in the National Toxicology Program's Technical Protocol for Sperm Morphology and Vaginal Cytology Evaluation in Toxicity Testing for Rats and Mice (NTP, 1987). Beginning 12 days prior to sacrifice, the vaginal vaults of 10 females from each exposure group were lavaged, and the aspirated lavage fluid and cells were stained with toluidine blue. Relative numbers of leukocytes, nucleated epithelial cells, and large

squamous epithelial cells were determined and used to ascertain estrous cycle stage (Le., diestrus, proestrus, estrus, or metestrus).

Sperm motility was evaluated at necropsy in the following manner. The left testis and epididymis were weighed. The tail of the epididymis (cauda epididymis) was then removed from the corpus epididymis and weighed. Test yolk (rats) or Tyrode's buffer (mice) was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers.

Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced and swirled, and the tissue was incubated and then heat fixed. Sperm density was then determined microscopically with the aid of a hemacyt ometer. To quantify spermatogenesis, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in 10% dimethyl sulfoxide in phosphate-buffered saline. Homogenization-resistant spermatid nuclei were counted using a hemacytometer.

Result : All mice survived to the end of the study. Mean body weights of both males and females in the three highest exposure groups (750, 1,500, and 3,000 ppm) were lower than those of the control groups especially during the latter part of the study. Thin appearance was the most frequently reported clinical sign for female mice and was most often observed in the three highest exposure groups. Thin appearance was also observed in male mice in the 3,000 ppm group. Other clinical signs observed in mice in the higher exposure groups included alopecia, abnormal posture, ptosis, and bristly hair.

The average amounts of feed consumed by males and females in all exposed groups were similar to the average amounts consumed by the control groups.

Significantly lower absolute organ weights and greater relative organ weights than controls were observed for several organs in the 1,500 or 3,000 ppm groups. These differences are not indicative of a specific toxic response but appear to be the result of the lower body weights of these groups.

No treatment-related gross or microscopic lesions were observed in male or female mice exposed to 1,3-diphenylguanidine.

Evaluation of male reproductive tissue from animals revealed greater numbers of spermatid heads and lower sperm motility than in the controls in the 3,000 ppm group. In females, estrous cycle length in the 3,000 ppm group was greater than controls.

Source : MLPC, Rion-des-Landes, France
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
06.09.2001 (74)

Type	:	
Species	:	rabbit
Sex	:	no data
Strain	:	no data
Route of admin.	:	oral unspecified
Exposure period	:	5.5 months
Frequency of treatm.	:	no data
Post exposure period	:	
Doses	:	50 mg/kg
Control group	:	no data specified
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	no data
Remark	:	No further information available
Result	:	Symptoms of toxicity: serious damage of the liver, associated with focal hepatitis; granular dystrophy of the cells of the convuluted kidney tubules; increased bilirubin levels; examination of the blood revealed no other changes (no details reported)
Source	:	MLPC, Rion-des-Landes, France
Reliability	:	(4) not assignable

27.12.2000 (52) (56)

Type	:	
Species	:	rabbit
Sex	:	no data
Strain	:	no data
Route of admin.	:	oral unspecified
Exposure period	:	no data
Frequency of treatm.	:	no data
Post exposure period	:	
Doses	:	10 % of LD100 (no further information available)
Control group	:	no data specified
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	no data
Remark	:	No further information available
Result	:	Symptoms of toxicity: decreased food consumption, decreased erythrocyte levels, increased serum-gamma-globulin levels
Source	:	MLPC, Rion-des-Landes, France
Reliability	:	(4) not assignable

27.12.2000 (52) (56)

Type	:	
Species	:	dog
Sex	:	no data
Strain	:	no data
Route of admin.	:	oral unspecified
Exposure period	:	no data
Frequency of treatm.	:	multiple (no further information available)
Post exposure period	:	
Doses	:	5 mg/kg
Control group	:	no data specified
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	no data

Remark : no further information available
Result : symptoms of toxicity: reduced levels of bile acids (no details reported)
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (77)

Type :
Species : dog
Sex : no data
Strain : no data
Route of admin. : oral unspecified
Exposure period : 24 days
Frequency of treatm. : 21 times
Post exposure period : no data
Doses : 10 mg/kg/day
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : no data

Result : A dosage of 10 mg/kg/d administered to 2 dogs in divided doses for a total of 21 doses in 24 days was reasonably well tolerated (no details reported).
Source : MLPC, Rion-des-Landes, France
Reliability : (3) invalid
27.12.2000 (75)

Type :
Species : rat
Sex : no data
Strain : no data
Route of admin. : inhalation
Exposure period : 15 days
Frequency of treatm. : 2 h/day
Post exposure period :
Doses : ca. 0.22 mg/l
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : no data

Remark : No further information available
Result : Symptoms of toxicity: marked disturbance in the intensity of oxidation-reduction processes; functional changes of nervous system; blood pressure rose briefly (for several days) and fell than to lower levels than the initial values (no details reported)
Source : MLPC, Rion-des-Landes, France
Reliability : (3) invalid
11.05.2001 (52) (56)

Type :
Species : other: not specified
Sex : no data
Strain : no data
Route of admin. : inhalation
Exposure period : 4 weeks
Frequency of treatm. : 30 min/day, every second day
Post exposure period :

Doses : dust, concentation not specified
Control group : no data specified
Method : other: no detail available
Year : 1949
GLP : no
Test substance : no data

Result : no toxic effects were observed
Source : MLPC, Rion-des-Landes, France
Reliability : (3) invalid
06.09.2001 (62)

Type :
Species : guinea pig
Sex : no data
Strain : no data
Route of admin. : inhalation
Exposure period : no data
Frequency of treatm. : repeated (no further information available)
Post exposure period :
Doses : 100 mg/l
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : no data

Result : all animals died (lethal, no details reported)
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (78)

Type :
Species : rabbit
Sex : no data
Strain : no data
Route of admin. : dermal
Exposure period : no data
Frequency of treatm. : 10 times
Post exposure period : no data
Doses : 1000 mg/kg
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : no data

Result : signs of systemic toxicity were not observed (no details reported)
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (75)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Salmonella typhimurium reverse mutation assay
System of testing : Strain TA98, TA100, TA1535 and TA1537
Test concentration : 1 to 10000 µg/plate
Cycotoxic concentr. :
Metabolic activation : with and without

Result : ambiguous
Method : other: Mortelman et al. (1986) Environ Mut, 8 (suppl. 7), 1-119.
Year : 1986
GLP : no data
Test substance : other TS: purity 98.9% +/- 0.6%

Result : 1,3-Diphenylguanidine (1 to 10,000 µg/plate) was weakly mutagenic or equivocal in Salmonella typhimurium strains TA98 and TA100 in the presence of induced hamster or rat liver S9, and an equivocal response was obtained in strain TA1537 with rat liver S9. No indication of mutagenic activity was noted in the absence of S9.
Source : MLPC, Rion-des-Landes, France
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
27.12.2000 (79) (74)

Type : Salmonella typhimurium reverse mutation assay
System of testing : Strains TA 1535, TA 1537, TA 1538, TA 98, TA 100
Test concentration : 0.1 to 500 µg/plate
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method : other: Litton Bionetics Inc. standard protocol
Year : 1976
GLP : no
Test substance : no data

Source : MLPC, Rion-des-Landes, France
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
06.09.2001 (80)

Type : Salmonella typhimurium reverse mutation assay
System of testing : Strains TA98, TA100, TA1535, TA1537 and TA1538
Test concentration : 2 to 500 µg/plate without S9, 20 to 5000 µg/plate with S9
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method : other: Standard procedure of the Japanese minister of Labour
Year : 1988
GLP : no data
Test substance : other TS

Source : MLPC, Rion-des-Landes, France
Test substance : Tokyo Kasei Kogyo Co. ltd, lot no. FBR01, garanteed reagent.
Reliability : (2) valid with restrictions
Flag : Critical s tudy for SIDS endpoint
06.09.2001 (81)

Type : Salmonella typhimurium reverse mutation assay
System of testing : Strains TA 98, TA 100
Test concentration : 0, 200, 1000 and 5000 (= highest non-toxic concentration) µg/plate
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method : other: Ames, B.N. et al., Mutat. Res. 31, 347-364 (1975)
Year : 1975
GLP : no data
Test substance : other TS: purity: 96.5 %

Source : MLPC, Rion-des-Landes, France

Reliability : (3) invalid
11.05.2001 (82) (83) (84)

Type : Salmonella typhimurium reverse mutation assay
System of testing : Strains TA 1535, TA 1537, TA 1538, TA 98, TA 100
Test concentration : 0.036 - 36 ug/plate
Cycotoxic concentr. :
Metabolic activation : with and without
Result : positive
Method : other: according to Ames, B.N. et al., Mutat. Res. 31, 347-364 (1975)
Year : 1975
GLP : no data
Test substance : other TS: several impurities were isolated from the sample used (no details reported)

Method : The standard plate-incorporation assay system contained approximately 1.5 x 10e8 bacteria with or without in vitro metabolic activation system. Mixed function oxidase (S-9) was prepared as previously described (Ames et al., 1975). The various experimental dosage levels and controls were plated in triplicate. Positive control used for the in vitro activation system was cyclophosphamide; for assays not incorporating S-9 liver fractions, MNNG was used. After 48 hr of incubation at 37°C, the number of his-revertant colonies was determined. The number of his-revenant plotted represents colonies in excess of control values.
Result : Lower dosage levels of DPG elicited more histidine revertants per plate in the absence of in vitro metabolic activation system than similar levels of the compound in the presence of mixed function oxidase. It may also be inferred from the data that Salmonella strains TA100 and 1525 showed stronger mutagenic response than strains TA98, 1537, and 1538. The level of mutagenic response elicited by higher levels of DPG in the presence of mixed function oxidase differs significantly from that generated by similar doses of DPG without metabolic activation. A comparison of dose-response profile of the two systems (with or without metabolic acti vation) clearly suggests that while direct incorporation without metabolic activation yielded less histidine revertants per plate as DPG levels were increased, with metabolic activation, however, there was a gradual but steady increase in the number of revenants per plate with an increase in the dosage level of DPG. The only exception was strain TA98.
Source : MLPC, Rion-des-Landes, France
Reliability : (3) invalid
The positive effect could be attributed to the impurities of DPG.
27.12.2000 (85) (86)

Type : Salmonella typhimurium reverse mutation assay
System of testing : Strains TA 1535, TA 1537, TA 1538, TA 98, TA 100
Test concentration : no data
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method : other: Ames, B.N. et al., Mutat. Res. 31, 347-364 (1975)
Year : 1975
GLP : no data
Test substance : other TS: purity: technical grade

Source : MLPC, Rion-des-Landes, France

Reliability : (3) invalid
27.12.2000 (87)

Type : Salmonella typhimurium reverse mutation assay
System of testing : Strains TA 98, TA 100
Test concentration : no data
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method : other: no data
Year :
GLP : no data
Test substance : no data

Remark : no further information available
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (88)

Type : Salmonella typhimurium reverse mutation assay
System of testing : Strains TA 98, TA 100
Test concentration : 1 - 100 ug/plate
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method : other: no data
Year :
GLP : no data
Test substance : no data

Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (89)

Type : Escherichia coli reverse mutation assay
System of testing : Strain WP2uvrA
Test concentration : 2 to 500 µg/plate without S9, 20 to 5000 µg/plate with S9
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method : other: Standard procedure of the Japanese minister of Labour
Year : 1988
GLP : no data
Test substance : other TS

Source : MLPC, Rion-des-Landes, France
Test substance : Tokyo Kasei Kogyo Co. ltd, lot no. FBR01, garanteed reagent.
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
06.09.2001 (81)

Type : Gene mutation in Saccharomyces cerevisiae
System of testing : Stain D4
Test concentration : 1 to 500 µg/plate
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method : other: Litton Bionetics Inc. standard protocol
Year : 1976
GLP : no
Test substance : no data

Source : MLPC, Rion-des-Landes, France
Reliability : (2) valid with restrictions
06.09.2001 (80)

Type : HGPRT assay
System of testing : V79 cells
Test concentration : 100, 200, 500 µg/ml
Cycotoxic concentr. :
Metabolic activation : without
Result : negative
Method : other: according to van Zeeland, A.A. & Simmons, J.W.I.M., Mutat. Res.35, 129-138 (1976)
Year : 1983
GLP : no data
Test substance : other TS: purity: technical grade

Remark : Survival rate were 95, 89 and 94% at 100, 200 and 500 µg/ml, respectively.
Source : MLPC, Rion-des-Landes, France
Reliability : (3) invalid
27.12.2000 (90)

Type : Mouse lymphoma assay
System of testing : Mouse lymphoma L 5178Y cells
Test concentration : 16.4 to 188 µg/ml without S9, 32.8 to 525 µg/ml with S9
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method : other: Litton Bionetics Inc. standard protocol
Year : 1978
GLP : no
Test substance : no data

Source : MLPC, Rion-des-Landes, France
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
06.09.2001 (91)

Type : Cytogenetic assay
System of testing : CHO cells
Test concentration : 0, 125, 250, 500 and 750 µg/ml
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method : other: 40 CFR 798
Year : 1990
GLP : yes
Test substance : other TS: purity 97.18%

Source : MLPC, Rion-des-Landes, France
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
27.12.2000 (92)

Type : other: see remarks
System of testing : HeLa-S3 cells
Test concentration : 7.5 ug/l
Cycotoxic concentr. :
Metabolic activation : without
Result :
Method : other
Year :

GLP	:	no data
Test substance	:	no data
Remark	:	ID50s = 50 % inhibition dose, determined from inhibition of colony formation of the indicator cells
Source	:	MLPC, Rion-des-Landes, France
Reliability	:	(4) not assignable

27.12.2000 (93)

5.6 GENETIC TOXICITY 'IN VIVO'

Type	:	Cytogenetic assay
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	single administration
Doses	:	300 mg/kg (maximum tolerated dose)
Result	:	negative
Method	:	OECD Guide-line 475 "Genetic Toxicology: In vivo Mammalian Bone Marrow Cytogenetic Test - Chromosomal Analysis"
Year	:	1983
GLP	:	yes
Test substance	:	other TS: purity 97.7%

Method : The potential for 1,3-diphenylguanidine to induce chromosomal aberrations in the bone marrow cells of Sprague-Dawley rats was tested.
In the range-finding experiment, male and female rats were treated with 1,3-diphenylguanidine at 50, 100, 200, 400, 600, 800, 1000 and 5000 mg/kg body weight.
1,3-Diphenylguanidine was found to be toxic to male rats at 400 mg/kg and higher, and toxic to female rats at 200 mg/kg and at 600 mg/kg and higher as indicated by clinical signs of toxicity and death. The combined male and female LD50 was determined to be 427.3 mg/kg by the Probit method.

Based on results from the toxicity range-finding experiments, 1,3-Diphenylguanidine was administered via oral gavage to male and female rats at a target dose of 300 mg/kg body weight (approximately 70% of the combined LD50). Control groups received 10 ml/kg of body weight of vehicle control (corn oil) or a 40 mg/kg of body weight dose of positive control (cyclophosphamide). Bone marrow was sampled at 6, 24 and 48 hours after dosing with the vehicle or 1,3-diphenylguanidine. A single sampling time of 24 hours after dosing was used for the cyclophosphamide control group. Slides were scored for increases in the proportion of aberrant metaphases and in the frequency of aberrations/cell.

Result : In the main cytogenetic experiment, 1,3-diphenylguanidine was toxic to male and female rats as evidenced by clinical signs of toxicity (hypoactive and nonresponsive) and death. Five male rats and six female rats were found dead within 24 hours of dosing. Statistically significant decreases in mean body weight were observed in the 1,3-diphenylguanidine treated male and female rats at 6 and 24 hours after treatment and in the positive control treated male rats 24 hours after treatment.

No statistically significant increases in the proportion of aberrant cells or aberrations/cell were observed at the 6, 24 and 48 hour time points. Significant induction of toxicity, measured as mitotic index depression, was observed at the 6 hour (35%) and 24 hour (31%) time points. No depression in mitotic index was observed at the 48 hour time point.

The positive control group (cyclophosphamide) yielded expected positive responses indicating the adequacy of the experimental conditions for the detection of clastogens.

Source : MLPC, Rion-des-Landes, France
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
29.10.2001 (94)

Type : Micronucleus assay
Species : mouse
Sex : male/female
Strain : B6C3F1
Route of admin. : oral feed
Exposure period : 13 weeks
Doses : 0, 250, 500, 750, 1500 and 3000 ppm
Result : negative
Method : other: McGregor et al. (1990) Fundam Appl Toxicol, 14, 513-522.
Year : 1990
GLP : no data
Test substance : other TS: purity 98.9% +/- 0.6%

Method : A modification of the technique described by MacGregor et al. (1990) was used. At the termination of the 13-week toxicity study, blood was obtained from male and female mice and smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. The frequency of micronuclei was determined in 2,000 normochromatic erythrocytes (NCEs) in each of 5 animals per dose group. The criteria of Schmid (1976) were used in defining micronuclei.
The frequency of micronucleated PCEs was analyzed by a statistical software package (ILS, 1990) that employed a one-tailed trend test across dose groups and a t-test for pairwise comparisons of each dose group to the concurrent control.

Result : No effect was noted in male mice, but in females, a significant increase in micronucleated normochromatic erythrocytes was noted in the 750 ppm group. Because the trend test for the female data did not yield a significant P value (P>0.025) and the increase in micronucleated normochromatic erythrocytes was noted in only one exposure group, the female mouse data were judged to be equivocal.

Frequency of Micronucleated Erythrocytes in Peripheral Blood of Male and Female Mice Admlnistered 1,3-Diphenylguanidine In Dosed Feed for 13 Weeks

Dose (ppm)	Micronucl. NCEs/1,000 NCEs	Number examined
MALE		
0	0.38 +/- 0.13	4
250	0.70 +/- 0.20	5

500	1.00 +/- 0.27	5
750	1.20 +/- 0.12	5
1,500	0.70 +/- 0.20	5
3,000	1.30 +/- 0.12	5

FEMALE

0	0.30 +/- 0.12	5
250	1.00 +/- 0.16	5
500	0.80 +/- 0.20	5
750	1.40 +/- 0.19*	5
1,500	1.20 +/- 0.12	5
3,000	1.30 +/- 0.20	5
	*P=0.005	

Source : MLPC, Rion-des-Landes, France
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
27.12.2000 (74)

Type : Micronucleus assay
Species : mouse
Sex : no data
Strain : no data
Route of admin. :
Exposure period : no data
Doses : no data
Result :
Method : other
Year :
GLP : no data
Test substance : no data

Remark : no further information available
Result : negative
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (86)

Type : other: Host mediated mutagenic assay
Species : mouse
Sex : no data
Strain : C57BL
Route of admin. : i.p.
Exposure period : single
Doses : 0.036 - 36 mg/kg
Result :
Method : other: Legator, M.S. et al., Mutat. Res. 26, 456-461 (1974)
Year : 1974
GLP : no data
Test substance : other TS: purity: several impurities were isolated from the sample used (nodetails reported)

Remark : To determine whether or not DPG was detoxified or converted to some active compound(s), 10 mice per each dosage level were given single i.p.injections of DPG at a final concentration of 0.036, 0.36, 3.6 or 36.0 mg/kg body weight. Control animais received single injections (0.2 ml) of 0.005% alcohol. The experimental and control animais were placed in metabolism cages in groups of three. Faeces, urine and peritoneal fluid from the two DPG-treated and two control animais were collected every 24 hr for a period of 4 days and directly assayed for mutagenic activity according

to Ames, B.N. et al., Mutat. Res. 31, 347-364 (1975).

Result : Since TA100 provided a greater mutagenic response in the presence of DPG, with or without metabolic activation, that strain was used in the analysis of body fluids and faecal material.

Incubation of Salmonella strain TA100 with peritoneal, urine or faecal material collected from animals given single intraperitoneal injections of DPG yielded sporadic results.

Time-dose-response testing of DPG with peritoneal fluid showed that in a three day recovery period, the number of revenants per plate increased with each advancing day when the initial dosage level was either 0.36 or 3.6 mg/kg. Peritoneal fluids from animals receiving 0.036 mg/kg generated time-dependent decreases in revenants, whereas incubation of TA100 in peritoneal fluid from animals previously exposed to DPG at 36 mg/kg was extremely toxic and thus produced a few revenants per plate.
It must be mentioned, however, that despite the time-dependent decrease in revertants per plate when peritoneal fluid from animals treated with 0.036 mg/kg of DPG was incubated with TA100, the mutagenic response of the treatment during the 3-day period was very high.

The urine from animals treated with 0.036 or 0.36 mg/kg generated steady inrease in revertants per plate with time; treatment with 3.6 mg/kg and 36 mg/kg on the other hand, showed time-and dose-dependent decrease in histidine revertants.

Unlike the sporadic mutagenic profile of peritoneal fluid and faeces of treated animals on TA100, the number of revenants generated by faecal material from similarly treated animals showed a definite dose-dependent response. The level of the mutagenic response of the faecal material is also influenced by the time elapsing between time of treatment and collection of faeces.
Faecal material collected within 24 hr post-treatment, and incubated with strain TA 100, resulted in moderate mutagenic responses. Faeces collected 48 or 72 hr post-treatment generated significantly higher number of revenants per plate. In addition to the mutagenic activity of the faecal material being time-dependent, histidine revertants per plate show slight increases with an increase in the dose level of DPG.
Dose-response curves based on analyses of peritoneal fluid and urine showed decreases in revenants per plate as the dosage levels of DPG were increased. Unlike the results obtained from the faecal material, histidine revenants produced by urine and peritoneal fluids were sporadic among the different treatment groups.

Analysis of peritoneal fluid showed that the lowest dosage level (0.036 mg/kg) produced the highest number of revenants in the three time-groups (24, 48 or 72 hr). 135, 170 and 210 revertants in excess of concurrent control were produced when peritoneal fluid removed from treated animais (0.036 mg/kg) at 24, 48 and 72 hr post-treated, were respectively incubated with TA 100 strain.
As the dosage level of DPG increased, histidine revenants produced by the three time-groups decreased. The 72-hr

treatment, however, generated the most number of revenants at higher concentrations of DPG.

Dose-response testing of urine from treated and control groups showed that 24 hr-treatment group produced the least revertants when urine from animals exposed to 0.036 or 0.36 mg/kg was used. The highest number or revertants (248/plate) was generated by the 72 hr-groups, with the 48-hr group producing 160. At higher concentrations of DPG (3.6 and 36 mg/kg) the 24 hr-groups produced more histidine revenants than the other groups.

Source : MLPC, Rion-des-Landes, France
Reliability : (3) invalid
27.12.2000 (85)

5.7 CARCINOGENICITY

Species : mouse
Sex : male/female
Strain : other: C57BLxDB hybrid
Route of admin. : oral feed
Exposure period : 32 weeks
Frequency of treatm. : continously in diet
Post exposure period : 10-16 weeks
Doses : 4 or 8 mg/kg
Result :
Control group : yes, concurrent no treatment
Method : other: no detail available
Year :
GLP : no data
Test substance : no data

Remark : 50 male and 50 female C57bl X DB2 hybrid mice/group were used.
Result : At the termination of dosing no tumors were observed; after the observation period 3/50 mice of the low dose group developed lymphatic adenocarcinomas, while in the high dose group and the control group no such tumors were observed; treatment also caused enlarged spleens, but this effect subsided after termination of treatment (no further details available)
Source : MLPC, Rion-des-Landes, France
Reliability : (3) invalid
27.12.2000 (95)

Species : mouse
Sex : male/female
Strain : other: ddy
Route of admin. : oral feed
Exposure period : 21 months
Frequency of treatm. : continuously in diet
Post exposure period : no data
Doses : 0, 20, 60, 180 or 540 ppm
Result :
Control group : yes
Method : other
Year :
GLP : no data
Test substance : no data

Remark	:	no further information available
Source	:	MLPC, Rion-des-Landes, France
Reliability	:	(3) invalid

27.12.2000 (96)

5.8.1 TOXICITY TO FERTILITY

Type	:	other: reproductive organ toxicity
Species	:	rat
Sex	:	male/female
Strain	:	Fischer 344
Route of admin.	:	oral feed
Exposure period	:	13 weeks
Frequency of treatm.	:	ad libitum
Premating exposure period		
Male	:	
Female	:	
Duration of test	:	13 weeks
No. of generation studies	:	
Doses	:	500, 750 and 1500 ppm
Control group	:	yes, concurrent no treatment
NOAEL parental	:	= 750 ppm
Method	:	other: NTP's Technical Protocol for Sperm Morphology and Vaginal Cytology Evaluation in Toxicity Testing for Rats and Mice
Year	:	1987
GLP	:	yes
Test substance	:	other TS: purity 98.9% +/- 0.6%

Method : As part of a 13-week toxicity study (NTP, 1995) groups of 10 male and 10 female rats were administered 0, 250, 500, 750, 1,500, or 3,000 ppm 1,3-diphenylguanidine in feed that was available ad libitum.

Rats were housed five per cage. Animal rooms were maintained at 69° to 75° F and 35% to 65% relative humidity, with 12 hours of fluorescent light per day and approximately 10 air changes per hour. Feed and water were available ad libitum. Because of the limited stability of the 1,3-diphenylguanidine feed mixtures, feeders were changed daily, 7 days per week, throughout the 2-week and 13-week studies.

At the end of the 13-week study, vaginal cytology and sperm motility evaluations were performed on all base-study rats in the 0, 500, 750, and 1,500 ppm groups. Methods were those outlined in the National Toxicology Program's Technical Protocol for Sperm Morphology and Vaginal Cytology Evaluation in Toxicity Testing for Rats and Mice (NTP, 1987). Beginning 12 days prior to sacrifice, the vaginal vaults of 10 females from each exposure group were lavaged, and the aspirated lavage fluid and cells were stained with toluidine blue. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, or metestrus).

Sperm motility was evaluated at necropsy in the following manner. The left testis and epididymis were weighed. The tail of the epididymis (cauda epididymis) was then removed

from the corpus epididymis and weighed. Test yolk was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers.

Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced and swirled, and the tissue was incubated and then heat fixed. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in 10% dimethyl sulfoxide in phosphate-buffered saline. Homogenization-resistant spermatid nuclei were counted using a hemacytometer.

Result : Gross necropsy observations related to 1,3-diphenylguanidine treatment were limited to thinness of the carcass in higher exposure rats. Microscopic changes associated with chemical administration were observed in the uterus, testes, prostate and gland/seminal vesicle. All of the gross and microscopic changes occurred in the two highest exposure groups and were attributed to the lower feed intake, reduced weight gains, and poor body condition of these animals.

An exposure-related effect in the uterus of females was characterized by an overall reduction in size and was diagnosed as hypoplasia. This finding occurred with greater incidence and severity in the three highest exposure groups. In general, this change was attributed to poor body condition and delayed development due to lower feed consumption; the younger age of those females which died or were killed during the study may have been a reason for the smaller size of the uterus.

Several lesions were noted sporadically in the reproductive organs of 3,000 ppm males. In two of ten 3,000 ppm males, lower numbers of mature spermatozoa were present in the seminiferous tubules than in the controls; lower numbers of spermatozoa were also noted in the epididymal tubules than in the controls. Secretory depletion of the prostate gland and seminal vesicles was obsetved in several 3,000 ppm males; this difference was characterized by alveolar size smaller than controls and smaller amounts of secretory material within the lumen. Decreased spermatogenesis and secretory depletion of the accessory sex glands were considered secondary to poor body condition. In the salivary glands of several 3,000 ppm males and females, a change diagnosed as cytologic alteration was observed, characterized by smaller size and increased basophilia of the secretory acini. This change was interpreted to be a reflection of physiological atrophy due to reduced feed intake. No specific cause of death could be determined for the early death animals from the 3,000 ppm groups.

Evaluation of male reproductive tissues in groups that received 500, 750, or 1,500 ppm revealed a significant reduction in sperm motility in 1,500 ppm males. Among 750 and 1,500 ppm group females the length of the estrous cycle was greater than the controls.
Summary of Reproductive Tissue Evaluations In Male F344/N

Rats in the 13-Week Feed Study of 1,3-Diphenylguanidine.

Study parameters				
Dose (ppm)	0	500	750	1,500
n	10	10	10	10
Weights (g)				
Necropsy body weight (g)				
	374±6	358±5	347±4**	300±7**
Left epididymis (mg)				
	480±10	482±9	496±10	464±8
Left cauda epididymis (mg)				
	196±5	199±5	198±6	186±4
Left testis (mg)				
	1550±20	1560±20	1510±30	1480±20
Spermatid measurements				
Spermatid heads (10e5/g testis)				
	1109±68	1046±29	1091±44	1084±41
Spermatid heads (10e5/testis)				
	1714±98	1631±40	1650±63	1600±54
Spermatid count (mean/10e-4 mL suspension)				
	85.70±4.90	81.55±1.98	82.48±3.13	80.0±2.71
Epididymal spermatozoal measurements				
Motility (%)				
	94.76±1.42	92.30±1.76	87.34±3.75	83.69±2.7**
Concentration (10e6/g caudal epididymal tissue)				
	331.5±33.2	259.0±29.62#	290.5±26.3	598.2±139

Data presented as mean ± standard error. Differences from the control group for epididymal, cauda epididymal, and testis weights, spermatid measurements, and sperm concentration are not significant by Dunn's test.
n=9.
** Significantly different (P<0.01) from the control group by Dunnett's test (necropsy body weight only) or Shirley's test.

Summary of Estrous Cycle Characterization In Female F344IN Rats in the 13-Week Feed Study of 1,3-Diphenylguanidine.

Study parameters				
Dose (ppm)	0	500	750	1,500
n	10	10	10	9
Necropsy body weight (g)				
	204±4	195±3	191±3**	177±2**#
Estrous cycle length (days)				
	4.95±0.05	5.00±0.00	6.00±0.33**	5.67±0.44"
Estrous stages (% of cycle)				
Diestrus	38.2	38.2	44.5	41.8
Proestrus	14.5	19.1	16.4	15.5
Estrus	30.0	24.5	24.5	22.7
Metestrus	17.3	18.2	14.5	20.0

Necropsy body weights and estrous cycle lengfhs are presented as mean ± standard error. By multivariate analysis of variance, exposed groups do not differ significantly from the control group in the relative length of time spent in the estrous stages.
n=10.
" Estrous cycle longer than 12 days or unclear in 1 of 10 animals.
** Significantly different (P<0.01) from the control group by Dunnett's test (necropsy body weight only) or Dunn's test.

Source : MLPC, Rion-des-Landes, France
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
27.12.2000 (74)

Type : other: reproductive organ toxicity
Species : mouse
Sex : male/female
Strain : B6C3F1
Route of admin. : oral feed
Exposure period : 13 weeks
Frequency of treatm. : ad libitum
Premating exposure period
Male :
Female :
Duration of test : 13 weeks
No. of generation studies :
Doses : 250, 750 and 3000 ppm
Control group : yes, concurrent no treatment
NOAEL parental : = 750 ppm
Method : other: NTP's Technical Protocol for Sperm Morphology and Vaginal Cytology Evaluation in Toxicity Testing for Rats and Mice
Year : 1995
GLP : yes
Test substance : other TS: purity 98.9% +/- 0.6%

Method : As part of a 13-week toxicity study (NTP, 1995) groups of 10 male and 10 female mice were administered 0, 250, 500, 750, 1,500, or 3,000 ppm 1,3-diphenylguanidine in feed that was available ad libitum.

Mice were housed individually. Animal rooms were maintained at 69° to 75° F and 35% to 65% relative humidity, with 12 hours of fluorescent light per day and approximately 10 air changes per hour. Feed and water were available ad libitum. Because of the limited stability of the 1,3-diphenylguanidine feed mixtures, feeders were changed daily, 7 days per week, throughout the 2-week and 13-week studies.

At the end of the 13-week studies, vaginal cytology and sperm motility evaluations were performed on all mice in the 0, 250, 750, and 3,000 ppm groups. Methods were those outlined in the National Toxicology Program's Technical Protocol for Sperm Morphology and Vaginal Cytology Evaluation in Toxicity Testing for Rats and Mice (NTP, 1987). Beginning 12 days prior to sacrifice, the vaginal vaults of 10 females from each exposure group were lavaged, and the aspirated lavage fluid and cells were stained with toluidine blue. Relative numbers of leukocytes, nucleated

epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, or metestrus).

Sperm motility was evaluated at necropsy in the following manner. The left testis and epididymis were weighed. The tail of the epididymis (cauda epididymis) was then removed from the corpus epididymis and weighed. Tyrode's buffer was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers.

Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced and swirled, and the tissue was incubated and then heat fixed. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in 10% dimethyl sulfoxide in phosphate-buffered saline. Homogenization-resistant spermatid nuclei were counted using a hemacytometer.

Result :

Summary of Reproductive Tissue Evaluations In Male B6C3F1 Mice in the 13-Week Feed Study of 1,3-Diphenylguanidine.

Study parameters				
Dose (ppm)	0	250	750	3,000
n	10	10	10	10
Weights (g)				
Necropsy body weight (g)	35.9±0.6	34.7±0.7	33.8±0.6*	29.1±0.3**
Left epididymis (mg)	55±2	61±1	56 ±2	54±2
Left cauda epididymis (mg)	22±1	24±1	22±1	21±1
Left testis (mg)	123±4	125±3	125 ±3	117±3
Spermatid measurements				
Spermatid heads (10e5/g testis)	1710±78	1733±94	1867±81	2052±104*
Spermatid heads (10e5/testis)	209±9	217±12	231±7	237±8
Spermatid count (mean/10e-4 mL suspension)	65.28±2.71	67.78±3.83	72.20±2.24	74.18±2.45
Epididymal spermatozoal measurements				
Motility (%)	84.84±3.43#	82.86±4.99	78.86±8.08	51.56±11.77*
Concentration (10e6/g caudal epididymal tissue)	1107±234	791±186	904±271	676±201

Data presented as mean ± standard error. Differences from the control group for epididymal, cauda epididymal, and tests weights, spermatid heads per tests, spermatid count, and sperm concentration are not significant by Dunn's test.

n=9.
* Significantly different (P<0.05) from the control group by Dunnett's test (necropsy body weight only) or Dunn's test.
** Significantly different (P<0.01) from the control group by Dunnett's test.

Summary of Estrous Cycle Characterization In Female B6C3F1 Mice in the 13-Week Feed Study of 1,3-Dlphenylguanldine.

Study parameters				
Dose (ppm)	0	250	750	3,000
n	10	10	10	10
Necropsy body weight (g)	29.3±0.7	28.5±0.6	27.4±0.5*	22.9±0.2**
Estrous cycle length (days)	4.30±0.13	4.45±0.16	4.10±0.07	5.15±0.27*
Estrous stages (% of cycle)				
Diestrus	33.3	26.7	30.0	28.3
Proestrus	20.8	21.7	20.0	20.0
Estrus	26.7	35.8	29.2	39.2
Metestrus	19.2	15.8	20.8	12.5

Necropsy body weights and estrous cycle lengths are presented as mean ± standard error. By multvariate analysis of variance, exposed groups do not differ significantly from the control group in the relative length of time spent in the estrous stages.
* Significantly different (P<0.05) from the control group by Dunnett's test (necropsy body weight only) or Dunn's test.
** Significantly different (P<0.01) from the control group by Dunnett's test
All mice survived to the end of the study (Table 7). Mean body weights of both males and females in the three highest exposure groups (750, 1,500, and 3,000 ppm) were lower than those of the control groups especially during the latter part of the study (Figure 2). Thin appearance was the most frequently reported clinical sign for female mice and was most often observed in the three highest exposure groups. Thin appearance was also observed in male mice in the 3,000 ppm group. Other clinical signs observed in mice in the higher exposure groups included alopecia, abnormal posture, ptosis, and bristly hair.

The average amounts of feed consumed by males and females in all exposed groups were similar to the average amounts consumed by the control groups.

Significantly lower absolute organ weights than controls were observed for seminal vesicles in the 3,000 ppm group. These differences are not indicative of a specific toxic response but appear to be the result of the lower body weights of these groups.

No treatment-related gross or microscopic lesions were observed in male or female mice exposed to 1,3-diphenylguanidine.

Evaluation of male reproductive tissue from animals revealed greater numbers of spermatid heads and lower sperm motility than in the controls in the 3,000 ppm group. In females,

estrous cycle length in the 3,000 ppm group was greater than controls.

Source : MLPC, Rion-des-Landes, France
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
27.12.2000 (74)

Type : other: testicular toxicity and male fertility study
Species : mouse
Sex : male/female
Strain : CD-1
Route of admin. : gavage
Exposure period : 8 weeks
Frequency of treatm. : 7 days/week
Premating exposure period
Male : 8 weeks
Female : none
Duration of test :
No. of generation studies :
Doses : 0.06, 0.25, 1, 4 or 16 mg/kg/day
Control group : yes, concurrent vehicle
NOAEL parental : >= 16 mg/kg bw
Method : other
Year : 1989
GLP : yes
Test substance : other TS: Purity = 99.9%

Method : An oral testicular toxicity and male fertility study with 1,3-diphenylguanidine was carried out in CD1 mice in two tiers respectively. In the first tier the test substance was administered daily to male mice (25 males/group) by daily oral intubation at dose levels of 0, 0.06, 0.25, 1, 4 and 16 mg/kg body weight per day during an 8-week premating period (7 days per week). Females were not treated during any period of the study. Within 24 hours after the last treatment day, 9 to 13 males, randomly taken from each group were killed and subjected to gross examination at autopsy. A selected number of organs were weighed and preserved in formalin. In addition, sperm abnormality evaluation was conducted in the selected males from the control and high dose group. In the second tier the remainder of the males of the control, 4 and 16 mg/kg group was mated (within 14 days after the 8-week dosing period) with untreated females. Reproductive performance, necropsy finding and litter data were recorded.

Result : During the treatment period a small number of animals of all groups but the control and 0.06 mg/kg group were found dead or were killed in moribund condition. The cause of death of these animals was not always discernible from gross necropsy observations. In addition, mortality that appeared to be related to dosing errors was observed in the control group (1 animal), the 0.06 mg/kg (3 animals), the 0.25 mg/kg group (4 animals), and the 4.0 mg/kg group (1 animal).

No differences in body weights between the various test groups and controls were found that could be related to the treatment.

Sperm abnormality evaluation showed a slight, but statistically significant increased incidence of sperm cells with folded tails in the high dose group (5% versus 2% in controls). However, since the total number of abnormal

sperm cells as well as the number of specified sperm abnormalities was similar in all groups, the observed increased number of sperm cells with folded tails is considered of doubtful significance.
Gross examination at necropsy of male mice did not reveal any treatment related changes. No significant differences in organ weights occurred between the groups. Microscopic examination of the testes did not show any effect of treatment with N,N'-dipherrylguanidine. on the basis of observed testis weights it was decided to continue the study with the second tier.
Male and female fertility as well as reproduction performance were comparable in all groups examined (0, 4.0 and 16.0 mg/kg).
Maternal autopsy findings and litter data did not reveal any treatment related effect.

Source : MLPC, Rion-des-Landes, France

Conclusion : Under the conditions of this study 1,3-diphenylguanidine did not exert any adverse
effects on fertility, reproduction capacity or embryonic/foetal development in CD1 mice when administered to male mice at levels up to 16 mg/kg body weight per day.

Reliability : (1) valid without restriction

Flag : Critical study for SIDS endpoint

14.11.2001 (97) (98)

Type : Fertility
Species : mouse
Sex : male/female
Strain : other: C57BL/6JxDBA2
Route of admin. : drinking water
Exposure period : up to 15 weeks
Frequency of treatm. : continously in drinking water
Premating exposure period
Male : up to 15 weeks
Female : no exposure
Duration of test : 15 weeks
No. of generation studies :
Doses : 4 or 8 mg/kg/day
Control group : yes, concurrent vehicle
Method : other
Year : 1983
GLP : no
Test substance : other TS: There were some impurities in the test material (Bempong, Jan. 21, 1987, personnal communication).

Method : Ten-week-old hybrid mice (C57BL/J6 X DBA2) weighing 25-30 g were used. They were maintained on Purina Lab Chow and water ad libitum. All mice used in the study were weighed before treatment and during chemical exposure.

Sperm morphology:
Three groups of mice received the following concentrations of DPG ad libitum: 0.00 (solvent control), 4.0, or 8.0 mg/kg/d. At the appropriate time, animals were sacrificed by cervical dislocation. Sperm were collected from 5 animals per group by dissecting the cauda epididymis to assess the sperm morphology. Groups were compared by using the chi-square and/or t-test.

uantitative sperm analysis:
Testes were weighed immediately after they were removed.

Solvent control and DPG-treated mice were sacrificed at the appropriate killing times. Suspensions of the content of cauda epididymis of each mouse were prepared by the method of Fiscor and Ginsberg (1980). The number of sperm per preparation was determined in a Makler sperm-counting chamber (Makler, 1978).

Histology:
Testes from mice sacrificed 1, 3, 5, 7, 9, and 15 wk after DPG treatment and from concurrent control animals were examined histologically.

Reproductive study:
Ten-week-old male mice were exposed to 4.0 or 8.0 mg/kg/d DPG prepared in acetic acid at a final concentration of 0.025%. Concurrent solvent control animals were exposed to 0.025% acetic acid prepared in deionized water. Exposure was ad libitum and the duration of exposure was 90 d. After 7 d of exposure, the animals were mated at weekly intervals to 12-wk-old virgin untreated females. All matings were monogamous. Pregnant animals, based on the presence of vaginal plugs, were isolated and housed one per cage. The fertility index, expressed as the ratio of the number of pregnant females to the number of females mated in a specified mating group, was determined. On day 13 of pregnancy the female animais were sacrificed by cervical dislocation, the uteri were removed, and the number of implants and frequencies of early (moles) and late fetal lethality per pregnancy were determined.

Result : The average levels of morphologically abnormal sperm among the control animals did not show significant differences during the 85 days. In the hybrid mice abnormal sperm morphology ranged from 1.8 to 5.3% with a mean of 3.5%. These figures were derived from 25 measurements of 200 sperm per measurement. A nonlinear increase in the frequency of sperm abnormalities was observed in the DPG-treated mice. The incidence of DPG-induced abnormal sperm in mice exposed to 4 mg/kg/d ranged from 16.2 to 42.4%. For the 8 mg/kg/d treatment group, the range was 38.6 to 75.1%.

Histopathological analysis of control and DPG-treated mice revealed that in the latter group the parietal peritoneum was saturated with fatty tissues and the mesentery and the greater omentum showed the greatest evidence of fatty tissue accumulation. Fatty tissue accumulation and attendant weight gains in DPG-treated mice were the antithesis of testicular growth. Testicular weights decreased significantly after 5, 7, 9, and 15 weeks of treatment, but were not different from the controls after 1 and 3 weeks of treatment. Sperm count in the two treatment groups decreased significantly 7, 9, and 15 wk after treatment. At the higher dose of DPG (8 mg/kg/d) significant differences in sperm counts were noted 5 weeks after treatment. Examination of the epididymis of treated mice showed the presence of germinal cells. With prolongation of DPG treatment, cytological preparations revealed more germinal cells than spermatozoa.

Changes In sperm Count and Testicular Weight in Mice after Continuous Exposure to 1,3-Diphenylguanidine ad libitum.

	Mean testis	Sperm	Suggested

Dose (mg/kg/d)	Exposure (week)	N	weight (mg)	count (10e4/ml)	meiotic stage at time of exposure
0			286	19.59	
4	1	10	293	16.72	Spermatozoa
8			278	16.23	
0			301	17.62	
4	3	8	285	19.43	Spermatids
8			269	16.75	
0			289	17.10	Preleptotene
4	5*	8	147	12.84	late sperma-
8			131	9.63	togonium
0			298	19.89	
4	7*	8	139	9.54	spermatogonium
8			124	7.61	
0			313	18.47	
4	9*	8	136	9.87	spermatogonium
8			121	4.68	
0			293	17.16	Spermatogonial
4	15*	10	139	7.45	stem cells
8			112	3.19	

* significantly different from control.

Histological preparations of testes from DPG-treated mice showed irregularly shaped seminiferous tubules with no defined basement membrane, loss of interstitial cells, and limited numbers of spermatids and spermatozoa in the lumen of the tubules. These observations were in contrast to the histological organization of the control testes. The developmental anomalies of germinal cells coupled with the decreased sperm count might have been responsible for the results obtained in the reproductive study.

Fertility indices, implants per pregnancy, and fetal mortalities per DPG-treated female were not significantly different from the control values during the first 4 weeks. However, after 5 weeks of DPG treatment significant differences ($p < 0.02$) were noted when the treated and control populations were compared. Differences between the two doses of DPG became evident in the 7th week. This trend continued until the 16th week of continuous DPG exposure (data not presented).

Effect of Chronic Exposure to 1,3-Diphenylguanidine on Fertility Index and Dominant Lethality in Mice.

Period (wk)	Treatment (mg/kg/d)	Number of pregnant mice	Number of implants: Per female	Number of implants: Total	Dead fetuses per pregnancy: Early	Dead fetuses per pregnancy: Late
	0	20/20°	11.8	236	0.54	0.35
1	4.0	18/120	10.3	186	0.44	0.39
	8.0	12/20	9.8	118	0.67	0.33

	0.0	20/19	12.4	248	0.40	0.35
3	4.0	16/20	11.1	178	0.05	0.44
	8.0	14/20	10.6	148	0.64	0.43
	0.0	19/20	12.2	232	0.53	0.37
5	4.0	16/20	10.9	175	0.75	0.69
	8.0	11/20	9.3	103	0.91	0.73
	0	20/120	11.3	236	0.45	0.25
7	4	17/20	10.4	177	1.35	1.06
	8	8/20	9.6	77	2.13	1.88

Source : MLPC, Rion-des-Landes, France
Reliability : (3) invalid
27.12.2000 (99) (100)

Type : other
Species : Syrian hamster
Sex : male
Strain : other
Route of admin. : drinking water
Exposure period : up to 80 days
Frequency of treatm. : continously in drinking water
Premating exposure period
Male :
Female :
Duration of test : 80 days
No. of generation studies :
Doses : 4 or 8 mg/kg/day
Control group : yes, concurrent vehicle
Method : other
Year : 1983
GLP : no
Test substance : other TS: There were some impurities in the test material (Bempong, Jan. 21, 1987, personnal communication).

Method : Twelve-week-old inbred golden Syrian hamsters with an average weight of 55 g were used. They were maintained on Purina Lab Chow and water ad libitum. Three groups of hamsters received the following concentrations of DPG ad libitum: 0.00 (solvent control), 4.0, or 8.0 mg/kg/d. At the appropriate time, animals were sacrificed by cervical dislocation. Sperm were collected from 5 animals per group by dissecting the cauda epididymis and examined for sperm morphology. Groups were compared by using the chi-square and/or t-test.

Result : The average levels of morphologically abnormal sperm among the control animais did not show significant differences during the 85 d. In the hybrid hamsters abnormal sperm morphology ranged from 2.0 to 13.6% with a mean of 9.2%. These figures were derived from 25 measurements of 200 sperm per measurement. Fluctuations in the levels of DPG-induced sperm abnormalities were observed in all preparations from day 30 to day 75. From day 75 to the end of the experiment, steady increases in the frequency of anomalous sperm were observed (up to 50 and 80% of abnormal sperm at 4.0 and 8.0 mg/kg/d, respectively). No further information available on testes weight, cytology and histology

Source : MLPC, Rion-des-Landes, France
Test substance : There were some impurities in the test material (Bempong, Jan. 21, 1987, personnal communication).

Reliability : (3) invalid
27.12.2000 (101) (100)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : rat
Sex : female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : day 6-15 of gestation
Frequency of treatm. : once daily
Duration of test : animals were sacrificed on day 20 of gestation
Doses : 10, 50, 100, 150 or 200 mg/kg/day
Control group : yes, concurrent vehicle
NOAEL maternal tox. : = 10 mg/kg bw
Method : other: range-finding study
Year : 1985
GLP : yes
Test substance : no data

Method : Potential maternal and embryotoxic effects of DPG were evaluated in a range-finding teratology study in rats. DPG was admixed in 0.5% aqueous Methocel and administered to five groups of five bred Sprague Dawley COBS CD rats once daily from gestation days 6 through 15. The route of administration was oral by gastric intubation. Dosage levels of 10, 50, 100, 150 and 200 mg/kg/day were selected. For comparative purposes, a concurrent control group, also composed of five bred females, was dosed with 0.5% aqueous Methocel the vehicle control, on a comparable regimen at 10 ml/kg. Throughout gestation all rats were observed twice daily for toxicity and body weights were recorded at appropriate intervals. All surviving animals were sacrificed on gestation day 20 for a scheduled uterine examination.

Result : CLINICAL OBSERVATIONS AND SURVIVAL
All of the animals in the 150 and 200 mg/kg/day study groups and four in the 100 mg/kg/day study group died between gestation days 7 and 11. The cause of death for these 14 rats was attributed to overt toxicity. All of the animals in the 0, 10 and 50 mg/kg/day study groups and one in the 100 mg/kg/day study group survived to the scheduled sacrifice. Clinical observations noted in the animals that did not survive to the scheduled sacrifice included lethargic behaviour, ataxia, prostrate behaviour, tachypne, body cool to the touch, salivation (both before and after dosing), lacrimation of both eyes, dried red material around the eyes and nose, gasping, shallow respiration, decreased defecation and urination, and wet yellow urogenital staining. Alopecia on various body surfaces was a primary clinical observation throughout the study and was not considered compound-related based on comparable frequencies in all treated groups. Convulsions were noted once in one animal in the 100 mg/kg/day on gestation day 8 (the animal died on gestation day 11).
In the 50 and 100 mg/kg/day dose groups, clinical observations noted in the surviving animals were similar to those that died. Lethargic behaviour and ataxia occurred in four animals in the 50 mg/kg/day dose group and one animal in the 100 mg/kg/day dose group, primarily during the initial dosing days (gestation days 6-9). Prostrate

behaviour and tachypnea were observed in one animal in each of the 50 and 100 mg/kg/day study groups, again primarily during gestation days 6-9. Dried red material around the nose and mouth, and wet yellow urogenital staining were noted once in the 100 mg/kg/day dose group. Dried red material around the nose was also seen twice in the 50 mg/kg/day study group. Salivation approximately one hour following dosing was observed once in the 50 and 100 mg/kg/day dose groups. Alopecia on various body surfaces was observed similarly in all of the treated groups as well as the control group. Based upon the frequencies in the treated groups, this was not considered compound-related.

BODY WEIGHTS
In the 200 mg/kg/day DPG-treated group, all animals died during the first three days of compound administration. In the 150 mg/kg/day dose group, the only measurement was a body weight loss prior to death.
A mean body weight loss occurred in the 100 mg/kg/day dose group during the first three days of compound administration (gestation days 6-9) as well as the last four days of dosing (gestation days 12-16). This resulted in a loss over the entire treatment period (gestation days 6-16). Following treatment, the mean body weight gain in this dose group was less than the corresponding control group gain (gestation days 16-20). Mean body weights were decreased on gestation days 9, 12, 16 and 20.
A mean body weight loss occurred in the 50 mg/kg/day study group during the first three days of DPG administration, resulting in a markedly decreased gain over the entire treatment period (gestation days 6-16). Following treatment, the mean body weight gain in this dose group was greater than the corresponding control group gain (gestation days 16-20). Mean body weights were decreased on gestation days 9, 12, 16 and 20.
In the 10 mg/kg/day treated group, slight differences in the group mean body weights and group mean body weight gains were not considered compound-related when compared to those in the control group.

GESTATION DAY 20 UTERINE EXAMINATION
No animals in the 150 and 200 mg/kg/day dose groups survived to the scheduled sacrifice and only one animal survived to the scheduled sacrifice in the 100 mg/kg/day group. Administration of DPG throughout the major period of organogenesis had no adverse effect on intrauterine survival in the 10 and 50 mg/kg/day study groups. In both of these treated groups, the mean post-implantation loss and the mean numbers of corpora lutea, implantation sites and viable foetuses were comparable to the control group.

NECROPSY EXAMINATIONS
Necropsy findings for the 14 rats which died were similar. The necropsy examinations of these animals showed congestion of the liver, kidneys, lungs, stomach and intestines, haemorrhagic intestines with loss of epithelium, enlarged adrenal glands, and meningeal or basal haemorrhage of the brain. No gross internal morphological changes were observed at the time of the uterine examination in the 10 and 50 mg/kg/day treated groups.

Source : MLPC, Rion-des-Landes, France

Conclusion : A total of fourteen animals in this study died. These

animals were in the 100, 150 and 200 mg/kg/day treated groups. Clinical signs of toxicity were observed in the 50, 100, 150 and 200 mg/kg/day dose groups. An overall reduction of body weight gain was evident in the 50 mg/kg/day dose group as well as a body weight loss during the first three days of DPG administration. Intrauterine survival was not affected by treatment at the 10 and 50 mg/kg/day dose levels. Based on the results of this study, dose levels of 5, 25, and 50 mg/kg/day were selected for the definitive teratology study with DPG.

Reliability : (1) valid without restriction
06.09.2001 (102)

Species : rat
Sex : female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : days 6-15 of gestation
Frequency of treatm. : once daily
Duration of test : animals were sacrificed at day 20 of gestation
Doses : 5, 25 or 50 mg/kg/day
Control group : yes, concurrent vehicle
NOAEL maternal tox. : = 5 mg/kg bw
NOAEL teratogen. : = 25 mg/kg bw
Method : other: EPA Health Effects Test Guidelines 560/6-82-001
Year : 1982
GLP : yes
Test substance : no data

Method : Potential maternal, embryotoxic and teratogenic effects of DPG were evaluated in this study in rats. DPG was admixed in 0.5% aqueous Methocel and administered orally by gavage to three groups of 25 bred Charles River COBS CD female rats as a single daily dose from days 6 through 15 of gestation. Dose levels of 5, 25 and 50 mg/kg/day were selected. For comparative purposes, 25 control females were concurrently dosed with 0.5% aqueous Methocel on a comparable regimen at 10 ml/kg/day. Throughout gestation, all females were observed twice daily for toxicity and body weights were recorded at appropriate intervals. On day 20 of gestation, all surviving females were sacrificed for Cesarean section; fetuses were weighed, sexed and examined for external, skeletal and soft tissue anomalies and developmental variations.

Result : CLINICAL OBSERVATIONS AND SURVIVAL
Alopecia on the forepaws and forelegs was observed in one animal in the 50 mg/kg/day group prior to dosing on gestation day 6 and in all study groups during the treatment period, with an increased incidence and duration noted in the 50 mg/kg/day group. During the treatment period, hair loss was extensive in the 50 mg/kg/day group in the pelvic, abdominal, thoracic, urogenital, inguinal, dorsal back and tail areas. All animals in this dose group were lethargic and had tachypnea and decreased limbtone during the treatment period and with one exception all animals were prostrate and ataxic. A few animals were hypersensitive to the touch, salivated and had piloerection during the treatment period. Clonic convulsions, lacrimation, clear nasal discharge, dried red material around the nose, red urogenital discharge and yellow urogenital matting were observed as single incidences in the 50 mg/kg/day group. Lethargic behavior, salivation prior to dosing, hair loss in

the pelvic and abdominal areas and dried brown material around the mouth were each noted once in different animals in the 25 mg/kg/day group and may be related to treatment with DPG. No clinical signs of toxicity were observed in the 5 mg/kg/day group.
One female in the 5 mg/kg/day group delivered 6 externally normal full-term pups on presumptive gestation day 19. Based on the size and development of the pups, there was an obvious error in the detection of mating. The dam was internally normal except for enlarged mesenteric l ymph nodes. All other females survived to the scheduled sacrifice.

BODY WEIGHTS
Mean maternal body weight gain in the 50 mg/kg/day dose group was significantly decreased at all intervals during the treatment period. The most severe decrease ($p < 0.01$) occurred during the last four days of treatment (gestation days 12-16). The mean body weight gain in the 50 mg/kg/day group was very slightly increased after the treatment period (gestation days 16-20) when compared to the vehicle control group. This resulted in significantly decreased ($p < 0.01$) body weight gains for the entire gestation period (days 0-20).

Group mean body weights were slightly decreased on gestation day 9 and significantly decreased at $p < 0.01$ on gestation days 12, 16 and 20 in the 50 mg/kg/day group.
Mean body weight gain in the 25 mg/kg/day group was very slightly decreased during the overall treatment period (gestation days 6-16) when compared to the vehicle control group. This effect may be related to treatment as there was also a very slight increase in body weight gain following treatment. However, mean body weights in the 25 mg/kg/day group were comparable to the vehicle control group throughout gestation. Body weights and body weight gains in the 5 mg/kg/day group were not affected by treatment with DPG.

GESTATION DAY 20 CESAREAN SECTION DATA
In all groups treated with DPG, foetal sex ratios, the mean numbers of viable foetuses, implantation sites and corpora lutea were comparable to the vehicle control group. Mean foetal weights in the 5 and 25 mg/kg/day groups were comparable to the vehicle control. Mean foetal weight in the 50 mg/kg/day group was significantly reduced ($p < 0.05$) when compared to the vehicle control group. Mean postimplantation loss was slightly increased in the 5 mg/kg/day group due to one female with twelve early resorptions. This increase was not considered biologically meaningful since the effect was not observed at the 25 mg/kg/day dose level. An increase in mean post-implantation loss was also apparent in the 50 mg/kg/day group. One female in the 50 mg/kg/day had all five of the late resorptions occurring in this study, which may be a secondary effect of maternal toxicity. Internal gross necropsy findings for females sacrificed at the scheduled laparotomy such as cystic ovaries, pitted kidneys, white foci or nodules on the lungs and hydronephrosis are considered normal for animals of this strain and age and could not be attributed to the compound.

FETAL MORPHOLOGICAL DATA

The infrequent occurrence of foetal malformations observed in this study was not indicative of a response to treatment with DPG; each study group, including the control, had one foetus with malformations. One foetus in the control group had multiple anomalies including vertebral agenesis, mandibular microagnathia, a dome-shaped head and microphthalmia. Situs inversus was observed in one foetus in the 5 mg/kg/day group, anophthalmia and internal hydrocephaly were observed in one foetus in the 25 mg/kg/day group and a thread-like tail with anal atresia was observed in one foetus in the 50 mg/kg/day group.
Developmental variations observed in the DPG groups were similar to those in the control group except for an increase in the number of fetuses with unossified sternebrae (#5 and/or #6), reduced ossification of the thirteenth ribs, 25 presacral vertebrae and bent ribs in the 50 mg/kg/day group. Reduced ossification would be expected in view of the foetal body weight inhibition at this dose level. The increased number of foetuses with bent ribs in the 50 mg/kg/day dose group is probably associated with maternal toxicity.
Although three foetuses from one dam in the 25 mg/kg/day group had bent ribs, the incidence is within the range of our historical control data. In addition, maternal toxicity was slight at this dose level and foetal body weight inhibition was not apparent. The expression of bent ribs at the 25 mg/kg/day dose level was not considered compound-related.

Source : MLPC, Rion-des-Landes, France

Conclusion : No deaths occurred in this study. Numerous clinical signs of toxicity were observed in many animals in the 50 mg/kg/day group such as extensive hair loss, tachypnea, decreased limbtone, prostrate and lethargic behavior, ataxia, hypersensitivity to touch, salivation and piloerection. Lethargic behavior, salivation prior to dosing, hair loss in the pelvic and abdominal areas and dried brown material around the mouth were each observed once in different animals in the 25 mg/kg/day group. No clinical signs of toxicity were observed in the 5 mg/kg/day dose group.
Mean body weight gain in the 50 mg/kg/day group was significantly decreased during the treatment period (gestation days 6-16) when compared to the vehicle control group. The most severely decreased mean body weight gain occurred during the last four days of treatment (12-16). The body weight gain after the treatment period (days 16-20) in this group was greater than in the control group. Mean body weights in the 25 mg/kg/day group were comparable to the vehicle control throughout gestation. However, mean body weight gain over the treatment interval (gestation days 6-16) in this dose group was slightly reduced and considered compound-related. Mean maternal body weights and body weight gains in the 5 mg/kg/day group were not affected by treatment with DPG. A slight increase in mean post implantation loss and a significant decrease in mean foetal body weight occurred in the 50 mg/kg/day group. No biologically meaningful differences in the mean numbers of viable foetuses, post-implantation loss, implantation sites, corpora lutea, foetal body weights and foetal sex ratios were apparent in the 5 and 25 mg/kg/day groups when compared to the control group. Maternal gross necropsy findings and foetal malformation data did not indicate an adverse response to treatment with DPG in any dose group. The increase in reduced ossification in the 50 mg/kg/day

foetuses would not be unexpected in view of the significantly reduced foetal weight at this dose level. Bent ribs are not associated with a teratogenic response in the absence of other malformations but are often associated with maternal stress and toxicity7. Bent ribs in the seven foetuses from three litters in the 50 mg/kg/day group are a result of maternal toxicity. Three foetuses from a single litter in the 25 mg/kg/day group had bent ribs, however, the data at this dose level suggests that this was a spontaneous expression of biological variation. The incidence of this particular variant is within the range of the historical control data and contrary to the results at the 50 mg/kg/day dose level, maternal toxicity was marginal and foetal body weight was not adversely affected.

In conclusion, DPG induced severe maternal toxicity at a dose level of 50 mg/kg/day. Foetotoxicity was also expressed at this dose level by a significantly reduced mean foetal body weight and by an increase in foetal variations. Maternal toxicity was slight at a dose level of 25 mg/kg/day although a foetotoxic response was not apparent. No sign of teratogenic induction was observed at any of the dose levels selected for investigation in this study.

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

06.09.2001 (103)

Species : mouse
Sex : female
Strain : ICR
Route of admin. : gavage
Exposure period : days 0-18 of gestation
Frequency of treatm. : once daily
Duration of test : until day 18 of pregnancy
Doses : 0.25, 1, 4 or 10 mg/kg/day
Control group : yes, concurrent vehicle
NOAEL maternal tox. : = 4 mg/kg bw
NOAEL teratogen. : >= 10 mg/kg bw
Method : other: similar to OECD Guide-line no 414
Year : 1980
GLP : no
Test substance : no data

Method : Mature ICR female mice were placed with males for 16 hours.
Mature ICR female mice were placed with males for 16 hours.
When a vaginal plug was found the following morning, it was considered to be day 0 of pregnancy, and these females were isolated. All animals were given standard pellets and water ad libitum.
DPG was suspended in 0.5 percent carboxymethyl cellulose solution and was given by gastric intubation. Since all non-pregnant mice given oral DPG in daily doses of 15 mg/kg of body weight died within six days, 10 mg/kg was chosen as the highest dose. Pregnant mice were given DPG daily orally in doses of 0.25, 1.0, 4.0, or 10 mg/kg of body weight from day 0 to day 18 of pregnancy. Control pregnant mice were given the vehicle alone. The volume of each treatment was 5 ml/kg. All treated mice were killed by cervical dislocation on day 18 of pregnancy. The uteri were removed and examined for site of implantation and foetal death. The live fetuses were weighed and examined for gross external malformations. About 50 percent of the foetuses per lifter were fixed in

Bouin's solution for soft tissue examination by the method of Barrow and Taylor (1968) under a dissecting microscope. The remaining fetuses were cleared in KOH and stained with alizarin red S by Dawson's method (1927) for detection of skeletal variations and the state of ossification.
Student's t-test was employed for comparison of the maternal body weight, litter size, foetal body weight, and number of ossified bones among five groups. Comparison of frequency of dead or malformed foetuses and of incidence of skeletal variations or anomalous ossification among the five groups was done with thé rank-sum test (Wilcoxon and Wilcox, 1965).

Result : No abnormalities were detectable in either the experimental or control mothers during pregnancy. There was no conspicuous difference in maternal body weight during pregnancy between treated and non-treated groups.
There were no significant differences in the percentage of dead foetuses, early or late in gestation, average litter size, sex ratio, and body weight between the experimental mice and the controls. The mean number of implants was significantly lower in the mothers treated with 10 mg/kg/day than in the control mothers.

Effect of Diphenylguanidine on Pregnant Mice and Their Fetuses

Dose (mg/kg)	No. of pregnant mice (average no. of implants)	Total No. of implants No.	Dead fetus Early (%)	Dead fetus Late (%)
0	20 (12.7±0.3)	253	4.3	2.8
0.25	19 (12.1±0.7)	229	6.1	1.3
1.0	20 (13.3±0.5)	266	5.3	1.9
4.0	20 (13.7±0.3)	261	5.7	8.0
10.0	7 (11.3 o_0.4)*	79	2.5	0.0

* Significant ifference from control (P<0.05).

Dose (mg/kg)	Live Fetus average No. in Litter ± SEM	Live Fetus ratio (M/F x100)	Live Fetus Sex Mean ± SEM	Live Fetus Body Weight (g) Mean ± SEM
0	11.8±0.4	153	1.42±0.02	1.34±0.02
0.25	11.1±0.7	121	1.46±0.03	1.38±0.02
1.0	12.4±0.5	89	1.36±0.04	1.33±0.03
4.0	11.3±0.7	109	1.40±0.02	1.31±0.05
10.0	11.0±0.5	126	1.40±0.02	1.32±0.02

There were no significant differences in the incidence of

malformed foetuses between the experimental and the control groups. As for the type of malformations, open eye lids were seen in both the control and the experimental groups except in the fetuses of mothers treated with 10 mg/kg/day. One case of postaxial polydactyly and one club foot were seen in foetuses of mothers treated with 1 mg/kg/day.
The incidence of anomalies of the sternebrae was significantly lower than normal in the foetuses from mothers treated with 0.25 mg/kg. Ossification of the talus was significantly retarded in the foetuses from mothers treated with 4.0 mg/kg/day. No significant abnormalities were detected in the soft tissues of either experimental or control foetuses.

Effect of Diphenylguanidine on Mouse Fetus at Term.

Dose (mg/kg)	Malformed fetus	
	Frequency (%)	Type (No.)
0	0.4	open eyelids (1)
0.25	0.4	open eyelids (1)
1.0	1.6	open eyelids (2) postaxial polydactyly (1) club foot (1)
4.0	0.4	open eyelids (1)
10.0	0	-----

Effects of Diphenylguanidine on Skeletal Development of Mouse Fetuses.

Dose (mg/kg)	No. of observed fetuses	No. of malformed fetuses	Type of variation		
			Sternebrae (%)	Cervical rib (%)	Lumbar rib (%)
0	115	0	7.8	1.7	16.5
0.25	101	0	1.0*	6.9	24.8
1.0	117	0	10.3	4.3	23.1
4.0	119	0	8.4	0.8	14.3
10.0	38	0	2.6	5.3	15.8

* Significantdifference from control (P<0.05).

Dose (mg/kg)	Ossification				
	No. of phalanges in forefoot	No. of phalanges in hindfoot	No. of ossified sacral vertebrae	Incidence of ossified calcaneus (%)	talus (%)
	Mean ± SEM	Mean ±SEM	Mean ±SEM		
0	6.05±0.20	5.82±0.30	12.86±0.32	73.9	10.4
0.25	6.08±0.27	6.22±0.32	12.81±0.41	70.3	4.0
1.0	6.10±0.16	5.77±0.27	12.29±0.40	70.1	3.4
4.0	6.27±0.15	6.01±0.26	12.80±0.33	63.0	2.5*

10.0	5.60±0.35	5.47±0.45	12.44±0.39	57.9	5.3

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* Significantdifference from control (P<0.05).

Source : MLPC, Rion-des-Landes, France
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
06.09.2001 (104)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

Remark : Toxicity:
occupational accidental exposure of workers to DPG can cause burning of the eyelids, reddening of the eyes, a bitter taste in the mouth and a painful sensation in the esophagus; flabbiness of the gums and a reduction in the acidity of the gastric juice, tending to achylia, were also reported (no further information available).
Occupational exposure
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.11.2000 (105)

Remark : Occupational medicine:
workers (29-58 years old) occupationally exposed for 3-15 years to DPG were checked up; from ca. 30 % of the examined subjects different complaints were reported, mostly gastritis, cholangitis, cholecystitis, neurological disturbances and dermatitis; beside these symptoms in some patients bronchial asthma, rhinitis, neuropathy, polyathritis, hypertonia and lithiasis were diagnosed and the liver function was disturbed: changes in the protein metabolism and increased bilirubin levels (no further information available)
Occupational exposure
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
24.11.2000 (76)

Remark : At an employee who was working in the area of weigh out- and preparation-procedure in a tyre factory, a work-place concentration of 0.26 mg/mE3 test substance was measured by the US Occupational Safety and Health Administration (1980)
Occupational exposure
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
24.11.2000 (106)

Remark : Sensitization
Result : Patch testing of 49 human volunteers with 70 % DPG in

petrolatum produced no positive rections following initial applicati on; 19 of the 49 subjects displayed positive reactions during subsequent exposures. two subjects displayed positive reactions upon rechallenge 2 weeks later.

Source : MLPC, Rion-des-Landes, France
Reliability : (1) valid without restriction
24.11.2000 (107)

Remark : Sensitization
Result : 74 cases of contact eczema caused by rubber were investigated; 2 were related to hypersensitivity to DPG.
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
24.11.2000 (108)

Remark : 5 patients with contact dermatitis caused by rubber filler in eyelash curlers were tested with DPG in crystals, and in dilutions of 0.01, 0.1 and 0.25 % in 90 % alcohol; patch tests were applied for 48 h, and test sites were observed at 72 h, 92 h and 1 w eek. 0/5 patients reacted positively. Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
24.11.2000 (109)

Remark : 24 patients with contact eczema were tested with a 1 % solution of DPG in petrolatum (48 h), reac tions were read 1 h after removal. 0/24 patients reacted positively. Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
24.11.2000 (110)

Remark : 5 patients with contact eczema caused by rubber products were tested; one patient showed hypersensitivity to DPG. Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (111)

Remark : 63 patients with contact eczema caused by rubber products were tested (24 h, readings: 30 min and 24 h after removal); 15 patients showed a positive reaction to DPG. Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (112)

Remark : 10 patients with contact dermatitis caused by rubber products were tested; 3 patients showed a positive reaction to DPG (1 % in Ungt. alc. lanae). Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (113)

Remark : 17 patients with contact dermatitis caused b y rubber products were tested (readings: 24 h and 48 h); 6 patients showed positive reactions to DPG (1 %).
Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (114)

Remark : 3 patients with contact der matitis due to Spandex (a synthetic polyurethane elastomer) were tested; one patient showed a positive reaction to DPG (2 % in petrolatum) with erythema, edema, papules and vesicles.
Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (115)

Remark : 59 patients with possible contact dermatitis to footwear were tested; no reactions to DPG (1 % in petrolatum) were observed.
Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (116)

Remark : 1/9 persons tested showed positive reactions to DPG.
Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (117)

Remark : 1600 patients of an allergy ambulatorium were tested; 25 showed positive reactions to DPG (1 % in petrolatum)
Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (118)

Remark : The patch tests were made on the first group of 4 patients with current contact dermatitis caused by rubber products, the second group of 5 subjects with hyperpigmentation in the skin in the past and the third group of 27 healthy subjects, namely 36 subjects in the total; all patients of group 1 reacted positive on DPG and the reactions were stronger than in the other groups, in group 2 with a history of dermatitis one patient developed a slight erythema and in the group of healthy subjects 2 reacted with slight erythema.
Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (119)

Remark : 35 patients with shoe dermatitis were tested (treatment: 48 h, readings: at 72 h and 96 h); 2 patients reacted positive to DPG (1 % in petrolatum).

Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (120)

Remark : 32 patients with different kinds of skin-disease were tested; none of these patients reacted to DPG (1 % in soft yellow paraffine).
Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (121)

Remark : 534 patients with hand eczema were tested (treatment: 48 h, readings on the third day); 6 patients reacted positive to DPG (1 and 2.1 % in ethanol).
Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (122)

Remark : A patient with facial contact dermatitis following scuba diving probably caused by the mask was tested (treatment: 20 min, 48 h) with 10 rubber chemicals; he did not react positive to DPG (1 % in petrolatum).
Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (123)

Remark : 15 patients with rubber boot dermatitis were tested (treatment: 48 h, readings: 20 min after removal and when negative 96 h to one week later); 1 patient reacted at DPG (1 %) with significant erythema.
Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (124)

Remark : 744 patients with contact dermatitis were tested (readings at 48 and 96 h); 74 patients showed positive reactions to DPG (1 % in yellow paraffin); because of possible primary skin irritation caused by DPG some positive readings were difficult to judge.
Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (125)

Remark : 47 bricklayers suffering from contact eczema were tested ; 6
Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (126)

Remark : 844 patients with contact dermatitis were tested (readings at 48 h and 96 h after removal); 44 showed positive reactions to DPG (1 % in petrolatum).
Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (127)

Remark : 1 patient probably suffering from nylon-clothes friction dermatosis was tested and showed a slight positive reaction to DPG.
Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (128)

Remark : 106 patients with contact dermatitis due to rubber articles were tested; DPG gave so many irritant reactions that it was impossible to assess whether or not it was also a sensitizer.
Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (129)

Remark : Workers exposed to DPG and suffering from various complaints were tested after scarification of the skin; 3 % showed positive reactions to DPG (no further information available).
Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (76)

Remark : 229 patients suffering from skin diseases, mostly eczematous eruptions were tested; 18 patients showed positive reactions to DPG (0.5 % in petrolatum).
Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (130)

Remark : 6 cattle farmers with occupational contact dermatitis due to rubber chemicals (main cause of rubber contact on the farms appeared to be the milking machine) were tested (readings at 48 h and 72 h, according to directions of the "International Contact Dermatitis Research Group"); 2 showed positive reactions to DPG (1 %).
Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (131)

Remark : 50 patients with occupational contact dermatitis were tested; 2 showed positive reactions to DPG.
Sensitization

Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (132)

Remark : 119 eczema patients were tested; 3 showed positive reactions to DPG (1 %).
Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (133)

Remark : 34 agricultural workers with contact dermatitis were tested; 4 showed positive reactions to DPG; as "control" group 244 patients with contact dermatitis were tested; 13 showed positive reactions to DPG; the authors supposed that the increased sensitivity to DPG in agricultural workers is caused by a possible cross sensitivity to some pesticides derived from guanidine (e.g. Cyprex = dodecylguanidine) and to other products with related structures (e.g. cyanamides).
Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (134)

Remark : 31 subjects were tested (readings: 72 h p.a.; 2 showed positive reactions to DPG (1 %)); no further information available.
Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (135)

Remark : 61 patients with atopic contact dermatitis were tested (readings: 48 h and 72 h p.a.); 2 showed positive reactions to DPG (1 %); the patients were subjected to a second patch test 3-15 years (average 7.3 years) following the first contact sensitivity examination; 3 showed positive reactions to DPG (1 %); 2 patients were positivized and one was negativized; the authors suggest that contact reactions in atopic patients are not linked to susceptibility to skin reactions and tend to increase with time.
Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (136)

Remark : 105 subjects were tested and 3 showed positive reactions to DPG (1 %).
Sensitization
Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (137)

Remark : A worker of a rubber factory had symptoms of allergic rhinitis only while working and breathing the special atmosphere of the factory area where tires are completed;

patch tes ts showed a positive urticarial immediate type reaction to DPG (1 % in petrolatum); the 48-h reactions of the patient were negative.
Sensitization

Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
27.12.2000 (138)

Remark : 1 patient with contact urticaria caused by rubber gloves was tested with DPG (scratch chamber test); patch test was applied for 48 h, and test was read at 20 and 60 min, 48 and 96 h. No reaction was observed.
Sensitization

Source : MLPC, Rion-des-Landes, France
27.12.2000 (139)

Remark : The purpose of this study was to examine the results of patch testing with the rubber components on a standard screening tray and compare them with the results of testing with a special series of 27 rubber components (rubber tray). 1670 patients were patch tested with the screening tray and 317 of these were also tested with the rubber tray. 4.4% of those tested with DPG had a positive response.
Sensitization

Source : MLPC, Rion-des-Landes, France
Reliability : (2) valid with restrictions
27.12.2000 (140)

Remark : Additional references cited in the summary table of section 7.1.
Sensitization

Source : MLPC, Rion-des-Landes, France
26.06.2001 (141) (142) (143) (144) (145) (146) (147) (148) (149) (150) (151) (152) (153) (154) (155)

5.11 ADDITIONAL REMARKS

Type : Toxicokinetics

Result : Absorption and disposition.
A comparison of DPG tissue distribution and excretion following oral vs iv administration of 15.15 µmol/kg indicates that gastrointestinal absorption of DPG was near complete and that tissue distribution and excretion were not significantly affected by the route of administration (Table 1). A comparison of tissue distribution and excretion over the 100-fold dose range studied indicates that absorption and disposition of DPG are not significantly affected by dose in the range studied (Table 1).

Table 1: Distribution and excretion of radioactivity 1 day after administration of [14C]-DPG to F344 male rats

	Percentage total dose	
	Intravenous	oral

Tissue	15.15	1.52	15.15	151.5 mmol/kg
Liver	1.37±0.08	1.31±0.09	1.23±0.11	0.92±0.09
Muscle	1.18±0.08	1.08±0.02	1.08±0.01	1.09±0.08
Adipose	0.56±0.07	0.62±0.03	0.47±0.03	0.49±0.03
Skin	0.52±0.07	0.40±0.41	0.41±0.05	0.39±0.02
Blood	0.24±0.01	0.27±0.01	0.23±0.01	0.24±0.02
Total excreted				
Urine	35.50±3.38	31.76±2.68	29.12±1.72	43.61±2.83
Feces	45.67±9.01	48.25±4.49	45.26±2.94	39.39±1.84
Total*	81.17±6.12	80.01±6.24	74.38±1.27	83.00±2.41

* DPG-derived radioactivity excreted in urine and feces in 24 hr. The remainder is still present in tissues and intestinal contents.

Tissue distribution vs time.
Major organ and tissue volumes were sampled for radioactive content at various time points following iv administration of a 15.15-µmol/kg DPG dose. Initially the highest concentration (% total dose/g tissue) of DPG-derived radioactivity was observed in liver followed by kidney and lung (Table 2). The peak concentration in liver was reached in 45 min after administration whereas the DPG-derived radioactivity in other tissues with the possible exception of testes and adipose tissues showed a decline. The concentration of DPG-derived radioactivity in liver was higher than in other tissues at every time point examined. At 24 hr post-exposure the concentration of DPG in liver was 5-10 times higher than in most other tissues. Interestingly, the brain and most lean tissues contained similar concentrations of DPG-derived radioactivity at comparable time points.

Table 2: Concentration of DPG-derived radioactivity in male F-344 rats vs time

	Percentage total dose#/g tissue				
Tissue	15 min	45 min	2 hr	6 hr	24 hr
Liver	2.20±0.23	2.44±0.41	1.21±0.33	0.42 0.09	0.17±0.02
Kidney	1.82±0.30	1.35±0.35	0.38±0.14	0.19±0.06	0.02±0.004
Muscle	0.44±0.04	0.26±0.08	0.08±0.04	0.02±0.01	0.01±0.009
Blood	0.24±0.02	0.18±0.03	0.07±0.02	0.03±0.006	0.02±0.001
Skin	0.20±0.05	0.18±0.02	0.08±0.02	0.03±0.01	0.02±0.002
Adipose	0.10±0.01	0.11±0.04	0.04±0.02	0.02±0.01	0.03±0.003
Lungs	1.05±0.16	0.35±0.12	0.19±0.06	0.06±0.03	<0.01
Spleen	0.49±0.02	0.27±0.04	0.09±0.03	0.04±0.02	<0.01
Heart	0.41±0.05	0.23±0.0 5	0.07±0.02	0.02±0.01	<0.0l
Brain	0.39±0.03	0.32±0.03	0.09±0.02	0.02±0.01	<0.01
Thymus	0.34±0.01	0.27±0.03	0.07±0.01	0.02±0.01	<0.01
Testes	0.10±0.02	0.19±0.05	0.14±0.02	0.06±0.002	<0.01
Adrenals		0.14±0.03	0.06±0.03		<0.01

#15.15 mmol/kg, iv.

The distribution of radioactivity in rat tissues at various time points following a single iv dose of DPG of 15.15 µmol/kg is presented in Table 3. DPG-derived radioactivity was readily cleared from all tissues so that within 24 hr after exposure the total tissue burden was approximately 10-fold lower than that observed at the earliest time point, 15 min (Table 3).

Table 3: DPG-DERIVED RADIOACTIVITY IN MAJOR F344 RAT TISSUES VS TIME

	Percentage total dose				
Tissue	15 min	45 min	2 hr	6 hr	24 hr
Blood	3.52±0.33	2.60±0.41	1.18±0.15	0.55±0.05	0.24±0.01
Liver	17.69±1.73	20.04±2.65	10.17±1.13	3.27±0.51	1.37±0.08
Kidney	3.05±0.61	2.24±0.72	0.62±0.21	0.30±0.08	0.03±0.01
Thymus	0.18±0.03	0.11±0.03	0.03±0.01	<0.01	<0.01
Skin	5.73±1.26	5.05±0.52	2.86±0.39	0.87±0.37	0.52±0.07
Adipose	1.89±0.13	2.08±0.87	0.80±0.12	0.44±0.34	0.56±0.07
Muscle	40.01±2.28	22.88±7.73	8.33±2.56	1.99±1.31	1.18±0.08
Brain	0.64±0.06	0.55±0.03	0.15±0.03	0.02±0.01	<0.01
Spleen	0.21±0.03	0.11±0.01	0.04±0.02	0.01±0.01	<0.01
Testes	0.24±0.05	0.43±0.11	0.32±0.04	0.15±0.01	<0.01
Lungs	0.96±0.10	0.39±0.12	0.22±0.08	0.06±0.03	<0.01
Heart	0.27±0.04	0.15±0.03	0.05±0.01	<0.01	<0.01
Small intest.	0.82±0.22	1.28±0.48	1.90±0.46	0.50±0.15	0.05±0.05
Small intest. cont.	1.31±0.46	7.15±2.15	11.43±2.17	3.57±1.89	0.17±0.10
Large intest.	0.40±0.21	0.70±0.30	0.32±0.39	0.51±0.25	0.15±0.12
large intest. cont.	0.08±0.03	0.10±<0.01	0.10±0.05	7.74±3.71	1.44±0.29

#iv dose of 15.15 µmol/kg.

Clearance of DPG-derived radio-activity from the tissues followed a biphasic curve. The initial phase of the curve was rapid and accounted for a major portion of the dose. The second component was much slower. The rapid and relatively nonspecific distribution of DPG front blood to the other tissues is well illustrated by the amount of DPG in muscle. Muscle accounts for approximately 50% of the tissue volume of the rat, has no apparent affinity for DPG, and contained approximately 40%, of the DPG dose within 15 min after an iv administration. This radioactivity was rapidly cleared from muscle so that by 24 hr after injection only 1.2% of the total injected dose was still present (Table 3). The data in Table 2 indicate that clearance of DPG-derived radioactivity front most lean tissues occurred at rates similar to those described for muscle. Skin and adipose tissue receive a lower portion of the blood supply than do the lean tissues and thus received less DPG following the iv dose. The lower perfusion rate of adipose tissue may account for the fact that the peak concentration of DPG-derived radioactivity in

this tissue was observed at 45 rather than 15 min.
The initial higher concentrations of DPG-derived radioactivity in liver and kidney may be accounted for by the higher perfusion of these tissues with blood. In kidney, this concentration decreases sharply from 6 to 24 hr which might suggest that DPG elimination through urine is near complete by 24 hr. On the other hand, DPG appears to have an affinity for liver as evidenced by the higher DPG concentrations in liver at all time points examined (Table 2). Clearance of most of the DPG-derived radioactivity from liver appears to be similar to that of other tissues. However, the concentration of DPG-derived radioactivity in liver remained high relative to other tissues because of the higher concentrations initially sequestered by liver. The initial higher concentration of DPG-derived radioactivity in kidney was cleared more rapidly than from other tissues.

Excretion.
Total excretion of [14C]DPG-derived radioactivity was determined by daily collection of urine and feces from each animal held from 1 to 3 days. Approximately equal amounts of radioactivity were excreted in both urine and feces and the relative amounts of excretion by these routes were not affected by the dose in the range studied or the route of administration (Table 1). Approximately 80% of the radioactivity is excreted in urine and feces 24 hr after an iv dose and total excretion approaches 100% in 3 days. Total clearance followed a single component exponential decay with a half-life of approximately 9.6 hr. These results indicate that DPG is not at all persistent in the rat.
The importance of the feces as a route of DPG elimination indicated that much of the DPG-derived radioactivity might be eliminated in bile. The elimination of DPG in bile was studied by cannulating the common bile duct of anesthetized rats. Approximately 50% of the injected dose was excreted within 2 hr and up to 75% of the total dose excreted in 6 hr. These results indicate that DPG excretion in feces (55% in 3 days) accounts for only a portion of the DPG excreted in bile. That portion of the DPG-derived radioactivity exereted in bile and not excreted in feces most probably undergoes extensive enterohepatic recirculation and is subsequently excreted in urine.

Metabolism.
The nature of the [14C]DPG derived radioactivity excreted in urine and bile was examined by direct HPLC analysis (Table 4). Bile contained only small amounts of parent compound at all time points examined. Most of the radioactivity in bile (95%) was in the form of a major metabolite (Peak II) of DPG with traces of another metabolite (Peak I). The major metabolite (Peak II) excreted in bile was resistant to hydrolysis by arylsulfatase, by strong acid, or by strong base. However, incubation of this metabolite with b-glucuronidase resulted in near complete hydrolysis to yield metabolite V. It is believe that this metabolite (Peak II) is in the form of a glucuronide, the position of glucuronidation has not been determined. DPG-derived radioactivity excreted in feces was primarily (94%) in the form of metabolite V. Therefore, it appears that the glucuronide present in bile (Peak II) was subsequently hydrolysed in the intestine, most probably by intestinal flora, to release metabolite V which accounted for most of

the radioactivity excreted in feces.
HPLC analysis of urine indicated that around 28% of the radioactivity excreted in urine was in the form of parent compound. The major metabolite (Peak II) in urine accounted for approximately 37% of the total radioactivity. Treatment of this metabolite with b-glucuronidase resulted in its hydrolysis to yield metabolite V. Comparison of excretion in bile versus feces indicates that as much as 30% of the total dose is reabsorbed from the intestine after excretion in bile. Since most of this material is metabolite V, reabsorption from the intestine and reconjugation may account for most of the metabolite II excreted in urine. Two other metabolites were detected in urine. Metabolite III which eluted from the column shortly after peak II accounted for approximately 32% of the radioactivity while the unconjugated metabolite V accounted only for 3% of the radioactivity.
The nature of radioactivity retained in some of the major tissue volumes was determined at several time points after an iv injection of 15.15 µmol/kg DPG. Tissues were extracted and the extracts analysed HPLC. With the exception of liver, all tissues examined at 45 min after DPG administration contained only the parent compound. In the liver, approximately 88% of the radioactivity was present as parent compound while the rest was present as a single metabolite, Peak II (Table 4). At the 2-hr time point, Peak II was present only in liver and muscle and accounted for approximately 18 and 10% of the radioactivity, respectively. At the 6-hr time point, Peak II increased slightly in liver, while in muscle and kidney this peak accounted for 50 and 60% of the radioactivity, respectively. In liver a major metabolite (Peak III) appeared 24 hr after DPG administration and accounted for approximately 60% of the extracted radioactivity (Table 4). Radioactivity extracted from lung, skin, and adipose tissue at the 45-min and 2-hr time points was present only in the form of the parent compound. The radioactivity extracted from other tissues at the 24-hr time point was insufficient for accurate metabolite determination.

Table 4: relative amounts of DPG and DPG-metabolites present in male F344 rat liver and excreta.

		DPG metabolite (%)					
Tissue	Time (hr)	I*	II	III	IV	V	DPG(%)
Liver	0.75		12±1.2				88±5.7
Liver	2.00		18±1.9				82±4.3
Liver	6.00		30±2.1				70±6.0
Liver	24.00		30±3.3		60±4.5		10±1.1
Bile#	6.00	2±1.2	95±1.7				3±0.5
Urine	24.00		37±1.6		32±1.4		3±0.8 28±0.8
Feces	24.00			2±1.0	94±3.5	4±1.4	

* DPG metabolites separated by HPLC.
Percentage represents only extractable radioactivity.
Collected continuously for 6 hr after an iv injection of 15.15 µmol/kg DPG.

The residual radioactivity present in the liver after

multiple exposures of rats to DPG was also extracted and analysed. The metabolites present were the same and at the same ratio as those metabolites (Peaks II and III) extracted from liver 24 hr after a single iv dose of DPG. The nature of the radioactivity retained in the tissues after extraction is not known.

Multiple exposures.
One, three, or nine daily doses of DPG were administered to groups of three rats each. Rats were sacrificed 24 hr after the last dose. Results of these studies indicated that most DPG-derived radioactivity was readily cleared from all tissues assayed and that DPG concentrations in all tissues except liver were as low or lower after nine daily doses as compared to a single dose. However, a minor portion of the dose in liver was cleared more slowly than observed for other tissues and the concentration of DPG-derived radioactivity in liver increased significantly relative to other tissues as the number of doses increased (Table 5). Extraction and analysis of the persistent radioactivity from liver demonstrated that it represented metabolites II and III (Table 4). An analysis for radioactivity covalently bound to liver macromolecules proved negative. Therefore, the mechanism which accounts for the slower clearance of this minor component from liver is unknown. Likewise, the relevance of this slower component to any toxicity which might be associated with DPG exposure is unknown.

Table 5: Effect of single and multiple DPG doses on tissue concentrations of DPG-derived radioactivity

	Concentration (nmol/g)		
Tissue	1 Dose*	3 Doses	9 Doses
Liver	4.86±0.282	7.38±0.363	11.89±0.811
sloud	0.44±0.022	0.10±0.012	0.20±0.041
Kidney	0.58±0.106	0.29±0.042	0.72±0.134
Skin	0.49±0.063	0.09±0.020	0.28±0.090
Adipose	0.77±0.091	0.03±0.011	0.10±0.024
Muscle	0.36±0.024	0.04±0.005	0.02±0.005

* Animals were administered DPG 15.15 µmol/kg orally and sacrificed 24 hr after last dose.

Source : MLPC, Rion-des-Landes, France
Reliability : (2) valid with restrictions
27.11.2000 (156)

Type : Toxicokinetics

Remark : Dermal absorption, distribution, and metabolism of 1,3-diphenylguanidine was studied in adult female Sprague-Dawley rats. Radiolabelled DPG (0.3 µmol = 0.063 mg/animal) was applied dermally and DPG showed 10% penetration through clipped back skin of the rats in 5 d. The first-order dermal absorption rate constant as determined by least square method was 0.021 ± 0.002 d-1 ($T_{½}$ = 33.6 d). Approximately 13% of the absorbed dose remained in the body in 5 d. Retention in skin, muscle, liver, intestine and fat contributed most to the body burden of DPG-derived radioactivity in 5 d. All tissues showed tissue to blood ratios greater than 1, with liver and intestine

ratios of 26 at 5 d. Approximately 61% of the absorbed dose was eliminated into urine and 27% into feces in 5 d showing rapid clearance of absorbed DPG from the body. High-pressure liquid chromatography (HPLC) analysis of urine revealed two major peaks (parent compound and metabolite(s)). Within 72 h, approximately 50% of the DPG-derived radioactivity excreted in the urine was parent compound. After 72 h, the DPG-derived radioactivity in the urine was present in the form of a single metabolite, and no parent compound was detected. No parent compound was detected in feces. Two metabolites, neither of which occurred in urine, were detected in feces. The HPLC analysis of the radioactivity at the application site showed only parent compound.

Source : MLPC, Rion-des-Landes, France
Reliability : (2) valid with restrictions
26.06.2001 (157)

Type : Toxicokinetics

Remark : A pharmacokinetic analysis of DPG did not reveal bioaccumulation of the DPG in the testes after ip administration. The amount of DPG recovered in mice testes after a single ip administration of 25 mg/kg was 0.46 µg at 5 min. Two or more hours after treatment, the amount of DPG in the testes was below the limit of detectability.

Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
24.11.2000 (158)

Type : Biochemical or cellular interactions

Remark : The influence of DPG on lipid metabolism in rats was investigated after oral application: one application of 375 mg/kg = LD50, 7 applications (once daily) of 188 mg/kg = 0.5 of LD50 and 20 applications (once daily) of 0.2 of LD50; in the livers of all treated animals increased levels of triglycerides and choesterol ethers were determined; in the plasma the levels of triglyceride, cholesterol and of all phospholipide fractions were lowered and the levels of free fatty acids increased; it was considered that these changes were based on an inhibition of the synthesis of phospholipids (especially lecithins) accompanied with an increased activity of lipase in the fatty tissue

Source : MLPC, Rion-des-Landes, France
Reliability : (4) not assignable
06.12.2000 (159) (160) (161) (162) (163)

Type : other: Chronic toxicity

Remark : The chronic exposure of C57Bl/J6 X DBA2 mice to DPG induced an enlargement of the spleen and a time-dependent increase of the DNA-synthesis in spleen cells (no further information available)

Source : MLPC, Rion-des-Landes, France
Reliability : (3) invalid
24.11.2000 (164)

Type : other: Toxicity in chicken embryos

Remark : 3 days old White Leghorn chicken embryos were injected by dropping the test substance (0.06-0.96 umol/egg) into the air chamber of the egg (20-30 eggs/dose); 10 control eggs

were injected with the vehicle (5 µl acetone) only; the ED50 (concentration that induced malformations or death in 50 % of the embryos) of DPG was 0.2 umol/egg, the total mortality (on day 14) LD50 was 0.29 umol/egg, the early deaths (days 3-5) LD50 was 0.31 umol/egg.

Source : MLPC, Rion-des-Landes, France
Test substance : purity: technical grade
Reliability : (3) invalid
27.12.2000 (165) (166)

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

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(2) MLPC Rion des Landes.

(3) Monsanto Chemical Company >99%

(4) Bayer AG, unpublished data

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10.1 END POINT SUMMARY

Memo : Acute oral toxicity

Remark : 5.1.1

Conclusion : The acute effects of DPG are compiled in the following table. The oral LD50 is given as 323-850 mg/kg bw in rats and 150-520 mg/kg bw in mice.

Results of Experiments on the Acute Oral Toxicity of DPG

Species	Dose (mg/kg)	Main Effects	Reference	Reliability
Rat	350	LD50	Monsanto, 1986	2
Rat	460 384	LD50 male LD50 female	Sumitomo, 1977	2
	> 170 mg/kg	dyspnea and ataxia		
Rat	850	LD50	Monsanto, 1954	2
Rat	375	LD50	Arkhangel'skaya and Roshchina; 1963, 1964	4
Rat	500	LD50	Dieke et al., 1947	3
Rat	250	maximum tolerated dose, spasms, liver weight	Arkhangel'skaya and Roshchina, 1963, 1968	4
Rat	500	minimum lethal dose	Arkhangel'skaya and Roshchina, 1963	4
Rat	323	LD50	Vlasyuk, 1978	4
Mouse	290	LD50	Arkhangel'skaya and Roshchina,1964	4
Mouse	250	maximum tolerated dose; spasms, liver weight	Arkhangel'skaya and Roshchina, 1963, 1968	4
Mouse	450	LD100	Arkhangel'skaya and Roshchina, 1963, 1968	4
Mouse	258	LD50	Vlasyuk, 1978	4
Mouse	520	LD50	Amer. Cyan. Co.; cited in McCormick (1971)	4
Mouse	150 211	LD50 male LD50 female	Hasegawa et al., 1989	4
Rabbit	246	LD50	Vlasyuk (1978)	4
Rabbit	250	minimum lethal dose	Smyth, 1931	3
Rabbit	250	LD50	Marhold, 1986	4
Guinea pig	250	minimum lethal dose	Smyth, 1931	4
Guinea pig	250	LD50	Marhold, 1986	4
Dog	10	emetic dose	Amer. Cyan. Co.; cited in McCormick (1971)	4

06.12.2000

Memo : Acute inhalation toxicity

Remark : The available data are not reliable to assess the acute toxicity of 1,3-diphenylguanidine (Valade et al., 1949; reliability 3).
5.1.2

06.12.2000

Memo : Acute dermal toxicity

Remark : 5.1.3
Conclusion : The dermal LD0 in rabbit is > 2000 mg/kg (Monsanto, 1992; reliability 1).

06.12.2000

Memo : Skin irritation

Remark : 5.2.1
Conclusion : DPG does not irritate the skin of rabbits (Monsanto, 1977; reliability 2).

06.12.2000

Memo : Eye irritation

Remark : 5.2.2
Conclusion : DPG is irritating to the rabbit eye (Monsanto, 1977; reliability 2).

06.12.2000

Memo : Sensitization

Remark : 5.3
Conclusion : According to the Magnusson and Kligman maximization test; DPG is not sensitizer in guinea-pigs (MLPC, 1995; reliability 1)).

06.12.2000

Memo : Repeated dose toxicity

Remark : 5.4
Conclusion : Subchronic feeding experiments in rats and/or mice have been performed according to OECD guidelines and GLP in the frame of the US National Toxicology Program (1995) and by Monsanto (1982).

In rats (F344/N) and mice (B6C3F1) which, in a dose-finding study, received DPG for 14 days in their feed in concentrations of 250, 500, 750, 1,500 and 3,000 ppm, only reduced feed intake and decreased body weights were observed as of 750 ppm (NTP, 1995). In the subsequent subchronic study, DPG was fed to the rat and mouse in the same concentrations as in the subacute study (equivalent to daily dose of 17/17, 32/32, 50/49, 100/95 and 181/184 mg/kg bw/d for male/female rats and 38/46, 75/93, 114/141, 231/285 and 457/577 mg/kg bw/d in male/female mice at 250, 500, 750, 1500 and 3000 ppm, respectively). Due to the poor palatability of the DPG-treated feed, a decreased feed consumption and reduced body weights were observed as of 750 ppm compared to controls (750 ppm (m/f): 92%/93%; 1,500 ppm: 79%/86%; 3,000 ppm: 52%/-). Increased mortality was observed for both sexes after receiving 3,000 ppm in feed, whereby all females of this concentration group died. Primarily substance-induced effects on the organs were not observed.

The diverse deviations from the controls, determined also for the hematological and clinical-chemical parameters particularly in both highest concentrations (1,500 and 3,000 ppm), are seen exclusively as the result of emaciation by reduced feed intake.
With the average feed intake comparable with the controls, body weight retardation was observed in the mice also as of 750 ppm. The reduced organ weights occurring as of 1,500 ppm were evaluated not as a specifically toxic response but rather were correlated to the clearly reduced body weights.
Histopathological changes and significant deviations from the controls for the hematological and clinical-chemical parameters were not observed.
The NOAEL for both species lies at 500 ppm (ca. 32 mg/kg b.w. and day for rats and ca. 75 mg/kg b.w. and day for mice) (NTP, 1995; reliability 1 (rat study) or 2 (mouse study)).

Dosing Sprague-Dawley rats with DPG in the diet at a concentration of 50, 150 or 500 ppm for 13 weeks (equivalent to daily dose of 4, 11 and 37 mg/kg bw/d), produced a marked reduction in growth rate of both males and females at 500 ppm, with respect to controls. The food consumption of these animals was also reduced, when compared to the controls. The effects were most pronounced over the first few weeks of dosing, suggesting that the cause may partly be due to the unpalatability of the test substance.
Dose levels of DPG up to 500 ppm did not cause an increase in mortality or the appearance of any abnormal clinical signs.
Laboratory investigations revealed small differences between rats receiving DPG and the controls in both haematological and clinical chemical parameters, but as the same effects were not present at both Weeks 6 and 13 they are considered to be of little or no significance. Male rats receiving 500 ppm DPG tended to produce more concentrated urine than the controls at Weeks 6 and 13, with a slightly reduced pH on the latter occasion. Female rats at the same dose level also showed a slight aciduria, but at Week 13 only. These effects were only slight and could not be attributed to any obvious structural change to the kidney as seen on histopathological examination. The changes seen lacked a dose response and were of marginal magnitude, thereby confirming their insignificance.
The terminal studies revealed no dose related lesions; those lesions found were spread across the dose groups with roughly equal incidence and are considered to be common findings in rats of this age and strain.
The NOAEL lies at 150 ppm (11 mg/kg bw/d) (Monsanto, 1982; reliability 1).

Additional subacute and subchronic studies are compiled in the following table. These are insufficiently documented in some cases; most do not meet current requirements (reliability 3 or 4), such as those concerning dose selection. Thus the results should be assessed critically.

Results on the Toxicity of DPG after Repeated Administration

Species (Sex/ Numb.)	Adminis- tration (Duration)	Dose/Day (mg/kg bw)	Main Effects	Reference

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Rat	feed (28 d)	7, 75	75 mg/kg: feed intake as result growth retardation	McCormick, 1971	
Rat	oral (4 mo)	32	reticulocytosis, eosinophilia, erythrocytes, hemoglobin, inhibited catalase and peroxidase, total bilirubin, threshold of nerve and muscle irrita-bility, mortality, no kidney findings	Orlov et al. (1973)	
Rabbit	oral (not given)	LD100	erythrocytes, serum t-globulin	Arkhangel'skaya and Roshchina, 1963, 1968	
Rabbit	oral (5.5 mo)	50	severely inflamed liver parenchyma, bilirubin, dystrophy of convoluted tubules, unchanged blood picture	Arkhangel'skaya and Roshchina 1963, 1968	
Dog	oral (24 d)	10 (21 administrations	"relatively well tolerated"	Amer. Cyan. Co.; cited in McCormick, 1971	
Dog	oral (not given)	5	bile acids	Burov (1964)	
Rabbit	dermal (10 administrations)	1000	no systemic effect	McCormick, 1971	
Rat	inhal.	about 220 mg/m3/2h	Disturbed "oxidation-reduction" processes, functionally disturbed nervous system, blood pressure	Arkhangel'skaya and Roshchina, 1963, 1968	
Guinea pig	inhal. (repeated)	100 mg/l	lethal	Verchovski, 1952	

06.12.2000

Memo : Genetic toxicology "in vitro"

Remark : 5.5

Conclusion : Results available on Ames tests are predominantly negative (Monsanto, 1976; JETOC, 1996; Crebelli et al., 1984, 1985; Rannug et al., 1984; You et al., 1982; Yamaguchi et al.,

1991). In one Ames test DPG caused a weak increased number of revertants after metabolic activation (S9 mix from hamster liver) (Mortelmans et al., 1986; NTP, 1995), while in another case the weakly positive result may have been due to contamination (Bempong & Mantley, 1985; Bempong, unpublished data).
All other in vitro investigations, gene mutation on Escherichia coli and Saccharomyces cerevisiae (JETOC, 1996; Monsanto, 1976), gene mutation assay on CHO (Donner, 1983) and mouse lymphoma cells (Monsanto, 1979) and cytogenetic assay on CHO cells (Monsanto, 1992) were consistently negative. DPG at a concentration of 7.5 ug/ml caused 50% inhibition of the colony formation in HeLa-S3 cells (Baba, 1980; reliability 4).

Test System	Metabol. Activ.	Concen. range	Result	Reference	Reliab.
Ames test on Salmonella typhimurium					
TA98,100	+(1)	1-100 µg/plate	weakly positive	Mortelmans et al, 1986, NTP, 1985	1
TA98,100,1535, 1537	+(2)/-	33-10 000 µg/plate	negative	Mortelmans et al., 1986; NTP, 1995	1
TA1535,1537	+(1)	100-10000 µg/plate	negative	Mortelmans et al, 1986, NTP, 1995	1
TA98,100, 1535,1537,1538	+/-	2-500 µg/plate	negative	JETOC, 1996	2
TA98, 100	+/-	200-5000 µg/plate	negative	Crebelli et al, 1984, 1985	2
TA98,100,1535, 1537, 1538	+/-	0.1-500 µg/plate	negative	Monsanto, 1976	2
TA98,100, 1535,1537,1538	-/+	0.036-36 µg/plate	weakly positive	Bempong and Mantley, 1985	3
TA98,100, 1535,1537,1538	+/-	not given	negative	Rannug et al., 1984	3
TA98,100,	+/-	not given	negative	You et al., 1982	4
TA98,100	+/-	1-100 µg/plate	negative	Yamaguchi et al. (1991)	4
E.coli WP2	-	2-500	negative	JETOC, 1996	2
	+	20-5000 µg/plate	negative		
HGPRT test V79 cells	-	100-500 mg/ml	negative	Donner et al., 1983	3

TK test - 16.4-188 negative Monsanto, 1979 2
L5178Y cells + 32.8-525 negative
µg/ml

S. cerevisiae +/- 1-500 negative Monsanto, 1976 2
D4 µg/plate

Cytogenetic +/- 125-750 negative Monsanto, 1992 2
assay, CHO cell µg/ml
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(1) 10 % hamster liver homogenate
(2) 10 % rat liver homogenate

06.12.2000

Memo : Genetic toxicity "in vivo"

Remark : 5.6

Conclusion : The micronucleus test on erythrocytes of the peripheral blood of mice, that obtained feed with a DPG-content of 250, 500, 750, 1,500 and 3,000 ppm in the subchronic test, led to a negative result in the male animals. The test result of the females was evaluated as "questionable" because of the statistically significant increase of the micronuclei-carrying normochromatic erythrocytes in the female animals of the middle concentration group (750 ppm) (NTP, 1995; reliability 1).

However, in a cytogenetic assay, DPG was administered via oral gavage to male and female rats at a target dose of 300 mg/kg body weight. Bone marrow was sampled at 6, 24 and 48 hours after dosing. No statistically significant increases in the proportion of aberrant cells or aberrations/cell were observed. Significant induction of toxicity, measured as mitotic index depression was observed at the 6 hour (35%) and 24 hour (31%) time points. No depression in mitotic index was observed at the 48 hour time point (Monsanto 1989; reliability 1).

A micronucleus test in mice was negative (further information not given; Bempong, unpublished data; reliability 4).

Bempong (1987) and Bempong and Mantley (1985) (reliability 3) administered a single i.p. dose of 0.036-36 mg DPG/kg bw to mice and then examined the peritoneal fluid, urine and feces daily for 4 days with S. typhimurium strain TA 100 (modified host-mediated assay). Only in the feces was a dose and time-related increase in the number of revertants seen. This result may have been due to contamination of the substance, as later investigations with the pure compound were negative. Further data are not available (Bempong, unpublished data).

06.12.2000

Memo : Carcinogenicity

Remark : 5.7

Conclusion : A carcinogenicity study which meets current requirements is not available.

Bempong & Myers (1985; reliability 3) report on the

induction of adenocarcinomas in C578L/J6xDBA2 mice through chronic exposure to DPG, without giving data on the experimental procedure. According to a personal communication by Bempong DPG was administered in this experiment in oral doses of 4 and 8 mg/kg bw for 32 weeks (7 days/week) to groups of 50 female and male mice. DPG had not caused the appearance of tumors by the end of the experiment. After a 10 to 16 week post-observation period, lymph gland adenosarcomas were determined in 3 of 50 animals in the 4 mg group (in 0 animals of the control group).

As data are missing on the number of accompanying control animals, contradictions occurred in the characterization of tumors, and tumors were seen in the low-dose group but not in the higher one, this study does not permit an assessment of a possible carcinogenic effect of DPG.

06.12.2000

Memo : Toxicity to reproduction

Remark : 5.8.1

Conclusion : In addition to the results of the subchronic studies on the rat and mouse decribed in Section 5.4, special studies for recognizing reproductive toxic effects were also performed (NTP, 1995; reliability 1).

Female rats which had been administered feed having a DPG-content of 250, 500, 750, 1,500 and 3,000 ppm for 13 weeks, exhibited uterine hypoplasia and a prolonged reproductive cycle in the 750 ppm -group (ca. 49 mg/kg b.w. and day) and 1,500 ppm -group (ca. 95 mg/kg b.w. and day) in comparison with the controls. All females of the 3,000 ppm -group died during the study, so that comparable studies of these animals could not take palce. After subchronic feeding of DPG, the male rats only in the 1,500 ppm -group (ca. 100 mg/kg b.w. and day) showed diminished sperm motility. Alterations in the reproductive organs (e.g. depletion of the prostate, hypospermia, reduced spermatogenesis) were occassionally found in the males of the 3,000 ppm-group (ca. 181 mg/kg b.w. and day).
Also for the mice which obtained up to 3,000 ppm DPG in feed for 13 weeks, a prolonged reproductive cycle was observed in the females of the highest concentration group and, in the male animals, a decreased sperm motility. The increased number of spermatid heads in the testes paired with the lower number of sperm in the epididymis is evidence against an effect on spermatogenesis, whereas an effect on the release of sperm into the epididymis cannot be excluded. Comparisons of the parameter changes determined after the DPG-feeding in the rat and mouse, which can be used for assessing a possible reproductive toxic effect, with the results of tests with feed withdrawal (Chapin et al., 1993; Levin et al., 1993) infer that the effects observed in the DPG-treated animals in high concentration groups are a result of the poor general state of health (malnutrition, exhaustion) of the animals.

After doses of 4 or 8 mg DPG/kg bw/day, the following symptoms were found in mice and hamsters: reduced testicular weights, oligospermia, sperm anomalies and a decreased fertility index after mating with untreated females (Bempong, 1983; Bempong & Hall, 1983). Information on the

purity of the DPG is not available (reliability 3).
In a later study with CD1 mice, 1,3-Diphenylguanidine (99.9%) was administered by daily gavage to male mice at dose levels of 0, 0.06, 0.25, 1, 4 and 16 mg/kg body wt. per day during an 8-week premating period. Females were not dosed at any time during the study. Sperm abnormality evaluation was performed in approximatively half the males, randomly selected from the control and 16 mg/kg dose group on completion of dosing. The remaining males in the control, 4 and 16 mg/kg body wt per day groups were mated with non-dosed females. Reproductive performance, necropsy findings and litter data were recorded. No differences were found between control and dosed groups in body weight gain during the dosing period, macroscopic observations and organ weights at necropsy. Microscopic examination of the testes and determination of the frequency of total sperm abnormalities in the 16 mg/kg body wt per day group, did not show any effect due to 1,3-diphenylguanidine dosing when compared to the control group, except for a slight increase in sperm with folded tails but normal heads. Male and female fertility as well as reproduction performance were comparable in the groups examined (0, 4 and 16 mg/kg body wt per day). Maternal necropsy findings and litter data did not reveal any dose-related effect.
At the opposite of Bempong's study, it was concluded that under the conditions of this study, 1,3-diphenylguanidine did not exert any significant adverse effects on fertility, reproductive capacity or embryonic/fetal development in CD-1 mice when administered to males at levels up to 16 mg/kg body wt per day (Koëter et al., 1992; WTR, 1989; reliability 1). The difference in these results may be explainable by contamination of the DPG employed in the studies carried out by Bempong.

26.06.2001

Memo : Developmental toxicity/Teratogenicity

Remark : 5.8.2

Conclusion : In female rats (Monsanto, 1986; reliability 1) and mice (Yasuda & Tanimura, 1980; reliability 2) fetotoxic, but not teratogenic, effects were seen after the oral administration of maternotoxic doses. In the rat study the NOEL was given as 5 mg/kg bw for the dams and 25 mg/kg bw for the fetuses. In the mouse study the NOEL was given as 4 mg/kg bw for the dams and > 10 mg/kg bw for the fetuses.

In 17 of 130 chicken embryos (control 1) DPG led to increased rates of mortality and malformation. The ED50 was 0.042 mg/egg (Korhonen et al., 1983; reliability 3). This test is not validated for reproduction toxicity.

01.12.2000

Memo : Toxicokinetics

Remark : Y09-069

Conclusion : After a single oral administration to male F344 rats, 14C-DPG was absorded almost completely from the gastrointestinal tract and distributed rapidly in the organism, as was also the case after a single intravenous administration (dose 15.15 µmol and 3.2 mg/kg bw respectively). No difference in distribution or excretion were seen. Single oral administrations of different doses

(1.52-151.1 pmol and 0.32-32 mg/kg bw) also brought about no change in the absorption or distribution (Ioannou & Matthews, 1984; reliability 2).

In female Sprague-Dawley rats, which had received a single dermal application of 0.063 mg 14C-DPG/animal (0.3 µmol), only 0.1-10% of the 14C activity penetrated the shaven skin of the back within 0.5-120 hours (T1/2. 33.6 days). Distribution throughout the entire organism also occurred here (Shah et al., 1985; reliability 2).

The highest 14C activities after intravenous or dermal administration were measured in the liver, kidneys, lungs, intestines with contents, and excreta; maximum tissue concentrations after intravenous administration were reached 15-45 minutes after the start of the experiment; after dermal application within 3-6 hours. With the exception of the liver, only 14C-DPG was detected in tissues 45 minutes after intravenous administration; the 14C activity in the liver was also higher than in other organs at all measurement times (Ioannou & Matthews, 1984; Shah et al., 1985).

Within 24 and 72 hours about 80 and >99% respectively of the 14C activity administered orally or intravenously was excreted about equally in the urine and feces (elimination half-life 9.6 hours). About 30% of the 14C activity eliminated in the bile was subjected to enterohepatic circulation and excreted in the urine (Ioannou & Matthews, 1984).
Within 6-120 hours after dermal application 34-64% of the absorbed 14C activity was excreted in the urine and <1-29% in the feces. Accumulation in the adipose tissue was not observed (Shah et al., 1985).

The following table gives an overview of the metabolites occurring, without identifying them specifically, however.

Relative Distribution (in% 14C-activity) of 14C-DPG or the metabolites (I-V) in Liver, Bile, Urine, Feces and Skin after Single Intravenous or Dermal Administration. The 14C-labelling was done by U-labelling on the phenyl rings (according to Ioannou & Matthews, 1984; Shah et al., 1985)

Excreta/ Organ	Time (h)	I	II	III	IV	V	14C-DPG
Liver(1)	0.75	-	12	-	-	-	88
	2	-	18	-	-	-	82
	6	-	30	-	-	-	70
	24	-	30	60	-	-	10
Bile (1)	6	2	95	-	-	-	3
Urine(1)	24	-	37	32	-	3	28
Urine(2)	24	-	50	-	-	-	50
	48	-	53	-	-	-	47
	120	-	100	-	-	-	-
Feces(1)	24	-	-	-	2	94	4
Feces (2)	24	-	-	-	-	100	-
	48	-	-	-	15	85	-
	120	-	-	-	26	74	-
Skin(2)	6-120	-	-	-	-	-	>95

---

(1) intravenous administration (3.2 mg/kg bw)
(2) dermal application (0.063 mg/animal)

Three and 9 oral administrations of 3.2 mg/kg 14C-DPG/kg/day (15.15 µmol) also caused no accumulation in the tissues (Ioannou & Matthews, 1984). In the liver there was a proportional 14C increase, the metabolites II and III being detected. Covalent binding to liver macromolecules was not determined (Ioannou & Matthews, 1984).

Additional, although insufficiently documented, studies in mice (Hunter & Scully; cited in Bempong & Hall, 1983) and rabbits (Kazarinova et al., 1975) indicate that DPG is excreted rather quickly and does not cumulate.

26.06.2001

Memo : Experience with human exposure

Remark : 5.10
Conclusion : Acute Poisoning
No data available.

Chronic Poisoning
Following unintentionally increased workplace exposure to DPG, due to inadequate safety measures, the following symptoms were reported: eyelid pain and eye redness, a bitter taste and a painful feeling in the esophagus. Reduced gastric juice acidity and achylia were also determined. Further data are not available (Arkhangel'skaya & Roshchina, 1963; reliability 4).

Epidemiological Data
The only results available are from a poorly documented study, whose validity cannot be judged due to the lack of data on possible previous employment, the possibilities of contact with other substances, concentrations and control groups.
Orlov et al. (1973; reliability 4) examined workers ranging in age from 29 to 58 years, who had come into contact with DPG during production (no further information) over 3-15 years. About 30% of the test subjects showed symptoms, most suffering from stomach and gall-bladder complaints, neurological disorders or skin diseases. Another finding was liver metabolism disorder (disturbed protein metabolism, increased bilirubin values).

Sensitization
In persons suffering from a contact dermatitis positive DPG patch tests have occasionally been described (see Table). The following main contact possibilities were named: shoes (Blank & Miller, 1952; de Vries, 1964; Hjorth & Fregert, 1972; Song et al., 1979; Lynde et al., 1982; Bajaj et al., 1988), articles of clothing (Bandmann, 1956; van Dijk, 1968; Song et al., 1979), rubberized protective clothing (Fegeler, 1963; Götz & Istvanovic, 1963; Höfer & Hänemann, 1967; Ross & Obst, 1969) and other rubber articles, e.g. gloves or the rubber parts of milking machines (Nater, 1975; Song et al., 1979).

A tire production employee, whose allergic rhinitis symptoms occurred only at the workplace, had a positive patch test reaction with 1% DPG (Camarasa & Alomar, 1978).

Garcia-Perez et al. (1984) found that Spanish agricultural workers with a contact dermatitis had a significantly higher sensitization to DPG than a contact dermatitis control group working in another profession (11.76% to 5.320. The authors attributed this to possible cross-reactions with pesticides, as some of these substances (e.g. Cyprex) are guanidine derivatives and others (e.g. the cyanamides) possess a similar chemical structure.

The available data are summarised in the following table.

Results of Patch Tests Performed with DPG

Subjects (n)	Pos. Reaction (n)	(%)	Concentration (%)	Reference
74	2	3	n.g. (1)	Bonnevie & Marcussen, 1944
5	0	0	0.25	Curtis, 1945
24	0	0	1	Blank & Miller, 1952
5	1	20	1	Bandmann, 1956
63	15	24	n.g.	Herrmann & Schulz, 1960
17	6	35	1	Götz & Istvanovic, 1963
4	3	75	n.g.	Takeda et al., 1964
9	1	11	n.g.	de Vries, 1964
10	3	30	n.g.	Höfer & Hönemann, 1967
3	1	33	2	van Dijk, 1968
524	6	0.01	1+2.1	Agrup, 1969
15	1	7	1+2	Ross & Obst, 1969
106	-(2)	-	1	Wilson, 1969
744	74(3)	9.9	1	Rudzki & Kleniewska, 1970
47	6	l3	n.g.	Rudzki & Kohutnicki, 1971
35	2	6	1	Adams, 1972
229	18	8	0.5	Baer et al., 1973
n.g.	n.g.	3(4)	n.g.	Orlov et al., 1973
32	0	0	1	te Lintum & Nater, 1973
59	0	0	1	Dahl, 1975
6	2	33	1	Nater, 1975
1600	25	1.6	1	Reifferscheid, 1979
844	44	5	1	Rajan & Khoo, 1980
49	2	4	70	Monsanto, 1982
50	2	4	n.g.	Kilpikari, 1982
119	3	3	1	Lynde et al., 1982
1	0	0	1	Tuyp & Mitchell, 1983
34	4	12	n.g.	Garcia-Perez et al., 1984
244	13	5	n.g.	Garcia-Perez et al., 1984
31	2	7	1	Kantoh et al., 1985
61	2	3	1	Lisi & Simonetti, 1985
61	3	5	1	Lisi & Simonetti, 1985
1	1	100	n.g.	Ruocco & Florio, 1986
105	3	3	3	Bajaj et al., 1988

1	1	100	1	Calan, 1978
1	1	100	n.g.	Bruze, 1994
1	1	100	1	Aguirre et al., 1994
1	1	100	n.g.	Helander & Mäkelä, 1983
1	0	0	1	Koch, 1996
2	1	50	n.g.	Roed-Petersen & Menne, 1976
5	1	20	1	Kanerva et al., 1994
11	0	0	1	Kanerva et al., 1996
15	0	0	1	Knudsen et al., 1993
20	7	35	n.g.	Jung, 1977
30	1	3.3	1	Koch et al., 1996
46	4	8.7	1	Kiec-Swierczynska, 1995
50	6	12	1	Saha et al.; 1993
502	7	1.4	2	Suskind, 1984
686	13	2.3	n.g.	Conde-Salazar et al., 1993
1377	5	0.4	n.g.	Meneghini et al., 1963

1 n.g. = not given
2 no evaluation possible due to a number of irritating reactions
3 possible irritating reactions could not be ruled out
4 scarification of skin

06.12.2000

10.2 HAZARD SUMMARY

Memo : Assessment of human health hazards

Source : MLPC, Rion-des-Landes, France

Conclusion : DPG is absorbed rapidly after oral uptake but only slowly after dermal application. The substance is metabolized quickly and eliminated in the urine and feces.

No information is available on the mode of action.

The oral LD50 is 323-850 mg/kg b.w. for the rat. The dermal LD0 is > 2,000 mg/kg b.w. in the rabbit. Intoxication is manifested by spasms, disturbed lipid metabolism, lung emphysema and kidney changes.

DPG is irritating to the eye and non-irritating to the skin.

DPG showed no sensitizing effect in the maximization test according to MAGNUSSON and KLIGMAN.

Three subchronic toxicity feeding studies in rats and mice have shown an increase of the mortality rate at high dose and a decrease of food consumption and body weight gain due to the poor palatability of the DPG-treated feed. Treatment-related effects on the organs and the hematological, clinical-chemical parameters and urinalysis were not observed. The NOAEL/LOAEL lies at 32/50 mg/kg bw/d and 11/37 mg/kg bw/d for rats and 75/114 mg/kg bw/day in mice. Based on these data the best estimate for the NOAEL was 32 mg/kg bw/d for rats.

Most of the in vitro and in vivo investigations available give no indication of a genotoxic effect.

A carcinogenicity study which would meet present standards is not available.

Previous and unreliable reproductive toxicity studies in male mice and hamsters indicated a negative influence on fertility, which may have been due to impurities in the test substance. However, these results could not be reproduced in a later study in mice, in which a higher dose was administered. In addition to the results of the subchronic studies on the rat and mouse, special studies for recognizing reproductive toxic effects were also perfonned. Comparisons of the parameter changes with the results of tests with feed withdrawal infer that the effects observed in the DPG-treated animals in high concentration groups are a result of the poor general state of health (malnutrition, exhaustion) of the animals and not a direct toxic effect on the reproductive organs

In female rats and mice fetotoxic, but not teratogenic, effects were seen after the oral administration of maternotoxic doses. In the rat study the NOEL was given as 5 mg/kg bw for the dams and 25 mg/kg bw for the fetuses. In the mouse study the NOEL was given as 4 mg/kg bw for the dams and > 10 mg/kg bw for the fetuses.

In man, earlier studies described the following symptoms after workplace exposures to DPG: eye and mucous membrane irritation, gastric and bilious complaints and disturbed liver metabolism. Occasionally patients with contact dermatitis reacted positively in the patch test.

27.12.2000

10.3 RISK ASSESSMENT

201-14886B2

ANNEX 2

COVER PAGE
SIDS INITIAL ASSESSMENT REPORT (SIAR)
For
SIAM

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03 DEC 10 AM 11:00

Chemical Name: 1,3-Diphenylguanidine

CAS No.: 102-06-7

Sponsor Country: France

National SIDS Contact Point in Sponsor Country:

Mme Laurence Musset
Ministère de l'Environnement et de l'Aménagement du Territoire
20, avenue de Ségur
75302 Paris 07 SP
France

History:

Testing:	No testing	(x)
	Testing	( )

Comments: The national peer review consisted of a presentation and critical discussion at a national panel of experts in toxicology and ecotoxicology from administration, university and industry and nominated by the ministry of environment. In parallel, a review was performed by the national institute on environmental and industrial risk (INERIS) by request from the ministry of environment. For this particular substance, the verification of most underlying study reports was not necessary as it had already been performed by the German authorities within the national German programme on existing chemicals (BUA).

Deadline for Circulation:

Date of Circulation:

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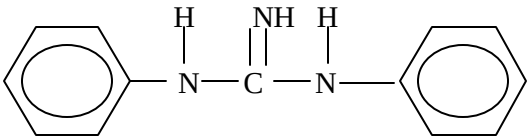
ANNEX : ROBUST SUMMARY

SIDS INITIAL ASSESSMENT REPORT

1. IDENTITY

1.1. Identity

Name (IUPAC) :	1,3-Diphenylguanidine
CA Index name :	Guanidine, N,N'-diphenyl-
Common Name:	Diphenylguanidine
DPG	
CAS Number:	102-06-7
EINECS n°:	203-002-1
Molecular Weight:	211.27 g/mol
Empirical Formula:	$C_{13}H_{13}N_3$
Structural Formula:	



1.2. Physico-chemical properties

Form:	solid
Boiling Point:	>170°C
Vapor Pressure:	174 x 10^{-6} kPa at 20° C
Melting Point:	145-150°C
Solubility in Water:	475 mg/l to 1 g/l at pH 7 and 25°C, to 519 g/l at strongly acid pH and 20°C
Log pKa:	Two protonation steps. First protonation 10.12
Log Kow:	1.69 (measured) pH of test unknown. Probably this result relates to the protonated molecule but whether in cationic or dicationic form unknown 2.9 (calculated SYRACUSE) 2.41 (calculated EPIWIN)
Flammability:	no data
Odor :	slight
Convertion factors :	1 ppm (v/v) = 4.1 mg/m3 1 mg/m3 = 0.24 ppm (v/v)

1.3. Composition of the technical product

Bayer AG (1989, cited in BUA, 1992) reported that the substance is marketed with a 1,3-diphenylguanidine content of 97.5%. Impurities in this product, which is considered to be typical, are aniline (<0.04%); nitrogenous polymers and other unknown compounds (about 0.7%), inorganic components (about 0.2%) and water (<0.1%). Solvent residues related to production (e.g. o-dichlorobenzene, toluene, xylene) amount to < 50 ppm. Formulation components also contained in the commercial product are 1% mineral oil and 0.5% emulsifier (e.g. fatty alcohol- or nonylphenolpolyglycol ether).

Acidimetric analysis performed routinely by MLPC (2001) on the formulated DPG (containing 1.5-2% mineral oil), indicate a purity of about 99% for the active material.

1.4. Production Volume/Uses:

The expected production volume of 1,3-Diphenylguanidine in year 2000 is 2400 tonnes/year in Europe, 2400 tonnes/year in the USA, an amount of 5300 tonnes/year for Asia and 11100 tonnes per year for the world.
The producers or importers and locations are listed below :

United States : none
Europe: MLPC (France), BAYER (Germany), FLEXYS (Belgium)
Other: PRONOVA (Russia), SUMITOMO CHEMICAL (Japan)

1.5. Uses and Functions

1,3-diphenylguanidine is used as a primary accelerator in vulcanisation of rubber, as secondary accelerator for sulphur-containing compounds such as thiazoles, sulfenamides and thiurams and as a minor use as a primary material for standardising acids.

Depending on the specific application, the concentration of 1,3-diphenylguanidine used in the production of rubber compounds may vary from 0.25% to 2.0% by weight. The main use is as a vulcanisation activator during which process it is incorporated in the rubber compound but much reverts after processing, leaching of DPG may occur from rubber.
DPG may be of concern locally in aqueous discharge from production and downstream use sites.

Rubber containing 1,3-diphenylguanidine has been used in footwear, tyres, and moulded goods.

2. GENERAL INFORMATION ON EXPOSURE

2.1. Environmental Exposure

2.1.1 General Discussion

1,3-Diphenylguanidine is soluble in water and is not expected to adsorb strongly to suspended solids or to bioaccumulate in biota. The substance hydrolyses at high pH and has been found to be inherently biodegradable in a closed bottle test using adapted activated sludge. Due to these properties and its low volatility 1,3-Diphenylguanidine is expected to be found mainly in the aquatic compartment.

1,3-diphenylguanidine has three forms: unionised, primarily protonated and secondarily protonated. The pKa at which the first protonation occurs is 10.12 while the pKa for the second protonation is unknown and as this will be less than 10.12 it is not known whether this state will be reached at normal environmental pHs between 6 and 8. This leads to problems in interpretation of the environmental fate of the substance.

2.1.2 Fate in Waste Water Treatment Plants

Based on the SIMPLETREAT model and its inherent biodegradability, 1,3-diphenylguanidine is expected to partially degrade in sewage treatment plants. The results using a calculated log Kow of 2.41 (EPIWIN) and a unitless H of 3.07×10^{-6} (EPIWIN) provide the following results:

Air: 0%
Water: 49%
Sludge: 1%
Degraded: 51%

While using a log Kow of 2.9 (SYRACUSE) the results are:
Air: 0%
Water: 46%
Sludge: 6%
Degraded: 48%

2.1.3 Distribution in Air, Water and Soil

Due to the physico-chemical properties of 1,3-diphenylguanidine (soluble in water at the gram per litre range, low volatility and low adsorption to suspended solids), this substance is expected to be found mainly in the aquatic compartment.

2.1.4 Abiotic and Biotic Degradation in Air, Water and Soil

2.1.4.1 Atmospheric degradation.

2.1.4.2 Biodegradation

1,3-Diphenylguanidine is not readily biodegradable (0% after 20 days in the OECD 301 D assay) using non-adapted inoculum. However, use of inoculum, pre-adapted for 14 days led to 76% degradation at 1,3-Diphenylguanidine concentrations of 2.4 mg/l and 74% at 0.8 mg/l (Bayer, 1990a). Furthermore, Chou et al. (1980) conducted a study of primary degradation of 1,3-Diphenylguanidine at a pH of 7.5 (measured at the beginning of the test) and found total loss of the parent substance within 14 days of exposure to unadapted river water. The substance can therefore be considered as inherently biodegradable.

2.1.5 Bioaccumulation

1,3-diphenylguanidine has a log P_{ow} of 1.69 (measured; Chou et al., 1980) and a measured BCF of <20 at 0.01 mg/l and <2 at 0.1 mg/l (limit of quantification) with an exposure period of 42 days. The substance is therefore not expected to bio-accumulate.

2.1.6 Predicted Environmental Concentration

1,3-Diphenylguanidine is produced on a scale estimated as 2400 tonnes in Europe in year 2000, 2400 tonnes in the USA 5300 tonnes in Asia and an amount of 11100 tonnes for the total world) and the vast majority is consumed as a vulcanising agent.
Due to its relatively high solubility and low partition coefficient 1,3-Diphenylguanidine may be present in surface water at very low concentrations.

Sources of release into the environment are multiple e.g. during production, during processing in rubber industry or during the use of rubber articles as well as during the elimination of rubber articles. In the rubber processing industry, waste water can occur during cleaning of equipment, vulcanisation (use of steam) and processing of used rubber.

During the use of rubber articles, releases to the environment may occur directly from the articles as well as from particle abrasions from these articles. Contributions to the emissions due to abrasion are especially relevant for tyres.

The Japanese Environmental Agency measured 42 water and sediment samples from non-industrial sites in Japan (cited in BUA report, 1992). No DPG was found at detection limits of 2 to 5 µg/l for water and 0.1 to 0.5 mg/kg in sediment.

2.2. Human Exposure

1,3-diphenylguanidine can be absorbed into the body by inhalation and by ingestion.

2.2.1 Occupational exposure

Exposure to 1,3-diphenylguanidine may occur during the manufacture of rubber and miscellaneous plastic.

No measurements are available on workplace concentration of 1,3-diphenylguanidine.

2.2.2 Consumer exposure

Exposure to 1,3-diphenylguanidine may occur as a result of contact with finished products.

2.2.3 Indirect exposure via the environment

Exposure is expected to be mainly local, from production and compoundingsites. Once DPG has been included in the vulacanisation process only residues are expected to be available for leaching from finished rubber compounds.

Little bioaccumulation is expected from bioavailable DPG due to the low bioconcentration factor found in fish.

3. HUMAN HEALTH HAZARDS

3.1. Effects on Human Health

3.1.1. Toxicokinetics & Metabolism

3.1.1.1 Oral administration

The absorption, distribution, metabolism and excretion of 1,3-diphenylguanidine was reported by Ioannou & Matthews (1984; reliability 2) after oral administration to male F344 rats.

A comparison of ^{14}C-1,3-diphenylguanidine (the ^{14}C-labelling was done by U-labelling on the phenyl rings) tissue distribution and excretion following single oral (dose levels 1.52 - 151.5 µmol/kg) versus intravenous (dose level 15.15 µmol/kg) administration to male F344 rats, indicates that gastrointestinal absorption of DPG was near complete and that tissue distribution and excretion were not significantly affected by the route of administration.

Within 24 and 72 hours about 80 and >99% respectively of the ^{14}C activity administered orally or intravenously was excreted about equally in the urine and faeces (elimination half-life 9.6 hours). About 30% of the ^{14}C activity eliminated in the bile was subjected to entero-hepatic circulation and excreted in the urine.

Distribution and excretion of radioactivity 1 day after administration of ^{14}C-1,3-diphenylguanidine to F344 male rats.

	Percentage total dose			
	Intravenous	Oral		
Tissue	15.15 µmol/kg	1.52 µmol/kg	15.15 µmol/kg	151.5 µmol/kg
Liver	1.37 ± 0.08	1.31 ± 0.09	1.23 ± 0.11	0.92 ± 0.09
Muscle	1.18 ± 0.08	1.08 ± 0.02	1.08 ± 0.01	1.09 ± 0.08
Adipose	0.56 ± 0.07	0.62 ± 0.03	0.47 ± 0.03	0.49 ± 0.03
Skin	0.52 ± 0.07	0.40 ± 0.01	0.41 ± 0.05	0.39 ± 0.02
Blood	0.24 ± 0.01	0.27 ± 0.01	0.23 ± 0.01	0.24 ± 0.02
Total excreted				
In urine	35.50 ± 3.38	31.76 ± 2.68	29.12 ± 1.72	43.61 ± 2.83
In feces	45.67 ± 9.01	48.25 ± 4.49	45.26 ± 2.94	39.39 ± 1.84
Total[a]	81.17 ± 6.12	80.01 ± 6.24	74.38 ± 1.27	83.00 ± 2.41

[a] DPG-derived radioactivity excreted in urine and faeces in 24 hr. The remainder is still present in tissues and intestinal contents

The following table gives an overview of the relative distribution (in % ^{14}C-activity) of ^{14}C-1,3-diphenylguanidine or the metabolites (without identifying them specifically, numbered I to V)) in liver, bile, urine and faeces after single intravenous administration.

Relative amounts of DPG and DPG-metabolites present in male F344 rat liver and excreta

		DPG metabolite (%)					^{14}C-DPG
Excreta or Organ[1]	Time(h)	I	II	III	IV	V	(%)
Liver	0.75	-	12 ± 1.2	-	-	-	88 ± 5.7
	2	-	18 ± 1.9	-	-	-	82 ± 4.3
	6	-	30 ± 2.1	-	-	-	70 ± 6.0
	24	-	30 ± 3.3	60 ± 4.5	-	-	10 ± 1.1
Bile	6	2 ± 1.2	95 ± 1.7	-	-	-	3 ± 0.5
Urine	24	-	37 ± 1.6	32 ± 1.4	-	3 ± 0.8	28 ± 0.8
Faeces	24	-	-	-	2 ± 1.0	94 ± 3.5	4 ± 1.4

[1] intravenous administration (15.15 µmol/kg)

Three or 9 oral administrations of 15.15 µmol/kg ^{14}C-1,3-diphenylguanidine/kg/day also caused no accumulation in the tissues. In the liver there was a proportional ^{14}C increase, the metabolites II and III being detected. Covalent binding to liver macromolecules was not determined.

3.1.1.2 Dermal administration

The absorption and disposition 1,3-diphenylguanidine was reported by Shah et al. (1985; reliability 2) after dermal administration to female Sprague-Dawley rats.

In female Sprague-Dawley rats, which had received a single dermal application of 0.063 mg ^{14}C-1,3-diphenylguanidine/animal (0.3 µmol), only 10% of the ^{14}C activity penetrated the shaven skin of the back within 5 days with an apparent first-order dermal absorption rate of 0.021 ± 0.002 d^{-1} and a $t_{½}$ of 33.6 days. Distribution throughout the entire organism also occurred here.

The highest ^{14}C activities after dermal administration were measured in the liver, kidneys, intestines and its content , and excreta. Maximum tissue concentrations after dermal application were reached 3-6 hours after the start of the experiment.

Within 120 hours after dermal application 64% of the absorbed ^{14}C activity was excreted in the urine and 29% in the faeces. Accumulation in the adipose tissue was not observed.

Relative amounts of DPG and DPG metabolites present in treated skin and excreta

		% metabolites			^{14}C-DPG
Excreta or Organ	**Time(h)**	**II**	**IV**	**V**	**(%)**
Urine	24	50 ± 5.3	-	-	50 ± 5.3
	48	53 ± 1.7	-	-	47 ± 1.5
	72	57 ± 5.2	-	-	43 ± 5.2
	96	100	-	-	0
	120	100	-	-	0
Faeces	24	-	-	100	-
	48	-	15	85	-
	72	-	20	80	-
	96	-	26	74	-
	120	-	26	74	-
Skin	6-120	-	-	-	>95

3.1.1.3 Other informations

Additional, although insufficiently documented (reliability 3), studies in mice (Hunter & Scully; cited in Bempong & Hall, 1983) and rabbits (Kazarinova et al., 1975) indicate that 1,3-diphenylguanidine is excreted rather quickly and does not accumulate.

Conclusion: 1,3-Diphenylguanidine is absorbed rapidly after oral uptake but only slowly after dermal application. The substance is metabolised quickly and eliminated in the urine and faeces. No information is available on the mode of action and the identity of the metabolites.

3.1.2. Acute toxicity

In three studies reliable with restriction (not GLP), oral LD_{50} values of rats were 350 mg/kg b.w. (Monsanto Company, 1977a; reliability 2), 384 - 460 (Sumitomo Chemical, 1977; reliability 2) and 850 mg/kg b.w. (Monsanto Chemical, 1954; reliability 2).

In one reliable study (OECD guideline and GLP), dermal LD_0 for rabbits was higher than 2000 mg/kg b.w. (Monsanto Company, 1992a; reliability 1).

The following table summarised the acute toxicity data performed following a protocol equivalent to OECD guidelines.

Acute toxicity data

Species	**Route**	**Result**	**Main Effects**	**Reference**	**Reliability**
Rat (male/female)	Oral	LD_{50} = 350 (290-420) mg/kg	Reduced appetite and activity, increasing weakness, collapse, and death. Gross autopsy of decedents: haemorrhagic areas of the lungs	Monsanto Company, 1977a	2
Rat	Oral	LD_{50} / male= 460 (320-662) mg/kg LD_{50} / female = 384 (309-477) mg/kg	Decrease of spontaneous motor activity, irregular respiration and hind limb, dyspnea	Sumitomo Chemical, 1977	2

Rat (male/female)	Oral	LD50 = 850 mg/kg	Prostration, coma, convulsion. Irritation of the mucous of the stomach and intestinal tract, dark liver and spleen	Monsanto Chemical, 1954	2
Rat (male/female)	Dermal	LD_0 > 2000 mg/kg	No mortality, transient dermal irritation. Red discoloration of the pancreas or pancreatic lymph nodes	Monsanto Company, 1992a	1

The other acute studies with a low reliability (3 or 4) are compiled in the following table.

Species	**Route**	**Dose (mg/kg)**	**Main Effects**	**Reference**
Rat	Oral	375	LD50	Arkhangel'skaya and Roshchina; 1963, 1964
Rat	Oral	c.a.500	LD50	Dieke et al., 1947
Rat	Oral	250	maximum tolerated dose, spasms, liver weight	Arkhangel'skaya and Roshchina, 1963, 1968
Rat	Oral	500	minimum lethal dose	Arkhangel'skaya and Roshchina, 1963
Rat	Oral	323	LD50	Vlasyuk, 1978
Mouse	Oral	290	LD50	Arkhangel'skaya and Roshchina,1964
Mouse	Oral	250	maximum tolerated dose; spasms, liver weight	Arkhangel'skaya and Roshchina, 1963, 1968
Mouse	Oral	450	LD100	Arkhangel'skaya and Roshchina, 1963, 1968
Mouse	Oral	258	LD50	Vlasyuk, 1978
Mouse	Oral	520	LD50	Amer. Cyan. Co.; cited in McCormick (1971)
Mouse	Oral	150	LD50 male	Hasegawa et al., 1989
Mouse	Oral	211	LD50 female	Hasegawa et al., 1989
Rabbit	Oral	246	LD50	Vlasyuk (1978)
Rabbit	Oral	250	minimum lethal dose	Smyth, 1931
Rabbit	Oral	250	LD50	Marhold, 1986
Guinea pig	Oral	250	minimum lethal dose	Smyth, 1931
Guinea pig	Oral	250	LD50	Marhold, 1986
Dog	Oral	10	emetic dose	Amer. Cyan. Co.; cited in McCormick (1971)
Rabbit	Dermal	>794	LD50	Monsanto Company, 1977b

Conclusion: 1,3-diphenylguanidine is moderately toxic by ingestion, the oral LD50 is 350-850 mg/kg b.w. for the rat. By dermal route, 1,3-diphenylguanidine is practically non toxic, the dermal LD0 is > 2,000 mg/kg b.w. in the rabbit. After oral administration, the symptoms were normally of a nervous character, but post mortem examination revealed liver effects (dark color) and severe irritation of the gastro-intestinal tract.

3.1.3. Repeated Dose Toxicity

3.1.3.1. Animal data

Sub-chronic feeding experiments in rats and/or mice have been performed according to OECD guidelines and GLP in the frame of the US National Toxicology Program (1995, reliability 1) and by the Monsanto Europe (1982, reliability1).

In F344/N rats and B6C3F1 mice which, in a dose-finding study, received 1,3-diphenylguanidine (98.9%) for 14 days in their feed in concentrations of 250, 500, 750, 1,500 and 3,000 ppm, only reduced feed intake and decreased body weights were observed as of 750 ppm (NTP, 1995; reliability 1).

In the subsequent sub-chronic 13-week study, 1,3-diphenylguanidine (98.9%) was fed to the F344/N rat and B6C3F1 mouse in the same concentrations as in the sub-acute study (equivalent to daily dose of 17/17, 32/32, 50/49, 100/95 and 181/184 mg/kg bw/d for male/female rats and 38/46, 75/93, 114/141, 231/285 and 457/577 mg/kg bw/d in male/female mice at 250, 500, 750, 1500 and 3000 ppm, respectively).
In rats, due to the poor palatability of the 1,3-diphenylguanidine-treated feed, a decreased feed consumption and reduced body weights were observed as of 750 ppm compared to controls (final weight relative to controls: 750 ppm (m/f): 92%/93%; 1,500 ppm: 79%/86%; 3,000 ppm: 52%/-). Increased mortality was observed for both sexes after receiving 3,000 ppm in feed, whereby all females of this concentration group died. Primarily substance-induced effects on the organs were not observed. The diverse deviations from the controls, determined also for the haematological and clinical-chemical parameters particularly in both highest concentrations (1,500 and 3,000 ppm), are seen exclusively as the result of emaciation by reduced feed intake.
With the average feed intake comparable with the controls, body weight retardation was observed in the mice also as of 750 ppm. The reduced organ weights occurring as of 1,500 ppm were evaluated not as a specifically toxic response but rather were correlated to the clearly reduced body weights. Histopathological changes and significant deviations from the controls for the haematological and clinical-chemical parameters were not observed.
Based on the secondary toxic effects, due to the poor palatability of the 1,3-diphenylguanidine-treated feed, the NOAEL for both species lies at 500 ppm (ca. 32 mg/kg b.w. and day for rats and ca. 75 mg/kg b.w. and day for mice) (NTP, 1995; reliability 1).

In an other sub-chronic toxicity study, dosing Sprague-Dawley rats with 1,3-diphenylguanidine (97.7%) in the diet at a concentration of 50, 150 or 500 ppm for 13 weeks (equivalent to daily dose of 4, 11 and 37 mg/kg bw/d), produced a marked reduction in growth rate of both males and females at 500 ppm, with respect to controls. The food consumption of these animals was also reduced, when compared to the controls. These effects were most pronounced over the first few weeks of dosing, suggesting that the cause may partly be due to the unpalatability of the test substance. Dose levels of 1,3-diphenylguanidine up to 500 ppm did not cause an increase in mortality or the appearance of any abnormal clinical signs.
Laboratory investigations revealed small differences between rats receiving DPG and the controls in both haematological and clinical chemical parameters, but as the same effects were not present at both weeks 6 and 13 they are considered to be of little or no significance. Male rats receiving 500 ppm DPG tended to produce more concentrated urine than the controls at weeks 6 and 13, with a slightly reduced pH on the latter occasion. Female rats at the same dose level also showed a slight aciduria, but at week 13 only. These effects were only slight and could not be attributed to any obvious structural change to the kidney as seen on histopathological examination. The changes seen lacked a dose response and were of marginal magnitude, thereby confirming their insignificance. The terminal macroscopic and histopathologic examinations revealed no dose related lesions; those lesions found were spread across the dose groups with roughly equal incidence and are considered to be common findings in rats of this age and strain. Based on the marked reduction of the growth rate at 500 ppm, due to the poor palatability of the 1,3-diphenylguanidine-treated feed, the NOAEL lies at 150 ppm (11 mg/kg bw/d) (Monsanto, 1982; reliability 1).

Additional subacute and subchronic studies are compiled in the following table. These are insufficiently documented in some cases; most do not meet current requirements (reliability 3 or 4), such as those concerning dose selection. Thus the results should not be assessed critically.

Species (sex/number)	Administration (duration)	Dose/Day (mg/kg b.w./d)	Main Effects	Reference
Rat	Feed (28 d)	7, 75 and 75	75 mg/kg: gowth retardation	McCormick, 1971
Rat	Oral (4 mo)	32	reticulocytosis, eosinophi lia, erythrocytes, hemoglobin, inhibited catalase and peroxidase, total bilirubin, threshold of nerve and muscle irrita bility, mortality, no kidney findings	Orlov et al. (1973)
Rabbit	Oral (not given)	10% LD100	feed intake, erythrocytes, serum t-globulin	Arkhangel'skaya and Roshchina, 1963, 1968
Rabbit	Oral (5.5 months)	50	severely inflamed liver parenchyma, bilirubin, dystrophy of convoluted tubules, unchanged blood picture	Arkhangel'skaya and Roshchina, 1963, 1968
Dog	Oral (24 d)	10 (21 administrations)	"relatively well tolerated"	Amer. Cyan. Co.; cited in McCormick, 1971
Dog	Oral (not given)	5	bile acids	Burov (1964)
Rabbit	Dermal (10 administrations)	1000	no systemic effect	McCormick, 1971
Rat	Inhalation	about 220 mg/m3/2h	Disturbed "oxidation-reduction" processes, functionally disturbed nervous system, blood pressure	Arkhangel'skaya and Roshchina, 1963, 1968
Guinea pig	Inhalation (repeated)	100 mg/l	Lethal	Verchovski, 1952

Conclusion : Three sub-chronic 13-week toxicity feeding studies in rats or mice have shown an increase of the mortality rate in rats at high dose (3000 ppm) and a decrease of food consumption in rats (as of 500-750 ppm) and body weight gain in rats and mice (as of 500-750 ppm) due to the poor palatability of the 1,3-Diphenylguanidine-treated feed. Treatment-related effects on the organs and the haematological, clinical-chemical parameters and urinalysis were not observed. The NOAEL/LOAEL lies at 500/750 ppm (32/50 mg/kg bw/d) and 150/500 ppm (11/37 mg/kg bw/d) for rats and 500/750 ppm (75/114 mg/kg bw/d) in mice.
Based on these data, a conservative NOAEL can be established at 32 mg/kg bw/d for rats and 75 mg/kg/d for mice.

3.1.3.2. Human data

The only results available are from a poorly documented study, whose validity cannot be judged due to the lack of data on possible previous employment, the possibilities of contact with other substances, concentrations and control groups. Orlov et al. (1973; reliability 4) examined workers ranging in age from 29 to 58 years, who had come into contact with 1,3-diphenylguanidine during production (no further information) over 3-15 years. About 30% of the test subjects showed symptoms, most suffering from stomach and gall-bladder complaints, neurological disorders or skin diseases. Another finding was liver metabolism disorder (disturbed protein metabolism, increased bilirubin values).

Following unintentionally increased workplace exposure to 1,3-diphenylguanidine due to inadequate safety measures, the following symptoms were reported: eyelid pain and eye redness, a bitter taste and a painful feeling in the oesophagus. Reduced gastric juice acidity and achylia were also determined. Further data are not available (Arkhangel'skaya & Roshchina, 1963; reliability 4).

3.1.4. Genetic Toxicity

In vitro assays

Results available on Ames tests are predominantly negative (8 out of 10 studies) (Monsanto, 1976; JETOC, 1996; Crebelli et al., 1984a, 1984b, 1985; Rannug et al., 1984; You et al., 1982; Yamaguchi et al., 1991). In one Ames test 1,3-diphenylguanidine caused a weak increased number of revertants after metabolic activation (S9 mix from hamster liver) (Mortelmans et al., 1986; NTP, 1995), while in another case the weakly positive result may have been due to contamination (Bempong & Mantley, 1985; Bempong, unpublished data).
All other in vitro investigations, gene mutation on Escherichia coli and Saccharomyces cerevisiae (JETOC, 1996; Monsanto, 1976), gene mutation assays on CHO (Donner, 1983) and mouse lymphoma cells (Monsanto, 1979) and cytogenetic assay on CHO cells (Monsanto, 1992b) were consistently negative. 1,3-diphenylguanidine at a concentration of 7.5 µg/ml caused 50% inhibition of the colony formation in HeLa-S3 cells (Baba, 1980; reliability 4).

In vitro genetic toxicity

Test System	**Metabolic activation**	**Concentration range**	**Result**	**Reference**	**Reliability**
Salmonella typhimurium TA98, 100, 1535, 1537	+(2)	33-10000 µg/plate	Negative	Mortelmans et al, 1986; NTP, 1995	1
Salmonella typhimurium TA98, 100, 1535, 1537, 1538	+/-	2-500 µg/plate	Negative	JETOC, 1996	2
Salmonella typhimurium TA98, 100, 1535, 1537, 1538	+/-	0.1-500 µg/plate	Negative	Monsanto, 1976	2
Salmonella typhimurium TA1535, 1537	+(1)	100-10000 µg/plate	Negative	Mortelmans et al, 1986; NTP, 1995	2
Salmonella typhimurium TA98, 100	+/-	200-5000 µg/plate	Negative	Crebelli et al, 1984, 1985	2
Salmonella typhimurium TA98, 100	+(1)	1-100 µg/plate	Weakly positive	Mortelmans et al, 1986; NTP, 1995	2
Salmonella typhimurium TA98, 100, 1535, 1537, 1538	+/-	0.036-36 µg/plate	Weakly positive	Bempong and Mantley, 1985	3
Salmonella typhimurium TA98, 100, 1535, 1537, 1538	+/-	No data	Negative	Rannug et al., 1984	3
Salmonella typhimurium TA98, 100	+/-	No data	Negative	You et al., 1982	4
Salmonella typhimurium TA98, 100	+/-	1-100 µg/plate	Negative	Yamaguchi et al., 1991	4
Escherichia coli WP2	- +	2-500 µg/plate 20-5000 µg/plate	Negative	JETOC, 1996	2
HGPRT test on V79 cells	-	100-500 mg/ml	Negative	Donner et al., 1983	3
TK +/- assay on L5178Y cells	- +	16.4-188 µg/ml 32.8-525 µg/ml	Negative	Monsanto, 1979	2
Saccharomyces cerevisiae D4	+/-	1-500 µg/plate	Negative	Monsanto, 1976	2
Cytogenetic assay on CHO cells	+/-	125-750 µg/ml	Negative	Monsanto, 1992b	2

(1) 10 % hamster liver homogenate
(2) 10 % rat liver homogenate

In vivo assays

The micronucleus test on erythrocytes of the peripheral blood of mice, that obtained feed with a 1,3-diphenylguanidine-content of 250, 500, 750, 1,500 and 3,000 ppm in the sub-chronic test, led to a negative result in the male animals. The test result of the females was evaluated as "questionable" because of the statistically significant increase of the micronuclei-carrying normochromatic erythrocytes in the female animals of the middle concentration group (750 ppm) (NTP, 1995; reliability 1).

However, in a cytogenetic assay, 1,3-diphenylguanidine was administered via oral gavage to male and female rats at a target dose of 300 mg/kg body weight. Bone marrow was sampled at 6, 24 and 48 hours after dosing. No statistically significant increases in the proportion of aberrant cells or aberrations/cell were observed. Significant induction of toxicity, measured as mitotic index depression was observed at the 6 hour (35%) and 24 hour (31%) time points. No depression in mitotic index was observed at the 48 hour time point (Monsanto 1989; reliability 1).

A micronucleus test in mice was reported negative (further information not given; Bempong, unpublished data; reliability 4).

Bempong and Mantley (1985) (reliability 3) administered a single i.p. dose of 0.036-36 mg 1,3-diphenylguanidine/kg bw to mice and then examined the peritoneal fluid, urine and faeces daily for 4 days with *Salmonella typhimurium* strain TA 100 (modified host-mediated assay). Only in the faeces was a dose and time-related increase in the number of revertants seen. This result may have been due to contamination of the test substance, as later investigations with the pure compound were negative. Further data are not available (Bempong, unpublished data).

Conclusion : Most of the *in vitro* and *in vivo* investigations available give no indication of a genotoxic effect.

3.1.5 Carcinogenicity

No relevant data available.

3.1.6. ReproductiveToxicity

In addition to the results of the sub-chronic studies on the rat and mouse described in Section 3.1.3 (NTP, 1995; Monsanto, 1982), special studies for recognising reproductive toxic effects were also performed (Koëter et al., 1992; WTR, 1989, NTP, 1995; reliability 1).

1,3-Diphenylguanidine (purity 99.9%) was administered by daily gavage to groups of 25 male CD-1 mice at dose levels of 0, 0.06, 0.25, 1, 4 and 16 mg/kg/d during an 8-week pre-mating period. Females were not dosed at any time during the study. Within 24 hours after the last treatment, 9 to 13 males, randomly taken from each group were killed and subject to gross examination at autopsy. A selected number of organs were weighted and preserved. Sperm abnormality evaluation was performed in the selected males from the control and 16 mg/kg dose group. The remaining males in the control, 4 and 16 mg/kg body wt per day groups were mated with non-dosed females. Reproductive performance, necropsy findings and litter data were recorded.
No differences were found between control and dosed groups in body weight gain during the dosing period, macroscopic observations and organ weights at necropsy. Microscopic examination of the testes in the 16 mg/kg/d group, did not show any effect due to 1,3-diphenylguanidine dosing when compared to the control group. Sperm abnormality evaluation in the 16 mg/kg/d group showed a slight but statistically significant increase (5% versus 2% in control) in sperm with folded tails but normal heads. However, since the total number of abnormal sperm cells as well as the number of specified sperm abnormalities was similar, the observed increased number of sperm cells with folded tails is considered of doubtful significance. Male and female fertility as well as reproduction performance were comparable in the groups examined (0, 4 and 16 mg/kg/d). Maternal necropsy findings and litter data did not reveal any dose-related effect.

It was concluded that under the conditions of this study, 1,3-diphenylguanidine did not exert any significant adverse effects on fertility, reproductive capacity or embryonic/foetal development in CD-1 mice when administered to males at levels up to 16 mg/kg/d (Koëter et al., 1992; WTR, 1989; reliability 1)..

Male and female F344/N rats and B6C3F1 mice had been administered feed having a 1,3-diphenylguanidine (purity 98.9%) content of 250, 500, 750, 1,500 and 3,000 ppm for 13 weeks (NTP, 1995; reliability 1).
Female rats exhibited uterine hypoplasia and a prolonged reproductive cycle in the 750 ppm-group (ca. 49 mg/kg b.w. and day) and 1,500 ppm-group (ca. 95 mg/kg b.w. and day) in comparison with the controls. All females of the 3,000 ppm-group died during the study, so that comparable studies of these animals could not take place. The male rats, only in the 1,500 ppm-group (ca. 100 mg/kg b.w. and day), showed diminished sperm motility. Alterations in the reproductive organs (e.g. depletion of the prostate, hypospermia, reduced spermatogenesis) were occasionally found in the males of the 3,000 ppm-group (ca. 181 mg/kg b.w. and day).
Also for the mice which obtained up to 3,000 ppm 1,3-diphenylguanidine in feed for 13 weeks, a prolonged reproductive cycle was observed in the females of the highest concentration group and, in the male animals, a decreased sperm motility. The increased number of spermatid heads in the testes paired with the lower number of sperm in the epididymis is evidence against an effect on spermatogenesis, whereas an effect on the release of sperm into the epididymis cannot be excluded.
Comparisons of the parameter changes determined after the 1,3-diphenylguanidine-feeding in the rat and mouse, which can be used for assessing a possible reproductive toxic effect, with the results of tests with feed withdrawal (Chapin et al., 1993a and 1993b; Levin et al., 1993) infer that the effects observed in the 1,3-diphenylguanidine-treated animals in high concentration groups are a result of the poor general state of health (malnutrition, exhaustion) of the animals.
In conclusion, no reprotoxic effect was observed in male and female rats up to 500 pmm (32 mg/kg bw/d) and in mice up to 1500 ppm (231-285 mg/kg bw/d) in feed. At higher concentrations, the effects on the reproductive parameters were due to reduced nutrient intake and are consistent with similar changes observed in other studies of feed restricted rats and mice.

Bempong analysed the effects of 1,3-diphenylguanidine (purity not reported, probably of low purity according to correspondence with the author) on seminal cytology, testicular development and fertility (Bempong, 1983a and 1983b; Bempong & Hall, 1983; reliability 3). Dose-levels of 4 and 8 mg/kg bw/day 1,3-diphenylguanidine, administered by the oral route up to 15 weeks, induced a time- and dose-dependent increase in the frequency of sperm abnormalities in both mice and hamsters from week 4, a significant decrease in sperm count and testes weight from week 5, and irregularly shaped seminiferous tubules in mice. The fertility index and the number of implants per pregnant female mice were decreased in a dose-dependent fashion, but the effect did not seem to be time-dependent. The frequency of early or late dead foetuses per litter was significantly increased at the 5th and 7th week of dosing at the high dose levels.
Taken into account the uncertainties related to the study protocol (lack of details on compound purity, mode of administration, number of treated animals/dose, food and water consumption, clinical signs and body weight, poor statistical evaluation and no GLP) and the conflicting results with the other available information, these studies were considered as not reliable (category 3) and not taken into account to evaluate the reprotoxicity of 1,3-diphenylguanidine. The difference in these results may be explainable by contamination of the 1,3-diphenylguanidine employed in the studies carried out by Bempong.)

The reliable available data on reprotoxicity of 1,3-diphenylguanidine are summarised in the following table.

Conclusion: Taken into account the reliable studies, 1,3-diphenylguanidine did not affect the fertility of male mice when administered by gavage up to the maximal tested dose level of 16 mg/kg/d. In addition to the results of the feeding sub-chronic studies on the rat and mouse, special studies for recognising reproductive toxic effects were also performed. Comparisons of the parameter changes with the results of tests with feed withdrawal infer that the effects observed in the 1,3-Diphenylguanidine-treated animals in high concentration groups are a result of the poor general state of health (malnutrition, exhaustion) of the animals and not a direct toxic effect on the reproductive organs. Very conservative NOAELs, based on the effects on the reproductive organs, secondary to

malnutrition and exhaustion, can be established at 32 mg/kg bw/d for rats and from 16 to 231 mg/kg bw/d for mice.

Reliable reprotoxicity studies available on 1,3-diphenylguanidine

	Koeter et al, 1992; WTR, 1989	**NTP, 1995**	**NTP, 1995**	**Monsanto, 1982**
QUALITY ASSESSMENT				
Product purity	99.9%, Industrial batch	98.9% ± 0.6%	98.9% ± 0.6%	97.7%
Check of DPG stability in vehicle	No. Solution prepared freshly once a week	Yes	Yes	Yes
GLP	Yes	Yes	Yes	Yes
Availability of raw data	Yes	Yes	Yes	Yes
Reliability	1	1	1	1
PROTOCOL				
Treated animals	8-week-old male CD-1 mice	6- to 7-week-old B6C3F1 male and female mice	6- to 7-week-old F344/N male and female rats	6-week old male and female Sprague-Dawley rats
Route of administration	Gavage	Dietary ad libitum	Dietary ad libitum	Dietary ad libitum
Dose levels	0, 0.06, 0.25, 1.0, 4.0, & 16.0 mg/kg/d	0, 250, 500, 750, 1500, & 3000 ppm (equivalent to 38, 75, 114, 231, & 457 m g/kg/d for the males and 46, 93, 141, 285 and 577 mg/kg/d for the females)	0, 250, 500, 750, 1500, & 3000 ppm (equivalent to 17, 32, 50, 100, & 181 mg/kg/d for the males and 17, 32, 49, 95 and 184 mg/kg/d for the females)	0, 50, 150, & 500 ppm (approx. 4, 11 or 37 mg/kg/day)
Duration of treatment	8 weeks	13 weeks	13 weeks	13 weeks
RESULTS				
Body weight gain	Not affected	↓ from 750 ppm and upward	↓ from 1500 ppm and upward	↓ at 500 ppm
Testis Organ weight Histology	 Not affected Not affected	 Not affected Not affected	 ↓ at 3000 ppm in relation with the body weight loss ↓ spermatogenesis at 3000 ppm secondary to body weight loss	 Not affected Not affected
Ovaries Organ weight Histology	 Not relevant Not relevant	 Not affected Not affected	 Not affected Not affected	 Not affected Not affected
Uterus Length of the	 Not relevant	 ↑ at 3000 ppm in relation with	 ↑ at > 750 ppm in relation with	 Not evaluated

estrous cycle		the body weight loss	the body weight loss	
Sperm Count Morphology Motility	 Not evaluated No effects on the total number of abnormal sperm Not evaluated	 Not affected Not evaluated ↓ at 3000 ppm secondary to poor body condition	 Not affected Not evaluated ↓ at 1500 ppm secondary to poor body condition	 Not evaluated Not evaluated Not evaluated
Male fertility after mating with untreated female	Not affected	Not evaluated	Not evaluated	Not evaluated
CONCLUSION				
	No testicular toxicity and no effects on fertility	**No direct toxicity on reproductive organs**	**No direct toxici ty on reproductive organs**	**No toxicity on reproductive organs**
NOAEL for reprotoxicity	**≥ 16 mg mg/kg/d**	**231 mg/kg/d**	**32 mg/kg/d**	**≥ 37 mg/kg/d**

3.1.7. Developmental Toxicity

Two studies, one in rats (Monsanto, 1986; reliability 1) and one in mice (Yasuda & Tanimura, 1980; reliability 2), are available for the evaluation of the effects of 1,3-diphenylguanidine on the development.

Potential maternal, embryotoxic and teratogenic effects of 1,3-diphenylguanidine were evaluated in rats. 1,3-diphenylguanidine was administered orally by gavage to three groups of 25 bred CD female rats as a single daily dose of 0, 5, 25 and 50 mg/kg/day from days 6 through 15 of gestation. Throughout gestation, all females were observed twice daily for toxicity and body weights were recorded at appropriate intervals. On day 20 of gestation, all surviving females were sacrificed for Cesarean section; foetuses were weighed, sexed and examined for external, skeletal and soft tissue anomalies and developmental variations.
No unscheduled deaths occurred in any study group. Severe clinical signs of toxicity, decreased maternal body weights and body weight gains, a slight increase in post-implantation loss and a significantly decreased mean foetal weight were evident in the 50 mg/kg/day dose group. A slight increase in foetuses with reduced ossification (associated with reduced foetal weights) and an increase in bent ribs (attributed to maternal toxicity in this group) were observed at the 50 mg/kg/day dose level. Scattered, infrequent clinical findings and a slightly reduced body weight gain over the treatment period (gestation days 6-16) occurred at the 25 mg/kg/day dose level. The 5 mg/kg/day group was comparable to the vehicle control group in all parameters measured. The infrequent occurrence and nature of the malformations were not indicative of a teratogenic response in any dose group.
In conclusion, 1,3-diphenylguanidine induced severe maternal toxicity at a dose level of 50 mg/kg/day. Fetotoxicity was also expressed at this dose level by a significantly reduced mean foetal body weight and by an increase in foetal variations. A dose level of 25 mg/kg/day was considered a marginal NOEL for maternal toxicity; a foetotoxic response was not apparent. A dose level of 5 mg/kg/day was considered a NOEL (Monsanto, 1986; reliability 1).

Groups of 20 pregnant mice of the ICR-JCL strain were given 1,3-diphenylguanidine orally in doses of 0.25, 1.0, 4.0, or 10.0 mg/kg of body weight/day throughout pregnancy. Control mice were fed the vehicle atone. On day 18 of pregnancy, all mice were killed and the foetuses were examined. Disturbances in implantation were seen in the mothers treated with 10 mg/kg/day (the highest dose) of DPG. Retarded ossification of the talus was seen in the foetuses of mothers treated with 4.0 mg/kg/day, but there was no dose-response relationship to this finding. Although malformations such as open eyelids or polydactyly were seen sporadically, these were categorised as spontaneous anomalies. Thus, DPG seems to have no detrimental effects on the development of mouse foetuses in doses of 4 mg/kg or less (Yasuda & Tanimura, 1980; reliability 2).

In 17 of 130 chicken embryos (control 1) 1,3-diphenylguanidine led to increased rates of mortality and malformation. The ED50 was 0.042 mg/egg (Korhonen et al., 1983a and 1983b; reliability 3). This test is not validated for reproduction toxicity.

Conclusion: In female rats and mice foetotoxic, but not teratogenic, effects were seen after the oral administration of maternotoxic doses. In the rat study the NOEL was given as 5 mg/kg bw for the dams and 25 mg/kg bw for the foetuses. In the mouse study the NOEL was given as 4 mg/kg bw for the dams and higher than 10 mg/kg bw for the foetuses.

3.1.8. Other: irritation, Sensitization

3.1.8.1. Irritation

In one study reliable with restriction (not GLP), no skin irritation was observed after a 24-hour occlusive application of 500 mg on the skin of 6 rabbits (Monsanto Company, 1977c; reliability 2). In two studies reliable with restriction (not GLP), slight to moderate irritation was observed after the instillation of 20 or 100 mg in the eye of 6 rabbits (Monsanto Company, 1977d and 1977e; reliability 2).

3.1.8.2. Sensitisation

Animal data

According to the Magnusson and Kligman maximization test performed according to GLP and OECD guidelines, 1,3-diphenylguanidine is not sensitizer in guinea-pigs (MLPC, 1995; reliability 1).

Human data

In persons suffering from a contact dermatitis positive 1,3-diphenylguanidine patch tests have **occasionally** been described (see the following table). The following main contact possibilities were named: shoes (Blank & Miller, 1952; de Vries, 1964; Hjorth & Fregert, 1972; Song et al., 1979; Lynde et al., 1982; Bajaj et al., 1988), articles of clothing (Bandmann, 1956; van Dijk, 1968; Song et al., 1979), rubberized protective clothing (Fegeler, 1963; Götz & Istvanovic, 1963; Höfer & Hänemann, 1967; Ross & Obst, 1969) and other rubber articles, e.g. gloves or the rubber parts of milking machines (Nater, 1975; Song et al., 1979).

A tire production employee, whose allergic rhinitis symptoms occurred only at the workplace, had a positive patch test reaction with 1% 1,3-diphenylguanidine (Camarasa & Alomar, 1978).

Garcia-Perez et al. (1984) found that Spanish agricultural workers with a contact dermatitis had a significantly higher sensitization to 1,3-diphenylguanidine than a contact dermatitis control group working in another profession (11.76% versus 5.32%). The authors attributed this to possible cross-reactions with pesticides, as some of these substances (e.g. Cyprex) are guanidine derivatives and others (e.g. the cyanamides) possess a similar chemical structure.

The available data are summarised in the following table.

Results of Patch Tests Performed with 1,3-diphenylguanidine

Subjects (n)	Positive (n)	+ve reaction (%)	Concentration (%)	Reference
35	2	6	1	Adams, 1972
524	6	0.01	1+2.1	Agrup, 1969
1	1	-	1	Aguirre et al., 1994
229	18	8	0.5	Baer et al., 1973
105	3	3	3	Bajaj et al., 1988
5	1	20	1	Bandmann, 1956
24	0	0	1	Blank & Miller, 1952
74	2	3	n.g.*	Bonnevie & Marcussen, 1944
1	1	-	n.g.	Bruze, 1994
1	1	-	1	Calan, 1978
686	13	2.3	n.g.	Conde-Salazar et al., 1993
5	0	0	0.25	Curtis, 1945
59	0		1	Dahl, 1975
9	1	11	n.g	de Vries, 1964
34	4	12	n.g.	Garcia-Perez et al., 1984
244	13	5	n.g.	Garcia-Perez et al., 1984
17	6	35	1	Götz & Istvanovic, 1963
1	1	-	n.g.	Helander & Mäkelä, 1983
63	15	24	n.g	Herrmann & Schulz, 1960
10	3	30	n.g.	Höfer & Hönemann, 1967
20	7	35	n.g.	Jung, 1977
5	1	20	1	Kanerva et al., 1994
11	0	0	1	Kanerva et al., 1996
31	2	7	1	Kantoh et al., 1985
46	4	8.7	1	Kiec-Swierczynska, 1995
50	2	4	n.g.	Kilpikari, 1982
15	0	0	1	Knudsen et al., 1993

Results of Patch Tests Performed with 1,3-diphenylguanidine (continued)

Subjects (n)	Positive (n)	+ve reaction (%)	Concentration (%)	Reference
30	1	3.3	1	Koch et al., 1996
1	0	-	1	Koch, 1996
61	2	3	1	Lisi & Simonetti, 1985
61	3	5	1	Lisi & Simonetti, 1985
119	3	3	1	Lynde et al., 1982
1377	5	0.4	n.g.	Meneghini et al., 1963
49	2	4	70	Monsanto, 1982
6	2	33	1	Nater, 1975
n.g.	n.g.	3****	n.g.	Orlov et al., 1973
844	44	5	1	Rajan & Khoo, 1980
1600	25	1.6	1	Reifferscheid, 1979
2	0	-	n.g.	Roed-Petersen & Menne, 1976
15	1	7	1+2	Ross & Obst, 1969
744	74***	9.9	1	Rudzki & Kleniewska, 1970
47	6	l3	n.g.	Rudzki & Kohutnicki, 1971
1	1	-	n.g.	Ruocco & Florio, 1986
50	6	12	1	Saha et al.; 1993
502	7	1.4	2	Suskind, 1984
4	3	75	n.g	Takeda et al., 1964
32	0	0	1	te Lintum & Nater, 1973
1	0	-	1	Tuyp & Mitchell, 1983
3	1	33	2	van Dijk, 1968
106	-**	-	1	Wilson, 1969

* n.g. = not given
** no evaluation possible due to a number of irritating reactions
*** possible irritating reactions could not be ruled out
**** scarification of skin

Conclusion: Human cases have shown that contact dermatitis patients, for whom a rubber intolerance was often present, occasionally reacted positively to 1,3-diphenylguanidine in the patch test. Taken into account the negative Guinea pig maximization assay, it can be infer that the positive reactions observed in human p atients with contact dermatitis reflected cross-reactions rather than a direct sensitizing effect of 1,3-diphenyl guanidine.

3.2 Initial Assessment for Human Health

3.2.1. Effects on human health

1,3-diphenylguanidine is absorbed rapidly after oral uptake but only slowly after dermal application. The substance is metabolised quickly and eliminated in the urine and faeces. No information is available on the mode of action and the identity of the metabolites.

The oral LD50 is 323-850 mg/kg b.w. for the rat. The dermal LD0 is > 2,000 mg/kg b.w. in the rabbit. Intoxication is manifested by spasms, disturbed lipid metabolism, lung emphysema and kidney changes.

Three subchronic toxicity feeding studies in rats and mice have shown an increase of the mortality rate at high dose and a decrease of food consumption and body weight gain due to the poor palatability of the 1,3-diphenylguanidine-treated feed. Treatment-related effects on the organs and the haematological, clinical-chemical parameters and urinalysis were not observed. The NOAEL/LOAEL lies at 32/50 mg/kg bw/d and 11/37 mg/kg bw/d for rats and 75/114 mg/kg bw/day in mice. Based on these data the best estimate for the NOAEL was 32 mg/kg bw/d for rats and 75 mg/kg/d for mice.

Most of the *in vitro* and *in vivo* investigations available give no indication of a genotoxic effect.

A carcinogenicity study which would meet present standards is not available.

1,3-diphenylguanidine did not affect the fertility of male mice when administered by gavage up to the maximal tested dose level of 16 mg/kg/d. In addition to the results of the feeding sub-chronic studies on the rat and mouse, special studies for recognising reproductive toxic effects were also performed. Comparisons of the parameter changes with the results of tests with feed withdrawal infer that the effects observed in the 1,3-Diphenylguanidine-treated animals in high concentration groups are a result of the poor general state of health (malnutrition, exhaustion) of the animals and not a direct toxic effect on the reproductive organs. Very conservative NOAELs, based on the effects on the reproductive organs, secondary to malnutrition and exhaustion, can be established at 32 mg/kg bw/d for rats and from 16 to 231 mg/kg bw/d for mice.

In female rats and mice foetotoxic, but not teratogenic, effects were seen after the oral administration of maternotoxic doses. In the rat study the NOEL was given as 5 mg/kg bw for the dams and 25 mg/kg bw for the foetuses. In the mouse study the NOEL was given as 4 mg/kg bw for the dams and higher than 10 mg/kg bw for the foetuses.

1,3-diphenylguanidine is irritating to the eye and non-irritating to the skin.

Human cases have shown that contact dermatitis patients, for whom a rubber intolerance was often present, occasionally reacted positively to 1,3-diphenylguanidine in the patch test. Taken into account the negative Guinea pig maximization assay, it can be infer that the positive reactions observed in human patients with contact dermatitis reflected cross-reactions rather than a direct sensitizing effect of 1,3-diphenyl guanidine.

In man, earlier and unconfirmed studies described the following symptoms after workplace exposures to 1,3-diphenylguanidine: eye and mucous membrane irritation, gastric and bilious complaints and disturbed liver metabolism.

3.2.2. Occupational exposure

No data available.

3.2.3. Consumer exposure

No data available.

3.2.4. Indirect exposure via environment

No data available.

4. HAZARDS TO THE ENVIRONMENT

4.1 Effects on the Environment

4.1.1 Aquatic Effects

1,3-Diphenylguanidine has been shown to be toxic to fish and algae and harmful to daphnia (fish : 96 h LC50 = 4.2-11 mg/l; algae : EC50 = 1.7-7.5 mg/l; daphnid : 48 h EC50 = 17-62.4 mg/l)

4.1.1.1 Acute toxicity to fish

Three studies on three fish species can be considered valid with restrictions. The lowest 96 h LC50 of 4.2 mg/l (ABC, 1979a) was observed for *Pimephales promelas* in a static test and based on nominal concentrations. The test is considered valid despite the lack of analysis as the test substance has been shown to be stable in water at the pH range required for this test. The remaining studies are not considered valid for use in hazard assessment due to the low study duration but the results support the valid studies.

species	duration	results	remarks	references	reliability
Pimephales promelas	96 h	LC50 = 4.2 mg/l	Static, no analysis	ABC laboratory (1979a)	2
Oncorhynchus mykiss	96 h	LC50 = 11 mg/l	Static, no analysis	ABC laboratory (1979b)	2
Lepomis macrochirus	96 h	LC50 = 9.6 mg/l	Static, no analysis, O2 concentration dropped below accepted limits at 96 h	ABC laboratory (1979c)	2
Oryzias latipes	48 h	LC50 = 10 mg/l	Static or semi-static	MITI (1992)	3
Leuciscus idus	48 h	LC100 = 10 mg/l	Static	Bayer (1975a)	3
Leuciscus idus	72 h	LC0 = 1 mg/l	Static	Bayer (1975b)	3
Cyprinus carpio			Fed by oral application of substance in gelatin capsule	Loeb & Kelly (1963)	3

4.1.1.2 Acute toxicity to daphnia

Two valid studies exist for daphnids but the lowest EC50 value was observed in the 48 h test (ABC, 1979d) and this is therefore considered to be the preferred study.

species	duration	results	remarks	references	reliability
Daphnia magna	48 h	EC50 = 17 mg/l	No analysis	ABC (1979d)	2
Daphnia magna	24 h	EC50 = 62.4 mg/l	No analysis	Bayer (1990b)	2

4.1.1.3 Acute toxicity to algae

There are two acute toxicity tests available for algae. While both studies are valid with restrictions, due to the lack of analysis, the test using *Selenastrum capricornutum* is preferred as it provides the lowest EC50 value and was the longer study of the two. Moreover, the 96 h result is calculated using the US EPA method which is based more on biomass (number of cells or chlorophyll with no logarithmic calculation to determine specific growth rate). While the NOEC for biomass from the Bayer (1990) study provides the lowest overall value, the concentration effect curve for biomass was sigmoid in shape, flattening out from 1 mg/l DPG and lower resulting in an abnormally low determination of EbC10. Examination of the log cell number against time leads to the conclusion that significant reduction in cell number compared to the control does not occur below 1 mg/l. Indeed, linear extrapolation of the concentration effect graph for biomass leads to a NOEC of 0.3 mg/l instead of 0.013 mg/l for the *S. subspicatus* study. The NOEC of 0.3 mg/l calculated from the *S. capricornutum* study is therefore considered the more appropriate value to be used in this case.

species	duration	results	remarks	references	reliability
Scenedesmus subspicatus	72 h	ErC50 = 7.5 mg/l NOEC = 2.1 mg/l EbC50 = 2.6 NOEC = 0.013	No analysis	Bayer (1990c)	2
Selenastrum capricornutum	96 h	EC50 = 1.7 mg/l NOEC = 0.3 mg/l	No analysis	BMRL (1979)	2

4.1.1.4 Acute toxicity to micro-organisms

Three studies exist but only one (Bayer, 1990d) is considered valid for use. The study followed OECD guideline 209 and determined an EC50 for sludge respiration inhibition of 147 mg/l.

4.1.1.5 Chronic toxicity to daphnids

One Daphnia reproduction test following the 1984 version of OECD 202 part b is available (Bayer, 1990e). The study is considered to be valid without restriction and comprises analytical measurements of 1,3-Diphenylguanidine and was performed to GLP.

The NOEC for immobilisation of the parent generation is 1.9 mg/l and of reproduction was 0.6 mg/l.

4.1.1.6 PNEC for the aquatic environment

Using the lowest aquatic toxicity result from three acute tests together with a factor of 1000 the PNEC would be 1.7 µg/l based on the *Selenastrum capricornutum* study. However, if the NOECs from the algae (0.3 mg/l) and daphnid chronic (1.9 mg/l) studies are used (excluding the EbC50 results), and applying a uncertainty factor of 50, the PNEC would be 6 µg/l.

4.2 Terrestrial Effects

Studies are available on terrestrial plant emergence, soil microorganism effects and bird acute oral toxicity.

One plant study (Kefford et al., 1965) examined germination induction of *Lactuca sativa* by 1,3-Diphenylguanidine rather than inhibition of emergence and so cannot be considered valid for hazard assessment. The second plant study examined the effect of the substance on the mitosis cycle of *Vicia faba* (Bempong & McCoy, 1972) and so no relevant end point for risk assessment can, be determined for this study either.

The soil micro-organism studies were also unconventional and cannot be used for hazard assessment. Both studies were performed by the same authors (Williams & Biodeter, 1984). In the first, inoculated aqueous soil extract (3.0×10^7 ind./ml) in nutrient agar containing the test substance (concentration not specified) was incubated for a period of 4-14 d. The 4 d EC50, compared to the growth rate of the control, was described as less than or equal to 0.1 % (1 g/l). In the second study the effect of test substance (concentration not specified) on growth rate of soil micro-organisms on a polycarbonate membrane or embedded in epoxy resin (Araldite) was determined. No colony formation was observed within the test period of 3 months.

The only plant study that can be considered valid was the 16 d plant study by Schallnass et al. 1995, following OECD 208 and using two plant species *Avena sativa* and *Brassica rapa*. All seedlings places in spiked or control soil emerged by day 9 of the test. All control plants emerged by day 3 of the test.
For *Avena sativa:*

EC50 = 1169 mg/kg based on plant fresh weight
LOEC = 1000 mg/kg based on plant fresh weight
NOEC = 316 mg/kg based on plant fresh weight

For *Brassica rapa:*

EC50 = 358 mg/kg based on plant fresh weight
LOEC = 316 mg/kg based on plant fresh weight
NOEC = 100 mg/kg based on plant fresh weight

The LOEC based on observed toxic effects = 100 mg/kg - dry leaf edges observed in 12 out of 20 plants, however, at 31.6 mg/kg this symptom was noted in 1 out of 20 plants. The LC50 could not be calculated as no plant mortality was found at any concentration.

4.3 Other Environmental Effects

The avian study (Schafer, 1983) reported acute oral toxicity up to a limit concentration of 100 mg/kg on three species of song birds *Agelaius phoeniceus, Sturnus vulgaris* and *Passer domesticus*. After a single oral application of test substance (dissolved in propyleneglycol) following a settling in-phase of 2-6 weeks, no acute effects were observed.

4.4 Initial Assessment for the Environment

1,3-diphenylguanidine has three forms: unionised, primarily protonated and secondarily protonated. The pKa at which the first protonation occurs is 10.12 while the pKa for the second protonation is unknown and as this will be less than 10.12 it is not known whether this state will be reached at normal environmental pHs between 6 and 8. This leads to problems in determining the environmental fate of the substance.

Due to the relatively high solubility, low octanol water partition coefficient and low volatility of 1,3-Diphenylguanidine the substance is not expected to adsorb to sediment and will mainly be present in the aqueous phase. A bioconcentration test on fish provided a BCF of <20 (LOQ). The substance is therefore likely to remain bioavailable and, although not readily biodegradable, has been shown to mineralise rapidly in the presence of adapted micro-organisms. Based on the above the substance can be considered inherently biodegradable while bioaccumulation in biota is not expected for this substance.

1,3-diphenylguanidine has been shown to be toxic to fish and algae and harmful to *Daphnia* in several acute studies (fish : 96 h LC50 = 4.2-11 mg/l; algae : EC50 = 1.7-7.5 mg/l; daphnid : 48 h EC50 = 17-62.4 mg/l).
The PNEC can be determined using the NOECs from the algae (0.3 mg/l) excluding the lowest EbC50 results, and daphnid chronic (0.6 mg/l) studies, by applying an uncertainty factor of 50. The resulting PNEC would be 6 µg/l.
A terrestrial plant study conducted on monocotyledons and dicotyledons did not show a high level of concern for DPG in these species
Due to its major use as a vulcanisation activator during which process it is incorporated in the rubber compound but much reverts after processing, leaching of DPG may occur from rubber compounds but the substance represents a relatively low percentage of content in the finished product (1-2%). DPG may be of concern locally in aqueous discharge from production and downstream use sites.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

1,3-diphenylguanidine has three forms: unionised, primarily protonated and secondarily protonated. The pKa at which the first protonation occurs is 10.12 while the pKa for the second protonation is unknown and as this will be less than 10.12 it is not known whether this state will be reached at normal environmental pHs between 6 and 8. This leads to problems in determining the environmental fate of the substance.

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In female rats and mice foetotoxic, but not teratogenic, effects were seen after the oral administration of maternotoxic doses. In the rat study the NOEL was given as 5 mg/kg bw for the dams and 25 mg/kg bw for the foetuses. In the mouse study the NOEL was given as 4 mg/kg bw for the dams and > 10 mg/kg bw for the foetuses. 1,3-Diphenylguanidine is irritating to the eye and non-irritating to the skin.

Human cases have shown that contact dermatitis patients, for whom a rubber intolerance was often present, occasionally reacted positively to 1,3-diphenylguanidine in the patch test. Taken into account the negative Guinea pig maximization assay, it can be infer that the positive reactions observed in human patients with contact dermatitis reflected cross-reactions rather than a direct sensitizing effect of 1,3-diphenyl guanidine.

In man, earlier and unconfirmed studies described the following symptoms after workplace exposures to 1,3-diphenylguanidine: eye and mucous membrane irritation, gastric and bilious complaints and disturbed liver metabolism.

5.2 Recommendations

Environment

Based on current information no clear conclusion can be drawn. While the fate properties suggest that the substance will not bioaccumulate in the environment and that degradation will occur, the PNEC, be it based on flora or fauna is relatively low and the downstream use is such that the substance is likely to be found (within or outside polymer matrix) in the environment mainly due to abrasion from car tyres.

In the absence of knowledge on the leaching behaviour of the substance from abraded rubber compounds, further work to provide a reasonable estimate of the environmental concentration is considered necessary.

Human Health

Low priority for further work.

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