PHYSICAL/CHEMICAL ELEMENTS

1. MELTING POINT

Test substance:	1,6-Hexamethylene bis (3,5-di-(tert)-butyl-4-hydroxyhydrocinnamate) CAS No. 35074-77-2
Method:	Not reported
GLP:	No
Year:	1989
Results:	104 - 108 °C
Remarks:	The melting point as reported in the MSDS from Ciba Specialty Chemicals Corp. The melting point was assigned a reliability code of 2g[1] (data from Handbook or collection of data).
References:	[1] Klimisch, H.J., Andreae, M and Tillman, U., A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

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References:	[1] Klimisch, H.J., Andreae, M and Tillman, U., A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

2. BOILING POINT

Test substance:	1,6-Hexamethylene bis (3,5-di-(tert)-butyl-4-hydroxyhydrocinnamate) CAS No. 35074-77-2
Method:	Since it is a solid, boiling point is estimated by the MPBPWIN Program (v. 1.40) using the adapted Stein and Brown Method).[1,2]
GLP:	No
Year:	2001
Results:	654.41 °C
Remarks:	In the absence of reliable experimental data, the boiling point was calculated using an accepted method and assigned a reliability code of 2f [3] (Accepted calculation method).
References:	[1]Syracuse Research Corporation, Syracuse, NY [2]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998. [3]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

3. VAPOR PRESSURE

Test substance:	1,6-Hexamethylene bis (3,5-di-(tert)-butyl-4-hydroxyhydrocinnamate) CAS No. 35074-77-2
Method:	Estimated by the MPBPWIN Program (v. 1.40) using the modified Grain method.[1, 2]
GLP:	No
Year:	2001
Results:	1.75×10^{-17} mm Hg
Remarks:	A vapor pressure of 1×10^{-10} mm Hg was reported in the MSDS from Ciba Specialty Chemicals Corp. Both estimates confirm that the substance has a very low vapor pressure. The vapor pressure was assigned a reliability code of 2f [3] (Accepted calculation method).
References:	[1]Syracuse Research Corporation, Syracuse, NY [2]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998 [3]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

4. PARTITION COEFFICIENT

Test substance:	1,6-Hexamethylene bis (3,5-di-(tert)-butyl-4-hydroxyhydrocinnamate) CAS No. 35074-77-2
Method:	KOWWIN Program (v. 1.66). [1, 2]
GLP:	No
Year:	2001
Results:	Log Kow > 11.74
Remarks:	The MSDS from Ciba Specialty Chemicals Corp. reported a partition coefficient of 6.0, but the method of determination was not provided. In the absence of this information, the partition coefficient was calculated using an accepted method. The estimate was assigned a reliability code of 2f [3] (Accepted calculation method).
References:	[1]Syracuse Research Corporation, Syracuse, NY [2]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998. [3]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

5. WATER SOLUBILITY

Test substance:	1,6-Hexamethylene bis (3,5-di-(tert)-butyl-4-hydroxyhydrocinnamate) CAS No. 35074-77-2
Method:	WSKOW v1.37 [1, 2]
Temperature:	20 °C
GLP:	No
Year:	2001
Results:	3.30×10^{-7} mg/L
Remarks:	The Ciba MSDS reported a water solubility of <0.01% at 20 °C (< 100 mg/L). In the absence of information at lower concentrations the calculated value is used. The water solubility calculated by an accepted method is assigned a reliability code of 2f [3] (Accepted calculation method).
References:	[1]Syracuse Research Corporation, Syracuse, NY [2]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998. [3]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

ENVIRONMENTAL FATE AND PATHWAYS ELEMENTS

6. PHOTODEGRADATION

Test substance:	1,6-Hexamethylene bis (3,5-di-(tert)-butyl-4-hydroxyhydrocinnamate) CAS No. 35074-77-2
Method:	Estimated by the AOP program (v. 1.87). [1,2] This model estimates rate constants and half-lives of atmospheric reactions of organic compounds with hydroxyl radicals and ozone in the atmosphere.
GLP:	No
Year:	2001
Results:	For reaction with hydroxyl radicals, the predicted half-life of the chemical was rapid. Rate constant: 47.0×10^{-12} cm^3/molecule-sec Half-life: 2.73 h
Remarks:	In the absence of reliable experimental data, the photodegradation was calculated using an accepted method and assigned a reliability code of 2f [3] (Accepted calculation method).
References:	[1]Syracuse Research Corporation, Syracuse, NY [2]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998 [3]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

7. STABILITY IN WATER

Test substance:	1,6-Hexamethylene bis (3,5-di-(tert)-butyl-4-hydroxyhydrocinnamate) CAS No. 35074-77-2
Method:	The HYDROWIN Program (v. 1.67) [1,2]
GLP:	No
Year:	2001
Results:	The HYDROWIN Program was unable to evaluate this chemical structure. Due to its low solubility, it is impractical to experimentally determine hydrolysis.

References:

[1]Syracuse Research Corporation, Syracuse, NY

[2]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998.

[3]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

8. THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

Test substance:	1,6-Hexamethylene bis (3,5-di-(tert)-butyl-4-hydroxyhydrocinnamate) CAS No. 35074-77-2
Method:	Estimated by EPIWIN Level III Fugacity Model.[1, 2]
Year:	2001
GLP:	No
Results:	Distribution using EQC Level III Fugacity Model

Air	0.0118 %
Water	1.1 %
Soil	41 %
Sediment	57.9 %

Persistence Time = 677 h

Remarks: In the absence of reliable experimental data, the fugacity was calculated using an accepted method and assigned a reliability code of code of 2f [3] (Accepted calculation method).

References:

[1]Syracuse Research Corporation, Syracuse, NY

[2]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998.

[3]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

9. BIODEGRADATION

Test substance:	1,6-Hexamethylene bis (3,5-di-(tert)-butyl-4-hydroxyhydrocinnamate) CAS No. 35074-77-2
Method:	This study was conducted under OECD Guideline 301 B "Ready Biodegradability: Modified Sturm Test (CO_2 Evolution)," 1981. Bacteria was collected from activated sludge of a sewage treatment plant. The preparation was carried out according to the guidelines, with the following exceptions: (1) the volume of test solution was reduced from 3 to 1.5 L**.** (2) an emulsifier, nonylphenol 10EO5PO, was added to enhance the solubility of the test material.[1]
Test Type:	Aerobic
Concentration of the chemical:	Test chemical: 10 mg/ L and 20 mg/ L. Reference chemical: aniline (Merck No.1261): 20 mg/ L
Vehicle:	Water as specified in the guideline containing 0.5 ml of the nonylphenol 10EO5PO solution.
Blank:	Water as specified in the guideline.
Inoculum:	Fresh sewage treatment plant sample (per guideline)
Medium:	Sewage sludge (per guideline)
GLP:	No
Year:	1989
Results:	Test chemical: 10 mg/L: 1 % degradation in 28 days 20 mg/L: 1 % degradation in 28 days Reference substance: 20 mg/L: 87.2 % in 28 days. Under the test conditions, no biodegradation was observed.
Conclusion:	Substance was not readily biodegradable according to OECD definition.
Remarks:	This study was assigned a reliability code of 2b [2] (guideline study with acceptable restrictions).
Reference:	[1]Report on the test for ready biodegradability of TK 10019 in the modified Sturm test, Ciba-Geigy Ltd., Basle, Switzerland, October 01, 1989. [2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

ECOTOXICITY ELEMENTS

10. ACUTE TOXICITY TO FISH

Test substance:	1,6-Hexamethylene bis (3,5-di-(tert)-butyl-4-hydroxyhydrocinnamate) CAS No. 35074-77-2
Method:	This study was conducted under OECD Guideline No. 203 (Paris, 1984). This study was performed as a limit test with a concentration of 100 mg/L (nominal). Glass aquarium (20 L) was filled with dechlorinated tap water. Fluorescent light was used for 16 hours daily. Oxygen, pH, temperature were measured daily. The exposed group consisted of 20 fish (in 2 tanks) and the control group consisted of 10 fish. [1]
Type of test:	Static
Species:	Zebra fish (*Brachydanio rerio*)
Length:	36 mm (23-28 mm)
Weight:	0.15 g (0.12 - 0.19 g)
Loading:	0.06 g/L
Exposure period:	96 h
Test Concentrations:	100 mg/ L
Controls:	Blank: water Vehicle: 91 mg DMF/ L water in the concentration used for the test concentration.
Analytical monitoring:	No
GLP:	No
Year:	1988
Results:	LC_0 (96 h) in test > 100 mg/L (nominal) LC_{50} (96 h) in test > 100 mg/L (nominal) Mortalities in Blank and Vehicle: 0% Mortalities in Treatment Group: 0%
Remarks:	This study was assigned a reliability code of 2b[2] (guideline study with acceptable restrictions).

Reference:

[1]Report on the test for acute toxicity of TK 10019 to zebra fish, Project No. 884461, Ciba-Geigy Ltd., Basel, Switzerland, December 2, 1988.

[2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

11. TOXICITY TO AQUATIC PLANTS

Test substance:	1,6-Hexamethylene bis (3,5-di-(tert)-butyl-4-hydroxyhydrocinnamate) CAS No. 35074-77-2
Method:	This study was conducted under test guideline: 87/302/EEC page 89-94, Algal growth inhibition test. The static *scenedesmus subspicatus* toxicity screen was conducted in 100-mL Erlenmeyer flasks containing 50 mL of algae nutrient media or test solution. Each test level and the controls were prepared in triplicate. The water quality parameters like temperature and pH were measured in each test solution at test initiation. The temperature for all the test solutions was 23 ^{o}C and the pH ranged from 7.4 to 7.6. Algal cell counts were conducted at 24, 48, 72 hours.[1]
Species:	Green Algae (*Scenedesmus subspicatus*)
Test Procedure:	Static
Age of Culture at Study Initiation:	3 days old
Test concentrations:	1.23, 3.7, 11, 33 and 100 mg/L (nominal)
Vehicle:	96.0 mg NMP (n-methyl- 2- pyrrolidone) and 4.0 mg Tween 80 (polyoxyethylene-sorbitan-mono-oleate) / liter
Blank:	Water
Exposure period:	72 h
Analytical monitoring:	No
GLP:	No
Year:	1992
Results:	EC_{50} (0-72 h) > 100 mg/L (cell density) NOEC (0-72 h) = 33 mg/L
Remarks:	This study is assigned a reliability code of 2b[2] (guideline study with acceptable restrictions).
Reference:	[1]Report on the growth inhibition test of Irganox 259 to green algae (Scenedesmus subspicatus); Dr. R. Grade, Dr. A.de Morsier; Ciba-Geigy, Ltd., Basel, Switzerland; December 18, 1992. [2] Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

12. ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Test substance:	1,6-Hexamethylene bis (3,5-di-(tert)-butyl-4-hydroxyhydrocinnamate) CAS No. 35074-77-2
Method:	This study was conducted under OECD Guideline No. 202 (Paris 1984). A stock solution was prepared by mixing 5.01 g of the test chemical with 40 mg alkylphenol-polyglycol-ether, and made up to 10 mL with tetrahydrofuran (THF). The study used 20 daphnia per concentration and control (4 replicates of 5 daphnia each). Lighting was for 16 hours daily. Oxygen, pH, temperature were measured daily. [1]
Species:	*Daphnia magna Straus 1820*
Type of test:	Static
Test concentration:	10, 18, 32, 58, 100 mg/L (nominal)
Controls:	Blank: Water Vehicle: 90 mg tetrahydrofuran and 0.8 mg alkylphenol-polyglycol-ether per liter water (the concentration used for the highest test concentration).
Exposure period:	24 hours
Analytical monitoring:	No
GLP:	No
Year:	1988
Results:	EC_{50} (24 h): > 100 mg/L EC_0 (24 h): > 100 mg/L No mortalities occurred at any of the test concentrations Immobilization in blank and vehicle = 0%
Remarks:	The study is assigned a reliability code of 2b[2] (guideline study with acceptable restrictions).
Reference:	[1]Test for acute toxicity of TK 10019 to Daphnia magna, Project No.: 884462, Ciba-Geigy Ltd., Basle, Switzerland, November 15, 1988. [2] Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

HEALTH ELEMENTS

13. ACUTE TOXICITY

A. Oral

Test substance:	1,6-Hexamethylene bis (3,5-di-(tert)-butyl-4-hydroxyhydrocinnamate) CAS No. 35074-77-2
Method:	The test substance was suspended in polyethyleneglycol (PEG 400). Before treatment the suspension was homogeneously dispersed with an ultra-turrax and during treatment it was kept stable with a magnetic stirrer. Healthy random bred mice of the Tif: MAG (SPF) strain raised on the premises were used. They were kept at room temperature of 22 ± 1° C, at a relative humidity of 55 ± 5% and on a 10 hours light cycle day. They received ad libitum mouse food and water. During the treatment and observation period the animals were housed in groups of 5 in Macrolon cages. Animals fastened overnight were treated by oral intubation. Physical condition and rate of deaths were monitored throughout the whole observation period. [1]
Species/strain:	Random bred mice of the Tif: MAG (SPF) strain
No. Animals/Group:	5 males and 5 females / dose level
Dose:	4640, 6000, 7750 mg / kg
GLP:	No
Year:	1978
Results:	LD_{50} (mouse) > 7,750 mg /kg Within 2 hours after treatment the animals in all dosage groups showed symptoms of sedation, dyspnoea, curved position and ruffled fur. Additionally in the highest dosage group ventral position was also observed. The animals recovered within 8 to 14 days. They were submitted at random to a necropsy at the end of the observation period. No substance related gross organ changes were seen. No mortalities were observed.
Remarks:	The study was assigned a reliability code of 2[2] (valid with restriction).

Reference:

[1]Acute Oral LD_{50} in Mouse, Ciba-Geigy Ltd., June 28, 1978.

[2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

B. Dermal

Test substance:	1,6-Hexamethylene bis (3,5-di-(tert)-butyl-4-hydroxyhydrocinnamate) CAS No. 35074-77-2
Method:	New Zealand white rabbits were kept in cages with a base of 0.3 m^2 at a room temperature of 21 ± 2 °C, at a relative humidity of 60 ± 3% and on a 12:12 light/dark cycle. Half of the animals underwent treatment of the intact skin, and the other half were further prepared by making epidermal abrasions at the application site. The application area was depilated and the test material was applied by brush to a spot of 15 x 16 cm with single exposure to intact and abraded back skin. They were held in contact with the skin by means of a fourfold layer of cotton gauze and a rubber sleeve. After 24 hours of exposure and during the 14 day observation period, the local and systemic reactions were observed. The body weight was determined weekly. At the end of the investigation all animals were sacrificed followed by microscopic examination of the tissue.[1]
Species/strain:	New Zealand white rabbits
Initial Body Weight Range:	2.5 and 3.0 kg
Dose level:	2500 and 10,000 mg/kg body weight in a 50% suspension
Control:	3 ml NaCl solution USP/ kg body weight
Total number of animals:	4 males and 4-females/ groups of 3 groups
Frequency of application:	One single dose
Exposure period:	24 hours
Post exposure observation period:	14 days
Year:	1970
Results:	LD50 > 10,000 mg/kg body weight.
Remarks:	This study is assigned a reliability code of $2e^2$ (meets generally accepted scientific standards, well documented and acceptable for assessment).

Reference:

[1]"Acute Dermal Toxicity (single exposure) of TA 1205 In New Zealand White Rabbits", February 2, 1970; Ciba-Geigy Limited, Additives Division, Basle, Switzerland.

[2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

C. Inhalation

Test substance:	1,6-Hexamethylene bis (3,5-di-(tert)-butyl-4-hydroxyhydrocinnamate) CAS No. 35074-77-2
Method:	Test material (20% suspension in ethanol) was sprayed into the exposure chamber by means of a pressure nozzle. The liquid was injected at a rate of 60 ml/hr into a stream of compressed air (2 atm.). The aerosol mist thus produced was discharged into the exposure chamber. Nine male and nine female rats were exposed for 4 h at a concentration of 1688 mg/m^3 (as determined by sampling in the breathing zone). They were kept at room temperature of 22 ± 1^o C, at a relative humidity of 50%. Animals were observed for 7 days after exposure, and monitored for clinical signs or toxicity and mortality[1].
Type:	Acute inhalation - mist
Species/strain:	Tif. RAI rats
Age at Initiation:	7 to 8 weeks old
Initial Body Weight Range:	180 and 185 g
Total number of animals:	9-animals/ cages
Dose level:	1688 ± 195 mg/m^3
Exposure time:	4 hours
GLP:	No
Year:	1973
Results:	LC_{50} (4 h) >1700 mg/m^3 air. After the 4-hour exposure the rats showed lateral position and apathy. All animals had recovered within 24 hours. They were killed and autopsied after an observation period of 7 days. No substance related gross organ changes were observed. By this route of administration TK-10019 has slight acute toxicity to the rat.
Remarks:	The study was assigned a reliability code of 2c[2] (comparable to guideline study with acceptable restrictions).

Reference:

[1]Acute inhalation toxicity study of TK 10019 in Rats; December 11, 1973; Ciba-Geigy Limited, Additives Division, Basle, Switzerland.

[2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

14. GENETIC TOXICITY IN VITRO

Test substance:	1,6-Hexamethylene bis (3,5-di-(tert)-butyl-4-hydroxyhydrocinnamate) CAS No. 35074-77-2
Method:	This study was conducted using the methods described by Ames *et al* (1973, 1975) [2,3,4]. The material was tested for mutagenic effects on histidine-auxotrophic mutants of Salmonella typhimurium (TA 98, TA 100, TA 1535 and TA 1537). The investigations were performed with and without microsomal activation. [1]
Type:	Bacterial mutagenicity
System of testing:	Salmonella typhimurium TA 98, 100, 1535, 1537
Concentration:	0.2, 2.0, 20, 200, 2000 µg/ plate
GLP:	No
Year:	1978
Results:	The test chemical did not increase mutations with or without metabolic activation.
Conclusion:	No mutagenic effects were observed.
Remarks:	This study was assigned reliability code of 2e (met generally accepted scientific standards, was well documented, and was acceptable for assessment).[5]
References:	[1]Report on a study of the mutagenic potential of TK 10019 (Ames Test). Ciba-Geigy Ltd, Basel, Switzerland, October 1978. [2]Ames, B.N., Lee, F.D., and Durston, W.E., "An improved bacterial test system for the detection and classification of mutagens and carcinogens, Proc. Natl. Acad. Sci. USA, 70, 782-786, 1973. [3]Ames, B.N., Durston, W.E., Yamasaki, E., and Lee, F.D., "Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection," Proc. Natl. Acad. Sci. USA, 70, 2281-2285, 1973.

[4]Ames, B.N., McCann, J., and Yamasaki, E., “Methods for detecting carcinogens and mutagens with the Salmonella / mammalian-microsome mutagenicity test,
Mutat. Res., 31, 347-364, 1975.

[5]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

15. GENETIC TOXICITY IN VIVO

Dominant Lethal Assay:

Test substance:	1,6-Hexamethylene bis (3,5-di-(tert)-butyl-4-hydroxyhydrocinnamate) CAS No. 35074-77-2
Method:	The experiment was done to evaluate any cytotoxic or mutagenic effects on the male germinal cells as expressed by the loss of pre-implantation zygotes as well as by the rate of deaths of post-implantation stages of embryonic development. The test material was administered orally in single dose by intubation to male albino mice, which were mated to untreated females from the same strain over a period of six weeks. At the end of each week the females were replaced by new ones. The animals were kept in an air-conditioned room at a temperature of 21 ± 1°c, humidity 60 ± 5 %. The room was illuminated for 10 hours daily. Water is tap water ad libitum. [1]
Species/strain:	Albino mice, NMRI derived (Tif : MAG f [SPF])
Age at Initiation:	Males: 2 ½ - 6 months Females: about 2 months
Initial Body Weight Range:	180 and 185 g
Total number of animals:	20 males / group
Dose level:	0, 1000, 3000 mg/ kg
GLP:	No
Year:	1978
Results:	No adverse effects were observed concerning mating behavior, pregnancy, pre-implantation loss, post- implantation loss or embryonic development.
Conclusion:	No evidence of dominant lethal effect was observed in the progeny of male mice treated with the test compound.
Remarks:	This study was assigned reliability code of 2c [2] (comparable to guideline study with acceptable restriction).

References:

[1] Dominant Lethal Study in Mice with TK 10019,
Ciba - Geigy (UK) Ltd., Genetic Toxicology. Project-no. 784821. September 26, 1978

[2] Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

16. REPEATED DOSE TOXICITY

A. Subchronic Toxicity:

i) 90-Day Dietary Toxicity study in Rats:

Test substance:	1,6-Hexamethylene bis (3,5-di-(tert)-butyl-4-hydroxyhydrocinnamate) CAS No. 35074-77-2
Method:	In this study, albino rats (15 males and 15 females/dose group of 4 groups) were given the test article at a concentration of 1,000, 3,000 and 10,000 ppm in the diet for 13 weeks. The rats were housed individually in standard, wire bottomed steel rat cages. Fresh diets were prepared each week. Each rat was offered an amount of diet sufficient for ad libitum feeding. Clinical observations and food consumption were made daily. Body weight was measured weekly. Abnormal reactions and deaths were recorded daily. Hematology, blood chemistry, and urinalysis were carried out on control and 3 test groups after 45 and 84 days of study. Pathological studies were conducted on liver, kidneys, spleen, gonads, heart, and brain[1].
Species/strain:	Charles River strain albino rats
Initial weight:	Male 130- 140 g, Female 120-130 g
No. of animals:	60 males and 60 females, 30 / group
Route of administration:	Dietary
Exposure period:	90 days (13 weeks)
Dose:	0, 1000, 3000, and 10,000 ppm in food
GLP:	No
Year:	1971
Results:	No mortality or symptoms of local and / or systemic toxicity were observed. There were no abnormalities in body weight gain, food consumption, survival rate, hematologic, blood chemistry and urologic studies. Also no gross effects were observed at necropsy; there were no effects on organ weights and ratios. *Hematologic Studies*: No outstanding differences between test and control rats were noted with respect to hematocrit value,

erythrocyte count, hemoglobin concentration, and total leukocyte count.

The statistical analyses conducted on absolute organ weights, organ to body weights and organ to brain weight ratios were noted and were considered to be normal for a random population of albino rats. The various organs examined were liver, kidney, spleen, gonads, heart, and brain.

Histopathological Studies: Organs examined for histopathological changes were esophagus, stomach (cardia, fundus and pylorus), small intestine (duodenum, jejunum and ileum), cecum, colon, liver, kidneys, spleen, pancreas, urinary bladder, pituitary gland, adrenal gland, testes, seminal vesicle, ovary, bone marrow, thyroid glands, parathyroid gland, salivary gland, prostate gland, heart, aorta, lungs, lymph node (cervical and mesenteric), skeletal, muscle, peripheral nerve, bone (femur), spinal cord, uterus, trachea, eye, optic nerve and brain (cerebrum, and cerebellum). The changes noted were those of spontaneous disease and are not unusual for the albino rats. The most frequent findings were lesions in the trachea and lungs, indicating chronic murine pneumonia, which also occurred in the control group.

Morphological changes were present in the thyroid of animals of the 10,000 ppm dose level, which were graded as minimal to mild in severity and attributed to the test material. The changes are confined to the follicles and consist of focal to diffuse hypertrophy and hyperplasia of the follicular epithelium. There was also a trend towards formation of increased numbers of small follicles within the thyroid of these animals. Inflammatory changes observed in the thyroid of two animals of the 10,000 ppm group are attributed to naturally occurring disease.

At levels of 1,000 and 3,000 ppm thyroid effects consisted of only diffuse hypertrophy (see Table 1). All other tissues examined showed no treatment effects.

The NOEL was < 1000 ppm.

Table 1

Treatment Related Histopathologic Changes

Thyroid

Group	Sex	Number of Animals Examined	Findings	Incidence	Grade*
Control	M	10	Focal hyperplasia and hypertrophy of follicular epithelium	2	1.0
	F	10	None	-	-
1,000 ppm	M	12	Focal hypertrophy of follicular epithelium	7	1.0
	F	13	Focal hypertrophy of follicular epithelium	8	1.0
3,000 ppm	M	11	Focal hypertrophy of follicular epithelium	4	1.0
			Diffuse hypertrophy of follicular epithelium	6	1.5
	F	11	Focal hypertrophy of follicular epithelium	7	1.0
			Diffuse hypertrophy of follicular epithelium	4	1.5
10,000 ppm	M	7	Focal hyperplasia and hypertrophy of follicular epithelium	1	2.0
			Diffuse hyperplasia and hypertrophy of follicular epithelium	6	1.5
	F	8	Focal hyperplasia and hypertrophy of follicular epithelium	6	1.5
			Diffuse hyperplasia and hypertrophy of follicular epithelium	2	1.5

*Grading system:

0.5 = minimal, 1 = slight, 2.0 = mild, 3.0 = moderate, 4.0 = severe, 5.0 = extreme

The details of reproductive organs examined in this study are specified and these results are relevant to evaluation of potential reproductive effects. The pathological and microscopic examination was carried out on testes, uterus and ovaries. No outstanding differences were noted between control and test groups. The results of the statistical analysis conducted on absolute organ weights, organ to body weight, and organ to brain weight are summarized in the table 2. Significant differences between a test group and the control are designated by asterisks. Overall, the test substance had no apparent effect on reproductive organs of either sex.

Table 2

Organ weight and ratio data
Summary of Mean Value
Organ: Gonads (testes and ovaries)

Dietary Level (ppm)	Organ Weight (g) Males	Females	Organ / Body Weight Ratio (g/100g) Males	Females	Organ / Brain Weight Ratio (g/g) Males	Females
Control	3.31	0.114	0.729	0.0418	1.63	0.0600
1000	3.43	0.086*	0.790	0.0334*	1.68	0.0465
3000	3.64	0.103	0.825	0.0390	1.82	0.0559
10,000	4.43	0.102	0.835	0.0425	1.74	0.0558

* Statistically significant difference at the 95% confidential level.

Remarks: This study was assigned a reliability code of 2c[2]
(comparable to guideline study with acceptable restrictions)

Reference:

[1]90-Day subacute oral toxicity study with TK 10019 in albino rats. Ciba - Geigy Limited, Basel, Switzerland, December 29, 1971.

[2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

ii) 90-Day Dietary Toxicity study in Rats:

Test substance:	1,6-Hexamethylene bis (3,5-di-(tert)-butyl-4-hydroxyhydrocinnamate) CAS No. 35074-77-2 Method: In this study, rats were housed five to a cage. Diet and water were allowed ad libitum. The compound was administered at a dose level of 2000, 10,000, and 30,000 ppm by mixing in the food. Clinical symptoms were recorded daily; food consumption and body weights were recorded weekly. An histopathological examination was carried out on the following organs: Kidneys, liver, lungs, pituitary, spleen thyroid, thymus, brain, gonads, adrenals, heart, aorta, colon, muscle, pancreas, seminal vesicles, spinal cord, tongue, bladder, eye, esophagus, rib junction, skin, stomach, uterus/prostate, bone marrow, lymph node, peripheral nerve, salivary gland, small intestine and trachea.[1]
Species/strain:	Sprague Dowley rats
Initial age:	8 to 9 weeks
No. of animals:	5/ cage
Route of administration:	Dietary
Exposure period:	90 days (13 weeks)
Dose:	0, 2000, 10,000, and 30,000 ppm in food
GLP:	No
Year:	1970
Results	Body weight gains, food consumption, and general health remained normal in control and group II (2000 ppm), whereas in group III and IV (10,000 and 30,000 ppm) the body weight and food consumption was significantly lower than controls. The animals in group IV appeared less lively than controls during week 3 and 4. Terminal autopsy results revealed consistent hypertrophy of the thyroid gland and liver in all groups except for the control. An ophthalmic examination showed no abnormalities in any group. Hematology: There is a statistically significant fall in group mean hemoglobin, RBC and PCV values in male animals in group 4 (30,000 ppm). There is a significant increase in the group mean prothrombin times in females in group 3 and 4 (10,000 and 30,000 ppm).

Biochemistry: There is a significant increase in the group mean values for SAP in group 4.

Urine Analysis: No important changes observed.

Histopathology: For the organs examined for histopathological changes, liver and thyroid showed effects.

Liver - In the high dose group (30,000 ppm), most livers showed hypertrophy. Many livers also showed concentric laminated bodies within the cytoplasm of the hepatocytes. These bodies probably represent a marked hypertrophy of the smooth endoplasmic reticulum. In this group, in addition, there is an abnormal fatty infiltration particularly into periportal cells.

In the mid-dose group (10,000 ppm), livers showed similar changes to those in high dose group (30,000 ppm) except for the fatty infiltration into periportal cells.

In the low-dose group (2,000 ppm), a few livers showed a mild hypertrophy of hepatocytes.

Thyroid - In the high dose and mid-dose groups, these organs showed a moderate to marked hypertrophy of the follicular epithelium.

In the low-dose group, there was a mild hypertrophy of follicular epithelial cells similar to, but less marked than, that seen in the higher treatment groups.

There were no reported histological effects on male or female reproductive organs.

The NOEL was less than 2000 ppm.

Remarks: This study was assigned a reliability code of 2c[2] (comparable to guideline study with acceptable restrictions).

Reference: [1]13-Week dietary study in rats, Final Report, 11 May 1970. Ciba Geigy Limited, Basel, Switzerland

[2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

iii) 90- Day Dietary Toxicity study in Rats:

Test substance:	1,6-Hexamethylene bis (3,5-di-(tert)-butyl-4-hydroxyhydrocinnamate) CAS No. 35074-77-2
Method:	In this study, the compound was administered in the feed over a period of 90 days. The room was partially air-conditioned at a temperature of 22-25 ^{o}C and relative humidity of 35-60%. The behavior and general condition of each rat was checked daily. The feed and water was available ad libitum. The behavior and the general condition of each rat were checked daily. Each animal was weighed once weekly. The animals were examined for corneal opacities and changes in the teeth and the oral mucosa. [1] Full autopsy was made on all animals surviving to the end of the trial and on those rats, which died during the test. The organs of all animals were inspected for gross changes and weighed.
Species/strain:	SPF Wistar rats of own breed
Initial average weight:	74 g (68-82 g) in males and 78 g (70-82 g) in females.
No. of animals per group:	10 males and 10 females of 4 groups and 1 control group.
Route of administration:	Dietary
Exposure period:	90 days
Dose:	0, 400, 2000, 10,000, and 30,000 ppm in food
GLP:	No
Year:	1975
Results	The general behavior of the animals in group I-IV was comparable to control group. Body weight gains and health remained normal in control and males treated with 30,000 and 10,000 ppm and controls. The male rats treated with 2,000 and 400 ppm showed higher body weights than the control throughout the experiment. In the females, only those of the group given 2,000 ppm did not differ in weight-gain from the controls. Hematological determination and urine analysis revealed no pathological changes after 90 days treatment. Corneal opacities and dental changes were not found. An increase in the weight of the liver was noted in both the males and females of group I (30,000 ppm) killed 24 hours after the

withdrawal of the substance. A similar increase in the thyroid gland weight was also noted 24 hours after the end of the experiment in the males and females of group I (30,000 ppm) and II (10,000 ppm).

These changes in organ weights proved reversible, at the end of the 13-day post-treatment observation period the values were again within the normal range of variation although dose-related differences were still discernible.

Histological examination of animals treated at 2,000, 10,000 and 30,000 ppm showed epithelial hyperplasia of the thyroid. Thyroids in the 400 ppm group were not significantly different from that of the controls. Other organs did not show microscopic effects.

No effects were noted in body weight gain, food consumption, survival, hematology, clinical blood chemistry, urology, gross pathology and organ weights and ratios.

The NOEL was 400 ppm.

Remarks: This study was assigned a reliability code of $2c^{2}$ (comparable to guideline study with acceptable restrictions)

Reference:

[1]90-day dietary toxicity study in rats, Final Report, 30 December 1975. Ciba Geigy Limited, Basel, Switzerland

[2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

iv) 90-Day Dietary Toxicity Study in Beagle Dogs:

Test substance:	1,6-Hexamethylene bis (3,5-di-(tert)-butyl-4-hydroxyhydrocinnamate) CAS No. 35074-77-2
Method:	In this study, the compound was administered in the feed over a period of 90 days. The room was partially air-conditioned at a temperature of 22-25^{o} C and relative humidity of 35-60%. The behavior and general condition of each rat was checked daily. At the termination of the experiment all animals were examined. The following tissues were taken for histopathological examination: Aorta arch, aorta abdominal, trachea, lung, heart, thymus, lymph nodes (cervical and mesenteric), liver, gall bladder, spleen, pancreas, kidney, urinary bladder, prostate, gonads, uterus, thyroids, adrenals, salivary gland, tongue, esophagus, stomach (body and antrum), duodenum, jejunum, ileum, upper colon, lower colon, skin, mammary gland, skeletal muscle, marrow smear, bone marrow (sternum), central nervous system (cerebral cortex, thalamic nuclei, mid-brain, cerebellum, medulla, spinal cord), pituitary, peripheral nerve, eye, and optic nerve. [1]
Species/strain:	Pure-bred Beagle dogs
Initial average weight:	74 g (68-82 g) in males and 78 g (70-82 g) in females
No. of animals per group:	5 males and 5 females of 2 groups and 4 males and 4 females of 2 groups.
Route of administration:	Dietary
Exposure period:	13 weeks, followed by a 4 week recovery period for 1 male and 1 female from each of the control and high dose group.
Dose:	0, 500, 1500, and 5,000 ppm in food
GLP:	No
Year:	1976
Results:	There were no mortalities. No clinical signs of toxicity were seen. No adverse effects were seen related to body weight gains, food consumption and health. Macroscopic post-mortem findings: The only findings which may possibly have been related to treatment were areas of congestion seen in the gastro-intestinal tract. Minimal congestion of the intestinal mucosa was seen in some dogs of 500, 1500, 5000 ppm dose level. Similar

observation was made for dogs of control-recovery group and one dog of 5000 ppm-recovery group. This congestion was mainly confined to the ileum and jejunum.

Additional findings were considered to be incidental.

Varying degrees of bile duct hyperplasia with an associated inflammatory reaction were seen in some dogs of 1500 and 5000 ppm dose group. This change was not seen in any of the controls or in the 500 ppm dose group. The degree of bile duct hyperplasia was greater than that previously identified in untreated beagle dogs. There was no evidence of bile duct hyperplasia in either of the two dogs receiving 5000 ppm after a recovery period of four weeks. These changes tended to be focal non-specific in nature, and probably related to parasitism. It is unlikely that they were related to treatment with test substance.

Liver: The significance of the intracytoplasmic, easinophillic globules in hepatocytes in a single dog was unknown.

Kidney: Treatment related changes were not detected. Minimal changes, commonly encountered in the kidneys of laboratory dogs, included the following: fat deposition in the epithelium of a variable number of cortical tubules in the majority of control and treated dogs from all groups, minimum calcification within a few medullary tubules, peri-pelvic chronic inflammation, cortical foci of intestinal chronic inflammation.

Intestine: There were no morphological findings corresponding to the mucosal congestion noted macroscopically at the post-mortem examination. Parasitic worms were seen within the lumen of the small intestine in some dogs of 1500 ppm group and minimal inflammatory changes, probably related to parasitic infestation, were identified in the mucosa or submucosa of the gastro-intestinal tract in some dogs of control-recovery group and 500 ppm group.

Other observations made and not considered to be significant included granulomatous lesions in a lymph node, a fibrous capsular adhesion in the spleen, unilateral testicular atrophy, and post-oestral changes in the uterus associated with corpora lutea in the ovaries.

No morphological changes of toxicological significance were seen in any of the other tissues examined.

High dose animals (5000 ppm) showed increased liver weights, which was the only apparent treatment-related effect.

The NOEL was found to be 1,500 ppm.

Remarks: This study was assigned a reliability code of $2c^2$
(comparable to guideline study with acceptable restrictions).

Reference:

[1]Toxicity study in Beagle dogs, Final Report, 21 October 1976. Ciba Geigy Limited, Basel, Switzerland

[2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

B. Chronic Dietary Toxicity Study In Rats:

Test substance: 1,6-Hexamethylene bis (3,5-di-(tert)-butyl-4-hydroxyhydrocinnamate)
CAS No. 35074-77-2

Method: Charles River CD rats of the Sprague-Dawley origin, were obtained for allocation to the following groups:

	Group and treatment	No. of rats	
		males	females
1.	Control (untreated diet)	60	60
2.	50 ppm	60	60
3.	150 ppm	60	60
4.	450 ppm	60	60

Throughout the investigation, the rats were housed five to a cage (unless the number was reduced by mortality) in suspended metal cages fitted with wire-mesh floors. Animal-room temperature and relative humidity were controlled at 21 ± 2°C and 55 ± 5% respectively; lighting was controlled to give 12 hours of light (8 a.m. to 8 p.m.) and 12 hours of darkness per 24 hours. All rats had free access to tap water and to quantities of powdered laboratory rat food. For the treated groups, the test substance was incorporated in this diet.[1]

Species/strain: Charles River CD rats of the Sprague-Dawley origin

Age: 28 ± 1 days old

Initial weight of animals age: 65-85 g

No. of animals/group: 60 males and 60 females/group

Route of administration: Dietary

Total duration of dietary intake: 104 weeks

Dose: 0, 50, 150, 450 ppm

GLP: No

Year: 1982

Results: Reactions to treatment at the various dietary levels are summarized as follows:

There was no indication of a treatment related effect on the number of rats exhibiting palpable masses.

Clinical Signs: There were no overt signs of reaction to treatment.

Mortality At the end of the 104-week treatment period the following number of rats had survived:

	Males				Females			
	Control	50 ppm	150 ppm	450 ppm	Control	50 ppm	150 ppm	450 ppm
No. of survivors	31	26	36	39	27	22	25	31
% of numbers at start of the study	52	43	60	65	45	37	42	52

The withdrawal period, during which all rats were fed control diet began at the end of the 104-week treatment period and continued until any one group within a sex had reached approximately the 20% survival point.

Bodyweight: Bodyweight gain for all treated rats was similar to that of control groups, during the treatment period.

Food Consumption: Food intake of female rats treated with 450 ppm was 4 to 5% lower than that of the controls during this study. Whereas for other groups was essentially similar to control. During the withdrawal period all female treated groups had a higher recorded food consumption than the controls.

Terminal Studies:
Macroscopic Pathology: In both control and treated groups there was a higher incidence in female rats, in comparison to males, of subcutaneous masses and enlarged and hemorrhagic pituitaries. In both control and treated male groups, there was a higher incidence of cortical scarring of kidneys, in comparison to that of the female rats. The incidence and distribution of macroscopic lesions suggestive of neoplasia were considered to fall within the expected background range of such lesions and therefore were considered unrelated to the treatment.

The macroscopic findings for reproductive organs are summarized in the table below. This data further demonstrates the lack of treatment effects on reproductive organs.

Summary of Macroscopic post-mortem findings

Macroscopic observation	Control		50 ppm		150 ppm		450 ppm	
	D*	T*	D*	T*	D*	T*	D*	T*
Reproductive System								
Testes								
Enlarged/swollen	-	2	-	1	-	-	-	-
Pale areas/mass	1	1	1	-	-	-	-	-
Small/flaccid	9	-	4	4	4	2	7	3
Epididymis								
Nodule/area	-	1	-	-	-	-	-	-
Seminal vesicles								
Pale raised area	-	-	1	-	-	-	-	-
Prostate								
mass	1	-	-	-	-	-	-	-
Scrotal sac								
Raised yellow nodule	-	-	-	-	-	-	1	-
Ovaries								
Cyst/peri-ovarian sac distended	5	5	4	3	4	1	2	8
No corpora lutea visible	3	-	-	-	1	-	2	-
Mass/ nodule	1	1	-	-	-	-	2	1
Uterus								
Area of distention/ fluid filled	3	1	3	1	4	3	4	-
Mass/swelling/nodule	-	3	1	2	1	3	2	4
Cervix								
Enlarged/swollen	-	-	1	-	-	-	-	1
Vagina								
Nodular swellings	-	-	1	-	-	-	-	-

*D = decedent animals
*T = terminal animals
- = No lesion observed

Organ Weight Analysis: The weights of the organs analyzed are heart, kidney, liver, thyroids, brain, gonads, adrenals, thymus and spleen. Although differences from control values in some organ weights attained statistical significance, these were not considered large enough to be of any biological significance. Statistical analysis of reproductive organ weights are summarized in the following table.

Statistical Analysis of Testes and Ovary Weights
(Mean Values)

Dose group (ppm)	Body-weight (g)	Testes (g)	Ovaries* (mg)
Control	816	5.48	85.10 (82.10)
50	805	4.76+	83.85 (81.87)

150	806	5.28	99.08 (100.33)
450	883	5.06	85.45 (89.17)

+ = students 't' test in comparison with control group: + P< 0.05

* = adjusted for final bodyweight as covariate: absolute value is in parenthesis

Histopathology: General histopathology of rats killed after 104 weeks of treatment showed no morphological abnormalities and variations from normal.

The tissues examined for histopathology are adrenals, aorta, abdominal nodule, adipose tissue/fat/nodule, bone marrow, brain, blood vessels, blood smear, bulbo-uretral gland, bile duct, caecum, duodenum, eye, eye-lid, extra-orbital lachrymal gland, epididymides, femur, feet/foot/limb/paw, heart, harderian gland, head, ileum, jejunum, jugular vein, kidneys, liver, lungs, lymph nodes, mammary gland, mesentry, colon, esophagus, optic nerve, ovaries, omental nodule, omentum, pancreas, pancreatic mesentry, pituitary, prostate, pinnae, salivary gland, sciatic nerve, skeletal muscle, skin, spinal cord, spleen, sternum, stomach, small intestine, seminal vesicles, scrotal sac nodule, testes, thymus, thyroid glands, tail, urinary bladder, ureter, and uterus.

Spontaneous, usually age-related changes were recorded in many organs and tissues. Such changes were most frequently seen in the heart, lungs, liver, thymus, kidneys, gonads and endocrine glands. However, they showed no treatment-related changes.

All neoplastic changes observed were not treatment related. There were neither treatment related effects on the incidence of any tumor type nor on the total number of rats with tumors (benign or malignant) per group and there was no deviation from the expected tumor profile.

Mammary tumors among females and pituitary tumors among males and females were the most commonly encountered tumors. They are expected to be a sex related feature. Although the incidence of pancreatic islet cell adenoma and adrenal phaeochromocytoma among male rats appears to be high, they are within the normal background range.

Subcutaneous tumors, mammary tumors, pituitary tumors and renal disease were among the most common causes of death. However, none of these factors were treatment related.

Neoplastc and non-neoplastic findings related to reproductive organs are summarized in the tables below.

Neoplastic findings in rats

Histopathology	Control		50 ppm		150 ppm		450 ppm	
	D*	T*	D*	T*	D*	T*	D*	T*
Reproductive System								
Testes								
Interstitial cell tumour	1	2 (1M)	1 (M)	1			1	
Ovaries								
Tubular adenoma				1			2	1
Adenocarcinoma		1						
Granulosa cell tumour							1	
Uterus								
Adenoma		2		1				1
Adenocarcinoma		1						1
Endometrial sarcoma					1			1
Leiomyoma				1 (M)				
Fibrosarcoma								1

*D = decedent animals
*T = terminal animals
(M) = Multiple tumours of same type

Non-neoplastic findings in rats

Histopathology	Control		50 ppm		150 ppm		450 ppm	
	D*	T*	D*	T*	D*	T*	D*	T*
Reproductive System								
Testes								
Peri-arteritis/polyarteritis	6	4	4	4	4	4	4	7
Atrophy	5	3	2	4	3	3	5	4
Arrest of /reduced spermiogenesis	2	-	-	-	-	-	-	-
Dilated tubules		1						
Granuloma								1
Mineralisation/calcification		1		2	2	1	1	
Mineralization of blood vessels/medial calcification			3				1	1
Mesothelial proliferation			1			1		
Increase in intertubular space							1	
Interstitial hyperplasia						1		1
Inflammation								1
Prostate								
Prostatitis/suppurative inflammation	4	2	6	2	4		6	1
Fibrosis	1		1			1	1	
Reduced secretion	1							
Inflammatory cells/ lymphocytes	1		2			1	2	
Fat necrosis	1							
Concretions			1					
Oedema			1					
Ovaries								
No corpora lutea	21	5	20	3	21	3	19	7
Tubular hyperplasia	5	5	2	2	5	2	4	5
Follicular cyst/ cysts	4	3	4	2	3	2	3	7
Dilated follicles	1							
Dilated/ distended bursa			3				1	
Uterus								
Dilated lumen	5	2	4	1	4	3	6	1
Cystic/dilated glands +/- inflammatory cells	2		2	1			2	
Stromal hyalinisation	2		1		1			
Endometrial hyperplasia		1				1	1	
Endometritis		2						
Squamous metaplasia			1					
Congested					1			
Polyp						3	2	
Glands with basophilic debris							1	

*D = decedent animals

*T = terminal animals

Conclusion:	Based on the above findings, it may be concluded that the test substance at a dietary level of 450 ppm (equivalent to an average daily intake of 15.4 mg/kg bodyweight for males and 20.0 mg/kg bodyweight for females) was not tumorigenic to rats. This treatment level also did not cause signs of toxicity or pathological changes in organ tissue. A conservative chronic NOEL of 150 ppm was demonstrated with the only effect being slightly reduced food intake in females at 450 ppm.
Remarks:	This study was assigned a reliability code of $2c^2$ (Comparable to guideline study with acceptable restrictions).
Reference:	[1]Long - Term Feeding of TK 10019 to Rats (Final Report 0 -104 weeks), Ciba - Geigy Limited, Basel, Switzerland, 5 April, 1982. [2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

17. DEVELOPMENTAL TOXICITY/ TERATOGENICITY

A. TERATOGENICITY IN RATS

Test substance:	1,6-Hexamethylene bis (3,5-di-(tert)-butyl-4-hydroxyhydrocinnamate) CAS No. 35074-77-2
Method:	The compound was administered by oral gavage on Days 6 through 15 of gestation. Throughout the experiment the successfully mated females were kept in groups of 5 in Macrolon cages in an air-conditioned room at a temperature of 21± 1°C and a humidity of 60 ± 5 %. The room was illuminated for 10 hours daily. Tap water was available at all times. During the treatment, general condition, and weight gain were checked daily. Food consumption was noted on days 6, 11, 16 and 21 of pregnancy. Dams were killed, and fetuses removed by Cesarean section on Day 21 of gestation.[1] The examinations were carried out in accordance with the World Health Organization recommendations (WHO, 1975) and the technique described by Wilson, 1965 and Dawson.[2,3,4]
Species/strain:	Sprague-Dawley derived (Tif : RAIf(SPF)) rats
Sex/ age:	Female, 2 months of age.
Body weight:	190 g
No. of animals:	25 females/dose/group
Route of administration:	Gavage
Exposure period:	Days 6 through 15 of pregnancy
Doses:	150, 750, 2000 mg/kg of body weight
Vehicle:	2% solution of sodium carboxymethylcellulose (2mL/100g of body weight)
Control group:	Concurrent vehicle
GLP:	No
Year:	1978
Results:	Throughout the period of treatment food intake was reduced in the three groups receiving the test material. At the 750 and 2000 mg/kg dose, the body weight gain

was diminished.

The implantation rates, embryo-lethality and/or feto-lethality (resorption) were comparable for all groups.

Hemorrhagic degeneration of implantation sites (deciduomata) were noted in one animal of the 750 and 2000 mg/kg dose groups and in one animal of the cumulative control group,

The average weight of the fetuses was slightly but significantly diminished in the 2000 mg/kg dose level.

Malformation (ophalocele) of 1 fetus in 2000 mg/kg and 2 fetuses in control group were seen.

No pathological changes of the viscera were found in experimental groups, including the vehicle control. A few anomalies were noted in the cumulative (historical) control. Sex ratios were not affected by administration of the test substance.

In skeletal assessment, the only deviation is an increased number of incompletely ossified phalangeal nuclei at the 2000 mg/kg dose. Some skeletal anomalies were observed in the cumulative control shown in Table 1 below.

Table 1

Skeletal Assessment

Dose Group (g/kg)	Number of Skeletons examined	Phalangeal Nuclei [a]		Calcaneus [a]	Sternebrae [c]	Vertebrae [d]	Skeletal anomalies		
		Fore-limb [b]	Hind-Limb [b]				Sternebrae [e]	Vertebrae [f]	Ribs [g]
150	206	0	10 (4.8)*	23 (11.2)	8 (3.9)	1 (0.5)	0	0	0
750	202	3 (1.5)	27 (13.4)	32 (15.8)	26 (12.9)	1 (0.5)	0	0	0
2000	194	11 (5.7)	34 (17.5)	29 (14.9)	15 (7.7)	0	0	0	0
Vehicle control	212	1 (0.5)	21 (9.9)	51 (24.0)	22 (10.4)	1 (0.5)	0	0	0
Cumulative control	2160	-	-	-	-	-	1 (0.05)	1 (0.05)	1 (0.05)

* Figures in parentheses refer to % skeletal anomalies

a) Ossification still absent
b) Proximal phalanges V
c) Particularly ossification centers of the 5th sternebra still incompletely ossified.
d) Some thoracic vertebral centers bipartite
e) Irregularly ossified, centers of ossification displaced
f) Synostosis of two lumbar vertebral arches. (unilat.)
g) Costal fusion

Conclusion: Test compound did not exhibit either a teratogenic potential or an increased embryo-lethality rate in the albino rats under the experimental conditions. The NOEL is 150 mg/kg bw per day.

Remarks: This study was assigned a reliability code of 2c[5] (comparable to guideline study with acceptable restrictions).

References:

[1]"Reproduction Study – TK 10019, Rat, Segment II (Test for Teratogenic or Embryotoxic Effects)",
Study No. 459778. Ciba Geigy Limited, Basel, Switzerland. October 25, 1978.

[2]World Health Organization Technical Report Service 563, 1975

[3]Wilson, J.G., in: Teratology, Principles and Techniques; J.G. Wilson and J. Warkany eds., The University of Chicago Press, Chicago, 1965, pp. 262-277.

[4]Dawson, A.B., Stain Tech. 1 (1926), 123-124.

[5]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

B. REPRODUCTIVE TOXICITY

Test substance:	1,6-Hexamethylene bis (3,5-di-(tert)-butyl-4-hydroxyhydrocinnamate) CAS No. 35074-77-2
	In the OECD SIDS program, the requirement for reproduction and developmental toxicity testing is fulfilled when: 1) a developmental study is available and 2) a 90-day repeat dose study is available that demonstrates no effects on reproductive organs. For this chemical, reproductive organs were analysed in 90-day repeat dose studies with rats and dogs, as well as in a 2-year chronic rat study. Treatment-related adverse effects on reproductive organs were not observed in these studies. Taken with the existing developmental toxicity study described in 17(A), the requirement for reproduction and developmental toxicity testing is fulfilled for CAS no. 35074-77-2.