

I U C L I D

D a t a S e t

Existing Chemical Substance ID: 128-37-0
CAS No. 128-37-0
EINECS Name 2,6-di-tert-butyl-p-cresol
EINECS No. 204-881-4
Molecular Weight 220.36
Molecular Formula C15H24O

Producer Related Part

Company: Bayer AG
Creation date: 03-MAR-1994

Substance Related Part

Company: Bayer AG
Creation date: 03-MAR-1994

Memo: X AKTUELL EG

Printing date: 29-JAN-2001
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Reliability (profile): Reliability: without reliability, 1, 2, 3, 4

Flags (profile): Flags: robust summary

1.1 General Substance Information

Substance type: organic

Physical status: solid

Purity: > 99 % w/w

Remark:

- Bayer AG is lead company for the substance.
- Great Lakes Chemicals France S.A. is another manufacturer/importer who has agreed on the above lead function.
- Derivados Fenolicos S.A. (Derfesa) is owned by Shell Espana S.A. For this submission both are represented by Shell International Chemical Company Ltd.
- Bayer AG, Great Lakes Chemicals France S.A. and Shell International Chemical Company Ltd. are cooperating companies of the CEFIC Sector Group European Butylated Hydroxytoluene Manufacturers Association (EBMA).

Flag: robust summary

10-JUN-1994

1.2 Synonyms

2,6-DI-TERT-BUTYL-4-METHYLPHENOL

Flag: robust summary

2,6-DI-TERT-BUTYL-P-CRESOL

Flag: robust summary

4-HYDROXY-3,5-DI-TERT-BUTYLTOLUENE

Flag: robust summary

BHT

Flag: robust summary

BUTYLATED HYDROXY TOLUENE

Flag: robust summary

BUTYLATED HYDROXYTOLUENE

Flag: robust summary

P-CRESOL, 2,6-DI-TERT-BUTYL-

Flag: robust summary

PHENOL, 2,6-BIS(1,1-DIMETHYLETHYL)-4-METHYL-

Flag: robust summary

1.3 Impurities

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1.4 Additives

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1.5 Quantity

Production during the last 12 months: yes

Quantity produced : 5 000 - 10 000 tonnes in 1993

Remark: 1992 5000 - 10000 t/a
1991 1000 - 5000 t/a
1990 1000 - 5000 t/a

Flag: robust summary

Quantity

Remark: no change of production volume 1999

Flag: robust summary

17-NOV-2000

1.7 Use Pattern

Type: type
Category: Wide dispersive use
Flag: robust summary

Type: industrial
Category: Fuel industry
Flag: robust summary

Type: industrial
Category: Polymers industry
Flag: robust summary

Type: industrial
Category: other: foodstuffs and feed industry
Flag: robust summary

Type: use
Category: Food/foodstuff additives
Flag: robust summary

Type: use
Category: Stabilizers
Flag: robust summary

1.9 Source of Exposure

Remark: human exposure by direct and indirect food additive
consumption

Flag: robust summary

1.15 Additional Remarks

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2.1 Melting Point

Value: 70 degree C
Reliability: (2) valid with restrictions
Flag: robust summary
02-NOV-2000 (1) (2) (3)

2.2 Boiling Point

Value: 265 degree C at 1013 hPa
Reliability: (2) valid with restrictions
Flag: robust summary
02-NOV-2000 (1) (4) (2) (3)

2.3 Density

Type: density
Value: 1.03 g/cm3 at 20 degree C
Reliability: (1) valid without restriction
Flag: robust summary
17-NOV-2000 (5)

Type: density
Value: 1.048 at 20 degree C
Reliability: (2) valid with restrictions
Flag: robust summary
18-OCT-2000 (4) (2)

2.4 Vapour Pressure

Value: .01 hPa at 20 degree C
Reliability: (1) valid without restriction
Flag: robust summary
02-NOV-2000 (6)

Value: .03 hPa at 25 degree C
Reliability: (1) valid without restriction
Flag: robust summary
02-NOV-2000 (6)

2.5 Partition Coefficient

log Pow: 5.1
Method: other (measured): no data
Year:
Flag: robust summary (7)

2.6.1 Water Solubility

Value: 1.1 mg/l at 20 degree C
Reliability: (2) valid with restrictions
Flag: robust summary
17-NOV-2000 (8)

Value: .4 mg/l at 20 degree C
Reliability: (2) valid with restrictions
Flag: robust summary
02-NOV-2000 (9)

2.12 Additional Remarks

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3.1.1 Photodegradation

Type: water
Light source: Sun light
Light spect.: 310 - 400 nm
Conc. of subst.: .6 mg/l
Method: other (measured)
Year: **GLP:** no data
Test substance: other TS: purity of 4-14CH3-BHT > 99%
Result: 25.2% of applied radiolabelled BHT was found after 8 days of exposure (volatiles amounted to ca. 1.4%)
Test condition: test duration: 8 days, 8 hours sunlight per day
Reliability: (2) valid with restrictions
study well documented, meets generally accepted scientific principles
Flag: robust summary
29-JAN-2001 (10)

Type:
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 500000 molecule/cm³
Method: other (calculated): acc. to Atkinson
Year: **GLP:**
Test substance:
Remark: Calculated half-life: t_{1/2} ca. 17 hours (0.5 x 10⁶ OH radicals/cm³, under conditions of Western Europe; rate constant 23.3 x 10⁻¹² cm³/molecule x s, sigma-value for meta position of OH group to H-atoms derived from Hammett)
Reliability: (2) valid with restrictions
accepted calculation method
Flag: robust summary
09-NOV-2000 (11)

3.1.2 Stability in Water

Type: abiotic
Method: other: (measured)
Year: **GLP:** no data
Test substance: other TS: purity of radiolabelled BHT > 99%
Result: 59.6% of radiolabelled BHT was recovered after 8 days in the
Test condition: test duration: 8 days; test medium: distilled water without irradiation
Reliability: (2) valid with restrictions
study well documented, meets generally accepted scientific principles
Flag: robust summary
29-JAN-2001 (10)

3.2 Monitoring Data (Environment)

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3.3.1 Transport between Environmental Compartments

Type: adsorption
Media: other: water-sediment
Method:
Year: 1978
Method: adsorption to river sediment calculated from measured test substance concentrations in river water and sediment; GC/MS analysis
Result: adsorption factor: 4000
Reliability: (2) valid with restrictions
Flag: robust summary
10-NOV-2000 (12)

3.3.2 Distribution

Media: air - biota - sediment(s) - soil - water
Method: Calculation according Mackay, Level I
Year:
Result:
Air: 81.2 %
Water: 0.9 %
Soil: 9.2 %
Sediment: 8.6 %
suspended Sediment: <0.1 %
Biota: <0.1 %
Reliability: (1) valid without restriction
accepted calculation method
Flag: robust summary
29-JAN-2001 (13)

3.5 Biodegradation

Type: aerobic
Inoculum: activated sludge
Concentration: .3 mg/l related to Test substance
Degradation: ca. 10 % after 56 day
Method: other
Year: **GLP:** no data
Test substance: other TS: purity of radiolabelled BHT > 99%
Remark: mineralization/elimination depended on ratio BHT/activated sludge; concentration (solubility) of test substance, and the presence of a dispersing agent (e.g. ethanol)
Result: after 56 days of exposure about 10% of ¹⁴C-phenyl-BHT were mineralized and about 99% eliminated altogether; half-life of disappearance: 3.4 days
Test condition: incubation at 25°C in the dark, ethanol as dispersing agent, sludge concentration: 100 mg/l, measurement of CO₂ evolution
Reliability: (2) valid with restrictions
study well documented, meets generally accepted scientific principles
Flag: robust summary
09-NOV-2000 (14)

Type: aerobic
Inoculum: activated sludge
Concentration: 50 mg/l related to Test substance
Degradation: 4.5 % after 28 day
Result: other: not readily biodegradable
Method: other: see remarks
Year: **GLP:** no data
Test substance: no data
Remark: The test was conducted in accordance with "Biodegradation test of chemical substance by microorganisms etc." stipulated in the Order Prescribing the Items of the Test Relating to the New Chemical Substance (1974, Order of the Prime Minister, the Minister of Health and Welfare, the Minister of International Trade and Industry No. 1). This guideline corresponds to "301C, Ready Biodegradability: Modified MITI Test I" stipulated in the OECD Guidelines for Testing of Chemicals (May 12, 1981)
Test condition: deviations from guideline:
sludge concentration: 50 mg/l
substance concentration: 50 mg/l
Reliability: (2) valid with restrictions
study conducted similar to guideline
Flag: robust summary
09-NOV-2000 (15)

3.7 Bioaccumulation

Species: Cyprinus carpio (Fish, fresh water)
Exposure period: 56 day
Concentration: .05 mg/l
BCF: 230 - 2500
Elimination:
Method: other: see remarks
Year: **GLP:** no data
Test substance: no data
Remark: The test was conducted in accordance with "Bioaccumulation test of chemical substance in fish and shellfish" stipulated in the Order Prescribing the Items of the Test Relating to the New Chemical Substance (1974, Order of the Prime Minister, the Minister of Health and Welfare, the Minister of International Trade and Industry No. 1). This guideline corresponds to "305C, Bioaccumulation: Degree of Bioconcentration in Fish" stipulated in the OECD Guidelines for Testing of Chemicals (May 12, 1981)
Reliability: (1) valid without restriction
guideline study
Flag: robust summary
09-NOV-2000 (15)

Species: Cyprinus carpio (Fish, fresh water)
Exposure period: 56 day
Concentration: .005 mg/l
BCF: 330 - 1800
Elimination:
Method: other: see remarks
Year: **GLP:** no data
Test substance: no data
Remark: The test was conducted in accordance with "Bioaccumulation test of chemical substance in fish and shellfish" stipulated in the Order Prescribing the Items of the Test Relating to the New Chemical Substance (1974, Order of the Prime Minister, the Minister of Health and Welfare, the Minister of International Trade and Industry No. 1). This guideline corresponds to "305C, Bioaccumulation: Degree of Bioconcentration in Fish" stipulated in the OECD Guidelines for Testing of Chemicals (May 12, 1981)
Reliability: (1) valid without restriction
guideline study
Flag: robust summary
09-NOV-2000 (15)

3.8 Additional Remarks

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AQUATIC ORGANISMS**4.1 Acute/Prolonged Toxicity to Fish**

Type: semistatic
Species: Brachydanio rerio (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** yes
LC0: > .57
Method: Directive 84/449/EEC, C.1 "Acute toxicity for fish"
Year: 1994 **GLP:** yes
Test substance:
Remark: only 1 test substance concentration was applied (1.0 mg/l; nominal); before starting the test, the test substance was crushed with a pestle. To accelerate the adjustment of the test concentration, 5 mg of the test substance was added to 1 litre of water, the resulting suspension was stirred on a magnetic stirrer for 24 hours, treated in an ultrasonic bath for 1 hour, and finally filtered to remove undissolved particels of the test substance; analyt. monitoring: GC
Result: LC0 related to effective test substance concentration measured after 24 h exposure (water change after 24 h).
Test condition: 21.4-21.9° C; pH 7.6-8.1; dissolved oxygen: 8.4-9.7 mg/l
Reliability: (2) valid with restrictions
 Guideline study, but recovery of test substance at end of test < 80 %
Flag: robust summary
 29-JAN-2001 (16)

4.2 Acute Toxicity to Aquatic Invertebrates

Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** yes
EC0: > .31
Method: other: Directive 67/548/EEC, C.2 "Acute Toxicity for Daphnia"
Year: 1994 **GLP:** yes
Test substance: as prescribed by 1.1 - 1.4
Method: only 1 test substance concentration was applied (1.0 mg/l; threshold of water solubility); before starting the test, the test substance was crushed with a pestle. To accelerate the adjustment of the test concentration, the test substance was added to 1 liter of Elendt medium, the resulting suspension was stirred on a magnetic stirrer for 24 hours, treated in an ultrasonic bath for 1 hour, and finally filtered to remove undissolved particels of the test substance; analytical monitoring: GC
Result: EC0 related to mean of the test substance concentration measured at beginning of test and after 48 hours of exposure
Reliability: (2) valid with restrictions
 Guideline study, but recovery of test substance at end of test < 80 %
Flag: robust summary
 25-JAN-2001 (17)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Scenedesmus subspicatus (Algae)
Endpoint: other: biomass and growth rate
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** yes
EC50: > .42
Method: other: Directive 67/548/EEC, C.3 "Algal inhibition test"
Year: 1994 **GLP:** yes
Test substance:
Method: only one test substance concentration applied (1 mg/l; nominal); before starting the test, the test substance was crushed with a pestle. To accelerate the adjustment of the test concentration, 5 mg of the test substance was added to 1 litre of water, the resulting suspension was stirred on a magnetic stirrer for 24 hours, treated in an ultrasonic bath for 1 hour, and finally filtered to remove undissolved particles of the test substance; analytical monitoring: GC
Remark: at a measured test concentration of 0.42 mg/l (= arithmetic mean of analytical values at start and end of the test) there was a slightly lower cell density at the end of test as compared to control (304000 and 358000 cells/ml, respectively); on the other hand, the cell density multiplied by a factor of 30 within 72 hours, which is much more than required for fullfilling the quality criteria with respect to the growth in the control (>= factor 16). For this reason, the slight differences of growth between control and test is regarded and not relevant to the result.
Result: EC50 is given as arithmetic mean of the measured test substance concentration at the beginning and end of test after 72 hours of exposure
Reliability: (2) valid with restrictions
Guideline study, but recovery of test substance at end of test < 80 %
Flag: robust summary
29-JAN-2001 (18)

4.5 Chronic Toxicity to Aquatic Organisms

4.5.2 Chronic Toxicity to Aquatic Invertebrates

Species: Daphnia magna (Crustacea)
Endpoint: reproduction rate
Exposure period: 21 day
Unit: mg/l **Analytical monitoring:** yes
NOEC: .14
Method: other: OECD Guide-line 202, part 2 "Daphnia sp., Reproduction Test" draft 1993
Year: 1994 **GLP:** yes
Test substance:
Method: semi-static test with 3 test substance concentration applied (0.1, 0.316 and 1 mg/l; nominal); before starting the test, the test substance was crushed with a pestle. To accelerate the adjustment of the test concentration 1 mg/l (= limit of water solubility), 5 mg of the test substance was added to 1 litre of Elendt medium, the resulting suspension was stirred on a magnetic stirrer for 24 hours, treated in an ultrasonic bath for 1 hour, and finally filtered to remove undissolved particles of the test substance; test medium renewed; analytical monitored by GC after 48 and 72 h of exposure
Remark: EC0 based on mean measured test substance concentrations (at the start and after 48 h and 72 h of exposure at water change)
Test condition: 20.0-21.6° C; pH 7.8-8.4; dissolved oxygen: 9.2-11.7 mg/l; irradiation: 7.5 uE/m³ x s; light/dark-cycle: 16/8 h
Reliability: (2) valid with restrictions
Guideline study, but recovery of test substance at end of test < 80 %
Flag: robust summary
29-JAN-2001 (19)

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Soil Dwelling Organisms

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4.6.2 Toxicity to Terrestrial Plants

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4.6.3 Toxicity to other Non-Mamm. Terrestrial Species

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4.9 Additional Remarks

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5.1 Acute Toxicity**5.1.1 Acute Oral Toxicity**

Type: LD50
Species: rat
Sex: male/female
Number of Animals:
Vehicle: other: an aqueous dispersion at 10% (W/V) of gum Arabic
Value: > 2930 mg/kg bw
Method: OECD Guide-line 401 "Acute Oral Toxicity"
Year: 1988 **GLP:** yes
Test substance: other TS: Rhodianox BHT AP5
Remark: NUMBER OF ANIMALS: 5/dose/sex
MORTALITY: 0/10 (2150 mg/kg); 1/5 (f)/0/5 (2510 mg/kg) death occurred 5th day after application; 0/10 (2930 mg/kg)
CLINICAL SIGNS: no
BODY WEIGHT: no effect
GROSS EXAMINATION: no effect
Reliability: (1) valid without restriction
Flag: robust summary
29-NOV-2000 (20)

Type: LD50
Species: rat
Sex: male/female
Number of Animals:
Vehicle: other: propyleneglycol
Value: > 10000 mg/kg bw
Method: other: 1 dose level; 14 days observation period
Year: 1978 **GLP:** no
Test substance: other TS: Vulkanox KB
Remark: NUMBER OF ANIMALS: 10/dose/sex
MORTALITY: 0/20 (10 g/kg)
CLINICAL SIGNS: no
BODY WEIGHT: no data
GROSS EXAMINATION: no effect
Reliability: (2) valid with restrictions
Flag: robust summary
14-NOV-2000 (21) (22)

5.1.2 Acute Inhalation Toxicity

-

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rat
Sex: male/female
Number of Animals:
Vehicle: other: an aqueous dispersion at 10% (W/V) of gum Arabic
Value: > 2000 mg/kg bw
Method: OECD Guide-line 402 "Acute dermal Toxicity"
Year: 1988 **GLP:** yes
Test substance: other TS: Rhodianox BHT AP5
Remark: NUMBER OF ANIMALS: 5/dose/sex
MORTALITY: 0/10 (2000 mg/kg)
CLINICAL SIGNS: no
LOCAL EFFECTS: no
BODY WEIGHT: no effect
Reliability: (1) valid without restriction
Flag: robust summary
14-NOV-2000

(23)

5.1.4 Acute Toxicity, other Routes

-

5.4 Repeated Dose Toxicity

Species: rat **Sex:** male
Strain: Fischer 344
Route of admin.: oral feed
Exposure period: 76 weeks
Frequency of treatment: daily
Post. obs. period: none
Doses: 100, 300, 1000, 3000 and 6000 ppm
(ca. 7.5, 23, 75, 225 and 450 mg/kg bw day)
Control Group: yes, concurrent no treatment
NOAEL: 3000 ppm
Method: other: see remark field
Year: 1990 **GLP:** no data
Test substance: other TS: purity: > 99 %
Remark: The study was not designed as definitive chronic bioassay. 21 rats /dose and 36 control rats; the diets were prepared every 4 weeks and stored at 40C until use (no analytical data available); interim kill at 12, 36 and 48 weeks of 4 randomly selected animals; observations of pathology: To demonstrate a deficiency in iron storage in cells of altered hepatocellular foci, rats were iron-loaded with sc injections of 12.5 mg elemental iron/100 g body weight in the inguinal regions, alternating sides 3 times/week for 2 weeks prior to killing. Complete autopsies livers were performed on all animals. At autopsy, livers were weighed and slices from each lobe were taken and fixed in 10% neutral buffered formalin. Sections were stained with haematoxylin and eosin and tested for iron to determine the presence of iron storage-deficient lesions.

Result: Tumors and lesions other organs were submitted for histology.
All scheduled rats survived for up to 76 weeks

6000 ppm:
BODY WEIGHT: decreased
LIVER WEIGHT: increased
HISTOPATHOLOGICAL EXAMINATION (liver): no altered foci by 36 weeks; slightly, but not significantly altered foci at 48 and 76 weeks; after 76 weeks slightly increased incidence of hepatic adenomas (33 %)

3000 ppm:
BODY WEIGHT: decreased
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): no altered foci by 36 weeks; slightly, but not significantly altered foci at 48 and 76 weeks

1000 ppm:
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): no altered foci by 36 weeks; slightly, but not significantly altered foci at 48 and 76 weeks

300 ppm:
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): no altered foci by 36 weeks; slightly, but not significantly altered foci at 48 and 76 weeks

100 ppm:
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): no altered foci by 36 weeks; slightly, but not significantly altered foci at 48 and 76 weeks

Reliability: (2) valid with restrictions
Flag: robust summary
17-NOV-2000 (24)

Species: rat **Sex:** male/female
Strain: Wistar
Route of admin.: oral feed
Exposure period: male: 14 weeks (P); 141-144 weeks (F1)
female: 20 weeks (P); 141-144 weeks (F1)

Frequency of treatment: daily
Post. obs. period: non
Doses: nominal: 0, 25 100 and 500 mg/kg bw (P); 0, 25, 100 and 250 mg/kg bw (F1)
Control Group: yes, concurrent no treatment
NOAEL: 25 mg/kg bw
Method: other: Two generation carcinogenicity study; the F1 generation being dosed for their entire lifespan (for further details see remark field and also chapter 5.8)

Year: 1986 **GLP:** no data
Test substance: other TS: purity > 99.5 %
Remark: ADMINISTRATION OF BHT: The BHT was mixed into a semi-synthetic powdered diet in concentrations adjusted according to food

consumption. Diet was prepared every second week. the stability of BHT in the diet was examined four times during each of the feeding periods for the F0 and F1 generations. The actual levels of BHT in the prepared diets were a few percent less than the added amounts.

NUMBER OF ANIMALS (F1): Control: 100/sex; 25 mg/kg: 80/sex; 100 mg/kg: 80/sex; 250 mg/kg: 100/sex

SERUM CHEMISTRY (only high dose F1, 20/sex):

glucose

blood urea nitrogen

free and total cholesterol

triglycerides

phospholipids

BLOOD ANALYSES (only high dose F1):

haematocrit

haemoglobin

red and white blood cell

differential white cell counts

PATHOLOGY (only F1): Specimens from the liver, kidneys, heart, lungs, brain, spleen, pituitary gland, thyroid, thymus (if any), pancreas, adrenals, testes, ovaries, seminal gland, uterus, mesenteric and axillary lymph nodes, salivary gland, gastro-intestinal tract (six levels), urinary bladder, spinal cord, peripheral nerve, skeletal muscle, bone, skin, mammary gland, eye and Harderian gland were fixed in 10 % neutral buffered formalin and embedded in paraffin, and sections were stained with haematoxylin and eosin for histological examination. Other appropriate staining methods were used for selected specimens.

SURVIVAL in Controls: 16 males and 17 females

EFFECTIVE NUMBERS: animals that survived beyond wk 43, the time when the first tumour appeared in the spleen of a male rat in the high-dose group

Result:

500 mg/kg (P):

BODY WEIGHT: decrease (m/f)

250 mg/kg (F1):

BODY WEIGHT: decrease (m: 21%; f:16%)

SURVIVAL: increase (m: 44 f: 39)

SERUM CHEMISTRY: decreased levels of triglyceride (f/m)

BLOOD ANALYSES: no effect (data not tabulated)

PATHOLOGY: increased number of liver adenomas in the males (18 animals with adenoma/99 (= "effective numbers"))

100 mg/kg (P):

BODY WEIGHT: no effect described

100 mg/kg (F1)

BODY WEIGHT: decrease (m: 11%; f:10%)

SURVIVAL: increase (m: 34; f: 26)

PATHOLOGY: no significant effect

25 mg/kg (P):

BODY WEIGHT: no effect described

25 mg/kg (F1):

BODY WEIGHT: decrease (m: 7%; f:5%)

SURVIVAL: (m: 44; f: 39)

PATHOLOGY: no significant effect

Reliability:

Flag:

21-NOV-2000

(2) valid with restrictions

robust summary

(25)

Species: rat **Sex:** male
Strain: Wistar
Route of admin.: gavage
Exposure period: 28 days
Frequency of treatment: daily
Post. obs. period: none
Doses: 0, 25, 250 and 500 mg/kg bw day
Control Group: yes, concurrent vehicle
NOAEL: = 25 mg/kg bw
Method: other: see remark field
Year: 1986 **GLP:** yes
Test substance: other TS: purity: 99.9 %; vehicle: arachis oil
Remark: EXPERIMENTAL DESIGN: Twenty rats were randomly to one of four groups and were given a dose of 25, 250 or 500 mg BHT/kg vehicle for 7 days. The rats in the 500 mg/kg group initially received doses of 750 mg BHT/kg for the first 3 days and a dose of 500 mg BHT/kg for the remaining days. After 7 days the rats were killed by cervical dislocation and autopsied. In the next phase of the experiment, groups of ten rats were treated with 0, 25, 250 or 500 mg BHT/kg daily for 28 days and were then killed and autopsied. Small samples of liver and epididymal adipose tissue were stored at -20°C and later analysed for BHT by HPLC.
EXPERIMENTAL TECHNIQUES USED TO EXAMINE LIVER TOXICITY:
BIOCHEMICAL ASSAYS:
Mitochondrial protein
Glucose-6-phosphatase
Epoxide hydrolase
Total cytochrome P-450
Cytochrome b5
Ethoxycoumarin o-deethylase
BHT oxidase
IMMUNOCYTOCHEMISTRY: sections of liver from rats killed after 28 days were stained immunocytochemically for cytochromes P-448 and P-450 using the three-layer PAP method of Sternburger (Immunocytochemistry, 2nd Ed. Raven Press, N.Y. (1979))
MICROSCOPIC EXAMINATION: samples of the 4 major lobes were fixed in 10% neutral buffered formalin; sections were stained with haematoxylin and eosin, with Van Gieson's stain for collagen and with Gordon and Sweet's method for reticulin
Result: 500 mg/kg:
BODY WEIGHT: weight loss reversed when dose was reduced (7 days); marginally lower than that of the control group (28 days)
LIVER WEIGHT: marked increase (7 or 28 days)
BHT CONTENT: very little (liver, 7 or 28 days)); 227.4 mg/kg wet weight (7 days), 168.4 mg/kg wet weight (28 days)
LIVER BIOCHEMISTRY: increase of proteins (7 or 28 days); decrease in glucose 6-phosphatase activity (7 or 28 days); increase in ethoxycoumarin o-deethylase- and epoxide hydrolase activity (7 or 28 days)
HISTOPATHOLOGICAL EXAMINATION:
After 7 days:
Periportal region

hepatocyte necrosis 2/5
 fibrosis 3/5
 hepatocyte hypertrophy 3/5
 hepatocyte hyperplasia 4/5
 glycogen accumulation 4/5

After 28 days:

Periportal region
 hepatocyte necrosis 6/10
 fibrosis 5/10
 bile-duct cell proliferation 4/10
 hepatocyte hypertrophy 2/10
 hepatocyte hyperplasia 3/10
 pigment-laden macrophages 3/10
 glycogen depletion 7/10
 glycogen accumulation 0/10

IMMUNOCYTOCHEMISTRY: moderately -increased staining intensity in the hypertrophied viable hepatocytes adjacent to the areas of damage

250 mg/kg:

BODY WEIGHT: no effect (7 or 28 days)

LIVER WEIGHT: moderate increase (7 or 28 days)

BHT CONTENT: very little (liver, 7 or 28 days); 66.6 mg/kg wet weight (7 days), 119.8 mg/kg wet weight (28 days)

LIVER BIOCHEMISTRY: increase of protein (28 days); decrease in glucose 6-phosphatase activity (28 days); increase in ethoxycoumarin o-deethylase- and epoxide hydrolase activity (7 or 28 days)

HISTOPATHOLOGICAL EXAMINATION: glycogen accumulation (7 days: (4/5) 28 days: (8/10));

IMMUNOCYTOCHEMISTRY: no effects

25 mg/kg:

BODY WEIGHT: no effect (7 or 28 days)

LIVER WEIGHT: slight increase (7 or 28 days)

BHT CONTENT: very little (liver, 7 or 28 days); 11 mg/kg wet weight (7 days), 15.5 mg/kg wet weight (28 days)

LIVER BIOCHEMISTRY: no effects (7 or 28 days)

HISTOPATHOLOGICAL EXAMINATION: no effects

IMMUNOCYTOCHEMISTRY: no effect

(1) valid without restriction

robust summary

Reliability:

Flag:

20-NOV-2000

(26)

Species: rat **Sex:** male/female
Strain: Wistar
Route of admin.: other: diet
Exposure period: male: 5 weeks (P); 4 weeks (F1), 6, 11, 16
and 22 months (F1)
female: 8 weeks (P)

Frequency of treatment: daily (during the period of mating, food pots were removed when male and females were mated)

Post. obs. period: no

Doses: nominal: 0, 25, 100 and 500 mg/kg bw (P); 0, 25, 100 and 250 mg/kg bw (F1)

Control Group: yes, concurrent no treatment

NOAEL: 25 mg/kg bw

Method: other: Two generation study with emphasis on hepatocellular changes in F1 generation (for further details see remark field and also chapter 5.8)

Year: 1994 **GLP:** yes

Test substance: other TS: purity: 99.96%

Remark: EXPERIMENTAL TECHNIQUES USED TO EXAMINE LIVER TOXICITY:
BIOCHEMICAL ASSAYS:
Glucose 6-phosphatase
Epoxide hydrolase
Glutathione S-transferase
Total cytochrome P450
Ethoxyresorufin O-deethylase
Pentoxyresorufin O-depentylase
Total glutathione
Total, microsomal and cytosolic protein
IMMUNOCYTOCHEMISTRY: Slides were stained with a three layer biotinylated streptavidin horseradish peroxidase method and the following polyclonal primary antibodies:
anti rat Cytochrome P450 1A subfamily
anti rat Cytochrome P450 2B subfamily
anti murine microsomal Epoxide Hydrolase
MICROSCOPIC EXAMINATION: light and electron microscopy were used; cellular proliferation using the technique of pulse labelling with osmotic pumps containing bromodeoxyuridine was only assessed in the high dose F1-animals beginning with 4 weeks after weaning
MICROSCOPIC EXAMINATION OF THE THYROID: The diagnostic criteria for hyperactivity are the presence of some or all of the following:
Reduction of the follicular size
Absence or reduction of colloid
Irregularities in the follicular outline
Hyperaemia
Increase in number of follicular cells
ADMINISTRATION OF BHT: the amount of BHT incorporated initially per unit weight of diet was calculated from the food consumption measured during acclimatisation and from normal growth rate of this strain of rats; throughout pregnancy and lactation no effort was made to adjust dietary BHT content in line with body weight gain during this time

Result: 500 mg/kg (P, females, 20 gestation day):
BODY WEIGHT: no effect

LIVER WEIGHT: increase
HISTOPATHOLOGICAL EXAMINATION (liver): 4/5 animals showed mild centrilobular enlargement and eosinophilia
LIVER BIOCHEMISTRY:
IMMUNOCYTOCHEMISTRY in the liver: no effect
500 mg/kg (foetuses):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): no effect
LIVER BIOCHEMISTRY: a trend towards an increase in glucose 6-phosphatase; activity; results for cytochrome P450 and its isoenzymes have not been presented
IMMUNOCYTOCHEMISTRY in the liver: no effect
500 mg/kg (male pups, 21 days post partum):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: decrease
LIVER WEIGHT: decrease
HISTOPATHOLOGICAL EXAMINATION (liver): no effect
LIVER BIOCHEMISTRY: increase in pentoxyresorufin O-depentylase; increase in total cytochrome P450; increase in glutathione S-transferase- and epoxide hydrolase activity
IMMUNOCYTOCHEMISTRY in the liver: no effect
250 mg/kg (F1, males 4 weeks post weaning):
LIVER TO BODY RATIO: increase
BODY WEIGHT: decrease
LIVER WEIGHT: decrease
HISTOPATHOLOGICAL EXAMINATION (liver): no effect (incl. cell proliferation)
LIVER BIOCHEMISTRY: statistical significant difference in pentoxyresorufin O-depentylase activity; increase in ethoxyresorufin O-deethylase; increase in glutathione S-transferase- and epoxide hydrolase activity
IMMUNOCYTOCHEMISTRY in the liver: no effect
250 mg/kg (F1, males 6 months post weaning):
LIVER TO BODY RATIO: increased
BODY WEIGHT: below that of controls
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): centrilobular enlargement and eosinophilia (4/5); no cell proliferation
LIVER BIOCHEMISTRY: statistical significant difference in pentoxyresorufin O-depentylase activity; increase in glutathione S-transferase- and epoxide hydrolase activity
IMMUNOCYTOCHEMISTRY in the liver: no effect
250 mg/kg (F1, males 11 months post weaning):
LIVER TO BODY RATIO: increase
BODY WEIGHT: decrease
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver, incl. histochemical staining): centrilobular enlargement and eosinophilia (10/10), single altered hepatic focus (2/10), periportal induction of GGT (8/10), no cell proliferation; (kidneys): chronic progressive nephropathy; (thyroid): hyperactivity (10/10); (adrenals): no effects
LIVER BIOCHEMISTRY: statistical significant difference in pentoxyresorufin O-depentylase activity; increase in total cytochrom P450; increase in glutathione S-transferase- and

epoxide hydrolase activity
IMMUNOCYTOCHEMISTRY in the liver: focal phenotypic or proliferative changes (2/19)
250 mg/kg (F1, males 16 months post weaning):
LIVER TO BODY RATIO: increase
BODY WEIGHT: decrease
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver, incl. histochemical staining): centrilobular enlargement and eosinophilia (12/13), periportal induction of GGT (13/13), no cell proliferation; (kidneys): chronic progressive nephropathy; (thyroid): hyperactivity (13/13); (adrenals): no effects
LIVER BIOCHEMISTRY: statistical significant difference in pentoxyresorufin O-depentylase activity; increase in total cytochrom P450; increase in glutathione S-transferase- and epoxide hydrolase activity
IMMUNOCYTOCHEMISTRY in the liver: focal phenotypic or proliferative changes (8/13)
TOTAL THYROXINE (T4): no effect
250 mg/kg (F1, males 22 months post weaning):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: below that of controls
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver, incl. histochemical staining): centrilobular enlargement and eosinophilia (18/19), nodules ((6/19) periportal induction of GGT (17/17), no cell proliferation (only one animal examined); (kidneys): chronic progressive nephropathy; (thyroid): hyperactivity (13/13); (adrenals): no effects
LIVER BIOCHEMISTRY: statistical significant difference in pentoxyresorufin O-depentylase activity; increase in total cytochrom P450; increase in glutathione S-transferase- and epoxide hydrolase activity
IMMUNOCYTOCHEMISTRY in the liver: focal phenotypic or proliferative changes (14/19)
TOTAL THYROXINE (T4): no effect
100 mg/kg (P, females, 20. gestation day):
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): no effect
100 mg/kg (foetuses):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): no effect
LIVER BIOCHEMISTRY: activity; results for cytochrome P450 and its isoenzymes have not been presented
100 mg/kg (F1, male pups, 21 days post partum):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): no effect
LIVER BIOCHEMISTRY: increase in pentoxyresorufin O-depentylase activity; increase in total cytochrome P450; increase in epoxide hydrolase activity
100 mg/kg (F1, males, 4 weeks post weaning):
LIVER TO BODY RATIO: no effect

BODY WEIGHT: below that of control
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): no effect
LIVER BIOCHEMISTRY: statistical significant difference in
pentoxyresorufin O-depentylase activity; increase in
ethoxyresorufin O-deethylase; increase in glutathione
S-transferase- and epoxide hydrolase activity
100 mg/kg (F1, males 6 months post weaning):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: below that of controls
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): centrilobular
enlargement and eosinophilia (3/5)
LIVER BIOCHEMISTRY: statistical significant difference in
pentoxyresorufin O-depentylase activity; increase in
glutathione S-transferase- and epoxide hydrolase activity
100 mg/kg (F1, males 11 months post weaning): LIVER TO BODY
RATIO: increased
BODY WEIGHT: below that of controls
LIVER WEIGHT: increased
HISTOPATHOLOGICAL EXAMINATION (liver, incl. histochemical
staining): centrilobular enlargement and eosinophilia (6/8);
single altered hepatic focus (2/10), periportal induction of
GGT (3/8); (kidneys): chronic progressive nephropathy;
(thyroid): hyperactivity (6/8); (adrenals): no effects
LIVER BIOCHEMISTRY: statistical significant difference in
pentoxyresorufin O-depentylase activity; increase in
glutathione S-transferase activity
100 mg/kg (F1, males 16 months post weaning):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: below that of controls
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver, incl. histochemical
staining): centrilobular enlargement and eosinophilia (0/9),
periportal induction of GGT (8/8); (kidneys): chronic
progressive nephropathy; (thyroid): hyperactivity (7/9);
(adrenals): no effects
LIVER BIOCHEMISTRY: statistical significant difference in
pentoxyresorufin O-depentylase activity; increase in
glutathione S-transferase- and epoxide hydrolase activity
TOTAL THYROXINE (T4): no effect
100 mg/kg (F1, males 22 months post weaning):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: below that of controls
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver, incl. histochemical
staining): centrilobular enlargement and eosinophilia (4/11),
periportal induction of GGT (7/11); (kidneys): chronic
progressive nephropathy; (thyroid): hyperactivity (9/11);
(adrenals): no effects
LIVER BIOCHEMISTRY: statistical significant difference in
pentoxyresorufin O-depentylase activity; increase in
glutathione S-transferase activity
TOTAL THYROXINE (T4): no effect
25 mg/kg (P, females, 20. gestation day):
BODY WEIGHT: no effect
LIVER WEIGHT: no effect

HISTOPATHOLOGICAL EXAMINATION (liver): 1/5 animals showed mild centrilobular enlargement and eosinophilia
25 mg/kg (foetuses):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): no effect
LIVER BIOCHEMISTRY: results for cytochrome P450 and its isoenzymes have not been presented
25 mg/kg (F1, male pups, 21 days post partum):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): no effect
LIVER BIOCHEMISTRY: increase in epoxide hydrolase activity
25 mg/kg (F1, males, 4 weeks post weaning):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): no effect
LIVER BIOCHEMISTRY: no effects
25 mg/kg (F1, males 6 months post weaning):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): centrilobular enlargement and eosinophilia (3/5)
LIVER BIOCHEMISTRY: increase in epoxide hydrolase activity
25 mg/kg (F1, males 11 months post weaning):
LIVER TO BODY RATIO: increased
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver, incl. histochemical staining): centrilobular enlargement and eosinophilia (0/8), single altered hepatic focus (1/8), periportal induction of GGT (1/8); (kidneys): chronic progressive nephropathy; (thyroid): no effect; (adrenals): no effects
LIVER BIOCHEMISTRY: no effects
25 mg/kg (F1, males 16 months post weaning):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver, incl histochemical staining): centrilobular enlargement and eosinophilia (3/9), no periportal induction of GGT; (kidneys): chronic progressive nephropathy; (thyroid): no effect; (adrenals): no effects
LIVER BIOCHEMISTRY: increase in epoxide hydrolase
TOTAL THYROXINE (T4): no effect
25 mg/kg (F1, males 22 months post weaning):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver, incl. histochemical staining): centrilobular enlargement and eosinophilia (1/13); no periportal induction of GGT; (kidneys): chronic progressive nephropathy; (thyroid): no effect (adrenals): no effects
LIVER BIOCHEMISTRY: no effects

TOTAL THYROXINE (T4): no effect

CONCLUSIONS:

No BHT effect was seen in F0 generation although the livers from lactating dams were much larger than those from respective controls and showed morphological evidence of considerable metabolic activity. The histological and biochemical changes seen in the F1 generation were similar to those reported by other workers on the hepatic effects of BHT and are consistent with the effects of an inducer of cytochromes P450. The nodules and glucose 6-phosphatase deficient AHF observed at Time Point 7 of this experiment were probably induced by BHT. No evidence of thyroid increased activity as a result of BHT administration was observed at a dose level of 25 mg/kg body weight/day BHT. Hyperactivity occurred at dose levels of 100 and 250 mg/kg body weight/day BHT. It appeared that BHT gave some protection against the development of chronic progressive nephropathy (CPN), because CPN was observed in all rats (incl. controls) at every time point, but the disease was less severe in rats treated with 250 mg/kg. No adverse effect of BHT was observed in the adrenals.

Reliability:**Flag:**

17-NOV-2000

(1) valid without restriction

robust summary

(27) (28)

5.5 Genetic Toxicity 'in Vitro'**Type:**

Bacterial gene mutation assay

System of**testing:**

S. typhimurium TA102 and TA2638; E. coli WP2/pKM101 and WP2 uvrA/pKM101

Concentration:**Metabolic****activation:****Result:**

negative

Method:**Year:**

1998

GLP:**Test substance:**

other TS: purity: > 99%

Remark:

In a large collaborative study has been performed using the four bacterial strains in order to compare the specific spectrum of response to chemicals and to evaluate the usefulness of each strain.

Reliability:

(2) valid with restrictions

Flag:

robust summary

27-NOV-2000

(29)

Type: Cytogenetic assay
System of testing: CHO cells
Concentration: 0.1; 0.25 and 0.5 ug/ml
Metabolic activation: without
Result:
Method: other: see remark field
Year: 1995 **GLP:** no data
Test substance: other TS: BHT from Sigma (no further information)
Remark: METHOD: CHO cells were cultered for 15-16 h in the presence of the different doses of BHT. Two hours before cell harvesting,, cultures were added with colchicine (0.1 ug/ml final concentration). Air dried slides were prepared following routine protocols. Each treatment was repeated 5 times and a total of 500 metaphases per treatment (100 per repetition) was scored in coded slides. Statistical analysis was performed using X2 test. Untreated cultures and DMSO terated cultures (0.1 ml DMSO per 10 ml culture medium) were used as controls. CYTOTOXICITY: mitotic index decreased to 71.5, 62.7 and 61.6% in relation to the mitotic index of untreated controls.
Result: Treatment with the three doses induced a significant increase of chromatid and isochromatid breaks with a corresponding increase of abnormal metaphases.
Reliability: (2) valid with restrictions
Flag: robust summary
 23-NOV-2000 (30)

Type: Sister chromatid exchange assay
System of testing: CHO cells
Concentration: 0.1, 0.25 and 0.5 ug/ml
Metabolic activation: without
Result: negative
Method: other: see remark field
Year: 1995 **GLP:** no data
Test substance: other TS: BHT from Sigma (no further information)
Remark: METHOD: For SCE analysis, culture medium was added with 10 ug/ml of 5'-bromo-2'-deoxyuridine (BrdU) and the cells were incubated in complete darkness. CHO cells were incubated for 30 h. Two hours before fixation, cells were treated with colchicine (0.1 ug/ml final concentration). For each treatment 5 repetitions were made. Air dried slides were prepared following routine protocols and differential staining of sister chromatids were obtained according to Wolff and Perry (1974). Cytogenetic analysis was performed on coded slides. Statistical analysis was performed using multifactorial ANOVA. Untreated cultures and DMSO terated cultures (0.1 ml DMSO per 10 ml culture medium) were used as controls. CYTOTOXICITY: >= 0.25 ug/ml; only a few metaphases could be analyzed in cells treated with 0.25 ug/ml (23 in relation to 180 of untreated and vehicle controls) and no cells at second mitosis after 0.5 ug/ml.
Reliability: (2) valid with restrictions
Flag: robust summary
 23-NOV-2000 (30)

Type: Sister chromatid exchange assay
System of testing: human lymphocytes (from umbilical cord)
Concentration: 0.1, 0.25 and 0.5 ug/ml
Metabolic activation: without
Result: negative
Method: other: see remark field
Year: 1995 **GLP:** no data
Test substance: other TS: BHT from Sigma (no further information)
Remark: METHOD: For SCE analysis, culture medium was added with 10 ug/ml of 5'-bromo-2'-deoxyuridine (BrdU) and the cells were incubated in complete darkness. Human lymphocytes were incubated for 72 h. Two hours before fixation, cells were treated with colchicine (0.1 ug/ml final concentration). For each treatment 5 repetitions were made. Air dried slides were prepared following routine protocols and differential staining of sister chromatids were obtained according to Wolff and Perry (1974). Cytogenetic analysis was performed on coded slides. Statistical analysis was performed using multifactorial ANOVA. Untreated cultures and DMSO treated cultures (0.1 ml DMSO per 10 ml culture medium) were used as controls.
CYTOTOXICITY: = 0.5%; a decrease of cells in second division with increasing concentration (26 cells scored in relation to 155 and 165 of untreated and vehicle controls).
Reliability: (2) valid with restrictions
Flag: robust summary
22-NOV-2000 (30)

Type: other: Anaphase-telophase test
System of testing: CHO cells
Concentration: 0.1, 0.25 and 0.5 ug/ml
Metabolic activation: without
Result: negative
Method: other: see remark field
Year: 1995 **GLP:** no data
Test substance: other TS: BHT from Sigma (no further information)
Remark: METHOD: CHO cells were cultured as monolayer in 24 x 36 mm cover glasses attached with a small drop of siliconized grease to the bottom of 90-mm Petri dishes. Three cover glasses were placed in each Petri dish. Each cover glass was seeded with 1.5 ml of culture medium containing about 50,000 cells. After 1 h, 8.5 ml of culture medium was added to each Petri dish. Cultures were incubated at 37°C in a humidified atmosphere of 5% CO₂. The set of cultures for each experiment was treated simultaneously for 8 h before fixation to avoid the detachment of cells from cover slides. Each treatment was repeated 5 times. Cell harvesting was accomplished by adding an equal volume of fixative (methanol-acetic 3:1) to the culture medium. After 10 min, two changes of fixative were made. Cover glasses were stained with Carbol fuchsin (Carr and Walker, 1961) and attached with DPX mounting medium to coded slides. Statistical comparisons were made by means of the Sokal and Rohlf G method (Sokal, 1979). Regression analyzes were

performed to evaluate the mitotic index variations. Untreated cultures and DMSO treated cultures (0.1 ml DMSO per 10 ml culture medium) were used as controls.
CYTOTOXICITY: mitotic index decreased to 62.3, 22.2 and 20.2% in relation to the mitotic index of untreated controls.

Reliability: (2) valid with restrictions
Flag: robust summary
22-NOV-2000 (30)

Type: other: DNA synthesis inhibition test
System of testing: HeLa S3 cells
Concentration: 0.4, 0.8, 1.5, 3 and 6 mM
Metabolic activation: without
Result:
Method: other: see remark field
Year: 1996 **GLP:** no data
Test substance: other TS: purity: > 98%
Remark: METHOD: In the DIT a culture of logarithmically growing HeLa S3 cells was transferred into a single cell suspension by gently detaching the cells with EDTA (250 mg/l PBS). Then the cells were seeded into 96-well microplates at a density of 2×10^4 cells/well. The next day, the monolayers of the HeLa cells were exposed for 90 min to the materials to be tested. All concentrations were tested in triplicate; with each set of experiments usually repeated three times. Thereafter, the cells were washed by two rinses with fresh, pre-warmed medium and allowed to recover for 2 h. This was followed by addition of BrdU in a final concentration of 20 μ M for 60 min. Subsequently, the cells were fixed with ethanol/acetic acid/water (90:5:5) for 30 min at room temperature. The alcohol was poured off and 4 N HCl was added to the fixed cells for 10 min to denature the DNA. Excess acid was washed away by rinsing the microplate twice with tap water. Then a 1:1500 dilution of a monoclonal anti-BrdU antibody was added to the cells for 30 min. After washing the cells three times with tap water, a 1:500 dilution of peroxidase-conjugated F(ab)2-sheep-anti-mouse IgG antibody was added for another 30 min. The cells were washed three times with tap water, and a freshly prepared peroxidase substrate solution was added. The color development was stopped with a stop solution (H₂SO₄). The extinction of the wells was measured at 495 nm using an ELISA reader. Cell counts were determined by sulforhodamine B (SRB) adsorption to total cell protein, followed by elution of the dye with Tris buffer and colorimetric measurement at 564 nm. In all experiments, the standard genotoxin 4-NQO was used as positive control. BHT was dissolved in DMSO at a stock concentration of 2M. This stock solution was serially diluted in 1:2 steps and transferred onto the microplate with the tester organisms using a laboratory workstation.
CYTOTOXICITY: ≥ 1.5 mM; cell count decreased to 32, 23 and 30% in relation to the vehicle control

Result: limited positive because higher degree of cytotoxicity (cell count < 40%) were observed at concentrations ≥ 1.5 mM
Reliability: (2) valid with restrictions
Flag: robust summary

23-NOV-2000

(31)

Type: other: Umu-test
System of testing: S. typhimurium TA 1535/pSK 1002
Concentration: 0.4, 0.8, 1.5, 3 and 6 mM
Metabolic activation: without
Result: negative
Method: other: see remark field
Year: 1996 **GLP:** no data
Test substance: other TS: purity: > 98%
Remark: METHOD: The umu test was performed by Reifferscheid et al., Mutat. Res. 253, 215-222 (1991). Salmonella from stock were grown in nutrient broth for the overnight culture. Logarithmically growing tester bacteria were exposed to varying concentrations of the test material. All concentrations were tested in triplicate; with each set of experiments usually repeated three times. After 2 h of exposure, the bacterial suspension was diluted 10-fold, followed by a subsequent additional incubation period of 2 h. Thereafter, bacterial growth was measured as turbidity (E600) with a microplate reader. The DNA damage induced expression of umuC was quantified via the determination of β -galactosidase activity at 420 nm using ONPG o-nitrophenyl- β -D-galactopyranoside; Sigma) as a substrate. In all experiments, the standard genotoxin 4-NQO (4-nitroquinoline N-oxide) was used as positive control. BHT was dissolved in DMSO at a stock concentration of 2M. This stock solution was serially diluted in 1:2 steps and transferred onto the microplate with the tester organisms using a laboratory workstation.
CYTOTOXICITY: no
Reliability: (2) valid with restrictions
Flag: robust summary

22-NOV-2000

(31)

Type: other: review of the mutagenicity/genotoxicity data up to 1991
System of testing:
Concentration:
Metabolic activation:
Result:
Method:
Year: **GLP:**
Test substance:
Result: A host of studies examining the potential of BHT to cause point mutations have been published. They include in vitro studies on various bacterial species and strains and on various types of mammalian cell lines. Together these studies convincingly show the absence of a potential for BHT to cause point mutations. A great number of studies on many cell types have also been carried out to examine the potential of BHT to cause chromosome aberrations. In vitro studies have been published using plant cells and the WI-38, CHL, CHO and V79 mammalian cell lines. Nearly all studies, especially those

using validated test systems, indicate that BHT lacks clastogenic potential. In vitro studies on bacterial, yeast and various mammalian cells including DON, CHO, CHL cells and primary hepatocytes demonstrate the absence of interactions with or damage to DNA.

Reliability: (2) valid with restrictions
Flag: robust summary
22-NOV-2000

(32)

5.6 Genetic Toxicity 'in Vivo'

Type: other: in vivo-in vitro replicative DNA synthesis test
Species: rat **Sex:** male
Strain: Fischer 344
Route of admin.: other: gavage or s.c. injection (no further information available)
Exposure period: single dose
Doses: 450 mg/kg and 900 mg/kg
Result: positive
Method: other: see remark field
Year: 1994 **GLP:** no data

Test substance: no data
Remark: METHOD: the vehicle used was corn oil; the numbers of animals treated and the number from which primary hepatocyte cultures were produced is not mentioned; production of primary hepatocyte cultures and assessment of RDS induction was performed using published procedures (Uno et al., Toxicol. Lett. 63, 191-199 and 201-209 (1992));
Judgement criteria for RDS incidence: RDS incidence was evaluated by our earlier documented judgement criteria. In the time-course experiment, when the maximum RDS incidence was 2.0% or above, it was considered to indicate a positive response. An incidence less than 1.0% was judged to be negative. an incidence between 1.0 and 2.0% was considered equivocal, and a dose-response experiment was subsequently performed. In this second experiment, when the incidence was 1.0% or above at any of the doses, a final judgement of positive was made, whereas a reponse of less than 1.0% was rated as negative.

Result: In the time course experiment BHT caused dose-related RDS induction; RDS incidence (%) after 450 mg/kg: 0.3 (24 h), 1.2 (39 h), 0.2 (48 h); RDS incidence (%) after 900 mg/kg: 2.5 (24 h), 9.2 (39 h), 0.8 (48 h) the hepatocyte viability did not vary from untreated control value

Reliability: (3) invalid
Flag: robust summary
23-NOV-2000

(33)

Type: other: liver DNA damage
Species: rat **Sex:** female
Strain: Sprague-Dawley
Route of admin.: gavage
Exposure period: first dose 21 h before killing; second dose 4 h before killing
Doses: among others 700 mg/kg bw and 140 mg/kg bw (no further information)

Result:
Method: other: see remark field
Year: 1994 **GLP:** no data

Test substance: no data

Remark: METHOD: the vehicle used for gavage was 2% gum tragacanth in water; the numbers of animals treated and the number from which hepatic DNA was obtained is not mentioned; the rat hepatic DNA damage assay (alkaline elution) was performed as described by Kitchin and Brown, Teratogenesis, Carcinogenesis and Mutagenesis 9, 61 (1989). The data was analyzed by analysis of variance, and where statistically significant differences were found, they were then evaluated with Student's t-test.

Result: As the highest dose did not show the DNA-damaging effects that one lower dose did, no dose response curve or regression model will fit
the highest tested dose that did not cause rat liver DNA damage to a statistically significant extent: 140 mg/kg
the lowest tested dose that caused rat liver DNA damage: 700 mg/kg

Reliability: (3) invalid

Flag: robust summary

23-NOV-2000

(34)

Type: other: review of the mutagenicity/genotoxicity data up to 1991
Species: **Sex:**

Strain:

Route of admin.:

Exposure period:

Doses:

Result:

Method:

Year:

GLP:

Test substance:

Result: A host of studies examining the potential of BHT to cause point mutations have been published. They include in vivo studies on *Drosophila melanogaster*, silk worms and also the mouse specific locus test (involving long-term exposure.) Together these studies convincingly show the absence of a potential for BHT to cause point mutations. A great number of studies on many species have also been carried out to examine the potential of BHT to cause chromosome aberrations. In vivo studies have been carried out on somatic and/or germ cells of *Drosophila melanogaster*, rats and mice. Nearly all studies, especially those using validated test systems, indicate that BHT lacks clastogenic potential.

Reliability: (2) valid with restrictions

Flag: robust summary

22-NOV-2000

(32)

5.8 Toxicity to Reproduction

Type: Two generation study
Species: mouse **Sex:** male/female
Strain: other: Crj:CD-1
Route of admin.: oral feed
Exposure Period: F0 and F1: during premating, mating, gestation and lactation (ca. 11 weeks)
Frequency of treatment: daily
Premating Exposure Period
male: no exact information given (probable during premating and mating period)
female: no exact information given (probable during premating, mating period, during gestation and lactation)
Duration of test: until postnatal day 21 of the F2 generation
Doses: 0.015, 0.045, 0.135 and 0.405 % in diet (ca. 22.5, 67.5, 202.5 and 607.5 mg/kg bw/day)
Control Group: yes, concurrent no treatment
NOAEL F1 Offspr.: .405 %
NOAEL F2 Offspr.: .405 %
Method: other: see remark field
Year: 1993 **GLP:** no data
Test substance: no data
Remark: METHOD: No. of mice/sex/dose: 10; mating period: 5 days; M/F ratio per cage: 1/1; length of cohabitation: no data; neurobehavioural procedure: The functional and behavioural developmental parameters were measured and scored for the individual pups in the lactation period in F1 and F2 generations, and were analyzed on a whole-litter basis. The measured parameters were as follows: surface righting on postnatal day 4 and 7, negative geotaxis on PND 4 and 7, cliff avoidance on PND 7, swimming behaviour (direction, head angle, and limb movement) on PND 4 and 14, and olfactory orientation on PND 14. Open field activity of mice was measured at 3 weeks of age in the F1 and F2 generations, both male and female. the apparatus used in this study was a square white board, 30 x 30 cm, divided by black lines into 25 equal squares. Ambulation, rearing, 180° turn, defecation, urination, and preening were recorded for 3 min in the apparatus. In the F1 generation, the following parameters were measured on postnatal (PND) 0: litter size, litter weight, and sex ration (m/f); the pups were weighed on PND 0,4,7,14 and 21 in the lactation period; the pups were removed from their dams at 4 weeks of age, and were selected at random to continue treatment; the F1 animals were mated at 9 weeks of age; in the F2 generation some parameter of the pups were measured identically to the F1 generation from birth to weaning. For the F0 generation only data on mortality are reported administration of BHT: no further information given
Result: F0 generation:
MORTALITY: Two dams died during the second week of the lactation period; one dam in the 0.015% group and one in the 0.045% group.
F1 generation:
0.015%:
MORTALITY: 1 dam died during 2nd week of lactation period

SURVIVAL INDEX (PND 21): 100% (control: 91.8%)
BODY WEIGHT: increased at PND 0,4 and 21
NO. of LITTERS: no effect
NO. of PUPS: no effect
LITTER SIZE: no effect
LITTER WEIGHT: no effect
SEX RATIO: no effect
NEUROBEHAVIOURAL PARAMETERS: increased surface righting at PND 7

0.045%:
SURVIVAL INDEX (PND 21): 90.3% (control: 91.8%)
BODY WEIGHT: no effect
NO. of LITTERS: no effect
NO. of PUPS: no effect
LITTER SIZE: no effect
LITTER WEIGHT: no effect
SEX RATIO: no effect
NEUROBEHAVIOURAL PARAMETERS: reduced ambulation in male mice

0.135%:
SURVIVAL INDEX (PND 21): 100% (control: 91.8%)
BODY WEIGHT: decreased at PND 14
NO. of LITTERS: no effect
NO. of PUPS: no effect
LITTER SIZE: no effect
LITTER WEIGHT: no effect
SEX RATIO: no effect
NEUROBEHAVIOURAL PARAMETERS: no effect

0.405%:
SURVIVAL INDEX (PND 21): 98.3% (control: 91.8%)
BODY WEIGHT: decreased at PND 7, 14 and 21
NO. of LITTERS: no effect
NO. of PUPS: no effect
LITTER SIZE: no effect
LITTER WEIGHT: no effect
SEX RATIO: no effect
NEUROBEHAVIOURAL PARAMETERS: no effect

F2 generation:
0.015%:
SURVIVAL INDEX (PND 21): 100% (control: 100%)
BODY WEIGHT: increased at PND 0,4, 7, 14 and 21
NO. of LITTERS: no effect
NO. of PUPS: no effect
LITTER SIZE: no effect
LITTER WEIGHT: no effect
SEX RATIO: no effect
NEUROBEHAVIOURAL PARAMETERS: reduced 180o turn (m)

0.045%:
SURVIVAL INDEX (PND 21): 99.1% (control: 100%)
BODY WEIGHT: no effect
NO. of LITTERS: no effect
NO. of PUPS: no effect
LITTER SIZE: no effect
LITTER WEIGHT: no effect
SEX RATIO: no effect
NEUROBEHAVIOURAL PARAMETERS: reduced 180o turn (m), reduced ambulation in both sex

0.135%:

SURVIVAL INDEX (PND 21): 99.1% (control: 100%)
 BODY WEIGHT: decreased at PND 14
 NO. of LITTERS: no effect
 NO. of PUPS: no effect
 LITTER SIZE: no effect
 LITTER WEIGHT: no effect
 SEX RATIO: no effect
 NEUROBEHAVIOURAL PARAMETERS: increased surface righting at PND 4, reduced 180o turn (m)
 0.405%:
 SURVIVAL INDEX (PND 21): 99.1% (control: 100%)
 BODY WEIGHT: decreased at PND 7, 14 and 21
 NO. of LITTERS: no effect
 NO. of PUPS: no effect
 LITTER SIZE: no effect
 LITTER WEIGHT: no effect
 SEX RATIO: no effect
 NEUROBEHAVIOURAL PARAMETERS: increased negative geotaxis at PND 4, reduced 180o turn (m)
 CONCLUSION:
 No effect on No. of litters, No. of pups, litter size, litter weight and sex ratio in any dose group of F1 and F2 animals; no effect on neurobehavioural parameters in F1 and F2 generation; the body weight of pups was increased in the 0.015% group at birth and during lactation period for each generation
Reliability: (2) valid with restrictions
Flag: robust summary
 21-NOV-2000 (35)

Type: other: two generation carcinogenicity study
Species: rat **Sex:** male/female
Strain: Wistar
Route of admin.: oral feed
Exposure Period: male: 14 weeks (P); 141-144 weeks (F1)
 female: 20 weeks (P); 141-144 weeks (F1)

Frequency of treatment: daily
Premating Exposure Period
male: 13 weeks
female: 13 weeks
Duration of test: 144 weeks
Doses: nominal: 0,25,100 and 500 mg/kg bw (P); 0, 25, 100 and 250 mg/kg bw (F1)

Control Group: yes, concurrent no treatment
Method: other: see remark
Year: 1986 **GLP:** no data
Test substance: other TS: purity: > 99.5 %
Remark: METHOD: No. of rats/sex/dose: 60 (control); 40 (25 mg/kg), 40 (100 mg/kg) and 100 (500 mg/kg); mating period was terminated within 1week; M/F ratio per cage: no data; length of cohabitation: no data; The only data reported are: gestation rate, No. of pups/litter and the body weight of pups at birth and at weaning

Result: F0 generation:
 No difference was found in food consumption between treated and control rats; body weight gain of males and females was

reduced significantly from week 6 of treatment with 500 mg/kg, persisting throughout the lifespan of the F0 rats. Gestation rate was not affected by treatment (or even slightly increased in the treated groups). The number of litters of ten or more pups at birth decreased significantly with increasing test substance dose.

F1 generation:
At weaning F1 rats had significantly lower body weights than the controls, the extent of the reduction being dose-related, although food consumption was not reduced in the treated groups. The effect was most pronounced in males.

500 mg/kg: decreased body weight (m/f) at weaning; the fraction of litters with ten or more pups decreased
100 mg/kg: decreased body weight (m/f) at birth and at weaning
25 mg/kg: no effects

The pathology findings (F1) including blood analysis and serum chemistry are presented in chapter 5.4 and 5.7 ("Repeated Dose Toxicity" and "Carcinogenicity").

Reliability: (2) valid with restrictions
Flag: robust summary
22-NOV-2000 (25)

Type: other: two generation study with emphasis on hepatocellular changes in F1 generation

Species: rat **Sex:** male/female

Strain: Wistar

Route of admin.: other: diet

Exposure Period: male: 5 weeks (P); 4 weeks (F1), 6, 11, 16
and 22 months (F1)
female: 8 weeks (P)

Frequency of treatment: daily (during the period of mating, food pots were removed when male and females were mated)

Premating Exposure Period

male: 3 weeks

female: 3 weeks

Duration of test: 22 months

Doses: nominal: 0, 25, 100 and 500 mg/kg bw (P); 0, 25, 100 and 250 mg/kg bw (F1)

Control Group: yes, concurrent no treatment

Method: other: see remark

Year: 1994

GLP: yes

Test substance: other TS: purity: 99.96%

Remark: NOEL PARENTAL:

The NOEL for clinical signs during pre-mating and mating phases, for both males and females, was 500 mg/kg. The NOEL for effects on body weight during pre-mating and mating phases was 500 mg/kg for the females and 100 mg/kg for the males.

The NOEL for maternal clinical signs and for effects on maternal body weight during gestation phase was 500 mg/kg.

The NOEL for maternal clinical signs and for effects on maternal body weight and food consumption during the lactation phase was 500 mg/kg.

NOEL F1 OFFSPRING:

The NOEL for pup clinical signs were 500 mg/kg; the NOEL for pup body weight during lactation phase were 100 mg/kg

METHOD: premating exposure period for males (7/dose) and females (50/dose): 3 weeks; mating exposure period for males (6/dose) and females (48/dose): 2 weeks; M/F ratio per cage: 1/8; length of cohabitation: 15 hours/day; number of animals allocated for each scheduled autopsy: 20 days gestation: 5 pregnant females/dose, 21 days after parturition: 5 mothers/dose and 20 pups/dose, 4 weeks after weaning: 5 male pups/dose, 6 months after weaning: 5 male pups/dose; 11 months after weaning: 8-10 male pups/dose, 16 months after weaning: 9-13 male pups/dose, 22 months after weaning: 10-19 male pups, administration of BHT: the amount of BHT incorporated initially per unit weight of diet was calculated from the food consumption measured during acclimatisation and from normal growth rate of this strain of rats; throughout pregnancy and lactation no effort was made to adjust dietary BHT content in line with body weight gain during this time

Result:

There were no differences in mating success. Pregnancy proceeded normally in all groups. There was no alteration in numbers of resorption sites. No statistically significant change was seen in the number of fetuses/dams. The number of pups per litter did not differ. There was a trend to an increase in the number of pups found dead or dying soon after birth with increase in dose but the actual number of deaths in affected litters influenced by treatment with BHT. The total litter weight was significantly decreased for dams treated with the high dose of BHT. The weight gain of pups from dams receiving the highest dose of BHT was consistently less than that of control pups or pups of dams receiving lower doses of BHT. The development was retarded in the high dose group. The pathology findings (P and F1), including liver-biochemistry, organ weights, gross and microscopic evaluations are presented in chapter 5.4 (Repeated Dose Toxicity).

Reliability:

(1) valid without restriction

Flag:

robust summary

20-NOV-2000

(27) (28)

5.9 Developmental Toxicity/Teratogenicity

Species: rat **Sex:** female
Strain: Sprague-Dawley
Route of admin.: gavage
Exposure period: 7th to 17th day of gestation
Frequency of treatment: daily
Duration of test: until day 20 on gestation
Doses: 100, 200, 300 and 400 mg/kg
Control Group: other: no data
Method: other: no data
Year: 1993 **GLP:** no data
Test substance: other TS: BHT (no further information) in corn oil
Remark: only abstract
Result: Pregnant performance and fetal developments were not affected; no significant differences were detected in maternal body weight gains and food intakes; a dose related increase in relative organ weight of liver at high doses; no significant fetal abnormalities in external and visceral observations; on skeletal examination sternebral retardation in BHT 300 mg/kg treated group were observed without dose dependence
Reliability: (4) not assignable
Flag: robust summary
 21-NOV-2000 (36)

Species: rat **Sex:** female
Strain: Wistar
Route of admin.: gavage
Exposure period: days 7 to 17 of pregnancy
Frequency of treatment: daily
Duration of test: until day 20 of gestation
Doses: 0, 93.5, 187 and 375 mg/kg bw
Control Group: other: no data
Method: other: see remark field
Year: 1990 **GLP:** no data
Test substance: no data
Remark: abstract, figures and tables in English
 METHOD: Number of animals per dose: 24 (control); 20 (93.5 and 187 mg/kg); 22 (375 mg/kg)
 MATERNAL PARAMETERS assessed: clinical signs, body weight, food consumption and mortality;
 REPRODUCTIVE PARAMETERS assessed: number of corpora lutea, number of implantation, number of live fetuses and sex ratio
 FETAL PARAMETERS assessed: body weight; postnatal survival; external abnormalities; visceral and skeletal abnormality
Result: In the dams at the two higher doses of 187 and 375 mg/kg, toxic signs such as hair fluffing and diarrhoea were observed, and their body weight gain and food consumption were suppressed. Two dams, which showed marked diarrhoea in the highest dose group, died. However, there was no evidence of fetal malformation attributable to treatment with the compound in any of the dose groups treated, although a slight increase in fetal death was found in the highest dose group.
 It is concluded that 2,2'-methylenebis (4-methyl-6-tert-butylphenol) has a weak lethal effect on fetal development but

not a teratogenic effect in the rat.

Reliability: (4) not assignable

Flag: robust summary

27-NOV-2000 (37)

Species: mouse **Sex:** female

Strain: other: JCL-ICR

Route of admin.: gavage

Exposure period: 7th to 13th day of gestation

Frequency of treatment: once a day

Duration of test: until the 18th day of gestation

Doses: 70, 240 and 800 mg/kg bw/day

Control Group: other: yes, concurrent vehicle and concurrent untreated

NOAEL Maternalt.: = 800 mg/kg bw

NOAEL Teratogen.: = 800 mg/kg bw

Method: other: see remark field

Year: **GLP:** no data

Test substance: other TS: food additive grade

Remark: BHT was dissolved in olive oil and was administered at a rate of 10 ml/kg/day
Age at study initiation: 8-13 week old
Number of animals per dose and vehicle control: 26
Number of animals in untreated control: 30
Mating: After keeping a pair of male and female mice together overnight, the female was examined in the next morning for the presence of vaginal plug. The mice with plug were considered as pregnant animal. The day where female mouse had virginal plug was designed as gestation day 0.
Body weight were measured everyday with the observation of general condition of the animal. The mice were sacrificed on 18th day of gestation by ether anesthetization. Immediately after sacrifice, abdomen of the dam was opened, then the number of implantation sites, corpus luteum absorbed embryos, dead or alive fetuses were counted. The alive fetuses were examined for their body weights, sex and external malformation. Major organs were weighed and the abnormality was observed grossly. Five dams were chosen at random and their alive fetuses were fixed with Bouin's fixative for observation of internal abnormalities. The remaining alive fetuses were fixed in 95% ethanol, then were stained with alizarin red S for examination of skeletal abnormalities.
MATERNAL PARAMETERS assessed: behavior; body weight; mortality; organ weights (liver, heart, spleen, kidneys, lung, adrenals and ovaries);
REPRODUCTIVE PARAMETERS assessed: gestation rate; number of corpora lutea, number of implantation and sex ratio
FETAL PARAMETERS assessed: body weight; postnatal survival; external abnormalities; skeletal deformity and abnormality

Result: 800 mg/kg:
MATERNAL PARAMETER: increased spleen weight; decreased liver weight (compared to the untreated control animals)
REPRODUCTIVE PARAMETERS: no effects
FETAL PARAMETER: no effects
240 mg/kg:
MATERNAL PARAMETER: no effects
REPRODUCTIVE PARAMETERS: no effects

FETAL PARAMETER: no effects
70 mg/kg:
MATERNAL PARAMETER: no effects
REPRODUCTIVE PARAMETERS: no effects
FETAL PARAMETER: no effects
Reliability: (2) valid with restrictions
Flag: robust summary
21-NOV-2000 (38)

Species: mouse **Sex:** female
Strain: other: JCL-ICR
Route of admin.: gavage
Exposure period: 9th day of gestation
Frequency of treatment: single administration
Duration of test: until the 18th day of gestation
Doses: 1200 and 1800 mg/kg bw
Control Group: yes, concurrent no treatment
NOAEL Maternalt.: < 1200 mg/kg bw
NOAEL Teratogen.: 1800 mg/kg bw
Method: other: see remark field
Year: **GLP:** no data
Test substance: other TS: food additive grade
Remark: BHT was dissolved in olive oil and was administered at a rate of 10 ml/kg/day
Age at study initiation: 8-13 week old
Number of animals per dose: 15
Number of animals untreated control: 19
Mating: After keeping a pair of male and female mice together overnight, the female was examined in the next morning for the presence of vaginal plug. The mice with plug were considered as pregnant animal. The day where female mouse had virginal plug was designed as gestation day 0.
Body weight were measured everyday with the observation of general condition of the animal. The mice were sacrificed on 18th day of gestation by ether anesthetization. Immediately after sacrifice, abdomen of the dam was opened, then the number of implantation sites, corpus luteum absorbed embryos, dead or alive fetuses were counted. The alive fetuses were examined for their body weights, sex and external malformation. Major organs were weighed and the abnormality was observed grossly. Five dams were chosen at random and their alive fetuses were fixed with Bouin's fixative for observation of internal abnormalities. The remaining alive fetuses were fixed in 95% ethanol, then were stained with alizarin red S for examination of skeletal abnormalities.
MATERNAL PARAMETERS assessed: behavior; body weight; mortality; organ weights (liver, heart, spleen, kidneys, lung, adrenals and ovaries);
REPRODUCTIVE PARAMETERS assessed: gestation rate; number of corpora lutea, number of implantation and sex ratio
FETAL PARAMETERS assessed: body weight; postnatal survival; external abnormalities; skeletal deformity and abnormality
Result: 1800 mg/kg:
MATERNAL PARAMETER: 5/20 died (11th day 3; 14th day 1 and 15th day 1), increased lung and spleen weights
REPRODUCTIVE PARAMETERS: no effects

FETAL PARAMETER: delay of progression of ossification
1200 mg/kg:
MATERNAL PARAMETER: 2/20 died (11th day 1 and 15th day 1),
increased lung weight
REPRODUCTIVE PARAMETERS: no effects
FETAL PARAMETER: delay of progression of ossification
(2) valid with restrictions
robust summary

Reliability:

Flag:

21-NOV-2000

(38)

5.11 Experience with Human Exposure

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7.1 Risk Assessment

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I U C L I D

D a t a S e t

Existing Chemical ID: 79-96-9
CAS No. 79-96-9
EINECS Name Phenol, 4,4'-(1-methylethylidene)bis
2-(1,1-dimethylethyl)-
Molecular Weight 340.51
TSCA Name Phenol, 4,4'-(1-methylethylidene)bis
2-(1,1-dimethylethyl)-
Molecular Formula C23H32O2

Producer Related Part
Company: Bayer Corporation
Creation date: 15-NOV-2001

Substance Related Part
Company: Bayer Corporation
Creation date: 15-NOV-2001

Printing date: 16-NOV-2001
Revision date:
Date of last Update: 16-NOV-2001

Number of Pages: 15

Chapter (profile): Chapter: 1, 2, 3, 4, 5, 7
Reliability (profile): Reliability: without reliability, 1, 2, 3, 4
Flags (profile): Flags: without flag, confidential, non confidential, WGK
(DE), TA-Luft (DE), Material Safety Dataset, Risk
Assessment, Directive 67/548/EEC, SIDS

1. General Information

1.0.1 OECD and Company Information

Type: lead organisation
Name: American Chemistry Council (formerly Chemical Manufacturers Association) Rubber and Plastics Additives (RAPA) HPV Panel
Street: 1300 Wilson Boulevard
Town: 22209 Arlington, VA
Country: United States
Phone: 703-741-5600
Telefax: 703-741-6091

15-NOV-2001

Type: cooperating company
Name: Bayer Corporation
Country: United States

15-NOV-2001

Type: cooperating company
Name: Ciba Specialty Chemicals Corporation
Country: United States

15-NOV-2001

Type: cooperating company
Name: Crompton Corporation
Country: United States

15-NOV-2001

Type: cooperating company
Name: Flexsys America L.P.
Country: United States

15-NOV-2001

Type: cooperating company
Name: Noveon, Inc. (formerly BF Goodrich)
Country: United States

15-NOV-2001

Type: cooperating company
Name: R.T. Vanderbilt Company, Inc.
Country: United States

15-NOV-2001

Type: cooperating company
Name: The Goodyear Tire & Rubber Company
Country: United States

15-NOV-2001

1. General Information

Type: cooperating company
Name: The Lubrizol Corporation
Country: United States

15-NOV-2001

Type: cooperating company
Name: UOP, LLC.
Country: United States

15-NOV-2001

1.0.2 Location of Production Site

-

1.0.3 Identity of Recipients

-

1.1 General Substance Information

Substance type: organic
Physical status:
15-NOV-2001

1.1.0 Details on Template

-

1.1.1 Spectra

-

1.2 Synonyms

Goodrite 3171
15-NOV-2001

Stabilox Intermediate
15-NOV-2001

1.3 Impurities

-

1.4 Additives

-

1.5 Quantity

-

1. General Information

1.6.1 Labelling

-

1.6.2 Classification

-

1.7 Use Pattern

-

1.7.1 Technology Production/Use

-

1.8 Occupational Exposure Limit Values

-

1.9 Source of Exposure

-

1.10.1 Recommendations/Precautionary Measures

-

1.10.2 Emergency Measures

-

1.11 Packaging

-

1.12 Possib. of Rendering Subst. Harmless

-

1.13 Statements Concerning Waste

-

1.14.1 Water Pollution

-

1.14.2 Major Accident Hazards

-

1.14.3 Air Pollution

-

1. General Information

1.15 Additional Remarks

-

1.16 Last Literature Search

-

1.17 Reviews

-

1.18 Listings e.g. Chemical Inventories

-

2. Physico-chemical Data

2.1 Melting Point

Value: 181.4 degree C
 Method: other: MPBPWIN (v1.31)
 Year: 1999
 GLP: no
 Remark: Melting Point: 349.84 deg C (Adapted Joback Method)
 Melting Point: 139.27 deg C (Gold and Ogle Method)
 Mean Melt Pt : 244.55 deg C (Joback; Gold,Ogle Methods)
 Selected MP: 181.38 deg C (Weighted Value)
 Accepted calculation method
 Flag: Critical study for SIDS endpoint
 15-NOV-2001 (1)

2.2 Boiling Point

Value: 433.2 degree C at 1013 hPa
 Method: other: MPBPWIN (v1.31) ; Adapted Stein & Brown Method
 Year: 1999
 GLP: no
 Testsubstance: other TS: molecular structure
 Reliability: (2) valid with restrictions
 Accepted calculation method
 Flag: Critical study for SIDS endpoint
 15-NOV-2001 (1)

2.3 Density

-

2.3.1 Granulometry

-

2.4 Vapour Pressure

Value: .00000000157 hPa at 25 degree C
 Method: other (calculated): MPBPWIN (v1.31) Modified Grain Method
 Year: 1999
 GLP: no
 Testsubstance: other TS: molecular structure
 Result: Vapor Pressure Estimations (25 deg C):
 (Using BP: 433.17 deg C (estimated))
 (Using MP: 181.38 deg C (estimated))
 VP: 8.63E-011 mm Hg (Antoine Method)
 VP: 1.18E-009 mm Hg (Modified Grain Method)
 VP: 7.58E-008 mm Hg (Mackay Method)
 Selected VP: 1.18E-009 mm Hg (Modified Grain Method)
 Reliability: (2) valid with restrictions
 Accepted calculation method
 Flag: Critical study for SIDS endpoint
 15-NOV-2001 (1)

2. Physico-chemical Data

2.5 Partition Coefficient

log Pow: 7.46 at 25 degree C
 Method: other (calculated): KOWWIN Program (v1.65)
 Year: 1999
 GLP: no
 Testsubstance: other TS: molecular structure
 Reliability: (2) valid with restrictions
 Accepted calculation method
 Flag: Critical study for SIDS endpoint
 15-NOV-2001 (1)

2.6.1 Water Solubility

Value: .01139 mg/l at 25 degree C
 Method: other: WSKOW (v1.36)
 Year: 1999
 GLP: no
 Testsubstance: other TS: molecular structure
 Remark: Log Kow used by Water solubility estimates: 7.46
 Equation Used to Make Water Sol estimate:

$$\text{Log S (mol/L)} = 0.796 - 0.854 \log \text{Kow} - 0.00728 \text{ MW} +$$
 Correction (used when Melting Point NOT available)

Correction(s):	Value
-----	-----
Phenol	0.580

Log Water Solubility (in moles/L) : -7.475
 Water Solubility at 25 deg C (mg/L): 0.01139

 Reliability: (2) valid with restrictions
 Accepted calculation method
 Flag: Critical study for SIDS endpoint
 15-NOV-2001 (1)

2.6.2 Surface Tension

-

2.7 Flash Point

-

2.8 Auto Flammability

-

2.9 Flammability

-

2.10 Explosive Properties

-

2. Physico-chemical Data

2.11 Oxidizing Properties

-

2.12 Additional Remarks

-

3. Environmental Fate and Pathways

3.1.1 Photodegradation

Type: air
 INDIRECT PHOTOLYSIS
 Sensitizer: OH
 Conc. of sens.: 1560000 molecule/cm3
 Rate constant: .000000000975473 cm3/(molecule * sec)
 Degradation: 50 % after 1.3 hour(s)
 Method: other (calculated):AOPWin (v1.88) Estimations Program
 Year: 1999 GLP: no
 Test substance: other TS: chemical structure
 Reliability: (2) valid with restrictions
 Accepted calculation method
 Flag: Critical study for SIDS endpoint
 15-NOV-2001 (1)

3.1.2 Stability in Water

-

3.1.3 Stability in Soil

-

3.2 Monitoring Data (Environment)

-

3.3.1 Transport between Environmental Compartments

Type: fugacity model level III
 Media: other: air - water - soil - sediment
 Air (Level I):
 Water (Level I):
 Soil (Level I):
 Biota (L.II/III):
 Soil (L.II/III):
 Method: other: EPIWIN Level III Fugacity Model
 Year: 1999

Result:	Media	Concentration (percent)	Half-Life (hr)	Emissions (kg/hr)	Fugacity (atm)
	Air	0.00509	2.63	1000	1.53e-014
	Water	2.15	1.44e+003	1000	9.07e-018
	Soil	39	1.44e+003	1000	1.3e-019
	Sediment	58.9	5.76e+003	0	8.84e-018

Media	Reaction (kg/hr)	Advection (kg/hr)	Reaction (percent)	Advection (percent)
Air	127	4.84	4.25	0.161
Water	98.2	204	3.27	6.8
Soil	1.78e+003	0	59.4	0
Sediment	673	112	22.4	3.73

Persistence Time: 3.17e+003 hr

3. Environmental Fate and Pathways

Reaction Time: 3.55e+003 hr
Advection Time: 2.96e+004 hr
Percent Reacted: 89.3
Percent Advected: 10.7

Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
15-NOV-2001

(1)

3.3.2 Distribution

-

3.4 Mode of Degradation in Actual Use

-

3.5 Biodegradation

-

3.6 BOD5, COD or BOD5/COD Ratio

-

3.7 Bioaccumulation

-

3.8 Additional Remarks

-

4. Ecotoxicity

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: other
 Species: other: fish
 Exposure period: 96 hour(s)
 Unit: mg/l Analytical monitoring: no
 LC50: .022
 Method: other: (calculated) ECOSAR v0.99e
 Year: 1999 GLP: no
 Test substance: other TS: molecular structure
 Remark: Chemical may not be soluble enough to measure this predicted effect.
 Reliability: (2) valid with restrictions
 Accepted calculation method
 15-NOV-2001 (1)

4.2 Acute Toxicity to Aquatic Invertebrates

Type: other: calculated
 Species: Daphnia sp. (Crustacea)
 Exposure period: 48 hour(s)
 Unit: mg/l Analytical monitoring: no
 EC50: .107
 Method: other: (calculated) ECOSAR v0.99e
 Year: 1999 GLP: no
 Test substance: other TS: molecular structure
 Remark: Chemical may not be soluble enough to measure this predicted effect.
 Reliability: (2) valid with restrictions
 Accepted calculation method
 15-NOV-2001 (1)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: other algae: green algae
 Endpoint: growth rate
 Exposure period: 96 hour(s)
 Unit: mg/l Analytical monitoring: no
 EC50: .002
 Method: other: (calculated) ECOSAR v0.99e
 Year: 1999 GLP: no
 Test substance: other TS: molecular structure
 Reliability: (2) valid with restrictions
 Accepted calculation method
 15-NOV-2001 (1)

4.4 Toxicity to Microorganisms e.g. Bacteria

-

4. Ecotoxicity

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

-

4.5.2 Chronic Toxicity to Aquatic Invertebrates

-

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Soil Dwelling Organisms

-

4.6.2 Toxicity to Terrestrial Plants

-

4.6.3 Toxicity to other Non-Mamm. Terrestrial Species

-

4.7 Biological Effects Monitoring

-

4.8 Biotransformation and Kinetics

-

4.9 Additional Remarks

-

5. Toxicity

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

-

5.1.2 Acute Inhalation Toxicity

-

5.1.3 Acute Dermal Toxicity

-

5.1.4 Acute Toxicity, other Routes

Type: LD50
Species: mouse
Strain:
Sex:
Number of
Animals:
Vehicle:
Route of admin.: i.p.
Value: 40 mg/kg bw
Method:
Year: GLP:
Test substance: other TS: CAS# 79-96-9; purity not noted
16-NOV-2001

(2)

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

-

5.2.2 Eye Irritation

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5.3 Sensitization

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5.4 Repeated Dose Toxicity

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5.5 Genetic Toxicity 'in Vitro'

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5.6 Genetic Toxicity 'in Vivo'

-

5. Toxicity

5.7 Carcinogenicity

-

5.8 Toxicity to Reproduction

-

5.9 Developmental Toxicity/Teratogenicity

-

5.10 Other Relevant Information

-

5.11 Experience with Human Exposure

-

6. References

(1) Meylan W. and Howard P. (1999) EPIWin Modeling Program.
Syracuse Research Corporation. Environmental Science Center, 6225
Running Ridge Road, North Syracuse, NY 13212-2510.

(2) NTIS Issue 99-3 (August, 1999) AD691-490

7. Risk Assessment

7.1 End Point Summary

-

7.2 Hazard Summary

-

7.3 Risk Assessment

-

I U C L I D

D a t a S e t

Existing Chemical ID: 85-60-9
CAS No. 85-60-9
TSCA Name 4,4'-Butylidenebis(6-tert-butyl-m-cresol)

Producer Related Part
Company:
Creation date: 08-NOV-2001

Substance Related Part
Company:
Creation date: 08-NOV-2001

Memo: RAPA Hindered Phenols

Printing date: 13-NOV-2001
Revision date:
Date of last Update: 13-NOV-2001

Number of Pages: 22

Chapter (profile): Chapter: 1, 2, 3, 4, 5, 7
Reliability (profile): Reliability: without reliability, 1, 2, 3, 4
Flags (profile): Flags: without flag, confidential, non confidential, WGK
(DE), TA-Luft (DE), Material Safety Dataset, Risk
Assessment, Directive 67/548/EEC, SIDS

1. General Information

1.0.1 OECD and Company Information

Type: lead organisation
Name: American Chemistry Council (formerly Chemical Manufacturers Association) Rubber and Plastics Additives (RAPA) HPV Panel
Street: 1300 Wilson Boulevard
Town: 22209 Arlington, VA
Country: United States
Phone: 703-741-5600
Telefax: 703-741-6091

09-NOV-2001

Type: cooperating company
Name: Bayer Corporation
Country: United States

09-NOV-2001

Type: cooperating company
Name: Ciba Specialty Chemicals Corporation
Country: United States

09-NOV-2001

Type: cooperating company
Name: Crompton Corporation
Country: United States

09-NOV-2001

Type: cooperating company
Name: Flexsys America L.P.
Country: United States

09-NOV-2001

Type: cooperating company
Name: Noveon, Inc. (formerly BF Goodrich)
Country: United States

09-NOV-2001

Type: cooperating company
Name: R.T. Vanderbilt Company, Inc.
Country: United States

09-NOV-2001

Type: cooperating company
Name: The Goodyear Tire & Rubber Company
Country: United States

09-NOV-2001

1. General Information

Type: cooperating company
Name: The Lubrizol Corporation
Country: United States

09-NOV-2001

Type: cooperating company
Name: UOP, LLC.
Country: United States

09-NOV-2001

1.0.2 Location of Production Site

-

1.0.3 Identity of Recipients

-

1.1 General Substance Information

-

1.1.0 Details on Template

-

1.1.1 Spectra

-

1.2 Synonyms

-

1.3 Impurities

-

1.4 Additives

-

1.5 Quantity

-

1.6.1 Labelling

-

1.6.2 Classification

-

1. General Information

1.7 Use Pattern

-

1.7.1 Technology Production/Use

-

1.8 Occupational Exposure Limit Values

-

1.9 Source of Exposure

-

1.10.1 Recommendations/Precautionary Measures

-

1.10.2 Emergency Measures

-

1.11 Packaging

-

1.12 Possib. of Rendering Subst. Harmless

-

1.13 Statements Concerning Waste

-

1.14.1 Water Pollution

-

1.14.2 Major Accident Hazards

-

1.14.3 Air Pollution

-

1.15 Additional Remarks

-

1.16 Last Literature Search

-

1. General Information

1.17 Reviews

-

1.18 Listings e.g. Chemical Inventories

-

2. Physico-chemical Data

2.1 Melting Point

Value: 210 degree C
Decomposition: no
Sublimation: no
Method: other: FF83.9-1 Initial and Final Melting Point of Organic Compounds.
Year: 1996
GLP: yes
Testsubstance: other TS: 4,4'-Butylidenebis(6-tert-butyl-m-cresol); purity not noted
Remark: Capillary method.
Reliability: (1) valid without restriction
GLP Guideline study
Flag: Critical study for SIDS endpoint
13-NOV-2001 (1)

2.2 Boiling Point

-

2.3 Density

Type: relative density
Value: 1.03
Method: other: FF97.8-1 Flexsys Standard Method
Year: 1997
GLP: yes
Testsubstance: other TS: 4,4'-Butylidenebis(6-tert-butyl-m-cresol); purity not noted
Remark: Density of solids by displacement
Reliability: (1) valid without restriction
GLP Guideline study
Flag: Critical study for SIDS endpoint
13-NOV-2001 (2)

2.3.1 Granulometry

-

2.4 Vapour Pressure

-

2. Physico-chemical Data

2.5 Partition Coefficient

log Pow: 9.09
Method: other (calculated): SRC LogKow (KowWin) Program
Year: 1995
GLP: no
Testsubstance: other TS: molecular structure
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
13-NOV-2001 (3)

2.6.1 Water Solubility

Value: < .1 other: mg/ml at 18 degree C
Qualitative: of very low solubility
Method: other
GLP: no data
Testsubstance: other TS: 4,4'-Butylidenebis(6-tert-butyl-m-cresol); purity
not noted
Flag: Critical study for SIDS endpoint
09-NOV-2001 (4)

2.6.2 Surface Tension

-

2.7 Flash Point

-

2.8 Auto Flammability

-

2.9 Flammability

-

2.10 Explosive Properties

-

2.11 Oxidizing Properties

-

2.12 Additional Remarks

-

3. Environmental Fate and Pathways

3.1.1 Photodegradation

Type: air
 INDIRECT PHOTOLYSIS
 Sensitizer: OH
 Conc. of sens.: 1560000 molecule/cm3
 Rate constant: .000000000206671 cm3/(molecule * sec)
 Degradation: 50 % after .6 hour(s)
 Method: other (calculated): AOP Program (v1.89)
 Year: 1999 GLP: no
 Test substance: other TS: molecular structure
 Reliability: (2) valid with restrictions
 Accepted calculation method
 Flag: Critical study for SIDS endpoint
 09-NOV-2001

(5)

3.1.2 Stability in Water

-

3.1.3 Stability in Soil

-

3.2 Monitoring Data (Environment)

-

3.3.1 Transport between Environmental Compartments

Type: fugacity model level III
 Media: other: air - water - soil - sediment
 Air (Level I):
 Water (Level I):
 Soil (Level I):
 Biota (L.II/III):
 Soil (L.II/III):
 Method: other: EPIWIN Level III Fugacity Model
 Year: 1999

Result:	Media	Concentration (percent)	Half-Life (hr)	Emissions (kg/hr)	Fugacity (atm)
	Air	0.0188	1.24	1000	2.95e-015
	Water	2.34	1.44e+003	1000	2.9e-019
	Soil	30.2	1.44e+003	1000	2.81e-021
	Sediment	67.4	5.76e+003	0	2.82e-019

Media	Reaction (kg/hr)	Advection (kg/hr)	Reaction (percent)	Advection (percent)
Air	826	14.8	27.5	0.494
Water	88.5	184	2.95	6.13
Soil	1.14e+003	0	38.1	0
Sediment	638	106	21.3	3.53

Persistence Time: 2.62e+003 hr

3. Environmental Fate and Pathways

Reaction Time: 2.92e+003 hr
 Advection Time: 2.58e+004 hr
 Percent Reacted: 89.8
 Percent Advected: 10.2
 Reliability: (2) valid with restrictions
 Accepted calculation method
 Flag: Critical study for SIDS endpoint
 09-NOV-2001 (5)

3.3.2 Distribution

-

3.4 Mode of Degradation in Actual Use

-

3.5 Biodegradation

Type: aerobic
 Inoculum: predominantly domestic sewage, adapted
 Concentration: 20.7 mg/l related to Test substance
 Degradation: 0 - 5 % after 35 day
 Result: under test conditions no biodegradation observed
 Method: other: Ultimate Biodegradation by Shake Flask CO2 Evolution;
 ASTM E35.24 Draft 3, 1980
 Year: GLP: yes
 Test substance: other TS: Santowhite Powder Lot#NM03-039, purity: >96%.
 Remark: Test run in triplicate. Biodegradation either unlikely or rate
 of mineralization is very slow.
 Reliability: (1) valid without restriction
 GLP Guideline study
 Flag: Critical study for SIDS endpoint
 13-NOV-2001 (6) (7)

3.6 BOD5, COD or BOD5/COD Ratio

-

3.7 Bioaccumulation

-

3.8 Additional Remarks

-

4. Ecotoxicity

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: static
 Species: Salmo gairdneri (Fish, estuary, fresh water)
 Exposure period: 96 hour(s)
 Unit: mg/l Analytical monitoring: no
 NOEC: 1000
 LC50: > 1000
 Method: other: EPA Methods for Toxicity Tests with Fish,
 Macroinvertebrates And Amphibians EPA-660/3-75-009 April 1979
 Year: 1979 GLP: yes
 Test substance: other TS: White powder., purity: 96.2%
 Remark: Working standard prepared in acetone. Water quality parameters
 monitored throughout test. No mortalities.
 Result: LC50 (24h) = >1000 mg/l
 LC50 (48h) = >1000 mg/l
 LC50 (72h) = >1000 mg/l
 LC50 (96h) = >1000 mg/l
 NOEC = 1000 mg/l
 LOEC = Not Determined
 Reliability: (1) valid without restriction
 GLP Guideline study
 Flag: Critical study for SIDS endpoint
 13-NOV-2001 (8)

Type: static
 Species: Lepomis macrochirus (Fish, fresh water)
 Exposure period: 96 hour(s)
 Unit: mg/l Analytical monitoring: no
 NOEC: 1000
 LC50: > 1000
 Method: other: EPA Methods for Toxicity Tests with Fish,
 Macroinvertebrates And Amphibians EPA-660/3-75-009 April 1979
 Year: 1979 GLP: yes
 Test substance: other TS: White powder, purity: 96.2%
 Remark: Working standard prepared in acetone. Water quality parameters
 monitored throughout test. No mortalities.
 Result: LC50 (24h) = >1000 mg/l
 LC50 (48h) = >1000 mg/l
 LC50 (72h) = >1000 mg/l
 LC50 (96h) = >1000 mg/l
 NOEC = 1000 mg/l
 LOEC = Not Determined
 Reliability: (1) valid without restriction
 GLP Guideline study
 Flag: Critical study for SIDS endpoint
 13-NOV-2001 (9)

4. Ecotoxicity

Type: static
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
NOEC: 1000
LC50: > 1000
Method: other: EPA Methods for Toxicity Tests with Fish,
Macroinvertebrates And Amphibians EPA-660/3-75-009 April 1979
Year: 1979 GLP: yes
Test substance: other TS: White powder, purity: 96.2
Remark: Working standard prepared in acetone. Water quality parameters
monitored throughout test. No mortalities.
Result: LC50 (24h) = >1000 mg/l
LC50 (48h) = >1000 mg/l
LC50 (72h) = >1000 mg/l
LC50 (96h) = >1000 mg/l
NOEC = 1000 mg/l
LOEC = Not Determined
Reliability: (1) valid without restriction
GLP Guideline study
Flag: Critical study for SIDS endpoint
13-NOV-2001 (10)

4.2 Acute Toxicity to Aquatic Invertebrates

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no
EC50: 16
Method: other: EPA Methods for Toxicity Tests with Fish,
Macroinvertebrates And Amphibians EPA-660/3-75-009 April 1979
Year: 1979 GLP: yes
Test substance: other TS: White powder purity: 96.2%
Remark: Working standard prepared in DMF. Water quality parameters
monitored throughout test. A NOEL was not observed for the
test article after 48 hours.
Result: EC50 (24h) = 24 mg/l
EC50 (48h) = 16 mg/l
NOEC = Not Observed
Reliability: (1) valid without restriction
GLP Guideline study
Flag: Critical study for SIDS endpoint
13-NOV-2001 (11)

4. Ecotoxicity

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Selenastrum capricornutum (Algae)
 Endpoint: biomass
 Exposure period: 96 hour(s)
 Unit: Analytical monitoring: no
 EC50: > 1000
 Method: other: EPA Selenastrum capricornutum Printz Algal Assay Test
 1978
 Year: 1978 GLP: yes
 Test substance: other TS: White powder, purity: 96.2%
 Remark: Working standard prepared in DMF. Water quality parameters
 monitored throughout test; pH was 7.5; closed system
 Result: EC50 (24 h) = >500<1000 ppm
 EC50 (96 h) = >1000 ppm
 LOEC = 125 ppm
 Reliability: (1) valid without restriction
 GLP Guideline study
 Flag: Critical study for SIDS endpoint
 13-NOV-2001 (12)

4.4 Toxicity to Microorganisms e.g. Bacteria

-

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

-

4.5.2 Chronic Toxicity to Aquatic Invertebrates

-

4. Ecotoxicity

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Soil Dwelling Organisms

-

4.6.2 Toxicity to Terrestrial Plants

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4.6.3 Toxicity to other Non-Mamm. Terrestrial Species

-

4.7 Biological Effects Monitoring

-

4.8 Biotransformation and Kinetics

-

4.9 Additional Remarks

-

5. Toxicity

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Strain: Sprague-Dawley
Sex: male/female
Number of Animals:
Vehicle: other: corn oil
Value: > 7940 mg/kg bw
Method: other: Defined Lethal Dose
Year: GLP: no data
Test substance: other TS: Santowhite Powder Lot# NB10-010, purity: >96%.
Remark: Santowhite Powder was fed to 2 groups of male and female rats as a 20.0% suspension in corn oil at dose levels of 6310 and 7940 mg/kg/body weight in a single oral dose study. Clinical signs of toxicity included reduced appetite and activity (one to three days in survivors), followed by increasing weakness, collapse and death. Gross autopsy findings were that all viscera appeared normal in all survivors; lung and liver hyperemia and gastrointestinal inflammation was noted in decedents.
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
13-NOV-2001 (13)

5.1.2 Acute Inhalation Toxicity

-

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rabbit
Strain: New Zealand white
Sex: male/female
Number of Animals:
Vehicle: other: corn oil
Value: > 7940
Method: other: Defined Lethal Dose
Year: GLP: no data
Test substance: other TS: Santowhite Powder Lot# NB10-101, purity: >96%
Remark: Santowhite Powder as a 40.0% suspension in corn oil was applied to the shaved skin of two groups of male and female rabbits in a single dermal application study at dose levels of 5010 and 7940 mg/kg/body weight. Clinical signs of toxicity included reduced appetite and activity for two or three days. There were no mortalities. All viscera appeared normal in the animals sacrificed after 14 days.

5. Toxicity

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented
and acceptable for assessment
Flag: Critical study for SIDS endpoint
13-NOV-2001 (13)

5.1.4 Acute Toxicity, other Routes

-

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

-

5.2.2 Eye Irritation

-

5.3 Sensitization

Type: Patch-Test
Species: human
Number of
Animals:
Vehicle:
Result: not sensitizing
Classification:
Method:
Year: GLP:
Test substance:
Remark: 50 human volunteers. No positive reactions following initial
application. No positive reactions following 15 serial
applications. No positive reactions on subsequent challenge
after 2 weeks.
Result: Not considered to be a primary irritant, a cumulative
irritant, or a sensitizing agent under test conditions.
13-NOV-2001 (14)

5. Toxicity

5.4 Repeated Dose Toxicity

Species: rat Sex: male/female
 Strain: Sprague-Dawley
 Route of admin.: oral feed
 Exposure period: 4 weeks
 Frequency of treatment: daily
 Post. obs. period:
 Doses: 0, 1000, 2500, 5000 and 10,000 ppm
 Control Group: yes, concurrent no treatment
 NOAEL: < 1000 ppm
 LOAEL: 1000 ppm
 Method: other: 28-Day Repeat Dose/OECD 407 equivalent
 Year: GLP: yes
 Test substance: other TS: Santowhite Powder Lot#N7E-009, purity: >95%
 Result: Santowhite Powder was fed to groups of ten male and female rats. There were no significant clinical signs, and all animals survived to terminal sacrifice. Reduced food intake and body weights in both sexes were noted at the three highest dose levels. Gross examination results were liver discoloration and increased absolute and relative hepatic weights for all animals at all dose levels. Microscopic findings were hepatocellular vacuolation at all dose levels. The three highest dose levels also showed hepatocellular degeneration/necrosis.
 Reliability: (1) valid without restriction
 GLP Guideline study
 Flag: Critical study for SIDS endpoint
 13-NOV-2001 (15)

Species: rat Sex: male/female
 Strain: Sprague-Dawley
 Route of admin.: oral feed
 Exposure period: 90 Days
 Frequency of treatment: Daily
 Post. obs. period:
 Doses: 0, 100, 500 and 1000 ppm.
 Control Group: yes, concurrent no treatment
 NOAEL: 100 ppm
 LOAEL: 500 ppm
 Method: other: 90-Day Repeat Dose / OECD 408 equivalent
 Year: GLP: yes
 Test substance: other TS: Santowhite Powder Lot#N7E-009, purity: >95%.
 Result: Groups of 15 male and female rats were fed Santowhite Powder for 90 days. All animals survived to terminal sacrifice. There were no clinical signs considered related to treatment. Highest-dose animals exhibited slightly reduced body weights and food consumption, altered serum enzymes (SGOT, SGPT), increased liver weights, and microscopic liver and lymph node changes. Mid-dose animals showed similar changes in SGOT and SGPT, in liver weights and in liver and lymph node tissue.

5. Toxicity

Reliability: (1) valid without restriction
GLP Guideline study
Flag: Critical study for SIDS endpoint
13-NOV-2001 (16)

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test
System of testing: Salmonella typhimurium TA-1535, TA-1537, TA-1538, TA-98, TA-100
Concentration: 0.1, 1.0, 10, 100 and 500 micrograms/plate
Cytotoxic Conc.:
Metabolic activation: with and without
Result: negative
Method: other: Ames Mutagenicity Plate Assay
Year: 1975 GLP: no data
Test substance: other TS: White powder, purity: 95+%
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
13-NOV-2001 (17)

Type: Yeast gene mutation assay
System of testing: Saccharomyces cerevisiae, D4
Concentration: 0.1, 1.0, 10, 100 and 500 micrograms/plate
Cytotoxic Conc.:
Metabolic activation: with and without
Result: negative
Method: other: Ames Mutagenicity Plate Assay
Year: 1975 GLP: no data
Test substance: other TS: White powder, purity: 95+%
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
13-NOV-2001 (17)

5. Toxicity

Type: Unscheduled DNA synthesis
System of testing: Primary rat liver cells
Concentration: 1,5,10, 50, 100 and 250 micrograms/L
Cytotoxic Conc.:
Metabolic activation: without
Result: negative
Method: other: according to Williams, G.M. 1977; Detection of Chemical Carcinogens by Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures.
Year: GLP: yes
Test substance: other TS: Santowhite Powder Lot# N6E-021, purity: >96%.
Remark: Negative - not a genotoxic agent under test conditions
Reliability: (1) valid without restriction
GLP study, meets generally accepted scientific standards, well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
13-NOV-2001 (18)

Type: Cytogenetic assay
System of testing: CHO Cells.
Concentration: 2, 4 and 8 micrograms/ml.in the absence of metabolic activation; 12.5, 25 and 50 micrograms/ml in the presence of metabolic activation
Cytotoxic Conc.: Precipitation conc:200 micrograms/ml.
Metabolic activation: with and without
Result: negative
Method: other: according to Preston et. al. 1981; Mammalian in vivo and in vitro.Cytogenetic Assays
Year: GLP: yes
Test substance: other TS: Santowhite Powder Lot# N6E-021, purity: >96%.
Remark: Negative - did not induce chromosomal aberrations in Chinese Hamster ovary cells (CHO) both in the presence or absence of rat S-9 metabolic activation.
The cells were evaluated via microscope for mitotic indices and for chromosomal aberrations.
Solvent and positive controls were included in the study.
Reliability: (1) valid without restriction
GLP study, meets generally accepted scientific standards, well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
13-NOV-2001 (19)

5.6 Genetic Toxicity 'in Vivo'

-

5.7 Carcinogenicity

-

5. Toxicity

5.8 Toxicity to Reproduction

Type: other
Species: rat Sex: male/female
Strain: Sprague-Dawley
Route of admin.: oral feed
Exposure Period: 90 days
Frequency of treatment:
Duration of test: 90 days
Doses: 0, 100, 500 and 1000 ppm
Control Group: yes, concurrent no treatment
NOAEL Parental: 100 ppm
Method: other: 90-Day Repeat Dose / OECD 408 equivalent
Year: GLP: yes
Test substance: other TS: Santowhite Powder Lot# N7E-009, purity: >95%.
Remark: OECD/SIDS program accepts adequate repeat dose 90-day studies that demonstrate no effect on reproductive organs. General parental toxicity: All animals survived. No clinical signs of treatment-related toxicity. Gross and microscopic examination of both male and female reproductive organs at sacrifice noted no significant differences in the organs of the control group vs. the treated groups. Reproductive system organs examined included testes with epididymides, ovaries and uterus. Testes with epididymides were weighed as well as examined.
Reliability: (2) valid with restrictions
GLP study, meets generally accepted scientific standards, well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
13-NOV-2001 (16)

5.9 Developmental Toxicity/Teratogenicity

-

5.10 Other Relevant Information

Type: Toxicokinetics
Method: A group of 5 male Sprague-Dawley rats were fed BBMC in the diet for one week at an exposure level of 1.135 mmol/100g of feed, with the average mean intake reported as 0.466 mmol/rat/day.
Remark: Authors noted that BBMC seemed to have "anticholinesteremic and antidibetic effects" and to produce hepatic fatty infiltration in rats fed 0.005% of the test substance in their diet for 90 days.
Result: A slight increase in the prothrombin index, increased relative liver weights, and changes in liver and plasma lipid concentrations were reported. Alterations in lipid levels included increases in triglycerides, diglycerides, non-esterified fatty acids, cholesterol and cholesterol esters in the liver and decreases in triglycerides, cholesterol and non-esterified fatty acids in plasma. The findings may

5. Toxicity

13-NOV-2001 suggest a decrease in fat excretion in the liver. (20) (21)

5.11 Experience with Human Exposure

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6. References

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- (1) ASTM D-1519./ Flexsys Physical Methods of Analysis FF83.9-1 Initial and Final Melting Point of Organic Compounds. 1996.
 - (2) FF97.8-1 Flexsys Standard Method 1997 - Density by Displacement
 - (3) Meylan, W.M. and. P.H. Howard, 1995 J. Pharm. Sci. 84: 83-92
KowWin Log P Calculations/Database
 - (4) NTP Chemical Repository
 - (5) Meylan W. and Howard P. (1999) EPIWin Modeling Program. Syracuse Research Corporation. Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212-2510.
 - (6) Monsanto ES-80-SS-42 Environmental Sciences Labs 1980
 - (7) Monsanto ES-80-SS-42 Environmental Sciences Labs 1980. Biodegradation Screening of Selected Rubber Chemicals - Ultimate Biodegradation by Shake Flask CO2 Evolution / ASTM E35.24 Draft 3 - 1980.
 - (8) Monsanto AB-80-536 Analytical BioChemistry Labs, July 1980. Acute Toxicity of Santowhite Powder to Rainbow Trout (*Salmo gairdneri*).
 - (9) Monsanto AB-80-538 Analytical BioChemistry Labs, July 1980. Acute Toxicity of Santowhite Powder to Bluegill Sunfish (*Lepomis macrochirus*).
 - (10) Monsanto AB-80-537 Analytical BioChemistry Labs, July 1980. Acute Toxicity of Santowhite Powder to Fathead Minnows (*Pimephales promelas*).
 - (11) Monsanto AB-80-543 Analytical BioChemistry Labs, November 1980. Acute Toxicity of Santowhite Powder to *Daphnia magna*.
 - (12) Monsanto BN-80-535 EG&G Bionomics August 1980. Toxicity of Santowhite Powder to the freshwater algae *Selenastrum capricornutum*
 - (13) Monsanto Y-73-289 Younger Laboratories Feb. 15, 1974. Toxicological Investigation of Santowhite Powder - Acute Oral LD50, Acute Dermal LD50, Acute Eye Irritation, Primary Skin Irritation
 - (14) Monsanto SH-66-6 Industrial Biology Laboratories May 1966. Repeat Insult Patch Test - Santowhite Powder Antioxidant

6. References

- (15) Monsanto ML-87-150 Monsanto Environmental Health Laboratory
February 17, 1988 Four Week Feeding Study of Santowhite
Powder in Sprague-Dawley Rats
- (16) Monsanto ML-87-311 Monsanto Environmental Health Laboratory
November 8, 1988. Three Month Study of Santowhite Powder
Antioxidant Administered to Feed in Sprague-Dawley Rats
- (17) Monsanto BIO-76-233 Litton Bionetics December 30, 1976.
Mutagenicity Evaluation of CP 3388 (Santowhite Powder) Final
Report
- (18) Monsanto SR-86-391 SRI International February 2, 1987.
Evaluation of the Potential of Santowhite Powder to Induce
Unscheduled DNA Synthesis in Primary Rat Hepatocyte Cultures
- (19) Monsanto SR-86-392 SRI International January 1987. An
Assessment of the Clastogenic Potential of Santowhite Powder
Utilizing the Mammalian Cell Cytogenics Assay with CHO Cells
- (20) Takahashi, O. and Hirage, K. (1981) Effects of Four
Bis-Phenolic Antioxidants on Prothrombin levels of Rat
Plasma. Toxicol. Lett. 7, 405-408
- (21) Takahashi, O. and Hirage, K. (1981) Effects of Four
Bis-Phenolic Antioxidants on Prothrombin levels of Rat
Plasma. Toxicol. Lett. 8, 77-86

7. Risk Assessment

7.1 End Point Summary

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7.2 Hazard Summary

-

7.3 Risk Assessment

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96-69-5
4,4-Thiobis(6-tert-butyl-m-cresol)

Molecular Formula: C22-H30-S-O2

Molecular Weight: 358.58

1.1 GENERAL SUBSTANCE INFORMATION

- A. Type of Substance:** Organic
B. Physical State: White Solid
C. Purity: 95-99+ % Typical for Commercial Products

1.2 SYNONYMS Santonox® TBMC
Santonox® R
Santowhite® Crystals
TBMC
TBBC
Lowinox® TBM-6

1.3 IMPURITIES 6-t-Butyl-3-methylphenol <1%

1.4 ADDITIVES None

2. PHYSICAL-CHEMICAL DATA

***2.1 MELTING POINT**

Value: 162°C
Decomposition: No
Sublimation: No
Method: FF83.9-1 Initial and Final Melting Point of Organic Compounds, 1996
GLP: Yes
Remarks: Instrumental - Capillary Tube Method. Typical range for initial to final melt point determinations is 158-164°C
Reference: ASTM D-1519 / Flexsys Physical Methods of Analysis
Reliability: (1) Valid without restriction

***2.2 BOILING POINT**

Value: Thermal decomposition begins at 207.4°C
Pressure: 1013 hPa
Decomposition: Yes
Method: Instrumental – Differential Scanning Calorimeter
GLP: No
Remarks: Volatile component identified by GC/MS as 6-t-butyl-m-cresol
Reference: Flexsys Lab Report DSC-AP00035.d03, March 2000
Reliability: (1) Valid without restriction

†2.3 DENSITY

Type: Density

Value: 1.09 Typical
Temperature: 25 °C
Method: Density of solids by displacement, 1997
GLP: Yes
Remarks: Density of solids by displacement in kerosene
Reference: FF97.8-1 Flexsys Standard Method 1997
Reliability: (1) Valid without restriction

***2.4 VAPOUR PRESSURE**

Value: 8.40E-007 hPa
Temperature: 70°C
Method: Measured
Perkin Elmer TGS, Weight Loss vs. Temperature plot
GLP: No
Remarks: Weight loss was linear with respect to time
Reference: Monsanto memo, May 20, 1976
Reliability: (1) Valid without restriction

***2.5 PARTITION COEFFICIENT $\log_{10}P_{ow}$**

Log Pow: 8.24
Temperature: Not Applicable
Method: calculated
SRC LogKow (KowWin) Program, 1995
GLP: No
Remarks: Accepted calculation model using molecular structure and measured melting point of 162°C
Reference: Meylan, W.M. and. P.H. Howard, 1995 J. Pharm. Sci. 84: 83-92
Reliability: (2) Valid with restrictions – modelling data

***2.6 WATER SOLUBILITY**

A. Solubility

Value: <0.1 mg/ml
Temperature: 25 °C
Description: Of very low solubility
Method: Not Specified
GLP: No data
Remarks: None
Reference: NTP Chemical Repository 4,4'-Thiobis(6-tert-butyl-m-cresol)
Reliability: (4) Not assignable - data from a secondary literature source

Value: 0.08%
Temperature: 20 °C
Description: Of very low solubility
Method: Not Specified
GLP: No data
Remarks: None
Reference: Practical Toxicology of Plastics, CRC Press, 1968
Reliability: (4) Not assignable - data from a secondary literature source

B. pH Value, pKa Value

pH Value: Not Applicable

2.11 OXIDISING PROPERTIES

†2.12 OXIDATION: REDUCTION POTENTIAL

2.13 ADDITIONAL DATA

A. Partition co-efficient between soil/sediment and water (Kd)

B. Other data – Henry's Law Constant

Results: 2.76E-012 atm-m³/mole
Remarks: Calculated at 25°C using measured melting point of 162°C
Reference: Environ Toxicol Chem 10: 1283-93 (1991)
EPIWIN/HENRYWIN v3.10
Reliability: (2) Valid with restrictions – Modelling data

3. ENVIRONMENTAL FATE AND PATHWAYS

*3.1.1 PHOTODEGRADATION

Type: air
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens. 1560000 molecule/cm³
Rate constant: .0000000001297621 cm³/(molecule * sec)
Degradation: 50 % after 1 hour(s)
Method: other (calculated): AOP Program (v1.89)
Year: 1999
GLP: No
Test substance: other TS: molecular structure and measured melting point of 162°C
Reliability: (2) Valid with restrictions - Accepted calculation method

*3.1.2 STABILITY IN WATER

Type: Abiotic (hydrolysis)
Half life: >168 hours at pH 7.0 and 23 °C
Method: Oxidative/Hydrolytic Stability
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: 95%
Remarks: The oxidative and/or hydrolytic stability of the test compound was determined by the following procedure: A borosilicate glass cylinder was filled with 1500 ml of purified water and adjusted to a 1 mg/l concentration of the test compound using 200:1 of a 7.5 mg/l acetone stock solution. The continuously stirred and aerated aqueous solution was then sampled as a function of time. Sample analysis was carried out on a methylene chloride extract of the solution, followed by concentration and analysis via gas chromatography. The test compound appeared to be reasonably stable in aerated water, with 63% remaining after 168 hours.
Reference: Monsanto ES-78-SS-28 Environmental Sciences Labs 1978
Reliability: (1) Valid without restriction

3.3 TRANSPORT AND DISTRIBUTION BETWEEN ENVIRONMENTAL COMPARTMENTS INCLUDING ESTIMATED ENVIRONMENTAL CONCENTRATIONS AND DISTRIBUTION PATHWAYS

*3.3.1 TRANSPORT

Type: Adsorption
 Media: Soil/Sediment
 Method: SRC Structure estimation method based on molecular connectivity indices, 1992
 Results: $K_{oc} = 4.297E+006$; $\log K_{oc} = 6.633$
 Remarks: Estimation based on molecular structure and measured melting point of 162°C
 Reference: EPIWIN/PCKOCWIN v1.66
 Reliability: (2) Valid with restrictions – Modelling data

Type: Volatility
 Media: Water
 Method: Estimation Method, 1990
 Results: Volatilization half-life from model river: 4.017E+008 hours
 Volatilization half-life from model lake: 4.382E+009 hours
 Remarks: Model river = 1 m deep flowing at 1 m/sec and wind velocity of 3 m/sec. Model lake = 1 m deep flowing at 0.05 m/sec and wind velocity of 0.5 m/sec.
 Reference: Handbook of Chemical Property Estimation Methods, 1990
 Reliability: (2) Valid with restrictions – Peer-reviewed published data from a generally accepted and validated estimation method

*3.3.2 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

Media: Air-biota-sediment-soil-water
 Method: Fugacity level III, 1999
 Results:

Media	Concentration (%)	Half-Life (hours)	Emissions (kg/hr)	Fugacity (atm)
Air	0.00224	1.98	1000	7.05e-01
Water	2.05	1.44e+003	1000	6.57e-019
Soil	39.20	1.44e+003	1000	9.51e-022
Sediment	58.7	5.76e+003	0	6.40e-020

Media	Reaction (kg/hr)	Advection (kg/hr)	Reaction (%)	Advection (%)
Air	76.0	2.17	2.53	0.0724
Water	95.7	199	3.19	6.63
Soil	1.83e+003	0	61.00	0
Sediment	684	114	22.8	3.79

Persistence Time: 3.23e+003 hr
 Reaction Time: 3.61e+004 hr
 Percent Reacted: 89.5
 Percent Advected: 10.5

Remarks: EPIWIN Level III Fugacity Model - accepted calculation method

Reference: using molecular structure and measured melting point of 162°C
EPIWIN Modeling Program, Syracuse Research Corp. 1999
Reliability: (2) Valid with restrictions – Modelling data

***3.5 BIODEGRADATION**

Type: aerobic
Inoculum: adapted
Concentration of the chemical: 3mg/l test substance
Medium: sewage treatment
Degradation: 11% +/- 7 % after 13 weeks
Results: Little biodegradation observed under test conditions
Method: Semi-Continuous Activated Sludge (Primary Biodegradation)
Thompson-Duthie-Sturm Procedure
Monsanto Shake Flask Procedure
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: 95%
Remarks: Analytical monitoring involved extraction with methylene chloride, sample concentration, and analysis via a gas chromatograph equipped with dual FID. The test compound showed significant resistance to primary degradation by either chemical or biological processes. Slight inhibition of the normal sludge growth was observed during the SCAS test
Shake Flask: 18.7% and 20.4% theoretical CO₂ in 35 days
T-D-S: 0.0% in 49 days
Reference: Monsanto ES-78-SS-28 Environmental Sciences Labs 1978
Reliability: (1) Valid without restrictions

3.6 BIOACCUMULATION

Species: Other
BCF: 453
Method: BCFWIN v2.14
GLP: No
Remarks: Calculated using molecular structure and measured melting point of 162°C.
Log BCF = 2.656
Reference: EPIWIN/BCFWIN v2.14
Reliability: (2) Valid with restrictions – modelling data

4. ECOTOXICITY

***4.1 ACUTE/PROLONGED TOXICITY TO FISH**

Type of test: static
Closed system
Species: Pimephales promelas (Fathead Minnow)
Exposure period: 96 hours
Results: LC₅₀ (24h) = 0.70 mg/l
LC₅₀ (48h) = 0.54 mg/l
LC₅₀ (96h) = 0.36 mg/l
NOEC = 0.10 mg/l
LOEC = Not Determined

Analytical monitoring: No
Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1972).
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 99%
Remarks: Test fish were obtained from Fattig Fish Hatchery in Brady, Nebraska. Test fish were held in culture tanks on a 16-hour daylight photoperiod and observed for at least 14 days prior to testing. A daily record of fish observations was maintained during the holding period, during which time the fish were fed a standard diet of commercial fish food until 48 hours prior to testing, when feeding was stopped. Concentrations to be tested were based on the results of previously completed acute assays using trout and bluegill. Test fish had a mean weight of 0.23 g and a mean standard length of 25 mm. The test was conducted in 5-gallon glass vessels containing 15 liters of laboratory well water. The 0-hour measured control water parameters of this dilution water were dissolved oxygen 9.3 ppm, hardness (CaCO₃) of 255 ppm and pH 8.2. The test vessels were kept in a water bath at 22°C. Test fish were acclimated to the dilution water and test temperature, and held without food for 48 hours prior to testing. Nanograde Acetone was used to prepare the test solutions and as the solvent control. Test concentrations were 0, 0.10, 0.18, 0.32, 0.56 and 1.0 mg/l for the test compound. Fish were placed in the testing vessels within 30 minutes of the addition of the test material aliquots. All concentrations were observed once every 24 hours for mortality and abnormal effects. Dissolved oxygen values and pH ranges were monitored during the testing and remained within acceptable limits of 40-100% saturation for dissolved oxygen and pH value consistent with control. The ammonia concentration was below the toxic limit. Water hardness (CaCO₃) was 255 ppm. As a quality check, test fish were challenged with Antimycin A. The estimated 96Hr LC₅₀ and 95% confidence limits were within the 95% confidence limits reported in the literature, indicating that the fish were in good condition. These values were obtained by employing the statistical methods described by Litchfield and Wilcoxon (A Simplified Method for Evaluating Dose-Effect Experiments, 1949) or Stephan (methods for calculating an LC₅₀, 1977).
Reference: Monsanto AB-79-1384322-3b, ABC Labs, 1979
Reliability: (1) Valid without restriction

Type of test: static
Closed system
Species: Salmo gairdneri (Rainbow Trout)
Exposure period: 96 hours
Results: LC₅₀ (24h) = 0.27 mg/l
LC₅₀ (48h) = 0.16 mg/l
LC₅₀ (96h) = 0.16 mg/l
NOEC = 0.10 mg/l
LOEC = 0.14 mg/l
Analytical monitoring: No

Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1972)

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: 99%

Remarks: The test material, in reagent-grade acetone, was introduced into 15 liters of diluent water in all-glass vessels. Test concentrations were 0, 0.10, 0.14, 0.18, 0.24, 0.32 and 0.42 mg/l for the test compound. Ten rainbow trout, standard length 3.7 cm, were added to each test vessel. The test fish were not fed for 48 hours prior to testing, nor during the exposure period. No aeration was provided during the test. Temperature was maintained at 12°C. Dissolved oxygen content ranged from 8.0 mg/l (75% of saturation) at the beginning of the test, to 3.4 mg/l (32% of saturation) at the end of the exposure period. Beginning pH was 7.6; ending pH was 6.9. Water hardness (CaCO₃) was 255 ppm. Observations and mortality counts were made every 24 hours during a 96-hour period following the initiation of exposure. Test concentrations and observed percentage mortality were converted to logarithms and probits, respectively, and these values were utilized in a least squares regression analysis. The LC₅₀ values and the 95% confidence intervals were calculated from the regression equation.

Reference: Monsanto BN-76-264 and BN-76-265 EG&G Bionomics 1977

Reliability: (1) Valid without restriction

Type of test: static
Closed system

Species: Lepomis macrochirus (Bluegill Sunfish)

Exposure period: 96 hours

Results: LC₅₀ (24h) = 0.73 mg/l
LC₅₀ (48h) = 0.29 mg/l
LC₅₀ (96h) = 0.24 mg/l
NOEC = 0.14 mg/l
LOEC = 0.18 mg/l

Analytical monitoring: No

Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1972).

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: 99%.

Remarks: The test material, in reagent-grade acetone, was introduced into 15 liters of diluent water in all-glass vessels. Test concentrations were 0, 0.14, 0.18, 0.24, 0.32, 0.56, 0.75 and 1.0 mg/l for the test compound. Ten bluegill, standard length 3.8 cm, were added to each test vessel. The test fish were not fed for 48 hours prior to testing, nor during the exposure period. No aeration was provided during the test. Temperature was maintained at 22°C. Dissolved oxygen content ranged from 8.6 mg/l (98% of saturation) at the beginning of the test, to 0.5 mg/l (6% of saturation) at the end of the exposure period. Beginning pH was 7.3; ending pH was 6.7. Water hardness (CaCO₃) was 255 ppm. Observations and mortality counts were made every 24 hours during a 96-hour period following the initiation of exposure. Test concentrations and observed percentage mortality were converted to logarithms

and probits, respectively, and these values were utilized in a least squares regression analysis. The LC50 values and the 95% confidence intervals were calculated from the regression equation.

Reference: Monsanto BN-76-264 and BN-76-265, EG&G Bionomics 1977
Reliability: (1) Valid without restriction

Type of test: static
Closed system

Species: Paratanytarsus parthenogenetica (Midge)

Exposure period: 48 hours

Results: LC₅₀ (24h) = >1000 mg/l
LC₅₀ (48h) = >1000 mg/l
NOEC = 100 mg/l
LOEC = 180 mg/l

Analytical monitoring: No

Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1972), and Methods of Conducting Acute Toxicity Tests with Midge (Paratanytarsus parthenogenetica) (1980)

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: 99%

Remarks: Test midge for this study were cultured at the ABC facilities. The adult midge were fed a suspension of trout chow and alfalfa daily until 24 hours prior to testing. The test was carried out using 3rd and 4th instar larvae, 8-10 days old. The static bioassay was conducted in 250 ml glass beakers containing 200 ml of ABC well water. The 0-hour measured control water parameters of this dilution water were dissolved oxygen 8.8 mg/l, hardness (CaCO₃) of 255 ppm and pH 8.1. The test vessels were kept in a water bath at 20°C. The photoperiod was controlled to give 16 hours of daylight and 8 hours of darkness. An initial range finding experiment preceded the definitive bioassay. Nanograde Acetone was used to prepare the test solutions, which ranged from 56-1000 mg/l, and as the solvent control. All concentrations were observed once every 24 hours for mortality and abnormal effects. Dissolved oxygen content ranged from 8.8 to 7.5 mg/l and pH ranged from 8.2 to 7.9 during the testing. Water quality parameters of temperature, dissolved oxygen content and pH were measured at the termination of the test and were within acceptable limits. The LC50 values were calculated via a computerized program performing the following statistical tests: binomial, moving average and probit tests.

Reference: Monsanto AB-81-981012, Analytical Bio-Chemistry Labs, 1981
Reliability: (1) Valid without restriction

Type of test: flow-through
Closed system

Species: Pimephales promelas (Fathead Minnow)

Exposure period: 14 Days

Results: LC₅₀ (24h) = 0.21 mg/l
LC₅₀ (48h) = 0.17 mg/l
LC₅₀ (72h) = 0.15 mg/l

LC₅₀ (96h) = 0.14 mg/l
 NOEC = Not determined
 LOEC = 0.031 mg/l

Analytical monitoring: Yes
 Method: EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians, 1975 and Methodology for Conducting Time Independent Flow-Through Toxicity Studies with Fish, Adams, et. al., 1979

GLP: Yes
 Test substance: As prescribed by 1.1-1.4, purity: 99%
 Remarks: The chronic toxicity of TBMC to fathead minnows was assessed in a 14-day flow-through study. The fish used for the study were obtained from EG&G Bionomics in Wareham, MA. Test fish were held in culture tanks at 18°C and observed for at least fourteen days prior to testing. During this time, the fish were fed a standard fish food once/day. Mortality during this observation time was less than 1%, indicating that the fish were in good condition. The fathead minnows used for this study had a mean weight of 0.39 g and a mean length of 34.5 mm. A continuous flow diluter system was used to deliver water and the test compound for six test concentrations and the solvent (DMF) control. The test tanks were 17 liter aquaria holding 15 liters of well water. The mean and range values for temperature (22.2°C, 22-23), dissolved oxygen (7.2 mg/l, 6.8-7.5), alkalinity (313 mg/l, 312-314) and hardness (292 mg/l, 290-294) were monitored and recorded throughout the study. Flow rate of 6 liters/hr provided six volume replacements/day. The stock solution of the test compound was prepared with dimethylformamide (DMF). The following nominal exposure concentrations were chosen for this study: 0, 0.04, 0.08, 0.16, 0.25, 0.40 and 0.60 mg/l. Thirty fathead minnows were placed in each tank. The test chemical was flowing through the system at least 24 hours before addition of the fish. Feeding was 1x/day. Mortality and behavioral observations were made once every 24 hours for the duration of the study. Dead fish, if present, were removed at each observation period. The actual concentrations of TBMC in the test aquaria were determined analytically via gas chromatography seven times during the study. No abnormal behavior symptoms or syndromes were noted during the study. Under test conditions, the test compound was found to be both highly toxic and an accumulative toxin to the test species. LC₅₀ on Day 14 = 0.054 mg/l. The LC₅₀ values were obtained by employing the statistical methods described by Litchfield and Wilcoxon (A Simplified Method for Evaluating Dose-Effect Experiments, 1949).

Reference: Monsanto ES-79-SS-17, MO-80-459 Environmental Sciences 1979
 Reliability: (1) Valid without restriction

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

*A. Daphnia

Type of test: static
 Closed system

Species: Daphnia magna
Exposure period: 48 hours
Results: EC₅₀ (24h) = 0.23 mg/l
EC₅₀ (48h) = 0.16 mg/l
NOEC = 0.06 mg/l
Analytical monitoring: No
Method: Standard Methods for Examination of Water and Wastewater (1975) Methods of Acute Toxicity Test with Fish, Macroinvertebrates and Amphibians (1975)
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 99%
Remarks: The Daphnia magna used in the test were cultured at the MIC Aquatic Laboratory. Adult Daphnia were fed an algae and trout chow solution daily. Daphnids known to be less than 24 hours old were separated and used for this test. The bioassay was conducted in 250 ml glass beakers containing 200 ml of well water. During the test, dissolved oxygen concentration ranged from 8.3-9.0 mg/l, pH range was 7.4-8.4, hardness (CaCO₃) was 220-306 mg/l, and alkalinity was 248-306 mg/l. Vessels were kept at room temperature in a temperature-controlled area. The average temperature was 21°C. Lighting was maintained at 50-70 foot-candles on a 16-hour daylight photoperiod. An initial range-finding experiment was carried out to determine the exposure concentrations for the definitive test. Dimethylformamide (DMF) was used as the solvent for the test solutions, and the experiment included both a control and a solvent control. Concentrations tested were 0, 0.063, 0.125, 0.25, 0.5 and 1.0 mg/l. Daphnia in all concentrations were observed once every 24 hours for mortality and abnormal effects. Water quality measurements were monitored throughout the testing and were considered adequate and equivalent to those measurements in the control chamber. The LC₅₀ values were obtained by employing the statistical methods described by Stephan (Methods for Calculating an LC₅₀, 1977).
Reference: Monsanto ES-82-SS-34, MIC Environmental Sciences, 1982
Reliability: (1) Valid without restriction

Type of test: static
Closed system
Species: Daphnia magna
Exposure period: 48 hours
Results: EC₅₀ (24h) = 1.10 mg/l
EC₅₀ (48h) = 0.70 mg/l
NOEC = 0.18 mg/l
Analytical monitoring: No
Method: Standard Methods for Examination of Water and Wastewater (1975) Methods of Acute Toxicity Test with Fish, Macroinvertebrates and Amphibians (1975)
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 99%
Remarks: The Daphnia magna used in the test were cultured at the ABC facilities. Adult Daphnia were fed an algae and trout chow mixture daily until 24 hours prior to testing. The bioassay was conducted in 500 ml glass beakers containing 250 ml of ABC

well water. During the test, dissolved oxygen concentration ranged from 8.6-7.7 mg/l, pH range was 7.7-8.2, hardness (CaCO₃) was 220 mg/l, and alkalinity was 210 mg/l. Vessels were kept in a water bath at 19°C. The photoperiod was controlled to give 16 hours of daylight and 8 hours of darkness. An initial range-finding experiment was carried out to determine the exposure concentrations for the definitive test. Acetone was used as the solvent for the test solutions, and the experiment included both a control and a solvent control. Concentrations of the test substance were 0, 0.18, 0.56, 1.0, 3.2, 5.6, 10 and 32 mg/ml. Ten daphnia, first instar less than 18 hours old, were placed in each test chamber. Daphnia in all concentrations were observed once every 24 hours for mortality and abnormal effects. Water quality measurements were monitored throughout the testing and were considered adequate and equivalent to those measurements in the control chamber. The LC₅₀ values were obtained by employing the statistical methods described by Litchfield and Wilcoxon (A Simplified Method for Evaluating Dose-Effect Experiments, 1949).

Reference: Monsanto AB-78-1384322-3a, ABC Laboratories, 1978

Reliability: (1) Valid without restriction

*4.3 TOXICITY TO AQUATIC PLANTS, e.g. algae

Species: Selenastrum capricornutum. (Freshwater Green Algae)

Endpoint: Biomass and Growth rate

Exposure period: 96 hours

Results: Chlorophyll a. EC₅₀ (96.h) = 90 mg/l
Cell Count EC₅₀ (96.h) = 126 mg/l
NOEC = 60 mg/l
LOEC = Not determined

Analytical monitoring: No

Method: US EPA Phytotoxicity Method - Algal Assay Procedure: Bottle Test (1971)
closed-system

GLP: No data

Test substance: As prescribed by 1.1-1.4, purity: 95%

Remarks: The test algae were obtained from the US EPA Environmental Research Laboratory in Corvallis, Oregon. Beginning cell numbers in the test flasks were 2.0 x 10⁴ cells/ml. Cultures were incubated at 24°C under approximately 4,000 lux illumination. Triplicate cultures were employed for each of the test concentrations and the control. Test containers were 125ml flasks containing 50ml of test medium. Concentrations for the definitive test were based on the results of a 96-hr range-finding study. These concentrations were 0, 60, 100, 320, 560 and 1000 mg/ml. No solvent was used to prepare test solutions. Instead, the test concentrations were prepared by adding the appropriate amount of weighed test compound to each test flask. There were no water quality measurements reported in this study. The toxicity of the test compound appeared to increase throughout the 96-hour exposure period. Statistical analysis involved converting each test concentration to a logarithm, and the corresponding percentage decrease of in vivo chlorophyll a or cell numbers was converted to

a probit (Finny, 1971). The EC50s and 95% confidence limits were then calculated by linear regression.

Reference: Monsanto BN-78-1384322, EG&G Bionomics 1978
Reliability: (2) Valid with restrictions – no water quality data

5. TOXICITY

*5.1 ACUTE TOXICITY

5.1.1 ACUTE ORAL TOXICITY

Type: LD₅₀
Species/strain: Rats, Sprague-Dawley Albino
Value: 4150 mg/kg bw
Sex: Male and female
of Animals: 20
Vehicle: Corn Oil
Doses: 2510, 3160, 3980 or 5010 mg/kg bw
Method: Single Oral Dose, Younger Laboratories Protocol, 1973
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: >96 %
Remarks: The test material was administered to four groups of male and female rats (5 animals/dose level) as a 25.0% suspension in corn oil. Male rats had initial average body weights of 210-230 grams; females had initial average body weights of 210-230 grams. Clinical signs of toxicity included reduced appetite and activity (three to five days in survivors), followed by increasing weakness, collapse and death. There were no deaths in males at the low and two mid-dose levels, while deaths occurred in females at all dose levels. Surviving animals were sacrificed on Day 14. Gross autopsy findings noted only slight lung congestion in some cases in the survivors; lung and liver hyperemia and gastrointestinal inflammation was noted in the decedents. 95% confidence limits 3940-4360 mg/kg.

<u>Dose mg/kg</u>	<u>Mortalities-Male</u>	<u>Mortalities-Female</u>	<u>Combined</u>
2510	0/2	1/3	1/5
3160	0/3	1/2	1/5
3980	0/2	2/3	2/5
5010	2/3	2/2	4/5

Reference: Monsanto Y-73-191 Younger Laboratories, 1973
Reliability: (2) Valid with restrictions – age of study, lack of method detail

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

Type: LD₅₀
Species/strain: Rabbits, New Zealand Albino
Value: >5010 mg/kg bw
Sex: Male and female
of Animals: 4
Vehicle: Corn Oil

Doses: 3160, 5010 or 7940 mg/kg bw
 Exposure Time: 24 Hours
 Method: Single Dermal Dose, Younger Laboratories Protocol, 1973
 GLP: No data
 Test substance: As prescribed by 1.1-1.4, purity: >96 %
 Remarks: The test substance, as a 40.0% suspension in corn oil, was applied to the shaved skin of three groups of male and female rabbits for 24 hours as single dermal application at dose levels of 3160, 5010 or 7940 mg/kg/body weight. Body weights of males were 2.5-2.6 kg, and females, 2.3 kg. The test material was held in place by means of an occlusive wrap of latex rubber and secured by bandaging and elastic tape. The occlusive wrap was removed after 24 hours and the excess material was wiped from the test animal. Clinical observations were made three times during the first eight hours after dosing, and twice daily thereafter until sacrifice. Clinical signs of toxicity included reduced appetite and activity for two or three days. There were no mortalities at the low and mid-dose levels. Surviving animals were sacrificed on Day 14. Gross autopsy findings on survivors were lung congestion and slight discoloration of the liver and kidneys. Findings on decedents included lung hyperemia, liver discoloration, enlarged gall bladder, discoloration of spleen and kidneys, and gastrointestinal inflammation.

<u>Dose mg/kg</u>	<u>Mortalities-Male</u>	<u>Mortalities-Female</u>	<u>Combined</u>
3160	0/1	----	0/1
5010	0/1	0/1	0/2
7940	1/1	----	1/1

Reference: Monsanto Y-73-191 Younger Laboratories, 1973
 Reliability: (2) Valid with restrictions – age of study, lack of method detail

5.2.1 SKIN IRRITATION/CORROSION

Species/Strain: Rabbits, New Zealand Albino
 Results: Slightly Irritating
 Classification: Not a Primary Skin Irritant
 Method: Draize, J.H., Woodard, G., and Calvery, H.O., 1944
 GLP: No data
 Test substance: As prescribed by 1.1-1.4, purity: >96%
 Remarks: 0.5 grams of the test substance, as a finely ground powder moistened with water, was applied to the shaved dorsal areas of six albino rabbits. The test material was applied to the skin under 1” square gauze patches and held in contact with the skin by means of an occlusive wrap of latex rubber secured by bandaging and elastic tape. The occlusive wrap and gauze patches were removed after 24 hours. Dermal irritation was scored by the Draize Method, and results were recorded 24, 48, 72 and 168 hours after topical application. The Primary Irritation Index was calculated by averaging the mean scores at 24 and 72 hours. The Primary Irritation Index was found to be 0.9 on a scale of 0.0-8.0. All animals scored “0” for edema at each observation period. All animals scored “1” for erythema at 48 hours. Five of six scored “1” after 72 hours, and all scored “0” after 168 hours.

Reference: Monsanto Y-73-191 Younger Laboratories, 1973
Reliability: (2) Valid with restrictions – age of study, lack of method detail

5.2.2 EYE IRRITATION/CORROSION

Species/strain: Rabbits, New Zealand Albino
Results: Slightly Irritating
Classification: Irritating
Method: Draize, J.H., Woodard, G., and Calvery, H.O., 1944
GLP: No data
Test substance: As prescribed in 1.1-1.4, purity: >96%
Remarks: 100 mg of the test substance, as a finely ground powder, was applied to one eye of six albino rabbits. The other eye was not treated and served as a control. The cornea, iris and conjunctiva were examined immediately after treatment, and then at intervals of 10 minutes, 1 hour, and then at 24, 48, 72 and 168 hours. The Draize Method was used for scoring eye irritation. Immediate findings were slight discomfort. At 10 minutes, moderate erythema slight edema and copious discharge were noted. At 1 hour, there was moderate to severe erythema, slight edema and copious discharge. At 24 hours, there was moderate to severe erythema, slight edema, and copious discharge containing a slight whitish exudate. At 48 hours there was gradual improvement, and at 72 hours, all scored 0. The average Draize score for 24, 48 and 72 hours was calculated for each animal and then averaged over the six animals. The average Draize score was 5.0 on a scale from 0-110. All signs of irritation had subsided by the third day after exposure.

Reference: Monsanto Y-73-191 Younger Laboratories, 1973
Reliability: (2) Valid with restrictions – age of study, lack of method detail

*5.4 REPEATED DOSE TOXICITY

Species/strain: Rats, F344N
Sex: Male and Female
Route of Administration: Dietary
Exposure period: 15 days
Frequency of treatment: Daily
Post exposure observation period: No recovery period
Dose: 0, 1000, 2500, 5000, 10000 or 25000 ppm
Males: 0, 95, 235, 335 or 365 mg/kg/day
Females: 0, 85, 220, 325, or 270 mg/kg/day
Control group: Yes
Concurrent no treatment
NOEL: Not determined
LOEL: 1000 ppm
Results: A 15-day toxicity study was run to provide a basis for identifying potential target organs and toxicities, and to assist in setting doses for a 13-week exposure study. Groups of 10 male and 10 female rats were fed diets containing the test compound in a controlled study. During the in-life portion of the study, animals were checked twice daily, at least six hours apart, for mortality and moribundity. Animals were weighed on Day 1, Day 7 and at sacrifice. A complete gross necropsy was performed on all treated

and control animals that either died during the study or were sacrificed. This included an external examination of the animal, including body orifices, and examination and fixation of organs and tissues from treated animals for histopathologic examination. Liver, thymus, right kidney, right testicle, heart, lung weights were recorded for all animals that survived until sacrifice. Histopathologic evaluation was done only on those organs or tissues that showed gross evidence of treatment-related lesions. The corresponding tissues were evaluated in control animals. Approximate doses for rats receiving 25,000 ppm could not be calculated due to early deaths. All 25,000 ppm rats and three male and four female 10,000 ppm rats died. Surviving rats in the 10,000 ppm groups exhibited significant weight loss, and the final mean body weights of the 5000 and 10,000 ppm male and female rats were significantly lower than those of the control animals. Both male and female rats in the three highest dose groups consumed markedly less feed than the controls. Diarrhea occurred in 5000, 10,000 and 25,000 ppm males and females. The principal lesions attributed to administration of the test compound were renal papillary and tubule necroses, which occurred in the 10,000 ppm rats. Focal necrosis or erosions of the glandular stomach also occurred in some, but not all, of the 10,000 ppm rats. Changes observed in the thymus and spleen were attributed to debilitation or stress; bone marrow depletion was attributed to nutrient deficiency accompanying weight loss.

Method: NTP 14-Day Standard Toxicity Study Protocol
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 99%
Reference: TR-435 NTIS# PB95-225751 Battelle Labs, December 1994
Reliability: (1) Valid without restriction

Species/strain: Mice, B6C3F1
Sex: Male and Female
Route of Administration: Dietary
Exposure period: 15 days
Frequency of treatment: Daily
Post exposure observation period: No recovery period
Dose: 0, 1000, 2500, 5000, 10000 or 25000 ppm
Males: 0, 285, 585, 475 mg/kg/day
Females: 0, 360, 950 or 1030 mg/kg/day

Control group: Yes
Concurrent no treatment

NOEL: Not determined

LOEL: 1000 ppm

Results: A 15-day toxicity study was run to provide a basis for identifying potential target organs and toxicities, and to assist in setting doses for a 13-week exposure study. Groups of 10 male and 10 female mice were fed diets containing the test compound in a controlled study. During the in-life portion of the study, animals were checked twice daily, at least six hours apart, for mortality and moribundity. Animals were weighed on Day 1, Day 7 and at sacrifice. A complete gross necropsy was performed on all treated and control animals that either died during the study or were

sacrificed. This included an external examination of the animal, including body orifices, and examination and fixation of organs and tissues from treated animals for histopathologic examination. Liver, thymus, right kidney, right testicle, heart, lung weights were recorded for all animals that survived until sacrifice. Histopathologic evaluation was done only on those organs or tissues that showed gross evidence of treatment-related lesions. The corresponding tissues were evaluated in control animals. Approximate doses for mice receiving 10,000 or 25,000 ppm could not be calculated due to early deaths. All 10,000 and 25,000 ppm mice died, as did eight males and eight females given 5000 ppm. A significant weight loss occurred in the surviving 5000 ppm males and females, and the final mean body weights of 2500 ppm females and 5000 ppm males and females were significantly lower than those of the control groups. Feed consumption by mice given 5000, 10,000 or 25,000 ppm was markedly reduced. Diarrhea occurred in all 25,000 ppm mice and in most, but not all, of both males and females given 5000 or 10,000 ppm. Renal tube necroses occurred in eight males and three females in the 5000 ppm groups. Lymphocytic depletion of lymphoid tissues in many 5000 ppm males and females was attributed to debilitation and stress, or to nutrient deficiency accompanying weight loss.

Method: NTP 14-Day Standard Toxicity Study Protocol
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 99%
Reference: TR-435 NTIS# PB95-225751 Battelle Labs, December 1994
Reliability: (1) Valid without restriction

Species/strain: Rats, F344/N
Sex: Male and Female
Route of Administration: Dietary
Exposure period: 13 Weeks
Frequency of treatment: Daily
Post exposure observation period: No recovery period
Dose: 0, 250, 500, 1000, 2500 or 5000 ppm
Males: 0, 15, 30, 60, 165 or 315 mg/kg/day
Females: 0, 15, 35, 70, 170 or 325 mg/kg/day

Control group: Yes
Concurrent no treatment

NOEL: Not determined

LOEL: 250 ppm

Results: In addition to obtaining toxicological data, the purpose of the study was to determine the doses for this particular strain and species to be used in a 2-year toxicology/carcinogenesis study. Groups of 10 male and 10 female rats were fed the test compound in a controlled study. During the in-life portion of the study, animals were checked twice daily, at least six hours apart, for mortality and moribundity. Animals were weighed on Day 1, Day 7, and then once a week thereafter, and again at sacrifice. Formal clinical observations were performed and recorded weekly. A complete gross necropsy was performed on all treated and control animals that either died during the study or were sacrificed. This included an external examination of the animal, including body

orifices, and examination and fixation of organs and tissues from treated animals for histopathologic examination. Liver, thymus, right kidney, right testicle, heart, lung weights were recorded for all animals that survived until sacrifice. A complete histopathologic evaluation, inclusive of gross lesions, was done on all control animals, all animals in the highest dose group with at least 60% survivors at time of sacrifice, and all animals in higher dose groups inclusive of early deaths and survivors. Chemical-related lesions (target organs) were identified, and these organs plus gross lesions were examined for all lower doses. Only those tissues designated as target tissues and gross lesions were evaluated in lower doses to a no-effect-level. A complete histopathologic evaluation was performed on all natural death/moribund sacrifice animals in lower dose groups. Blood was collected from both sexes. Sperm morphology and vaginal cytology evaluations were performed.

Findings: All rats survived until the end of the study. The final mean body weight of the 5000 ppm males was 40% lower than that of the controls; the final mean body weight of the 5000 ppm females was 27% lower than that of controls. Feed consumption by both 5000 ppm males and females was markedly lower than that of controls throughout the study. The absolute and relative liver weights of 5000 ppm females were significantly greater than those of the controls. Serum alkaline phosphatase (ALP) levels were significantly higher in 2500 and 5000 ppm males, and slightly higher in 5000 ppm females. Serum alanine aminotransferase levels were significantly higher in 2500 and 5000 ppm males and females. Hematocrit and hemoglobin concentrations and mean erythrocyte volume (MCV) were significantly lower in 1000, 2500 and 5000 males than in controls; MCV values were also significantly lower in 5000 ppm females. A dose-related significant increase in forelimb and hindlimb grip strength was observed in exposed male and female rats. Histopathologic findings in the liver of 2500 and 5000 ppm males and females included hypertrophy of Kupffer cells, bile duct hyperplasia, and individual cell necrosis of hepatocytes; centrilobular hepatocyte hypertrophy also occurred in 5000 ppm males and females. Macrophages were increased in size and number in the mesenteric lymph nodes of 5000 ppm males and females, and to a lesser extent in 2500 ppm males and females. Pigmentation and degeneration of the renal cortical tubule epithelial cells was also present in both males and females in the 2500 and 5000 ppm groups. Cortical tubule necrosis occurred in 5000 ppm males and females.

Method:	NTP 13-Week Standard Toxicity Study Protocol
GLP:	Yes
Test substance:	As prescribed by 1.1-1.4, purity: 99%
Reference:	TR-435 NTIS# PB95-225751 Battelle Labs, December 1994
Reliability:	(1) Valid without restriction

Species/strain:	Mice, B6C3F1
Sex:	Male and Female
Route of Administration:	Dietary

Exposure period: 13 Weeks
Frequency of treatment: Daily
Post exposure observation period: No recovery period
Dose: 0, 100, 250, 500, 1000 or 2500 ppm
Males: 0, 15, 30, 65, 145 or 345 mg/kg/day
Females: 0, 10, 35, 60, 165 or 340 mg/kg/day
Control group: Yes
Concurrent no treatment
NOEL: 250 ppm
LOEL: 500 ppm
Results: In addition to obtaining toxicological data, the purpose of the study was to determine the doses for this particular strain and species to be used in a 2-year toxicology/carcinogenesis study. Groups of 10 male and 10 female mice were fed the test compound in a controlled study. During the in-life portion of the study, animals were checked twice daily, at least six hours apart, for mortality and moribundity. Animals were weighed on Day 1, Day 7, and then once a week thereafter, and again at sacrifice. Formal clinical observations were performed and recorded weekly. A complete gross necropsy was performed on all treated and control animals that either died during the study or were sacrificed. This included an external examination of the animal, including body orifices, and examination and fixation of organs and tissues from treated animals for histopathologic examination. Liver, thymus, right kidney, right testicle, heart, lung weights were recorded for all animals that survived until sacrifice. A complete histopathologic evaluation, inclusive of gross lesions, was done on all control animals, all animals in the highest dose group with at least 60% survivors at time of sacrifice, and all animals in higher dose groups inclusive of early deaths and survivors. Chemical-related lesions (target organs) were identified, and these organs plus gross lesions were examined for all lower doses. Only those tissues designated as target tissues and gross lesions were evaluated in lower doses to a no-effect-level. A complete histopathologic evaluation was performed on all natural death/moribund sacrifice animals in lower dose groups. Blood was collected from both sexes. Two unstained blood smears were prepared from the test mice at study termination for micronuclei determinations. Sperm morphology and vaginal cytology evaluations were performed.
Findings: All mice survived to the end of the study. The final mean body weights of the 2500 ppm males, and of 500, 1000 and 2500 ppm females were significantly lower than those of controls. Feed consumption by 2500 ppm males averaged 24% lower than controls through Week 3, and was similar to that of controls for the remainder of the study. Feed consumption by females receiving 2500 ppm averaged 27% less than that of controls during most of the study. The absolute and relative liver weights of males and females at the 2500 ppm level was slightly, but statistically significantly greater than those of controls. Males given 500, 1000 and 2500 ppm and females given 2500 ppm had significantly increased absolute and relative spleen weights. No clinical findings in mice were considered chemical related. Hematocrit concentrations and erythrocyte counts of males given

1000 and 2500 ppm were significantly less than those of controls; hemoglobin concentration and mean erythrocyte volume was significantly less in 2500 ppm males. Females in the 1000 and 2500 ppm groups had significantly decreased hematocrit concentrations and erythrocyte counts: 2500 ppm females also had significantly decreased hemoglobin concentrations and mean erythrocyte volumes. Kupffer cell hypertrophy, bile duct hyperplasia and an increase in size and number of macrophages in mesenteric lymph nodes were present in the 2500 ppm males and females.

Method: NTP 13-Week Standard Toxicity Study Protocol
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 99%
Reference: TR-435 NTIS# PB95-225751 Battelle Labs, December 1994
Reliability: (1) Valid without restriction

Species/strain: Rats, F344/N
Sex: Male and Female
Route of Administration: Dietary
Exposure period: 2 Years
Frequency of treatment: Daily
Post exposure observation period:
Dose: 0, 500, 1000 or 2500 ppm
Males: 0, 20, 40 or 100 mg/kg/day
Females: 0, 20, 45 or 120 mg/kg/day

Control group: Yes
Concurrent no treatment

NOEL: 500 ppm
LOEL: 1000 ppm

Results: Doses selected for this study were based on the lower body weights and liver and kidney toxicity observed in rats given 5000 ppm in the 13-week study. Groups of 115 male and 75 female rats were fed the test compound for 104 weeks. Individual animal body weights of all test animals are recorded on Day 1, and then at 4-week intervals thereafter. During the in-life portion of the study, animals were checked twice daily, at least six hours apart, for mortality and moribundity. Formal examinations for clinical signs of toxicity were made and recorded at 4-week intervals. A complete necropsy was performed on all treated and control animals that either died or were sacrificed. All tissues required for complete histopathology were trimmed, embedded, sectioned and stained with hematoxylin and eosin for histopathologic examination. All animals in all dose groups that died and those that completed the 104-week exposure were subjected to a complete necropsy and slides of all tissues required for a complete histopathologic evaluation were prepared and evaluated. Hematology, clinical chemistry and urinalysis evaluations were performed on 15 male and 15 female rats from each group at 3, 9 and 15 months. Also at 15 months, an additional 10 male and 10 female rats from each group were evaluated for histopathology, hematology and clinical chemistry. 40 male rats per group were evaluated for neurotoxic effects.

Survival, Body Weights, Feed Consumption and Clinical Findings:

Two-year survival rates and mean body weights of exposed male and female rats were generally similar to those of controls. The mean body weights of 2500 ppm males were slightly lower than that of control animals throughout the study. At Week 65, the mean body weight of the 2500 ppm females was 14% lower than that of the controls, but the final mean body weight was only 6% lower than the control group. Feed consumption, behavior, and general health and appearance of exposed males and female rats were similar to those of the controls.

Hematology and Clinical Chemistry:

Results of the hematology evaluation were not uniformly consistent at 3, 9 and 15 months in one set of rats, nor were they consistent between the two sets of rats evaluated at 15 months. Slight, but significant decreases in hematocrit levels, hemoglobin concentrations, and erythrocyte counts were observed in the 1000 and 2500 ppm groups in one set of males at 15 months. Similar significant decreases in hematocrit level and hemoglobin concentration occurred in the 2500 ppm females at 9 months. Mean erythrocyte hemoglobin and mean erythrocyte hemoglobin concentration of 2500 ppm females were also significantly lower than those of controls at 9 months, and in both sets of female rats evaluated at 15 months. Platelet counts were also slightly, but significantly increased in 2500 ppm males in one set evaluated at 15 months, and in 2500 ppm females of the second set evaluated at 15 months. Serum activities of alkaline phosphatase, alanine aminotransferase and sorbitol dehydrogenase in 2500 ppm males were significantly greater than those in controls at 3, 9 and 15 months. Alkaline phosphatase activities in both sets of 1000 ppm males evaluated at 15 months were also significantly greater than those of controls. Serum activities of alanine aminotransferase and sorbitol dehydrogenase in 2500 ppm females were also significantly greater than those of controls at 3, 9 and 15 months.

Neurotoxicity Findings:

There were no significant inhibitory effects in dose animals on motor nerve excitability or conduction, neuromuscular transmission or muscle contractility. There were no microscopic lesions in the sciatic nerve, quadriceps muscle, or teased nerve preparations of sciatic nerve that could be attributed to administration of the chemical.

Pathology Findings:

At the 15-month interim evaluation, the absolute and relative liver weights of the 2500 ppm female rats were significantly greater than those of controls. At 15 months, and at the end of the study, the incidences of Kupffer cell hypertrophy, hepatocyte cytoplasmic vacuolization, and mixed cell foci were also significantly increased. At the end of the study, the incidence of hepatocellular fatty change was significantly increased in 2500 ppm females. The incidence of Kupffer cell hypertrophy was significantly increased in 2500 ppm males at 15 months and at 2 years. The incidence of cytoplasmic vacuolization was significantly increased in all exposed males at 15 months, but only moderately increased in 1000 and 2500 ppm males at 2 years. The incidence

of basophilic foci was significantly increased in 2500 ppm males at 15 months, and the incidence of mixed cell foci was significantly increased in the 1000 and 2500 ppm males at 2 years. The incidences of hepatocellular adenoma or carcinoma (combined) in exposed male rats were not significantly greater than that in the controls [0 ppm, 1/50; 500 ppm, 3/50, 1000 ppm, 3/50; 2500 ppm, 5/49], were within the historical control range, and were not considered chemical related. The severity of nephropathy was significantly increased in the 2500 ppm female rats. There was a significant negative trend in the instances of mammary gland fibroadenoma, adenoma, or carcinoma (combined) in female rats [0 ppm, 32/50; 500 ppm, 24/50; 1000 ppm, 11/50; 2500 ppm, 16/50], and the incidences of fibroadenoma in the 1000 and 2500 ppm females were significantly less than controls.

CONCLUSIONS: NO EVIDENCE of carcinogenic activity in male or female F344/N rats at the study dose levels.

Method: NTP Toxicology and Carcinogenesis Standard Study
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 99%
Reference: TR-435 NTIS# PB95-225751 Battelle Labs, December 1994
Reliability: (1) Valid without restriction

Species/strain: Mice, B6C3F1
Sex: Male and Female
Route of Administration: Dietary
Exposure period: 2 Years
Frequency of treatment: Daily
Post exposure observation period:
Dose: 0, 250, 500 and 1000 ppm
Males: 0, 30, 60 or 145 mg/kg/day
Females: 0, 45, 110 or 255 mg/kg/day
Control group: Yes
Concurrent no treatment
NOEL: Not Determined
LOEL: 250 ppm
Results: Doses selected for this study were based on the lower body weights, the increase in liver and spleen weights, and the accompanying histopathologic changes in the liver of 2500 ppm male and female in the 13-week study. Groups of 80 male and 80 female mice were fed the test compound for 104 weeks. Individual animal body weights of all test animals are recorded on Day 1, and then at 4-week intervals thereafter. During the in-life portion of the study, animals were checked twice daily, at least six hours apart, for mortality and moribundity. Formal examinations for clinical signs of toxicity were made and recorded at 4-week intervals. A complete necropsy was performed on all treated and control animals that either died or were sacrificed. All tissues required for complete histopathology were trimmed, embedded, sectioned and stained with hematoxylin and eosin for histopathologic examination. All animals in all dose groups that died and those that completed the 104-week exposure were subjected to a complete necropsy and slides of all tissues required for a complete histopathologic evaluation were prepared and

evaluated. Hematology, clinical chemistry and urinalysis evaluations were performed on nine or ten animals from each group at 3, 9 and 15 months.

Survival, Body Weights, Feed Consumption and Clinical Findings:

Two-year survival rates of exposed male and female mice were similar to those of the controls. The final mean body weights of male and female mice exposed to 1000 ppm were 8% and 18% lower than those of the controls, respectively. The final mean body weights of females exposed to 250 or 500 ppm were 8-9% lower than those of the controls. Feed consumption by exposed males was similar to that by controls, and there were no clinical findings attributed to administration of the test compound.

Hematology and Clinical Chemistry:

Hematocrit level, hemoglobin concentration, and erythrocyte count in 1000 ppm male mice were significantly lower than those in controls as the 15-month interim evaluation. Serum alkaline phosphatase activities in 1000 ppm males was slightly but significantly greater than those in controls at 3 and 9 months, as was the serum alkaline phosphatase activity in 1000 ppm females at 9 months. Serum levels of total bilirubin in all groups of exposed males were significantly greater than those in controls at 9 and 15 months.

Pathology Findings:

In the liver of male mice, negative trends in the incidences of fatty change, clear cell foci, and adenoma or carcinoma combined occurred at the end of the 2-year study. There were no test compound-related increased incidences of neoplasms or non-neoplastic lesions in mice receiving the test compound for 2 years. A negative trend in the incidence of fatty change in the liver of male mice also occurred at 15 months.

CONCLUSIONS: NO EVIDENCE of carcinogenic activity in male or female B6C3F1 mice at the study dose levels.

Method: NTP Toxicology and Carcinogenesis Standard Study
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 99%
Reference: TR-435 NTIS# PB95-225751 Battelle Labs, December 1994
Reliability: (1) Valid without restriction

***5.5 GENETIC TOXICITY IN VITRO**

A. BACTERIAL TEST

Type: Ames Bacterial Reverse Mutation Assay
System of testing: Salmonella typhimurium TA-1535, TA-1537, TA-1538, TA-98, TA-100
Concentration: 0.1 to 500 micrograms/plate
Metabolic activation: With and without
Results:
Cytotoxicity conc: With metabolic activation: 500 ug/plate
Without metabolic activation: 100 ug/plate
Precipitation conc: None
Genotoxic effects:
With metabolic activation: Negative

Without metabolic activation: Negative

Method: Ames Mutagenicity Plate Assay 1975 OECD 471 Equivalent

GLP: No data

Test substance: As prescribed by 1.1-1.4, purity: 99%

Remarks: The test compound was evaluated for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations. The *Salmonella typhimurium* strains used for this experiment were obtained from Dr. Bruce Ames. The activation system used was S-9 homogenate from Aroclor 1254-induced adult male Sprague-Dawley rat livers. The metabolizing system contained 10% S-9 and cofactors according to the Ames method. The mutagenesis assay was carried out as the plate-incorporation test according to the Ames protocol. Chemicals used as positive controls for the non-activation assays were methylnitrosoguanidine (MNNG), 2-nitrofluorene (NF) and quinacrine mustard (QM). Positive control chemicals used for the activation assays were 2-anthramine (ANTH), 2-acetylaminofluorene (AAF) and 8-aminoquinoline (AMQ). Dimethylsulfoxide (DMSO) was used as the solvent and the solvent control. Statistical analysis included Bartlett's test for homogeneity of variance, and comparison of treatments with controls using within-levels pooled variance and a one-sided t-test. Grubbs' test was performed to determine if outliers were present. The test compound did not demonstrate mutagenic activity in any of the assays conducted and was considered not mutagenic under the test conditions.

Reference: Monsanto BIO-76-236 Litton Bionetics December 30, 1976

Reliability: (1) Valid without restriction

B. NON-BACTERIAL IN VITRO TEST

Type: Mammalian Chromosome Aberration Test

System of testing: Chinese hamster lung (CHL/IU) cells

Concentration: 1.25, 2.5 and 5.0 mg/ml (duplicate)

Metabolic activation: With and without

Results:

 Cytotoxicity conc: With metabolic activation: 5.0 mg/ml
 Without metabolic activation: 5.0 mg/ml

 Precipitation conc: None

 Genotoxic effects:

 With metabolic activation: Negative

 Without metabolic activation: Negative

Method: OECD 473 In vitro Mammalian Chromosome Aberration Test, 1997

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: >97%

Remarks: The CHL/IU cells were obtained from the Japanese Cancer Resources Bank. Cells were cultured with Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum. The 2x10⁴ cells were plated in 5ml medium on 60mm plate and cultured for 72 hours at 37°C in a humidified incubator before treatment. The test substance was dissolved in DMSO. The proliferating cells were treated with the test substance for 6 hours in serum-free MEM with and without S9 mix, then cultured an

additional 18 hours in fresh MEM with serum. S9 and co-factors were mixed immediately before use and then applied to the cultures to expose the cells to the S9 mix at 5% (v/v). Cells were also treated for either 24 or 48 hours continuously, in the absence of S9 mix. Duplicate cultures were used for each dose. A preliminary growth inhibition test was conducted to determine the cytotoxicity of the test chemical. A maximum dose was set for the chromosome aberration test at 50% or more of the cytotoxic dose determined by the growth inhibition test. As cytotoxicity was 50% or less, 5 mg/ml was set as the maximum dose, with the lesser doses as sequential half dilutions. Before harvest, the cells were treated with 0.1 ug/ml of colcemid for 2 hours, then chromosome specimens were made by the standard air-dry method. Chromosome specimens were stained with 3% Giemsa solution for 8 minutes. The number of cells with chromatid- and chromosome-type breaks were scored per 200 cells at each dose level. Polyploid cells were also scored per 800 cells at each dose. Results: The test substance did not induce chromosome aberration at any dose or time, with or without activation. There was no induction of polyploidy, suggesting that treatment did not affect the mitotic apparatus.

Reference: H. Kusakabe et. al., Mutation Research 517, 187-198, 2002
Reliability: (1) Valid without restriction

Type: Mitotic Recombination Assay
System of testing: Saccharomyces cerevisiae Strain D4
Concentration: 0.1 to 500 micrograms/plate
Metabolic activation: With and without
Results:
 Cytotoxicity conc: With metabolic activation: Not toxic
 Without metabolic activation: Not toxic
 Precipitation conc: None
 Genotoxic effects:
 With metabolic activation: Negative
 Without metabolic activation: Negative
Method: Ames Mutagenicity Plate Assay 1975 OECD 471 Equivalent
GLP: No data
Test substance: As prescribed by 1.1-1.4, purity: 95%
Remarks: The test compound was evaluated for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations. The activation system used was S-9 homogenate from Aroclor 1254-induced adult male Sprague-Dawley rat livers. The metabolizing system contained 10% S-9 and cofactors according to the Ames method. The mutagenesis assay was carried out as the plate-incorporation test according to the Ames protocol. The chemical used as the positive control for the non-activation assay was methylnitrosoguanidine (MNNG) at 10 ug/plate. Positive control chemical used for the activation assay was DMNA at 100 micromoles/plate. Dimethylsulfoxide (DMSO) was used as the solvent and the solvent control. Statistical analysis included Bartlett's test for homogeneity of variance, and comparison of treatments with controls using within-levels pooled variance and a

one-sided t-test. Grubbs' test was performed to determine if outliers were present. The test compound did not demonstrate mutagenic activity in any of the assays conducted and was considered not mutagenic under the test conditions.

Reference: Monsanto BIO-76-236 Litton Bionetics December 30, 1976
Reliability: (1) Valid without restriction

* 5.6 GENETIC TOXICITY IN VIVO

Type: Mammalian Bone Marrow Chromosomal Aberration Test
Species/strain: Rat, Fisher 344
Sex: Male and Female
Route of Administration: Oral gavage in corn oil vehicle
Exposure period: 6, 18 and 30 Hours
Doses: 700 mg/kg and 1400 mg/kg
Results:
 Effect on mitotic index or P/N ratio: No effect
 Genotoxic effects: Negative
Method: In vivo Bone Marrow Cytogenetics Rat Metaphase Analysis 1981 OECD 475 Equivalent
GLP: Yes
Test substance: As prescribed by 1.1-1.4, purity: 99%
Remarks: The test compound was evaluated in a preliminary study at doses of 1500 and 2075 mg/kg bw. Due to the pharmacotoxic signs observed at 1500 mg/kg, and the mortalities occurring at 2075 mg/kg, 1400 mg/kg was selected as the maximum tolerated dose. An additional dose level of 700 mg/kg was also evaluated as one-half of the maximum tolerated dose. In the definitive test, 65 adult male and 65 adult female rats (5 male and 5 female rats/group) were dosed with the test article in a controlled study. All animals exhibited decreased body tone, diarrhea, abnormal gait, piloerection and brown discoloration around the oral-nasal region and forepaws. The pharmacotoxic signs indicated that the test article was at or near the maximum tolerated dose. Animals from each group and dose level were sacrificed at 6, 18 and 30 hours after dosing. Control groups received either 10 ml/kg bw of vehicle control (corn oil), or 20 mg/kg bw of the positive control cyclophosphamide (CP). Two to three hours prior to sacrifice, each animal was given a single intraperitoneal dose of colchicine at 4 mg/kg bw to arrest dividing cells in metaphase. Bone marrow was sampled at 6, 24 and 48 hours after dosing with the vehicle or the test substance. A single sampling time of +24 hours was used for the positive control group. A total of 500 (if possible) well spread, intact metaphase cells were scored for the presence of chromosome aberration per experimental treatment point (50/animal) by two investigators (25 each/animal). Slides were scored for increases in the proportion of aberrant metaphases by Chi-square analysis and in the frequency of aberrations/cell by a one-way analysis of variance (ANOVA). No statistically significant increases in the proportion of aberrant cells or aberrations/cell were observed at the 6, 24 and 48 hour time points. No statistically significant differences from the vehicle controls were detected by this analysis in animals treated with the

test compound. The positive control group (CP) yielded the expected positive responses, indicating the adequacy of the experimental test conditions for the detection of clastogens. The test compound was judged negative in its ability to induce structural chromosomal aberrations to the hemopoietic cells of the rat bone marrow under the experimental conditions of this assay.

Reference: Monsanto PK-87-344 Pharmakon Research June 10, 1988
 Reliability: (1) Valid without restriction

***5.8 TOXICITY TO REPRODUCTION**

Type: Other
 Species/strain: Rats, Fisher 344
 Sex: Male and Female
 Route of Administration: Dietary
 Exposure period: 2 Years
 Frequency of treatment: Daily
 Post exposure observation period: None
 Premating exposure period: Not applicable
 Duration of the test:
 Doses: 2500 ppm
 Control group: Yes
 Concurrent no treatment
 Results: See Remarks section
 Method: NTP Toxicology and Carcinogenesis Study
 GLP: Yes
 Test substance: As prescribed by 1.1-1.4, purity: 99%
 Remarks: Adequate repeat dose studies that demonstrate no effects on reproductive organs, in particular the testes, can be considered as an adequate test for reproductive/developmental effect. Target Organs and Levels of Evidence for NTP Technical Report Number 435 data indicates examination of the reproductive organs of the female rats (Clitoral Gland, Ovary, Uterus, Vagina) and male rats (Epididymus, Preputial Gland, Prostate, Seminal Vesicle, Testes) showed no statistical effects from the test article.

Fischer 344 rats, Female, dose = 2500 ppm for 2 years

Genital System	
Clitoral Gland	49
Adenoma	1 (2%)
Carcinoma	2 (4%)
Ovary	50
Uterus	50
Polyp Stromal	9 (18%)
Sarcoma Stromal	1 (2%)

Fischer 344 rats, Male, dose = 2500 ppm for 2 years

Genital System	
Epididymis	49
Preputial gland	49
Adenoma	4 (8%)
Prostate	48
Carcinoma, Metastatic, Kidney	1 (2%)
Seminal Vesicle	48

Testes 49

Bilateral, Interstitial Cell, Adenoma 31 (63%)

Interstitial Cell, Adenoma 13 (27%)

Reference: TR-435 NTIS# PB95-225751 Battelle Labs, December 1994

Reliability: (2) Valid with restrictions - adequate repeat dose studies that demonstrate no effect on reproductive organs

*5.9 DEVELOPMENTAL TOXICITY/ TERATOGENICITY

Species/strain: Rabbits, New Zealand White

Sex: Female

Route of Administration: Oral gavage in corn oil vehicle

Duration of the test: 13 Days

Exposure period: Days 6-18 of gestation

Frequency of treatment: 1x/day

Doses: 0, 0.2, 2.0 or 20.0 mg/kg/day

Control group: Yes

Concurrent vehicle

NOEL Maternal Toxicity: 0.2 mg/kg/day

NOEL teratogenicity:

Results: Groups of 13 female rabbits were dosed with the above levels of the test article and observed for general appearance, behavior, weight gain and food intake during the life phase of the study. Fetuses were delivered via cesarean section following sacrifice and observed for visceral abnormalities and skeletal anomalies. Maternal general toxicity: Clinical signs of toxicity were anorexia, marked weight loss and abortion in one animal at 2.0 mg/kg/day and in four animals at 20.0 mg/kg/day. Rabbits at the two lowest dose levels exhibited mild decreased weight gains. Rabbits at the highest dose level exhibited weight loss. Pregnancy/litter data: Five animals (1/13 at 2 mg, 4/13 at 20 mg) experienced total litter loss. Litter size was reduced in the high dose animals. If animals with total litter loss are included, the incidence of embryonic death was markedly increased at the high dose level. Foetal data: The incidence of visceral abnormalities was higher at 20.0 mg/kg than in controls, and the pups at this dose level had a slightly higher incidence of skeletal anomalies, but these differences were judged to be not statistically significant (p>0.05).

Method: OECD 421 Repro/Tox Screening Test equivalent

GLP: Yes

Test substance: As prescribed by 1.1-1.4, purity: >95%

Remarks: TOXLINE citation

Reference: Proctor & Gamble Co. Huntingdon Research Center Ltd. 1992

Reliability: (4) Not assignable - data from a secondary literature source

5.10 OTHER RELEVANT INFORMATION

A. Specific toxicities

Type: Neurotoxicity

Results: From the NTP 2-year feeding study on 115 male and 75 female F334/N.rats, there were no significant inhibitory effects of TBBC on motor nerve excitability or conduction, neuromuscular transmission or muscle contractility. There were no microscopic lesions in the sciatic nerve, quadriceps muscle or teased nerve preparations of sciatic nerve that could be attributed to the test article

Remarks: NTP Study

Reference: TR-435 NTIS# PB95-225751 Battelle Labs, December 1994

Reliability: (1) Valid without restriction

Type: Reproductive Hazards Screening

Results: 4,4'-Thiobis(6-tert-butyl-m-cresol) was screened for the potential to cause reproductive effects using a postnatal mouse screening test. Experiments were designed to determine the appropriate dose level, and the reproductive effects were studied. The predicted median lethal dose level for the test article was determined to be 485 mg/kg/day. All animals received a constant volume of 10 ml/kg/day. The test article caused an increase in maternal mortality and a decreased percentage of surviving pups. There was no effect on the number of viable litters, litter size, birth weight, or the weight gain of pups.

Remarks: NIOSH-sponsored test

Reference: Environmental Health Research and Testing, 1989

Reliability: (4) Not assignable - data from a secondary literature source

Type: Immunotoxicity – Human Skin Patch

Results: TBMC was one of 13 common commercial antioxidants tested on patients who exhibited symptoms of rubber allergy and/or contact dermatitis. No positive responses to the test article were noted at concentrations of 0.1%, 1% and 10%.

Remarks: None

Reference: Toho University, Tokyo Japan, Toho Igakkai Zasshi 1999

Reliability: (4) Not assignable - data from a secondary literature source

Type: Immunotoxicity – Repeated Insult Skin Patch

Results: Patch tests were conducted on 50 human volunteers. The test material was applied to linteen discs and taped to the subjects' upper arms with Blenderm tape. After 24 hours, the patches were removed and the reactions graded and recorded. After a 24-hour rest period, the process was repeated until 15 successive patches had been applied. A two-week rest period followed, and then a challenge application was made to the same site. There were no reactions produced by any of the primary applications or by the challenge application. The test article was judged as neither a primary irritant nor a skin fatiguing agent. There was no evidence of skin sensitization.

Remarks: Shelanski and Shelanski Method

Reference: MonsantoSH-66-7 Industrial Biology Laboratories, August 1966

Reliability: (1) Valid without restriction

B. Toxicodynamics, toxicokinetics

Type: Absorption, distribution, metabolism and excretion in rats

Results: Metabolic fate of C14-labeled TBBC was studied in male rats. Oral treatment showed a dose-related decrease in the rate of absorption due to a dose-related increase in stomach retention time. The test article was

completely absorbed after oral treatment and rapidly distributed throughout the body, with the liver being the major tissue depot. Significant accumulations of the test article were also present in blood, muscle, skin and adipose tissue. The test article was rapidly cleared from all tissue except adipose, although a small percentage of the total dose tended to persist in liver and skin. >50% was excreted on Day 1, primarily via bile into feces. Little of the C14 labeled compound was detected in the urine. Metabolites of the test article were detected in tissues shortly after administration, but all were rapidly excreted. The major metabolites were identified as glucuronide conjugates of the test article.

Remarks: None
References: National Institute of Environmental Health Sciences 1983
Reliability: (4) Not assignable - data from a secondary literature source

Type: Extractability/Migration from plastics
Results: The migration of antioxidants in packaging materials or utensils made from polystyrene and polypropylene was studied using the following pilot foods: Water, 3% Acetic Acid, 15% Ethanol, 50% Ethanol, Heptane and Sunflower Seed Oil. The conditions of exposure were 200 cm²/250 ml test solution for 10 days at 45°C. For the polystyrene compounds containing a maximum of 0.5% 4,4'-Thiobis(6-tert-butyl-m-cresol), there was little tendency to migrate to the water, acid, 15% alcohol or vegetable oil. For polypropylene, major amounts of the test article was extracted by the vegetable oil. Migration amounts were also high with heptane.

Remarks: TBMC is approved for use in several food-contact applications
References: Zentralinst. Ernaehr., Dtsch. Akad. Wiss. Berlin, Germany 1971
Reliability: (4) Not assignable - data from a secondary literature source

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I U C L I D

D a t a S e t

Existing Chemical ID: 7786-17-6
EINECS Name 2,2'-methylenebis(6-nonyl-p-cresol)
EINECS No. 232-092-5
Molecular Formula C33H52O2

Producer Related Part
Company: Epona Associates, LLC
Creation date: 04-DEC-2001

Substance Related Part
Company: Epona Associates, LLC
Creation date: 04-DEC-2001

Printing date: 06-DEC-2001
Revision date:
Date of last Update: 06-DEC-2001

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Chapter (profile): Chapter: 2.1, 2.2, 2.4, 2.5, 2.6.1, 3.1.1, 3.1.2, 3.3.1,
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5.6, 5.8, 5.9

Reliability (profile): Reliability: without reliability, 1, 2, 3, 4
Flags (profile): Flags: without flag, confidential, non confidential, WGK
(DE), TA-Luft (DE), Material Safety Dataset, Risk
Assessment, Directive 67/548/EEC, SIDS

2. Physico-chemical Data

2.1 Melting Point

Value: = 251.8 degree C
Method: other
GLP: no
Testsubstance: other TS
Test substance: Phenol, 2,2'-methylenebis 4-methyl-6-nonyl-
04-DEC-2001 (1)

2.2 Boiling Point

Value: = 584 degree C
Method: other
GLP: no
Testsubstance: other TS
Test substance: Phenol, 2,2'-methylenebis 4-methyl-6-nonyl-
04-DEC-2001 (1)

2.4 Vapour Pressure

Value: = .8332648 hPa at 25 degree C
Method: other (calculated)
GLP: no
Testsubstance: other TS
Test substance: Phenol, 2,2'-methylenebis 4-methyl-6-nonyl-
04-DEC-2001 (1)

2.5 Partition Coefficient

log Pow: = 13.1
Method:
Year:
GLP: no
Testsubstance: other TS
Test substance: Phenol, 2,2'-methylenebis 4-methyl-6-nonyl-
06-DEC-2001 (1)

2.6.1 Water Solubility

Value: = 0 mg/l at 25 degree C
Method: other
GLP: no
Testsubstance: other TS

Test substance: Phenol, 2,2'-methylenebis 4-methyl-6-nonyl-
04-DEC-2001

(1)

- 1/6 -

Date: 06-DEC-2001
ID: 7786-17-6

3. Environmental Fate and Pathways

3.1.1 Photodegradation

Type: air
DIRECT PHOTOLYSIS
Halflife t1/2: = 1.9 hour(s)
Method:
Year: GLP: no
Test substance: other TS
Test substance: Phenol, 2,2'-methylenebis 4-methyl-6-nonyl-
04-DEC-2001

(1)

3.1.2 Stability in Water

-

3.3.1 Transport between Environmental Compartments

Type: fugacity model level III
Media:
Air (Level I):
Water (Level I):
Soil (Level I):
Biota (L.II/III):
Soil (L.II/III):
Method: other
Year:
Result: Air 0.0911 %, 3.85 hr half-life, 1000 kg/hr
Sediment 67.3%, 3.6E+3 hr half-life, 1000 kg/hr
Soil 29.2%, 900 hr half-life, 1000 kg/hr
Water 3.39%, 900 hr half-life, 1000 kg/hr

04-DEC-2001

(1)

Date: 06-DEC-2001
ID: 7786-17-6

3. Environmental Fate and Pathways

3.5 Biodegradation

Type:

Inoculum:

Degradation: =

Method:

Year:

GLP: no

Test substance: other TS

Result: BIOWIN (v3.67) Program Results:

```
=====
SMILES : Oc(c(cc(c1)C)Cc(c(O)c(cc2C)CCCCCCCC)c2)c1CCCCCCCC
CHEM   : Phenol, 2,2'-methylenebis 4-methyl-6-nonyl-
MOL FOR: C33 H52 O2
MOL WT : 480.78
```

----- BIOWIN v3.67 Results

```
Linear Model Prediction      : Biodegrades Fast
Non-Linear Model Prediction: Biodegrades Fast
Ultimate Biodegradation Timeframe: Weeks-Months
Primary Biodegradation Timeframe: Days-Weeks
```

Test substance: Phenol, 2,2'-methylenebis 4-methyl-6-nonyl-

05-DEC-2001

(1)

4. Ecotoxicity

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

-

4.2 Acute Toxicity to Aquatic Invertebrates

-

4.3 Toxicity to Aquatic Plants e.g. Algae

-

5. Toxicity

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

-

5.1.2 Acute Inhalation Toxicity

-

5.1.3 Acute Dermal Toxicity

-

5.1.4 Acute Toxicity, other Routes

-

5.4 Repeated Dose Toxicity

-

5.5 Genetic Toxicity 'in Vitro'

-

5.6 Genetic Toxicity 'in Vivo'

-

5.8 Toxicity to Reproduction

-

5.9 Developmental Toxicity/Teratogenicity

-

- 5/6 -

Date: 06-DEC-2001
ID: 7786-17-6

6. References

(1) EPIWIN

