AR 201-12966

Ciba Specialty Chemicals
North America
Additives
Legal and Regulatory Affairs

Ciba

February 20, 2001

Christine Todd Whitman, Administrator
U. S. Environmental Protection Agency
P. O. Box 1473
Merrifield, VA 22116

Attn: HPV Program

Dear Administrator Whitman,

Ciba Specialty Chemicals Corporation, Additives Division (HPV Registration #) acknowledges receipt of EPA's comments on the robust summaries and test plans that we submitted for the following chemicals:

- Octadecyl 3,5-di(tert)-butyl-4-hydroxyhydrocinnamate, CAS No. 2082-79-3
- Tetrakis-(methylene-(3,5-di-(tert)-butyl-4-hydrocinnamate))methane, CAS No. 6683-19-8
- Tris(2,4-di-(tert)-butylphenyl)phosphite, CAS No. 31570-04-4

We have addressed EPA's comments, and are pleased to submit the revised robust summaries. The documents are being provided as hardcopies and electronically as Microsoft Word files. We expect that the revised documents will be acceptable to EPA. Please feel free to contact me if you have additional comments or questions regarding our submissions. Ciba remains committed to supporting EPA's HPV Challenge Program, and looks forward to our continued participation.

Sincerely,

David La
Senior Toxicologist

MR 45009

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AR 201-12966B2

IRGANOX 1010

Tetrakis-(methylene-(3,5-di-(tert)-butyl-4-hydrocinnamate))methane

CAS No. 6683-19-8

Name of Sponsoring Organization:	Ciba Specialty Chemicals Corporation
HPV Registration Number:	
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Date:	May 2000; revised February 2001

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SUMMARY TABLE

CAS No. 6683-19-8 PHYSICAL/CHEMICAL ELEMENTS	DATE	RESULTS	FULFILLS REQUIREMENT'
Melting Point	1997	115 - 118 °C	Yes
Boiling Point	2000	1130.4 °C	Yes
Vapor Pressure	2000	7.1×10^{-31} mm Hg	Yes
Partition Coefficient	1985	log P = 23	Yes
Water Solubility	1985	$< 10^{-4}$ g/L	Yes
ENVIRONMENTAL FATE ELEMENTS			
Photodegradation	2000	For reaction with hydroxyl radical, predicted rate constant = 106.3×10^{12} cm^3/molecule-sec predicted half-life = 1.2 h	Yes
Stability in Water	2000	$t_{1/2}$ at pH 8 = 75.4 days $t_{1/2}$ at pH 7 = 2.1 years	Yes
Fugacity	2000	Predicted distribution using Level III fugacity model Air 1.54×10^{-5} % Water 5.28×10^{-3} % Soil 99.4 % Sediment 0.61 % Persistence = 3.5×10^{6} h	Yes
Biodegradation	1985	Not biodegradable 4 – 5% after 28 days	Yes
ECOTOXICITY ELEMENTS			
Acute Toxicity to Fish	1985	Zebra fish (Brachydanio rerio): LC_{50} (24 – 96 h) => 100 mg/L NOEC = 100 mg/L	Yes
Toxicity to Aquatic Plants	1992	Green algae (Scenedesmus subspicatus): EC_{50} (0 – 72 h) => 100 mg/L $NOEC_b$ = 100 mg/L	Yes
Acute Toxicity to Aquatic Invertebrates	1985	Daphnia magna: EC_0 (24 h) = 31 mg/L EC_{50} (24 h) => 86 mg/L EC_{100} (24 h) => 86 mg/L	Yes

SUMMARY TABLE (CONTINUED)

CAS No. 6683-19-8 HEALTH ELEMENTS	DATE	RESULTS	FULFILLS REQUIREMENT
Acute Toxicity	1974	Rat: LD_{50} (Oral) > 12,250 mg/kg	Yes
	1980	Rat: LC_{50} (Inhalation, 4 h) > 1951 mg/m^3	Yes
	1964	Rabbit: LD_{50} (Dermal) > 3160 mg/kg	Yes
Genetic Toxicity in vivo	1975	Mouse: No evidence of dominant lethal effects (single gavage dose of 1000 or 3000 mg/kg). No effect on mating ratio, implantations, or embryonic death	Yes
	1977	Chinese hamster: Nonmutagenic in somatic mutation assay (exposed by gavage 500, 1000, or 2000 mg/kg/day for 2 days)	Yes
	1978	Chinese hamster: No evidence of chromosomal aberrations (exposed by gavage 500, 1000, or 2000 mg/kg for 2 days)	Yes
Genetic Toxicity in vitro	1977	Salmonella typhimurium: No increase in mutations with (at doses of 5 - 100 µg/0.1 mL) or without metabolic activation (at doses of 10 – 250 µg/0.1 mL)	Yes
Repeated Dose Toxicity	1981	Dog: NOEL = 10000 ppm (13-week exposure, diet)	Yes
Reproductive Toxicity	1984	Rat: NOEL parental = 10000 ppm NOEL F_1 offspring = 10000 ppm NOEL F_2 offspring = 10000 ppm	Yes
Developmental Toxicity/Teratogenicity	1975	Rat: NOEL maternal toxicity = 1000 mg/kg NOEL teratogenicity = 1000 mg/kg	Yes
	1975	Mouse: NOEL maternal toxicity = 1000 mg/kg NOEL teratogenicity = 1000 mg/kg	Yes

1. MELTING POINT

Test substance:	Tetrakis-(methylene-(3,5-di-(tert)-butyl-4-hydrocinnamate))methane CAS No. 6683-19-8
Method:	From Aldrich.'
GLP:	No
Year:	1997
Results:	115 - 118 °C
Remarks:	A similar melting point (110 - 125 °C) was reported in the MSDS from Ciba Specialty Chemicals Corp. The method of determination by Aldrich or Ciba was not reported. The melting point was assigned a reliability code of 2g (data from handbook or collection of data).[2]
References:	'Sigma-Aldrich.com.

[2]See general reference, p. 48.

2. BOILING POINT

Test substance:	Tetrakis-(methylene-(3,5-di-(ert)-butyl-4-hydrocinnamate))methane CAS No. 6683-19-8
Method:	Estimated by the MPBPWIN Program (v. 1.40)[1,2] using the adapted Stein and Brown method.
GLP:	No
Year:	2000
Results:	1130.4 °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]
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]See general reference, p. 48.

3. VAPORPRESSURE

Test substance:	Tetrakis-(methylene-(3,5-di-(ert)-butyl-4-hydrocinnamate))methane CAS No. 6683-19-8
Method:	Estimated by the MPBPWIN Program (v. 1.40),[1,2] using the modified Grain method.
GLP:	No
Year:	2000
Results:	7.1 x 10^{-31} mm Hg (25 °C)
Remarks:	The MSDS from Ciba Specialty Chemicals Corp reported a vapor pressure of approximately 2 x 10^{-9} mm Hg at 20 °C, but the method of determination was not reported. In the absence of this information, the vapor pressure was calculated using an accepted method and assigned a reliability code of 2f.[3]
References:	'Syracuse Research Corporation, Syracuse, NY. *Pollution Prevention (P2) Assessment Framework, US. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998. [3]See general reference, p. 48.

4. PARTITION COEFFICIENT

Test substance:	Tetrakis-(methylene-(3,5-di-(ert)-butyl-4-hydrocinnamate))methane CAS No. 6683-19-8
Method:	Directive 84/449/EEC, A.8 "Partition coefficient", 1985
Temperature:	25°C
GLP:	Yes
Year:	1985
Results:	Log Pow = 23
Remarks:	The partition coefficient was calculated using the computer program CLOGP.3. A partition coefficient value of 19.6 was estimated by KOWWIN (v. 1.66).[2,3] Due to the high values, this difference was not considered significant. This study was assigned a reliability code of 1 (reliable without restriction) as it was conducted under relevant guidelines!
References:	"'Report on Partition Coefficient", Ciba Geigy Ltd., Basel, Switzerland. Dr. P. Moser, 06/14/85. [2]Syracuse Research Corporation, Syracuse, NY. [3]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998. [4]See general reference, p. 48.

5. WATER SOLUBILITY

Test substance:	Tetrakis-(methylene-(3,5-di-(ert)-butyl-4-hydrocinnamate))methane CAS No. 6683-19-8
Method:	Directive 84/449/EEC, A.6, "Water Solubility", 1989
Temperature:	20 °C
GLP:	No
Year:	1985
Results:	$< 10^{-4}$ g/L
Remarks:	Results were consistent with a calculated value of 2.3 x 10^{-16} mg/L (25 "C) using the WSKOW Program (v.1.37).[2,3] This study was assigned a reliability code of 2 (reliable with restrictions) as it was not conducted under GLP guidelines.[4]
References:	'"Report on water solubility, Irganox 1010," Ciba-Geigy Limited, Basel, Switzerland. 1985 'Syracuse Research Corporation, Syracuse, NY 'Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998. [4]See general reference, p. 48.

6. PHOTODEGRADATION

Test substance:	Tetrakis-(methylene-(3,5-di-(ert)-butyl-4-hydrocinnamate))methane CAS No. 6683-19-8
Method:	Estimated by the AOP program (v. 1.90),[1,2] which estimates rate constants and half-lives of atmospheric reactions of organic compounds with hydroxyl radicals and ozone in the atmosphere.
GLP:	No
Year:	2000
Results:	For reaction with hydroxyl radicals, the predicted rate constant and half-life are as follows: Rate constant: 106.3 x 10^{-12} cm^3/molecule-sec Half-life: 1.2 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]
References:	Syracuse Research Corporation, Syracuse, NY 'Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998. [3]See general reference, p. 48.

7. STABILITY IN WATER

Test substance:	Tetrakis-(methylene-(3,5-di-(ert)-butyl-4-hydrocinnamate))methane CAS No. 6683-19-8
Method:	Estimated by the HYDROWIN Program (v. 1.67).[1,2]
GLP:	No
Year:	2ooo
Results:	At 25 °C $t_{1/2}$ (pH 8) = 75.4 days $t_{1/2}$ (pH 7) = 2.1 years
Remarks:	In the absence of reliable experimental data, the stability in water was calculated using an accepted method and assigned a reliability code of 2f.[3]
References:	'Syracuse Research Corporation, Syracuse, NY 'Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998. [3]See general reference, p. 48.

8. THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

Test substance:	**Tetrakis-(methylene-(3,5-di-(ert)-butyl-4-hydrocinnamate))methane** CAS No. 6683-19-8
Method:	Estimated by EQC Level III Fugacity Model.'
Year:	2000
GLP:	No
Results:	Distribution using EQC Level III Fugacity model

Air	1.54 x 10^{-5} %
Water	5.28 x 10^{-3} %
Soil	99.4 %
Sediment	0.61 %

Persistence = 3.5 x 10^{6} h

Remarks:	In the absence of reliable experimental data, the fugacity was **caculated** using an accepted method and assigned a reliability code of **2f.**[2]
References:	'Environmental Modelling Centre, Trent University, Peterborough, Ontario

[2]**See** general reference, **p.** 48.

9. BIODEGRADATION

Test substance:	Tetrakis-(methylene-(3,5-di-(ert)-butyl-4-hydrocinnamate))methane CAS No. 6683-19-8 Batch No. EN 97149.44
Method:	OECD Guideline 301 B "Inherent Biodegradability: Modified Sturm Test" (Paris, 1981). The only deviation from the guideline related to the volume of the test solution which was reduced from 3.0 to 1.5 L. Bacteria was collected from a sewage treatment plant. Biodegradation was calculated on the basis of the theoretical carbon content of the test substance and the cumulative quantities of carbon dioxide determined on the days of measurement.
Test Type:	Aerobic
Inoculum:	Fresh sewage treatment plant sample (per guideline)
Medium:	Sewage sludge (per guideline)
Concentration of the chemical:	10 mg/L and 20 mg/L for the test substance 20 mg/L reference chemical (aniline)
GLP:	Yes
Year:	1985
Results:	Degradation: 10 mg/L: 5 % after 28 days 20 mg/L: 4 % after 28 days
Conclusion:	Substance is not biodegradable according to OECD definition.
Remarks:	This study was considered reliable (reliability code 1) as it was conducted under OECD and GLP Guidelines.[2]
Reference:	"'Report on the Test for Ready Biodegradability of TK 10042 in the Modified Sturm Test", Ciba Geigy, Basel, Switzerland. Dr. A. de Morsier, 04/11/85.

[2]See general reference, p. 48.

10. ACUTE TOXICITY TO FISH

Test substance:	**Tetrakis-(methylene-(3,5-di-(ert)-butyl-4-hydrocinnamate))methane** CAS No. 6683-19-8 Batch No. EN 97 149.44 Purity: Commercial grade
Method:	OECD Guideline No. 203 (Paris 1981). Aquaria of 20 L (filled with 15 L) were used. The water source was dechlorinated tap water (carbon filtered). Hardness was 180 mg $CaCO_3$/L; pH was 8.0; O_2 ranged from 65 to 100% saturation; water temperature was 23 $\pm$ 1 "C. The study was performed as a limit test with a concentration of 100 mg/L (nominal). The test substance precipitated immediately at concentrations > 100 mg/L. The vehicle contained 950 mg DMF and 4 mg Marlopon AT50 per liter water. Samples for analysis were taken at 0 and 96 h exposure. There were 10 fish per concentration and control groups and 10 fish per aquarium. Tests were conducted in duplicate.
Type of test:	Static
Species:	Zebra Fish (Brachydanio rerio)
Length:	25 mm (20-28 mm)
Weight:	0.13 g (0.09-o. 18 g)
Loading:	100 mg /L
Exposure period:	96 h
Analytical monitoring:	**Yes**
GLP:	**Yes**
Year:	1985
Results:	There were no mortalities in the controls or treated groups. LC_{50} (24 h) = > 100 mg/L (nominal) LC_{50} (48 h) = > 100 mg/L LC_{50} (72 h) = > 100 mg/L LC_{50} (96 h) = > 100 mg/L NOEC = 100 mg/L

Table 1. Analytical data of test concentrations

Nominal Concentration	Measured concentration, mg/L	
	Oh	96 h
100 mg/L	113	64
100 mg/L	97	58

Remarks: This study was assigned a reliability code of 1 (reliable without restrictions) according the criteria established by Klimisch *et al* (1997), as it was conducted under OECD and GLP Guidelines.[2]

Reference: "'Report on the test for acute toxicity of TK 10042 to Zebra Fish", Ciba Geigy, Limited, Basel, Switzerland. Dr. A. de Morsier, 04/01/85.

[2]See genera1 reference, p. 48.

11. TOXICITY TO AQUATIC PLANTS

Test substance:	Tetrakis-(methylene-(3,5-di-(ert)-butyl-4-hydrocinnamate))methane CAS No. 6683-19-8 Batch No. EN 146553.4; purity: 99%
Method:	87/302/EEC pp 89-94 Algal Inhibition Test Closed-system There were no deviations from the above stated guideline. Tests were conducted in 100 mL Erlenmeyer flasks containing 50 mL test solution. The vehicle contained 4 mg alkylphenol-polyglycolether (ARKOPAL)/L. Nominal test concentrations were 1.23, 3.7, 11, 33, and 100 mg/L. Each test concentration was tested in 3 replicates and the blank control in 6 replicates. Samples for analysis were taken immediately before exposure and after 72 h exposure. The temperature was 23 ± 2 °C; pH of the test solution was 7.8 at time 0 and ranged up to 9.8 at 72 h, other information, such as water hardness, TOC and O_2 was not provided. Continuous illumination was provided by cold white fluorescent light (122 µE/m2 sec). Cell densities were measured at 24, 48, and 72 h, and the EC values calculated according to Berkson, JASA 48 (1953), 569-599.
Species:	Green Algae (Scenedesmus subspicatus)
Endpoint:	Growth rate
Exposure period:	72 h
Initial Cell Density:	10^4 cells/mL
Test Concentrations:	1.23, 3.7, 11, 33 and 100 mg/L (nominal)
Vehicle:	Alkylphenol-polyglycolether
Analytical monitoring:	Yes
GLP:	Yes
Year:	1992
Results:	EC_{50} (0 – 72 h) = > 100 mg/L $NOEC_b$ (0 – 72 h) = 100 mg/L

Table 1. Analytical data of test concentrations

Nominal Concentration	Measured concentration, mg/L 0 h	72 h
Blank	< 0.19	< 0.19
Vehicle	< 0.19	< 0.19
1.23 mg/L	1.0	1.2
3.7 mg/L	4.2	0.9
11 mg/L	12.1	12.9
33 mg/L	38.1	45.3
100 mg/L	110.4	171.5

Remarks: This study is assigned a reliability code of 1 (reliable without restrictions) according the criteria established by Klimisch *et al* (1997).[2]

Reference: "'Report on the Growth Inhibition Test of Irganox 1010 to Green Algae (Scenedesmus subspicatus)", Ciba-Geigy, Limited, Basel, Switzerland. Dr. A. von Schulthess, 1 1/12/92.

[2]See general reference, p. 48.

12. ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Test substance:	Tetrakis-(methylene-(3,5-di-(ert)-butyl-4-hydrocinnamate))methane CAS No. 6683-19-8 Batch No. 97149.44 Purity: Commercial grade
Method:	OECD Guideline No. 202 (Paris 1981). Tests were conducted in 20 mL test tubes containing 10 mL solution. Reconstituted water was prepared by dissolving 65 mg $NaHCO_3$, 294 mg $CaCl_2$ (2 H_2O), 123 mg $MgSO_4$ (7 H_2O), 6 mg KC1 per liter bidistilled water. Total hardness was 240 mg $CaCO_3$/L; pH ranged from 7.2 to 7.9; O_2 ranged from 87 to 96% saturation; temperature was 20 ± 1 °C. The vehicle consisted of 950 mg DMF and 4 mg MARLOPON AT50 per liter water (vehicle concentration deviated from the OECD Guideline). The nominal concentrations of the test compound were 10, 18, 32, 58 and 100 mg/L. The test substance appeared homogeneously distributed at all test concentrations except at 100 mg/L, where a slight deposit was observed. Samples for analysis were taken after 0 and 24 h exposure. The organisms used in this study were 0 - 22 h old and unfed. 20 Daphnia were used per concentration and control (4 replicates of 5 daphnia). EC values were graphically determined.
Type of test:	Static
Species:	Daphnia magna Straus 1820
Exposure period	24 hours
Analytical monitoring:	Yes
GLP:	No
Year:	1985
Results:	EC_{50} (24 h) = > 86 mg/L EC_0 (24 h) = 31 mg/L EC_{100} (24 h) = > 86 mg/L

Table 1. Analytical data of test concentrations

Nominal Concentration	Measured concentration, mg/L 0 h	24 h
10 mg/L	5	4
18 mg/L	11	8
32 mg/L	28	25
58 mg/L	35	26
100 mg/L	93	78

Remarks: This study was assigned a reliability code of 2a (reliable without restrictions) according to the criteria established by Klimisch *et al* (1997).[2] This study was conducted under OECD, but not GLP guidelines.

Reference: "Report on the Test for Acute Toxicity of TK 10042 to Daphnia Magna", Ciba-Geigy Limited, Basel, Switzerland. Dr. A. de Morsier, 03/29/85.

[2]See general reference, p. 48.

13. ACUTE TOXICITY

A. ORAL

Test substance:	Tetrakis-(methylene-(3,5-di-(ert)-butyl-4-hydrocinnamate))methane CAS No. 6683-19-8 Batch Nos. MA-l 1 and MA-12
Method:	Two batches of the test material (MA1 1 and MA12) were tested at each dose level. The test material was administered to 4 rats (2 male, 2 female) per dose by gavage. Animals were observed for clinical signs of toxicity and mortality daily for 14 days. Initial and final body weights were recorded. Necropsy was performed at the end of the observation period.
Species/strain:	Sprague Dawley Rats
Sex:	Males and Females
No. Animals/Group:	Usexigroup
Doses:	4556, 6834 or 10250 mg/kg body weight
Vehicle:	25% (w/v) suspension in corn oil
Post dosing observation period:	14 days
GLP:	No
Year:	1974
Results:	LD_{50} > 10,250 mg/kg body weight No animals died during the study. No significant adverse effects were noted, except for hypoactivity and ruffled fur at all dose groups; labored breathing and diuresis were observed at the high dose group. The animals returned to normal by Day 2. No gross pathologic alterations were observed at necropsy.
Remarks:	This study was assigned a reliability code of 2 (reliable with restrictions) based on the criteria described by Klimisch et *al* (1997).[5] This study was not conducted under GLP or OECD guidelines. However, these findings validated several earlier studies which found the LD_{50} to be greater than 10,000 mg/kg in rats. [2-4]

Reference:

"'Acute Oral Toxicity Studies with Two Samples in Albino Rats", IBT No. 601-04522. Industrial Bio-Test Labs, Inc., M.L. Keplinger, 02/15/74 .

[2]"Acute Oral Toxicity Study in Albino Rats", IBT Report No. A2060, Industrial Bio-Test Labs, Inc. M.L. Keplinger, 09/19/72.

[3]"Acute Oral Administration (LD50) in Male Rats", Geigy Pharmaceuticals, Dept. of Toxicology, Technical Report. 02/23/68.

"'Acute Oral Administration (LD.50) in Female Rats", Geigy Pharmaceuticals, Dept. of Toxicology, Technical Report. 02/23/68.

[5]See general reference, p. 48.

B. INHALATION

Test substance:	Tetrakis-(methylene-(3,5-di-(ert)-butyl-4-hydrocinnamate))methane CAS No. 6683-19-8
Method:	Inhalation toxicity was tested according to the method of Sachsse et *al.*[2] Rats, weighing 197 to 252 g, were placed in a nose-only exposure system and exposed to an aerosol of the test compound for 4 h. During the exposure, concentration and particle size were monitored. The chamber atmosphere was sampled 5 times during the test, and the concentrations were determined gravimetrically. Animals were observed during exposure at 1, 2, and 4 hours as well as 2 hours post-exposure and daily for 14 days for physical condition and incidence of death. Gross pathologic evaluation was conducted on all animals dying within the observation period as well as the survivors killed at 14 days. Particular attention was given to the respiratory tract.
Type:	LC_{50}
Species/strain:	Rat
No. animals/group:	10 Males/ 10 Females/group
Doses:	0,762, and 1951 mg/m^3
Exposure time:	4 hours
Post exposure observation period:	14 days
GLP:	No
Year:	1980
Results:	$LC_{50} >$ 195 1 mg/m^3 No mortality was observed during the 14 day observation period. Slight dyspnea and ruffled fur were noted in both treatment groups. All animals recovered within 6 days. There were no differences in body weight and weight gain among exposure groups. No pathological changes were observed at necropsy.
Remarks:	This study was assigned a reliability code of 2e.[3] This study was not conducted under GLP or OECD guidelines, but does meet generally accepted scientific standards, is well documented, and is acceptable for assessment.
Reference:	'"Acute aerosol inhalation toxicity in the rat of TK-10042," Ciba-Geigy

Limited, Experimental Toxicology, Project 801603, 1980.

[2]Sachsse, K., Ulhnann, L., Voss, G., and Hess, R., "Measurement of inhalation toxicity of aerosols in small laboratory animals." In Proceedings of the European Society of the Study of Drug Toxicity, Vol. XV, pp. 239-251, 1973.

[3]See general reference, p. 48.

C. DERMAL

Test substance:	Tetrakis-(methylene-(3,5-di-(ert)-butyl-4-hydrocinnamate))methane CAS No. 6683-19-8
Method:	The test material was applied to albino rabbits (4/group) at a dose of 100, 316, 1000, or 3160 mg/kg body weight. The material was applied to the closely-clipped abdominal skin. Exposure areas of 2 animals/dose were abraded and the other 2 remained intact. The test material was moistened with corn oil and spread evenly on a nonabsorbent paper backing which was then applied to the skin. The trunks of the animals were wrapped securely with gauze and adhesive tape. After an exposure period of 24 h, the binders were removed and the area of exposure was sponged with warm tap water to remove any sample residue. The animals were observed for toxic effect and mortality at the following intervals after exposure: immediately, at 1.4, and 24 h, and daily for 14 days.
Type:	LD_{50}
Species/strain:	Rabbit
Doses:	100 mg/kg 316 mg/kg 1000 mg/kg 3 160 mg/kg
Exposure period:	24 hours
Post exposure observation period:	14 days
GLP:	No
Year:	1964
Results:	LD_{50} > 3 160 mg/kg body weight There were no deaths at any of the dose groups. All animals exhibited normal appearance and behavior throughout the study and showed normal body weight gains. At the end of the exposure period, slight erythema was observed in all animals. This dermal response completely subsided between Days 2 and 5. At termination, there were no signs of dermal irritation in any animal. There were no gross pathologic findings at necropsy.
Remarks:	This study was assigned a reliability code of 2e.[2] This study was not conducted under GLP or OECD guidelines, but does meet generally

accepted scientific standards, is well documented, and is acceptable for assessment.

Reference: "Acute Dermal Application - Albino Rabbits," Ciba-Geigy Limited, Basel, Switzerland, 5/19/64.

[2]See general reference, p. 48.

14. GENETIC TOXICITY IN VIVO

A. DOMINANT LETHAL ASSAY

Test substance:	Tetrakis-(methylene-(3,5-di-(ert)-butyl-4-hydrocinnamate))methane CAS No. 6683-19-8 Batch No. EN 28303
Method:	This study was not conducted under OECD guidelines. Male mice (20/group) were administered a single gavage dose of 0, 1000 or 3000 mg/kg in aqueous carboxymethylcellulose (0.2 mL/kg body weight). Males were mated with females for up to 6 weekly mating periods. Each group consisted of 20 males, each of which was placed in a cage with 2 untreated females immediately after treatment. At the end of 1 week, the females were replaced by another group of 2 females. The procedure was continued for 6 consecutive weeks. The females were examined daily for successful mating, as indicated by the occurrence of a vaginal plug. The day the vaginal plug was observed was designated as Day 0 of gestation. Pregnant females were necropsied on Day 14 of pregnancy. The number of live embryos and embryonic deaths were recorded. In addition, uteri were examined for early embryonic resorptions.
Type:	Dominant lethal assay
Species/strain:	Albino mice (NMRI derived)
Sex:	Male
Route of Administration:	Gavage
Exposure period:	Single exposure
Doses:	1000 and 3000 mg/kg
Vehicle:	Aqueous carboxymethylcellulose (0.2 mL/kg body weight)
Control:	Concurrent Positive: None Negative: Vehicle only
GLP:	No
Year:	1975

Results: No evidence of dominant lethal effects was noted. There were no differences in mating ratio, number of implantations or embryonic deaths between controls and treated.

Table 1. Reproductive parameters

Dose group (mg/kg)	Mating Ratio	Number Pregnant	Mean Implantations	Live Embryos	Embryonic Deaths
Mating period 1					
0	35/40	29	11.97	91.4%	8.6%
1000	36/40	30	11.20	93.8%	6.2%
3000	36/40	36	11.72	92.2%	7.8%
Mating period 2					
0	37/40	30	11.30	93.5%	6.5%
1000	31/40	29	12.34	93.3%	6.7%
3000	39/40	35	12.29	95.1%	4.9%
Mating period 3					
0	35/40	33	12.55	93.7%	6.3%
1000	38/40	34	12.62	95.3%	4.7%
3000	40/40	37	12.59	92.3%	7.7%
Mating period 4					
0	39/40	34	11.56	92.9%	7.1%
1000	37/40	35	11.91	91.4%	8.6%
3000	36/40	33	13.36	90.7%	9.3%
Mating period 5					
0	35/40	31	11.65	87.0%	13.0%
1000	34/39	28	12.68	90.7%	9.3%
3000	37/40	33	11.48	93.7%	6.3%
Mating period 6					
0	34/40	30	11.43	89.8%	10.2%
1000	38/40	33	11.39	93.6%	6.4%
3000	37/40	31	12.45	95.1%	4.9%

Remarks: This study was assigned a reliability code of 2e (meets generally accepted scientific standards, is well documented, and acceptable for assessment).[2]

Reference: '"Dominant Lethal Study on TK 10042, Mouse (Test for Cytotoxic or Mutagenic Effects on Male Germinal Cells), Experiment No. 327539. Ciba Geigy, Limited,Basel, Switzerland. Dr. H. Fritz, 09/12/75.

[2]See general reference, p. 48.

B. SOMATIC MUTATION ASSAY

Test substance:	Tetrakis-(methylene-(3,5-di-(ert)-butyl-4-hydrocinnamate))methane CAS No. 6683-19-8 Batch No. EN 1343
Method:	This study was not conducted under OECD or GLP guidelines. Chinese hamsters of either sex and weighing 23-33 g were used in this study. Animals (b/group) were gavaged with either 500, 1000 or 2000 mg/kg test material in 0.5% carboxymethylcellulose (CMC) (20 mL/kg). Positive controls animals were administered 128 mg/kg cyclophosphamide in 0.5% CMC (20 mL/kg) and negative controls were administered 0.5% (CMC 20 mL/kg). Treatment consisted of daily administration on 2 consecutive days. Twenty-four hours after the second application the animals were sacrificed and bone marrow was harvested from the shaft of both femurs. The bone marrow cells were transferred onto slides, stained with May-Grunwald solution and Giemsa solution, and scored for chromosomal anomalies. Bone marrow cells (1000 cells per animal) were scored and the following anomalies were registered: single Jolly bodies, fragments of nuclei in erythrocytes, micronuclei in erythroblasts, micronuclei in leucopoietic cells, and polyploid cells.
Type:	Somatic mutation assay
Species/strain:	Chinese hamster
Sex:	Male/Female
Route of Administration:	Gavage
Exposure period:	2 Days
Doses:	500, 1000 and 2000 mg/kg
GLP:	No
Year:	1977
Results:	In all groups, the percentage of cells displaying anomalies of nuclei did not differ significantly from the negative control. The test material was considered to benonmutagenic.

Table 1. Percent of cells with anomalies of nuclei

Dose	Animal No.	% Cells with Anomalies of Nuclei
Control	1	0.1
	2	0.2
	3	0
	A	0
	5	0.2
	6	0.1
Cyclophosphamide	1	5.6
		3.9
		2.8
	4	4.4
	5	5.3
		3.6
500 mg/kg	1	0
		0.1
	3	0.1
	4	0.1
	5	0.3
	6	0.2
1000 mg/kg	1	0
	2	0.4
	3	0.1
	4	0
		0.2
	6	0
2000 mg/kg	1	0
	2	0.1
	3	0.2
	4	0.3
	5	0.1
	6	0

The data represent the sum of single Jolly bodies, fragments of nuclei in erythrocytes, micronuclei in erythroblasts, micronuclei in leucopoietic cells, bizarre forms of nuclei, andpolyploid cells. The study reported separate incidences for each endpoint, but only the total is represented in this table.

Remarks: This study was assigned a reliability code of 2e (meets generally accepted scientific standards, is well documented, and acceptable for assessment).[2]

Reference: "'Nucleus Anomaly Test on Somatic Interphase Nuclei, TK 10042, Chinese Hamster (Test for Mutagenic Effects on Bone Marrow Cells)", Ciba Geigy, Limited,Basel-Switzerland. Dr. D. Muller, 10/26/77.

[2]See general reference, p. 48.

C. SOMATIC MUTATION ASSAY

Test substance:	Tetrakis-(methylene-(3,5-di-(ert)-butyl-4-hydrocinnamate))methane CAS No. 6683-19-8 Batch No. EN 1343
Method:	This study was not conducted under OECD guidelines. Chinese hamsters of either sex, weighing 22 to 29 g, were used. Animals (4/sex/dose group) were gavaged once daily for 2 consecutive days with either 0, 500, 1000 or 2000 mg/kg body weight test material in 2% aqueous solution of sodium carboxymethylcellulose (CMC) (20 mL/kg). Positive controls were administered 64 mg/kg cyclophosphamide in 2% CMC (20 mL/kg). The animals were injected with 10 mg/kg colcemide 2 hours after administration of the second dose and sacrificed 4 hours later. Bone marrow was harvested from the shaft of both femora, fixed, and transferred onto slides. The slides were stained with acetic orcein and analyzed for chromosomal aberrations (100 metaphases/animal). The following aberrations were recorded: chromatid-type aberrations, chromosome-type aberrations, chromatid gaps, and chromosome pulverations.
Type:	Somatic mutation assay
Species/strain:	Chinese Hamsters (Cricetulus griseus)
Sex:	Male/Female
Route of Administration:	Gavage
Exposure period:	2 Days
Doses:	500, 1000 and 2000 mg/kg
GLP:	No
Year:	1978
Results:	The chromosome displays from the negative control group and the intermediate and high dose groups showed no aberrations. In the animals of the low dose group, one metaphase per 400 cells with chromatid-type aberration in the form of a break was detected. This incidence was within the frequency observed in historical controls and was therefore considered to be spontaneous in origin. The test material was considered to benonmutagenic.

Remarks: This study is assigned a reliability code of 2e (meets generally accepted scientific standards, is well documented, and acceptable for assessment).[2]

Reference: "'Chromosome Studies in Somatic Cells, TK 10042, Chinese Hamster (Test for Mutagenic Effects on Bone Marrow Cells." Experiment No. 764028. Ciba Geigy, Limited, Basel, Switzerland. Dr. D. Muller, 09127178.

[2]See general reference, p. 48.

15. GENETIC TOXICITY IN VITRO

Test substance:	Tetrakis-(methylene-(3,5-di-(ert)-butyl-4-hydrocinnamate))methane CAS No. 6683-19-8
Method:	This study was not conducted under OECD guidelines, but was conducted using the methods described by Ames et *al.*[2-4] The material was tested for mutagenic effects on histidine auxotrophic mutants of Salmonella typhimurium. Cultures were prepared from frozen stock and on the following day the standard plate test was carried out. The concentrations of the test substance used without microsomal activation were: 10, 25, 50, 100 and 250 µg/0.1 mL; concentrations with microsomal activation were: 5, 10, 25, 50 and 100 µg/0.1 mL. Dose selection was limited by solubility. In the experiments in which the substance was metabolically activated, 0.5 mL of the activation mixture (S9 fraction of liver from rats induced with Aroclor 1254 plus co-factors) were added. Positive controls included *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (T A 1535), 9(5)-aminoacridine hydrochloride monohydrate (TA 1537), daunoblastin (TA 98), 4-nitroquinoline-n-oxide (TA 100). The activation mixture was tested with TA 100 and cyclophosphamide. In experiments without the addition of microsomal activation mixture, 3 Petri dishes were prepared per strain and per dose. In the experiments with activation mixture, 6 Petri plates were used per strain and per group. Three of each set of 6 were preincubated. Details of incubation time and temperature were not provided, but were based on the method reported by Ames*et al.*
Type:	Reverse mutation assay
System of testing:	S. typhimurium TA 98, TA 100, TA 1535, TA 1537
Concentration:	10-250 µg/0.1 mL
Vehicle:	DMSO
Metabolic activation:	With and without S9 fraction of liver from rats induced with Aroclor 1254 plus co-factors
GLP:	No
Year:	1977
Results	No increase in reverse mutations with or without metabolic activation. Cytotoxicity conc: Not reported. Precipitation conc: 100 µg/0.1 mL

Table 1. Mean number of revertant colonies **from experiments without metabolic activation**

	TA 98	TA 100	TA 1535	TA 1537
Control (DMSO)	28	115	11	8
10 μg/0.1 mL	24	118	11	8
25	24	151	14	5
50	24	149	17	6
100	24	136	13	7
250	24	163	16	7

Table 2. Mean number of revertant colonies from experiments with metabolic activation (without/with pre-incubation)

	TA 98	TA 100	TA 1535	TA 1537
Control (DMSO)	18/21	173/165	18/20	8/7
5 μg/0.1 mL	23/25	1791202	17/17	9/9
10	22/25	181/188	21/20	8/5
25	23/21	190/217	26/20	7/8
50	23/23	1771189	21/19	7/7
100	23/22	157/155	17/18	7/7

Remarks: This study followed methods described by Ames et *al* and was considered sound. The study was assigned a reliability code of 2e (meets generally accepted scientific standards, is well documented, and acceptable for assessment).[5]

References: '"Salmonella/Mammalian Microsome Mutagenicity Test with TKA 10042 (Test for Mutagenic Properties in Bacteria." Ciba Geigy, Limited, Basel, Switzerland. Dr. P. Arni, 04/20/77.

*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]See general reference, p. 48.

16. REPEATED DOSE TOXICITY

Test substance:	Tetrakis-(methylene-(3,5-di-(ert)-butyl-4-hydrocinnamate))methane CAS No. 6683-19-8 Batch No. EN 29040, An. No. 4689
Method:	Although this study was not formally conducted under OECD guidelines, the method paralleled OECD Guideline 409 "Subchronic Oral Toxicity – Non-Rodent: 90-Day study". In this study, Beagle dogs (6 males and 6 females/dose group) were given the test article in the diet for 13 weeks. After the administration period of three months, one animal per sex per dose group was fed the control diet for an additional 4 weeks. Clinical observations and food consumption were made daily. Body weight and auditory perception were measured weekly. Hematology, blood chemistry, and urinalysis were carried out on weeks -1, 4, 9 and 13, and on week 17 for recovery animals. Eye examinations were performed pre-test and at weeks 13 and 17.
Species/strain:	Beagle Dogs
Age at initiation:	24-31 weeks
Sex:	Male/Female
No. animals/group:	6/sex
Route of administration:	Dietary
Exposure period:	13 weeks
Frequency of treatment:	Daily
Post exposure observation period:	4 weeks
Dose:	1000, 3000 and 10000 ppm
Control group:	Concurrent (diet without admixing the test article)
GLP:	Yes
Year:	1981
Results:	**NOEL:** 10000 ppm There were no adverse effects that could be related to treatment. No clinical symptoms and no signs of systemic toxicity were observed. Ophthalmic inspection revealed no changes related to treatment. No

impairment of auditory perception was found. No animals died during the study. Food consumption, body weight gain and mean food conversion were unaffected by treatment. The results of the hematology, blood chemistry and urine analysis were unremarkable. An increase in total bilirubin concentration was observed at weeks 4 and 9, but not at week 13. As no other bilirubin linked parameters were changed, this observation was considered to be incidental and of no toxicological significance. Neither macroscopic nor microscopic changes that could be related to treatment were found. Organ weights and ratios for the compound treated dogs were comparable to those in the control animals.

Table 1. Total bilirubin (μmol/L)

	Pretest	Week 4	Week 8	Week 12	Recovery Wk 1
Male					
Control	2.3	2.2	2.1	2.5	1.0
1000	2.8	2.7	4.1*	3.2	1.9
3000	1.9	2.9	4.0*	3.6	1.8
10000	2.1	3.9*	4.5*	3.0	2.6
Female					
Control	2.4	2.4	2.8	2.7	2.5
1000	2.7	2.5	4.3	3.3	2.3
3000	2.4	3.4"	4.1	3.3	2.2
10000	2.5	3.8	4.7	3.2	2.4

*Statistically significant difference from control

Remarks: This study was assigned a reliability code of 1 (reliable without restriction) according to the guidelines described by Klimisch et *al* (1997).[2]

Reference: "'Final Report: TK 10042, 3-Month Toxicity Study on Dogs (Project No. 790539)", Ciba Geigy Limited, Basel, Switzerland. Dr. med R. Hess, 06/06/8 1.

[2]See general reference, p. 48.

17. REPRODUCTIVE TOXICITY

Test substance: Tetrakis-(methylene-(3,5-di-(ert)-butyl-4-hydrocinnamate))methane
CAS No. 6683-19-8

Method: Although not formally conducted under OECD guidelines, the methods in this study paralleled OECD Guideline 416 "Two-Generation Reproduction Toxicity Study." Diets containing 0 (control), 1000, 3000, or 10000 ppm of the test material were fed continuously to both sexes throughout two generations. Animals of the F_0 generation were approximately 6 weeks of age at the commencement of treatment, and were maintained on their treatments for 10 weeks prior to mating. One male and one female were paired for mating for a period of 20 days and vaginal smears were taken daily throughout the mating period. Dams were allowed to rear their young to Day 21 postpartum; 24 male and 24 female pups were retained as the F_1 generation. Following selection of the F_1 generation, a male and a female from each litter were selected for organ weight analysis and preservation of tissues. The remaining animals were sacrificed and examined macroscopically and discarded.

F_0 females that failed to produce a litter at the first mating were remated for a second 20-day period. Resultant litters were sacrificed on Day 8 postpartum, With the exception of the occasional animals that were involved in the remate, F_0 parents were sacrificed following weaning of the F_1 litters. Organ weight analysis and preservation of tissues was performed on all F_0 parents.

Animals of the F_1 generation were kept on their respective diets after weaning for 12 weeks prior to mating, which was carried out as described above. Dams were allowed to rear the pups until Day 21 post partum Analysis of the F_2 generation and F_1 parents was carried out as described above.

Throughout the study animals were observed for any clinical signs of toxicity. Food consumption, water intake and body weight gain was also monitored throughout the study.

Type: Two-generation study

Species/strain: CrL:COBS CD (SD) BR Rats

Sex: Male/Female

Route of Administration: Oral

Exposure period: 2 generations, 10 months

Frequency of treatment:	Daily dietary exposure
Premating exposure period:	Male: 10 weeks; Female: 10 weeks
Duration of the test:	2 generations, 10 months
Doses:	1000, 3000 and 10000 ppm
Control group:	Concurrent (standard diet)
GLP:	Yes
Year:	1984
Results:	NOEL Parental: 10000 ppm NOEL F_1 Offspring: 10000 ppm NOEL F_2 Offspring: 10000 ppm

General Parental Toxicity: No deaths occurred among animals of either the F_0 or F_1 generation nor were there any consistent effects which could be attributed to treatment, including any clinical signs of toxicity, food consumption, body weight gain and efficiency of food utilization, reproductive capacity as assessed by mating performance, pregnancy rate and duration of gestation, and findings at terminal autopsy.

Toxicity to offspring: There were no adverse effects on litters of treated parents in either generation, as assessed by: (1) the incidence of total litter loss; (2) mean values of litter size, pup mortality, sex ratio, litter and mean pup weights; (3) findings at terminal necropsy. A slightly faster growth rate was apparent among offspring at 10000 ppm in both generations, an observation which appeared to be independent of litter size and was confirmed by the noticeably higher mean litter weight in this group at termination.

The findings of this study indicated that, under the conditions of the test procedure, animals administered Irganox 1010 at levels of 1000, 3000 and 10000 ppm in their diet showed no substantial differences from their control counterparts and that their reproductive capacity was not impaired.

Table 1. Group mean litter data, F_0 generation

Dose	Number animals		At birth					At Day 4			
	Mated	Pregnant	Total litter size	Live litter	% Loss	Litter wt, g	Mean pup wt, g	Litter size	% Cumulative loss	Litter wt, g	Mean pup wt, g
Control	28	A=28	13.5	13.4	0.5			12.6	6.9		
		B=27	13.6	13.5	0.6	75.5	5.7	13.1	3.4	106.1	8.3
1000	28	A=B=25	11.7*	11.5*	1.2	67.7	6.0	11.3*	2.9	102.5	9.3*
3000	28	A=B=26	13.7	13.5	1.7	73.8	5.5	12.9	5.7	103.7	8.1
10000	28	A=27	12.0	11.9	0.9			10.9	7.4		
		A=26	11.8*	11.7	0.9	67.3	5.9	11.3*	3.9	102.9	9.4*

*Statistically significant difference from control

Table 1. Group mean litter data, F_0 generation (continued)

Dose	At Day 8				At Day 12				At Day21			
	Total litter size	% Cumulative loss	Litter wt, g	Mean pup wt, g	Total litter size	% Cumulative loss	Litter wt, g	Mean pup wt, g	Total litter size	% Cumulative loss	Litter wt, g	Mean pup wt, g
Cont	12.4	8 . 0			12.1	10.4			11.9	11.3		
	12.9	4.5	165.7	13.2	12.5	7.11	223.1	18.2	12.4	8.0	412.3	34.1
1000	11.2*	3.8	162.0	14.9*	11.1	4.5	223.4	20.7	11.1	4.8	419.5	39.2
3000	12.5	8.8*	161.0	12.9	12.3	9.8	224.9	18.2	11.7	11.5	417.0	34.7
10000	IO.8	8.4			10.7	9.0			10.7	9.0		
	11.2*	4.9	165.1	15.2*	11.1	5.5	226.8	21.2*	11.1	5.5	430.6	40.3*

Table 2. Group mean litter data, F_1 generation

Dose	Number animals		At birth					At Day 4			
	Mated	Pregnant	Total litter size	Live litter	% Loss	Litter wt, g	Mean pup wt, g	Litter size	% Cumulative loss	Litter wt, g	Mean pup wt, g
Control	24	A=B=22	11.5	11.3	1.6	65.2	5.8	11.0	4.0	102.3	9.4
1000	24	A=B=23	11.4	11.3	1.6	67.3	6.1	11.1	2.6	105.9	9.9
3000	24	A=22	12.0	11.9	1.3			11.5	4.6		
		B=21	12.0	11.9	1.4	69.6	5.9	11.6	3.3	108.6	9.5
10000	24	A=B=22	11.3	11.1	1.2	68.2	6.2*	11.0	1.9	106.9	9.9

*Statistically significant difference from control

Table 2. Group mean litter data, F_1 generation (continued)

Dose	At Day 8				At Day 12				At Day 21			
	Total litter size	% Cumulative loss	Litter wt, g	Mean pup wt, g	Total litter size	% Cumulative loss	Litter wt, g	Mean pup wt, g	Total litter size	% Cumulative loss	Litter wt, g	Mean pup wt, g
Cont	10.9	5.2	162.5	15.0	10.9	5.2	231.2	21.3	10.9	5.2	429.1	40.2
1000	11.0	3.2	172.7	16.2	11.0	3.8	250.7	23.7	10.9	4.4	459.0	43.7
3000	11.0	8.1			11.0	8.1			11.0	8.8		
	11.6	3.7	173.2	15.2	11.6	3.7	250.9	22.1	11.5	4.5	468.5	41.5
10000	11.0	1.9	175.8	16.5*	11.0	2.3	254.3	23.9*	11.0	2.3	475.5	44.7

Remarks: This study was assigned a reliability code of 1 (reliable without restrictions).[2] This study was conducted under GLP guidelines. The report contains all of the information necessary to evaluate the adequacy and results.

Reference: '"Effects of TK 10042 on Reproductive Functions on Two Generations of the Rats", Huntingdon Research Centre, Huntingdon, Cambridgeshire, England.Audry M. Bottomley, 09/05/84.

[2]See general reference, p. 48.

18. DEVELOPMENTAL TOXICITY/ TERATOGENICITY

Two studies were conducted to assess the teratogenic potential. Both studies are given equal weight, and taken together are adequate to meet the necessary requirements for this endpoint.

A. RAT STUDY

Test substance:	Tetrakis-(methylene-(3,5-di-(ert)-butyl-4-hydrocinnamate))methane CAS No. 6683-19-8
Method:	Female rats, weighing approximately 240 g, were mated overnight with males of proven fertility in a ratio of one male to three females. The day on which spermatozoa were found in the vaginal smear was designated as Day 0 of pregnancy. Throughout the experiment, successfully mated females were housed in groups of 5 in an air-conditioned room at a temperature of 22 °C ± 5 °C and a humidity of 56% ± 5%. The room was illuminated for 12 hours daily. Animals were provided standard diet and tap water ad libitum The compound was administered by oral gavage on Days 6 through 15 of pregnancy. During the treatment, general condition, weight gain, food consumption, and symptomology were checked daily. Dams were necropsied and fetuses were removed by Caesarean section on Day 21 of pregnancy. The examinations were carried out in accordance with the World Health Organization (WHO) recommendations (WHO, 1967) and the technique described by Wilson, 1965.[2,3]
Species/strain:	Sprague-Dawley Rats
Sex:	Female
Route of administration:	Oral gavage
Duration of the test:	10 Days
Exposure period:	Days 6 through 15 of gestation
Frequency of treatment:	Daily
Doses:	150,500 and 1000 mg/kg in 1 mL/100 g body weight
Control group:	Concurrent
GLP:	No
Year:	1975

Results: No teratogenic effects or maternal toxicity observed.

NOEL maternal toxicity: 1000 mg/kg
NOEL teratogenicity : 1000 mg/kg

Maternal general toxicity: At the low and intermediate dose levels, an increase in food consumption was noted during the treatment period. However, there was no effect on body weight gain. Numerical data were not provided in the study report.

Pregnancy/litter data: The rates of implantation and resorptions, as well as the average weights of the fetuses, were comparable for all groups.

Fetal data: Embryonic development was not adversely affected by treatment. In both the low and intermediate dose groups, phalangeal nuclei of the hind limb and calcanei displayed higher rates of ossification than in the controls. This effect on the physiological growth may be associated with the above mentioned increase in food consumption by the dams. It was not observed in the high-dose group.

Table 1. Reproductive parameters

	Dose group			
	Control	150 mg/kg	500 mg/kg	1000 mg/kg
Number of dams	30	25	25	25
Spontaneous deaths	0	0	0	0
# of females with implantations	26	24	22	21
Mean # implantations	13.28	13.17	13.0	12.05
% Embryonic resorptions	7.8	8.5	7.3	5.9
% Fetal resorptions	0	0.3	0	0.4
% Dead fetuses	0			
% Live fetuses	92.2	91.1	92.7	93.7
Live fetuses with malformations	0	1	1	0
Weight of live fetuses, g	5.25	5.32	5.24	5.27

Table. 2. Skeletal assessment data

Dose group	# Skeletons examined	Phalangeal nuclei, hind limb, %	Calcaneus, %
Control	206	29.6	20.9
150 mg/kg	193	8.8*	4.7*
500 mg/kg	177	17.5"	11.3"
1000 mg/kg	157	29.6	28.7

*Statistically significant difference from control

Remarks: This study was assigned a reliability code of 2 (reliable with restrictions) based on the criteria described by Klimisch et *al* (1997).[4] This study was not conducted under formal guidelines but the methods were standard.

References:

"'Reproductive Study - TK 10042, Rat, Segment II (Test for Teratogenic or Embryotoxic Effects)", Ciba Geigy Limited, Basel, Switzerland, Dr. H. Fritz, 06/19/75.

[2]World Health Organization Technical Report Service 364, 1967

[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]See general reference, p. 48.

B. MOUSE STUDY

Test substance:	Tetrakis-(methylene-(3,5-di-(ert)-butyl-4-hydrocinnamate))methane CAS No. 6683-19-8
Method:	Female mice, weighing approximately 30 g, were mated overnight with males of proven fertility in a ratio of one male to four females. The day on which the sperm plug was found was designated as Day 0 of pregnancy. Throughout the experiment, successfully mated females were housed in groups of 5 in an air-conditioned room at a temperature of 22 °C ± 5 °C and a humidity of 56% ± 5%. The room was illuminated for 12 hours daily. Animals were provided standard diet and tap water ad libitum. The compound was administered by oral gavage on Days 6 through 15 of pregnancy. During the treatment general condition, weight gain, food consumption, and symptomology were checked daily. Dams were necropsied and fetuses were removed by Caesarean section on Day 18 of pregnancy. The examinations were carried out in accordance with the World Health Organization (WHO) recommendations (WHO, 1975) and the technique described by Wilson, 1965.[2,3]
Species/strain:	NMRI derived Albino Mice
Sex:	Female
Route of Administration:	Oral gavage
Duration of the test:	10 Days
Exposure period:	Days 6 through 15 of gestation
Frequency of treatment:	Daily
Doses:	150,500 and 1000 mg/kg in 0.1 mL/10 g body weight
Control group:	Yes Concurrent vehicle (2% aqueous carboxymethyl cellulose)
GLP:	No
Year:	1975
Results:	No teratogenic effects or maternal toxicity observed. **NOEL maternal toxicity:** 1000 mg/kg **NOEL teratogenicity:** 1000 mg/kg

Maternal general toxicity: No reactions to treatment were noted. Body weight gain and food consumption were comparable for all groups.

Pregnancy/litter data: The rates of implantation and resorptions, as well as the average weights of the fetuses were comparable for all groups.

Fetal data: Skeletal assessment revealed minor deviations from controls in the low and high dose groups. At the 150 mg/kg dose level, the incidences of ossification of the phalangeal nuclei of the hind limb and calcanei were significantly different than controls. An increase in the number of still incompletely ossified sternebrae was observed at the high dose. However, since there were no dose relationships and incidences generally display great variability, no special significance should be attached to these findings. The compound was considered to be nonteratogenic in the mouse

Table 1. Reproductive parameters

	Dose group			
	Control	150 mg/kg	500 mg/kg	1000 mg/kg
Number of dams	60	30	30	30
Spontaneous deaths	0	0	0	0
# of females with implantations	51	23	26	27
Mean # implantations	11.57	11.61	10.69	11.67
% Embryonic resorptions	7.3	10.1	8.3	5.7
% Fetal resorptions	1.0	1.9	1.1	0.3
% Dead fetuses	0.2	0.4	0.4	0
% Live fetuses	91.6	87.6	90.3	94.0
Live fetuses with malformations	1/540	1/234	1/251	1/296
Weight of live fetuses, g	1.13	1.16	1.11	1.12

Table 2. Skeletal assessment data

Dose group	# Skeletons examined	Phalangeal nuclei, hind limb, %	Calcaneus, %	Sternebrae, %
Control	359	5.0	36.8	17.5
150 mg/kg	157	1.9*	47.8*	17.8
500 mg/kg	166	3.0	39.2	22.3
1000 mg/kg	198	4.0	36.9	27.8*

*Statistically significant difference from control

Remarks: This study was assigned a reliability code of 2 (reliable with restrictions) based on the criteria described by Klimisch et *al* (1997).[4] This study was not conducted under formal guidelines but the methods were standard.

References: '"Reproductive Study - TK 10042, Mouse, Segment II (Test for Teratogenic or Embryotoxic Effects)", Ciba Geigy Limited, Basel, Switzerland, Dr. H. Fritz, 08/28/75.

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

'Wilson, J.G., in: Teratoloev. Principles and Techniques; J.G. Wilson and J. Warkany eds., The University of Chicago Press, Chicago, 1965, pp. 262-277.

[4]See general reference, p. 48.

GENERAL REFERENCE

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.

Definition of codes

1 = Valid without restriction
la: GLP guideline study
lb: Comparable to guideline study
lc: Meets national standard methods (AFNOR/DIN)
1d: Meets generally accepted scientific standards and is described in sufficient detail

2 = Valid with restriction
2a: Guideline study without detailed documentation
2b: Guideline study with acceptable restrictions
2c: Comparable to guideline study with aceptable restrictions
2d: Meets national standard methods with acceptable restrictions
2e: Meets generally accepted scientific standards, well documented and acceptable for assessment
2f: Accepted calculation method
2g: Data from Handbook or collection of data

3 = Invalid
3a: Documentation insufficient for assessment
3b: Significant methodological deficiencies
3c: Unsuitable test system

4 = Not assignable
4a: Abstract
4b: Secondary literature
4c: Original reference not yet available
4d: Original reference in foreign language
4e: Documentation in sufficient for assessment