

# **HPV Robust Summaries**

**For**

**1,3,5-tris(3,5-di-tert-butyl-4-hydroxybenzyl)-1,3,5-triazine-2,4,6  
(1H,3H,5H) -trione**

**CAS No. 27676-62-6**

Rubber and Plastic Additives Panel of  
The American Chemistry Council

**July 11, 2003**

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**CAS No. 27676-62-6**

**SUMMARY TABLE**

<b>CAS No. 27676-62-6</b>	<b>DATE</b>	<b>RESULTS</b>	<b>FULFILLS REQUIREMENT</b>
<b>PHYSICAL/CHEMICAL ELEMENTS</b>			
Melting Point	2002	219.5-225.5 °C	Yes
Boiling Point	2001	960.98 °C	Yes
Vapor Pressure	2002	5 x 10 <sup>-15</sup> mm Hg	Yes
Partition Coefficient	2002	log P > 6.0	Yes
Water Solubility	2002	< 1 ppm	Yes
<b>ENVIRONMENTAL FATE ELEMENTS</b>			
Photodegradation	2001	For reaction with hydroxyl radical, predicted rate constant = 66.5 x 10 <sup>12</sup> cm <sup>3</sup> /molecule-sec predicted half-life = 1.93 h	Yes
Stability in Water	2001	Hydrolysis rate extremely slow	Yes
Fugacity	2001	Predicted distribution using Level III fugacity model Air 0.02 % Water 1.15 % Soil 38.4 % Sediment 60.4 %  Persistence = 6.4 x 10 <sup>3</sup> h	Yes
Biodegradation	1985	Not biodegradable 0-7 % after 28 days	Yes
Bioaccumulation	2001	Estimated log BCF = 0.50 (BCF = 3.16)	
<b>ECOTOXICITY ELEMENTS</b>			
Acute Toxicity to Fish	1988	Zebra fish (Brachydanio rerio): LC <sub>50</sub> (24 - 96 h) => 100 mg/L	Yes
Toxicity to Aquatic Plants	1992	Green algae (Scenedesmus subspicatus): EC <sub>50</sub> (0 - 72 h) => 100 mg/L NOEC (0 - 72 h) = 33 mg/L	Yes
Acute Toxicity to Aquatic Invertebrates	1988	Daphnia magna: EC <sub>0</sub> (24 h) = > 100 mg/L EC <sub>50</sub> (24 h) = 32 mg/L EC <sub>100</sub> (24 h) => 100 mg/L	Yes

**1,3,5–tris(3,5-di-tert-butyl-4-hydroxybenzyl)-1,3,5-triazine-2,4,6  
(1H,3H,5H) -trione**

**CAS No. 27676-62-6**

**SUMMARY TABLE (CONTINUED)**

<b>CAS No. 27676-62-6</b>	<b>DATE</b>	<b>RESULTS</b>	<b>FULFILLS REQUIREMENT</b>
<b>HEALTH ELEMENTS</b>			
Acute Toxicity	1986	Rat: LD <sub>50</sub> (Oral) > 5000 mg/kg	Yes
	1992	Rabbit: LD <sub>50</sub> (Dermal) > 2000 mg/kg	Yes
Genetic Toxicity in vivo	1987	Chinese hamster: Nonmutagenic in somatic mutation assay (exposed by gavage 5000 mg/kg)	Yes
Genetic Toxicity in vitro	1986	Salmonella typhimurium: No increase in mutations with or without metabolic activation (at doses of 20 – 5000 µg/0.1 mL)	Yes
	1978	Salmonella typhimurium: No increase in mutations with or without metabolic activation (at doses of 25 – 2025 µg/0.1 mL)	Yes
Genetic Toxicity in vitro (non-bacterial)	1991	Chinese hamster V79 cells: No increase in mutations with or without metabolic activation (at doses of 27.5 – 550 µg/0.1 mL)	Yes
Cytogenetic test	1991	Chinese hamster ovary cells: No clastogenic effects	Yes
Repeated Dose Toxicity	1990	Albino Rats: NOEL = 3000 ppm (males) NOEL = 800 ppm (females) (90 days exposure, diet)	Yes
	1970	Albino Rats: NOEL = 10,000 ppm (92-93 days exposure, diet)	
	1970	Dog: NOEL = 10,000 ppm (90 days exposure, diet)	
Developmental Toxicity	NA	NA	Study proposed – OECD 414
Toxicity to Reproduction	1970-90	90-day repeat dose studies provide appropriate data on reproductive organs	Yes
Chronic Toxicity / Carcinogenicity	1978	2 year rat study: Not carcinogenic at 100 ppm	Yes

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## 1.0 GENERAL INFORMATION

### 1.0.1 SUBSTANCE INFORMATION

A. **CAS Number** 27676-62-6

**Name (IUPAC name)** 1,3,5-tris(3,5-di-tert-butyl-4-hydroxybenzyl)-1,3,5-triazine-2,4,6  
(1H,3H,5H)-trione

B. **Molecular Formula** C<sub>48</sub> H<sub>69</sub> N<sub>3</sub> O<sub>6</sub>

C. **Structural Formula** (indicate the structural formula in smiles code, if available)

n1(C(c2cc(C(C)(C)C)c(O)c(C(C)(C)C)c2))c(=O)n(C(c3cc(C(C)(C)C)c(O)c(C(C)(C)C)c3))c(=O)n(C(c4cc(C(C)(C)C)c(O)c(C(C)(C)C)c4))c1(=O)

D. **Molecular Weight** 784

E. **Type of Substance**

element [ ]; inorganic [ ]; natural substance [ ]; organic [ X ]; organometallic [ ];  
petroleum product [ ]

F. **Physical State (at 20°C and 1.013 hPa)**

gaseous [ ]; liquid [ ]; solid [X]

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## 2.0 PHYSICAL-CHEMICAL DATA

### 2.0.1 MELTING POINT

Value: 219.5 – 225.5 °C

Decomposition: Yes  No  Ambiguous

Sublimation: Yes  No  Ambiguous

Method: From Ciba<sup>1</sup>

GLP: Yes  No  ?

Remarks: The melting point was reported in the MSDS from Ciba Specialty Chemicals Corp. The method of determination by Ciba was not reported. The melting point was assigned a reliability code of 2g (data from handbook or collection of data)<sup>2</sup>.

Reference: <sup>1</sup> Ciba MSDS data sheet No. 85, Tarrytown, New York.

<sup>2</sup>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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## 2.0.2 BOILING POINT

Value: 960.98 °C

Method: Estimated by the MPBPWIN Program (v. 1.40)<sup>1,2</sup> using the adapted Stein and Brown method.

GLP: Yes  No  ?

Remarks: In the absence of reliable experimental data, the boiling point was calculated using an accepted method and assigned a reliability code of 2f<sup>3</sup> (Accepted calculation method). A measured value is not required if the calculated value is > 300 °C (OECD TG 103).

Reference: <sup>1</sup>Syracuse Research Corporation, Syracuse, NY.

<sup>2</sup>Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998.

<sup>3</sup>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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### 2.0.3 VAPOR PRESSURE

Value:  $5 \times 10^{-15}$  mm Hg at 25°C

Temperature: 25 °C

Method: calculated ; measured   
The vapor pressure was reported from Ciba MSDS. <sup>1</sup>

GLP: Yes  No  ?

Remarks: The vapor pressure of 4.68E-028 mm Hg was estimated by the MPBPWIN Program(v.1.40) using the modified Grain method,<sup>2,3</sup> which is comparable to MSDS data. The study and was assigned a reliability code of 2g (data from handbook or collection of data)<sup>4</sup>.

References: <sup>1</sup>Ciba MSDS data sheet No. 85, Tarrytown, New York.

<sup>2</sup>Syracuse Research Corporation, Syracuse, NY.

<sup>3</sup>Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998.

<sup>4</sup>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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#### 2.0.4 PARTITION COEFFICIENT

Log Pow: > 6.0

Method: calculated ; measured   
Ciba MSDS report<sup>1</sup>

GLP: Yes  No  ?

Remarks: A log P value of 15.18 was calculated by KOWWIN (v. 1.66)<sup>2,3</sup>. The calculated log P confirms the high measured value. The partition coefficient was assigned a reliability code of 2g (data from handbook or collection of data)<sup>4</sup>.

Reference: <sup>1</sup>Ciba MSDS data sheet No. 85, Tarrytown, New York.

<sup>2</sup>Syracuse Research Corporation, Syracuse, NY.

<sup>3</sup>Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998.

<sup>4</sup>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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## 2.0.5 WATER SOLUBILITY

Value: < 1 ppm

Temperature: 25 °C

Description: Miscible [ ]; Of very high solubility [ ];  
Of high solubility [ ]; Soluble [ ]; Slightly soluble [ ];  
Of low solubility [X]; Of very low solubility [ ]; Not soluble [ ]

Method: calculated [ ]; measured [X]  
Ciba MSDS report.<sup>1</sup>

GLP: Yes [ ] No [X] ? [ ]

Remarks: Ciba MSDS reported the solubility as < 1 ppm in water at 20 °C. The water solubility value was 3.998e-012 mg/L estimated by WSKOW Program (v. 1.37) <sup>2,3</sup> which confirms the low solubility. The method was assigned a reliability code of 2g (data from handbook or collection of data)<sup>4</sup>.

Reference: <sup>1</sup>Ciba MSDS data sheet No. 85, Tarrytown, New York.  
<sup>2</sup>Syracuse Research Corporation, Syracuse, NY.  
<sup>3</sup>Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998.  
<sup>4</sup>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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### 3.0 ENVIRONMENTAL FATE AND PATHWAYS

#### 3.0.1 PHOTODEGRADATION

Type: Air [ **X** ]; Water [ ]; Soil [ ]; Other [ ]

Half life: 1.93 hours.

Rate constant (radical): 66.5 E-12 cm<sup>3</sup>/molecule-sec

Method: calculated [ **X** ]; measured [ ]  
Estimated by the AOP program (v. 1.90) <sup>1,2</sup> which estimates rate constants and half-lives of atmospheric reactions of organic compounds with hydroxyl radicals and ozone in the atmosphere.

GLP: Yes [ ] No [ **X** ] ? [ ]

Test substance: 1,3,5-tris(3,5-di-tert-butyl-4-hydroxybenzyl)-1,3,5-triazine-2,4,6(1H,3H,6H)-trione.

Remarks: In the absence of reliable experimental data, the photodegradation was calculated using an accepted method and assigned a reliability code of 2f.<sup>3</sup> (Accepted calculation method)

Reference: <sup>1</sup>Syracuse Research Corporation, Syracuse, NY.

<sup>2</sup>Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998.

<sup>3</sup>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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### 3.0.2 STABILITY IN WATER

Type: Abiotic (hydrolysis) ; biotic (sediment)

Results: Hydrolysis rate extremely slow. Calculation not performed.

Method: Estimated by the HYDROWIN Program (v. 1.67) <sup>1,2</sup>

GLP: Yes  No  ?

Test substance: 1,3,5-tris(3,5-di-tert-butyl-4-hydroxybenzyl)-1,3,5-triazine-2,4,6(1H,3H,6H)-trione.

Remarks: The stability in water was calculated using an accepted method and assigned a reliability code of 2f.<sup>3</sup> (Accepted calculation method)

References: <sup>1</sup>Syracuse Research Corporation, Syracuse, NY  
<sup>2</sup>Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998.  
<sup>3</sup>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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### 3.0.3 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

Media: Air-biota [ ]; Air-biota-sediment-soil-water [ ]; Soil-biota [ ];  
Water-air [ ]; Water-biota [ ]; Water-soil [ ]; Other [ ]  
Method: Fugacity level I [ ]; Fugacity level II [ ]; Fugacity level III [ X ]; Fugacity  
level IV [ ]; Other (calculation) [ X ]; Other (measurement) [ ]

Estimated by EPIWIN Level III Fugacity Model <sup>1,2</sup>

Results: Distribution using level III fugacity model

Air	0.02 %
Water	1.15 %
Soil	38.4 %
Sediment	60.4 %

Persistence Time:  $6.4 \times 10^3$  hr.

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

References: <sup>1</sup>Syracuse Research Corporation, Syracuse, NY

<sup>2</sup>Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998.

<sup>3</sup>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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### 3.0.4 BIODEGRADATION

Type: aerobic [  ]; anaerobic [  ]

Concentration of the chemical: 10 mg and 20 mg of test substance /l.

Medium: water [  ]; water-sediment [  ]; soil [  ]; sewage treatment [  ]

Vehicle: Water as specified in the guideline containing 0.5 ml of the Nonylphenol 10E05P0 solution.

Inoculum: Fresh sewage treatment plant sample (per guideline)

Degradation: The biodegradation calculated as percentage of measured amount of carbon dioxide was:  
10 mg test substance/ L = 7 % in 28 days (time)  
20 mg test substance/ L = 0 % in 28 days (time)

Results: Readily biodeg. [  ]; inherently biodeg. [  ]; under test condition no biodegradation observed [  ], other [  ]

Method: *OECD Guideline for testing of Chemicals No. : 301B (May 1981)*  
The EEC Directive 79/831 Annex V part C 5.2 was established according to the OECD Guideline for testing of chemicals No. : 301 E ( May 1981).  
The only deviation from the guideline method is the volume of the test solution was reduced from 3.0 L to 1.5L. The carbon dioxide formed by biodegradation was absorbed with NaOH and determined on a carbon analyser. Due to the poor solubility of the test material in water, an emulsifier was used to achieve a better distribution in the medium.<sup>1</sup>

GLP: Yes [  ] No [  ] ? [  ]

Test substance: 1,3,5-tris(3,5-di-tert-butyl-4-hydroxybenzyl)-1,3,5-triazine-2,4,6(1H,3H,6H)-trione.

Remarks: This study was assigned a reliability code of 2b<sup>2</sup> (guideline study with acceptable restrictions) according the criteria established by Klimisch *et al* (1997).

Reference: <sup>1</sup>Report on the test for ready biodegradability of Irganox 3114 in the modified sturm test. Project No.: 88 43 81, November 01, 1988. Ciba-Geigy Ltd., Basle, Switzerland.  
  
<sup>2</sup>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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### 3.0.5 BIOACCUMULATION

BCF: Estimated log BCF = 0.50 (BCF = 3.16)

Elimination: Yes  No  ?

Method: Estimated by EPIWIN BCF Program (v2.14)<sup>1,2</sup>

Type of test: calculated ; measured   
static ; semi-static ; flow-through ; other (*e.g. field test*)

GLP: Yes  No  ?

Test substance: 1,3,5-tris(3,5-di-tert-butyl-4-hydroxybenzyl)-1,3,5-triazine-2,4,6(1H,3H,6H)-trione.

Remarks: In the absence of reliable experimental data, the bioaccumulation was calculated using an accepted method and assigned a reliability code of 2f.<sup>3</sup> (Accepted calculation method).

References: <sup>1</sup>Syracuse Research Corporation, Syracuse, NY  
<sup>2</sup>Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998.  
<sup>3</sup>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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## 4.0 ECOTOXICITY ELEMENTS

### 4.0.1 ACUTE/PROLONGED TOXICITY TO FISH

Type of test: static ; semi-static ; flow-through ; other (*e.g. field test*)   
open-system ; closed-system

Species: Zebra-Fish (*Brachydanio rerio*)

Number of fishes: 20 fish in test concentration, tested in 2 separate tanks  
10 fish in control  
10 fish per aquarium

Control: Water

Vehicle: 4 mg alkylphenol-polyglykol-ether per liter water

Exposure period: 96 - hours

Results: LC<sub>50</sub> (24h) = > 100 mg/l  
LC<sub>50</sub> (48h) = > 100 mg/l  
LC<sub>50</sub> (72h) = > 100 mg/l  
LC<sub>50</sub> (96h) = > 100 mg/l

Values are based on nominal concentrations.

Analytical monitoring: Yes  No  ?

Method: OECD-Guideline No. 203, Paris 1984 (static procedure)

Test solution containing 5.0 g of test material and 200 mg alkylphenol-polyglykol-ether were mixed with and made up to 1 L with water and stored at room temperature. Glass aquaria of 20 litres was filled with 15 litres of dechlorinated tap water. The temperature is maintained at 23 ± 1°C and was lighted for 16 hours with fluorescent light. Daily measurements of oxygen, pH, temperature were taken. Desired test concentrations were homogeneously distributed into the water. A slight deposit was observed at concentration of 100 mg/ L (nominal) after 24 hour exposure<sup>1</sup>.

GLP: Yes  No  ?

Test substance: 1,3,5-tris(3,5-di-tert-butyl-4-hydroxybenzyl)-1,3,5-triazine-2,4,6(1H,3H,6H)-trione. Purity: commercial grade

Remarks: This study was assigned a reliability code of 2b (guideline study with acceptable restrictions) according the criteria established by Klimisch *et al* (1997)<sup>2</sup>.

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Reference: <sup>1</sup>Test for Acute Toxicity of TK 10730 to Zebra Fish (*Brachydanio rerio*), Project No.: 884382, Ciba-Geigy Ltd., Basel, Switzerland, December 2, 1988.

<sup>2</sup>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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#### 4.0.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type of test: static [ **X** ]; semi-static [ ]; flow-through [ ]; other (*e.g. field test*) [ ]; open-system [ ]; closed-system [ ]

Species: Daphnia Magna Straus 1820

Number of Daphnia: 20 daphnia per concentration and control.  
4 replicates of 5 daphnia each

Control: Blank: water  
Vehicle: 4 mg alkylphenol-polyglykol-ether per litre water

Test Concentration: 10, 18, 32, 58, 100 mg/L

Exposure period: 24 hours

Results: EC<sub>50</sub> (24h) = > 100 mg/l  
EC<sub>0</sub> (24h) = 32 mg/l  
EC<sub>100</sub> (24h) = > 100 mg/l  
10% immobilization was observed at 58 mg/L and 20% at 100 mg/L.

Analytical monitoring: Yes [ ] No [ **X** ] ? [ ]

Method: OECD Guideline No. 202, Paris 1984. Tests were conducted in beakers containing 100 mL solution. Reconstituted water was prepared by dissolving 65 mg NaHCO<sub>3</sub>, 294 mg CaCl<sub>2</sub> (2 H<sub>2</sub>O), 123 mg MgSO<sub>4</sub> (7H<sub>2</sub>O), 6 mg KCl per liter distilled water. Total hardness was 240 mg CaCO<sub>3</sub>/L; pH ranged from 7.2 to 7.9; O<sub>2</sub> ranged from 87 to 96% saturation; temperature was 20 ± 1 °C. ). 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 58-100 mg/L, where a slight deposit was observed. Samples for analysis were taken after 0 and 24 h exposure for water quality measurements.<sup>1</sup>

GLP: Yes [ ] No [ **X** ] ? [ ]

Test substance: 1,3,5-tris(3,5-di-tert-butyl-4-hydroxybenzyl)-1,3,5-triazine-2,4,6(1H,3H,6H)-trione

Remarks: This study was assigned a reliability code of 2b (guideline study with acceptable restrictions) according the criteria established by Klimisch *et al* (1997)<sup>2</sup>.

Reference: <sup>1</sup>Test for Acute Toxicity to Daphnia magna, Project No.: 884383, Ciba-Geigy Ltd., Basel, Switzerland, November 16, 1988.  
<sup>2</sup>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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#### 4.0.3 TOXICITY TO AQUATIC PLANTS

Species: Green Algae ( *Scenedesmus subspicatus*)

Endpoint: Biomass [X]; Growth rate [ ]; Other [ ]

Exposure period: 72 hours

Test concentrations: 1.23, 3.7, 11, 33 and 100 mg/ L (nominal)

Controls: Blank: water  
Vehicle: 4.0 mg Arkopal/L (alkylphenol-polyglykoether)

Results: EC<sub>50</sub> (72 h) = > 100 mg/l  
NOEC (72 h) = 33 mg/l

Analytical monitoring: Yes [ ] No [X] ? [ ]

Method: 87/302/EEC, Algal growth inhibition test.

Tests were conducted in 100 mL Erlenmeyer flasks containing 50 mL test solution. The vehicle contained 4 mg alkylphenol-polyglycoether (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  $23 \pm 2$  °C, other information, such as pH, water hardness, TOC and O<sub>2</sub> was not provided. Continuous illumination was provided by cold white fluorescent light (117 µE/m<sup>2</sup> sec). Cell densities were measured at 24, 48, and 72 h, and the EC values calculated.<sup>1</sup>

GLP: Yes [ ] No [X] ? [ ]

Test substance: 1,3,5-tris(3,5-di-tert-butyl-4-hydroxybenzyl)-1,3,5-triazine-2,4,6(1H,3H,6H)-trione. Batch 313377.12, Purity: > 95%.

Remarks: This study was assigned a reliability code of 2b (guideline study with acceptable restrictions) according the criteria established by Klimisch *et al* (1997).<sup>2</sup>

Reference: <sup>1</sup>Report on the growth inhibition test of Irganox 3114 to green algae (*Scenedesmus subspicatus*), Test No.: 928149, Ciba-Geigy Ltd., Basel, Switzerland, december 17, 1992.

<sup>2</sup>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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## 5.0 HEALTH ELEMENTS

### 5.1 ACUTE TOXICITY

#### 5.1.1 ACUTE ORAL TOXICITY

Type: LD<sub>0</sub> [ ]; LD<sub>100</sub> [ ]; LD<sub>50</sub> [ X]; LDL<sub>0</sub> [ ]; Other [ ]

Species/strain: Rat, Tif : Raif (SPF), F3 – hybrid of RII 1/ Tif x RII 2/ Tif

Dose Level: 5000 mg/kg bw. (limit test)

Number of animals: 5 males and 5 females

Initial age: 7 – 8 weeks

Body weight: 168 to 201 g

Vehicle: distilled water containing 0.5% carboxymethylcellulose and 0.1% polysorbate 80

Administration: oral, by gastric intubation (gavage)

Observation period: 14 days

Results: LD50 > 5000 mg/kg b.w.

There were no mortalities during the study. Dyspnea, ruffled fur, and curved body position were noted. (see table 1). These are common symptoms in acute tests. The animals recovered within 11 days. The animals were submitted to a gross necropsy at the end of the observation period. Autopsy revealed no deviations from normal morphology. Treated rats had a slight loss of body weight. The results are summarized in table 2.

#### Signs and Symptoms

Table 1

Observations	Exposure day: Hours				Days of post-exposure period													
	1	2	3	5	1	2	3	4	5	6	7	8	9	10	11	12	13	>13
Dose	5000 mg / kg																	
Dyspnea	X	X	X	X	X	X	X	X	X	X	X							
Ruffled fur	X	X	X	X	X	X	X	X	X	X	X	X	X					
Body position - curved	X	X	X	X	X	X	X	X	X									

X = slight, XX = moderate, XXX = marked

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**Body weights and Standard deviations**

**Table 2**

Dose mg/ kg	Males			Females		
	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14
5000	193/ 6.6*	260/ 7.6*	307/11.7*	179/10.1*	211/10.1*	222/ 4.3*

\* mean / standard deviation

Method: OECD Guideline No. 401

The animals were caged in groups of 5. The animal room was air conditioned at a temperature of  $22 \pm 3$  °C, relative humidity of  $55 \pm 15$  %, 12 hours light / day, with approximately 15 air changes/h. Food and water were provided ad libitum. The animals were observed for body weight changes, clinical symptoms, and mortalities for 14 days. Necropsy was performed at the end of the observation period.<sup>1</sup>

GLP: Yes [ ] No [ X ] ? [ ]

Test substance: 1,3,5-tris(3,5-di-tert-butyl-4-hydroxybenzyl)-1,3,5-triazine-2,4,6(1H,3H,6H)-trione. Batch EN 30169.62, Purity 97.5%.

Remarks: The study is assigned a reliability code of 2b ( guideline study with acceptable restrictions).<sup>2</sup>

Reference: <sup>1</sup>Acute Oral Toxicity in the Rat, GU Project No.: 860786, Ciba-Geigy Limited, Basle, Switzerland, September 8, 1986.

<sup>2</sup>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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### 5.1.2 ACUTE DERMAL TOXICITY

Type: LD<sub>0</sub> [ ]; LD<sub>100</sub> [ ]; LD<sub>50</sub> [ **X** ]; LD<sub>L0</sub> [ ]; Other [ ]

Species/strain: Albino rats / Tif: RAI f (SPF)

Number of animals: 5 males and 5 female / dose level

Initial age: 7 – 8 weeks

Body weight: 207 to 273 g

Dose Level: 2000 mg/kg bw. (limit test)

Vehicle: 0.5% carboxymethylcellulose in 0.1% ( w/v) aqueous polysorbate 80

Observation period: 14 days

Results: LD50 > 2000 mg/kg b.w.

No mortalities occurred in this study. Piloerection and hunched posture were seen, being common symptoms in acute dermal tests. The animals recovered within 2 days. No mortalities occurred in this study. At necropsy, no deviations from normal morphology were found. Individual body weights, their group means and standard deviations are shown in table 1.

**Table 1**

**Body Weight and Necropsy Findings**

Animal number (male)	Body weights (g)			Necropsy findings
	Day 0	Day 7	Day 14	
1	264	307	350	No abnormalities
2	273	301	333	No abnormalities
3	256	290	319	No abnormalities
4	265	291	317	No abnormalities
5	260	288	316	No abnormalities
Mean deviation	<b>264</b>	<b>295</b>	<b>327</b>	
Standard deviation	<b>6.3</b>	<b>8.2</b>	<b>14.6</b>	

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**Table 1 (continued)****Body Weight and Necropsy Findings**

Animal number (female)	Body weights (g)			Necropsy findings
	Day 0	Day 7	Day 14	
1	254	253	291	No abnormalities
2	232	241	252	No abnormalities
3	229	247	240	No abnormalities
4	207	212	235	No abnormalities
5	221	226	235	No abnormalities
Mean deviation	<b>229</b>	<b>236</b>	<b>251</b>	
Standard deviation	<b>17.2</b>	<b>16.7</b>	<b>23.6</b>	

Method: OECD Guideline 402/ 84/ 449 EEC, B.3, "Acute Dermal Toxicity", adopted February 24,1987. The animals were kept in an air conditioned room, at a temperature of  $22 \pm 3$  °C, relative humidity of  $55 \pm 15$  %, with 12 hours light /day, and approximately 15 air changes/h. Food and water were provided ad libitum. The dose group consisted of 10 rats. During and after exposure, the animals were placed in their cages. The test article was evenly dispersed on the skin. The only deviation from the protocol is, due to the physical-chemical properties, test material had to be applied by weight.<sup>1</sup>

GLP: Yes [**X**] No [ ] ? [ ]

Test substance: 1,3,5-tris(3,5-di-tert-butyl-4-hydroxybenzyl)-1,3,5-triazine-2,4,6(1H,3H,6H)-trione. Batch EN 313377.12, Purity >95%.

Remarks: The study is assigned a reliability code of 2b (guideline study with acceptable restrictions).<sup>2</sup>

Reference: <sup>1</sup>Acute Dermal Toxicity in the Rat, Test No. 924064, Ciba-Geigy Limited, Basle, Switzerland, June 22, 1992.

<sup>2</sup>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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## 5.2 GENETIC TOXICITY IN VITRO

### A. BACTERIAL TEST

Type: *Bacterial reverse mutation assay*  
System of testing: *Salmonella typhimurium* TA 98, TA 100, TA 102, TA 1535 and TA 1537  
Concentrations: 0.08 – 5000 ug/ 0.1 ml, range in the toxicity test  
20, 78, 313, 1250 and 5000 ug/ 0.1 ml in the mutagenicity test  
Metabolic activation: With ; Without ; With and Without ; No data   
Vehicle: Acetone  
Results:

Precipitation conc: 1250 ug/ 0.1 ml

Genotoxic effects: + ? -  
With metabolic activation:     
Without metabolic activation:

In the experiments performed without and with microsomal activation, comparison of the number of back-mutants in the controls and the cultures treated with the various concentrations of test material revealed no marked deviations. No evidence of the induction of point mutations in the strains of *S.typhimurium* by the test substance or by the metabolites.

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

Strain	TA 98	TA 100	TA 102	TA 1535	TA 1537
Control ( Acetone)	37	160	322	17	7
20 µg/0.1 mL	25	140	311	17	7
78	28	152	329	15	7
313	26	133	282	16	7
1250	26	158	217	16	7
5000	18	111	245	13	3

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

Strain	TA 98	TA 100	TA 102	TA 1535	TA 1537
Control ( Acetone)	37	135	334	20	13
20 µg/0.1 mL	44	115	348	11	16
78	56	113	253	15	12
313	39	115	237	16	9
1250	39	110	286	16	7
5000	31	109	256	10	5

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Method:	OECD Guideline 471 (with the exception of statistical analysis) and methods described by Ames <i>et al</i> <sup>2,3,4</sup>
	A preliminary toxicity test was carried out with the concentrations ranging from 0.08 to 5000 ug/ 0.1 ml. Thereafter, the concentration range of 20 to 5000 ug/ 0.1 ml was used in the mutagenicity test with and without metabolic activation. 1 ml of metabolic activation mixture contained 0.3 ml S9 fraction of liver from rats (Tif : RAIF (SPF)) induced with Aroclor 1254 and 0.7 ml of a solution of co-factors. The substance was dissolved in acetone. Positive control experiments were carried out simultaneously. Positive controls included sodium azide ( for TA 1535), 9(5)-aminoacridine hydrochloride monohydrate ( for TA 1537), daunorubicin (for TA 98), 4-nitroquinoline-N-oxide (for TA 100), mytomyacin (for TA 102). In the experiments without and with the addition of microsomal activation mixture, three petri dishes were prepared per strain and per group (i.e. per concentration or per control group). The plates were incubated for about 48 hours at $37 \pm 1.5^{\circ} \text{C}$ in darkness. <sup>1</sup>
GLP:	Yes <input type="checkbox"/> No <input checked="" type="checkbox"/> ? <input type="checkbox"/>
Test substance:	1,3,5-tris(3,5-di-tert-butyl-4-hydroxybenzyl)-1,3,5-triazine-2,4,6(1H,3H,6H)-trione. Batch EN 30169.62, Purity 97.5%.
Remarks:	The study was assigned a reliability code of 2b ( guideline study with acceptable restrictions). <sup>5</sup>
References:	<p><sup>1</sup>“Salmonella/Mammalian Microsome Mutagenicity Test with TKA 10730.” Test No.: 860790, Ciba Geigy, Limited, Basel, Switzerland. August 13, 1986.</p> <p><sup>2</sup>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.</p> <p><sup>3</sup>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.</p> <p><sup>4</sup>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.</p> <p><sup>5</sup>Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. <i>Regulatory Toxicology and Pharmacology</i>. 25:1-5, 1997.</p>

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**B. NON-BACTERIAL IN VITRO TEST**

Type: *Gene mutation test with V79 Chinese Hamster Cells*  
System of testing: Chinese hamster V79 cells  
Concentration: Cytotoxicity test: 0.27 – 550.0 ug/mL  
Mutagenicity test: 27.5 – 550.0 ug/mL  
Vehicle: Dimethylsulfoxide  
Metabolic activation: With [ ]; Without [ ]; With and Without [ X]; No data [ ]  
Results:

Mutagenic effects:  
+ ? -  
With metabolic activation: [ ] [ ] [ X]  
Without metabolic activation: [ ] [ ] [ X]

In this mutagenicity test, both preliminary and confirmatory experiments were performed with and without microsomal activation. In both experiments comparison of the number of mutant colonies in the controls and in the cultures treated with the various concentrations of the test material revealed no significant deviations of the mutant frequencies. The test material is non- mutagenic.

Summary of Mutagenic Experiment with microsomal activation

Treatment	Mean of Survivor II colonies per dish	Mean of mutants per dish	Normalized mean of Mutants/dish
Negative control	56.83	0.17	0.29
Negative control	66.0	0.28	0.42
Positive control DMN, 1.00 ul/ml	35.67	7.33	20.56
Test substance: (ug/ml)			
550.00	75.83	0.44	0.59
440.00	76.67	0.39	0.51
330.00	67.17	0.28	0.41
220.00	70.00	0.11	0.16
110.00	74.67	0.22	0.30
55.00	54.50	0.39	0.71
27.50	70.67	0.33	0.47

Summary of Mutagenic Experiment without microsomal activation

Treatment	Mean of Survivor II colonies per dish	Mean of mutants per dish	Normalized mean of Mutants/dish
Negative control	71.50	0.33	0.47
Negative control	66.83	0.33	0.50
Positive control EMS, 300.00 ul/ml	31.17	32.00	102.67
Test substance: (ug/ml)			
550.00	61.50	0.22	0.36
440.00	59.33	0.44	0.75
330.00	87.67	0.50	0.57
220.00	71.33	0.39	0.55
110.00	68.00	0.17	0.25
55.00	69.50	0.22	0.32
27.50	84.83	0.17	0.20

Method: *OECD Guideline 476 (April 4, 1984)*<sup>2</sup>  
*EPA Guidelines (1987)*<sup>3</sup>  
*EPA Guidelines (1988)*<sup>4</sup>

A cytotoxicity test was performed on V79 cells as a preliminary test to determine the highest concentration of the test substance. The test was carried out with and without microsomal activation. The activation mixture, rat liver microsomal fraction S9 was prepared from Aroclor 1254 induced livers of male RAI rats. The co-factors used were NADP, glucose-6-phosphate, Ca<sup>2+</sup>, and Mg<sup>2+</sup>. The activation mixture was added to the medium at a concentration of 10% in both the cytotoxicity and mutagenicity test and the final concentration of S9 fraction was 2% in the treatment medium. In the microsomal activated and non-activated cultures 7 concentrations of test substance, 2 negative controls and 1 positive control were included. The high density cultures were subjected to mutant selection procedure. The number of colonies formed in these dishes after a period of 7–8 days were measured with Fisher Count-All™ colony counter.<sup>1</sup>

GLP: Yes  No  ?

Test substance: 1,3,5-tris(3,5-di-tert-butyl-4-hydroxybenzyl)-1,3,5-triazine-2,4,6(1H,3H,6H)-trione, Batch EN 142096.82, Purity 98.3%.

Remarks: The study is assigned a reliability code of 2b (guideline study with acceptance restrictions)<sup>5</sup>

Reference: <sup>1</sup>Gene Mutation Test with Chinese Hamster Cells V79 in Vitro, Test No.: 904299, Ciba–Geigy Limited, Basle, Switzerland, , March 26, 1991.

<sup>2</sup>OECD (April 1984), Genetic Toxicology: In vitro Mammalian Cell Gene Mutation Tests. OECD Guideline for testing of chemicals 476.

<sup>3</sup>EPA (May 20, 1987), Detection of gene mutations in somatic cells in culture. Environmental Protection Agency Health Effects Testing Guidelines, 52 FR 19072 (Corr. 52 FR 26150, July 13, 1987); 798.5300.

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<sup>4</sup>EEC (May 30, 1988), Mutagenicity testing and screening for carcinogenicity – In vitro mammalian cell gene mutation test. Official Journal of the European Comm. No L 133 61-63.

<sup>5</sup>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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**C. CYTOGENETIC TEST**

Type: *Chromosomal studies on Chinese hamster ovary cell line in vitro*

System of testing: Cell line: ATCC (American Type Culture Collection)  
CCL 61 (ovary, Chinese hamster)

Concentration: Cytotoxicity test: 0.22 – 28.0 ug/mL  
Mutagenicity test: 0.22 – 28.0 ug/mL

Vehicle: Dimethylsulfoxide

Metabolic activation: With [ ]; Without [ ]; With and Without [ **X**]; No data [ ]

Results: The chemical was tested for clastogenic effects on Chinese hamster ovary cells in vitro. In the studies performed without microsomal activation using 18 and 42 hours incubation no significant increase in the number of chromosome aberrations was observed. In the studies performed in the presence of metabolic activation system (3 hours treatment and harvest time after treatment is 15 and 39 hours), there were no marked increase in the number of specific chromosome aberrations observed. The number of chromosome aberrations was within the historical control range at all doses assessed. The test substance is considered to be non- clastogenic.

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The effect on Chinese Hamster Ovary Cells without Metabolic Activation ( 18 h Treatment)

Table 1

	Vehicle Control	Test substance* (ug/ml)			Positive control Mitomycin-C 0.2 ug/ml
		7.0	14.0	28.0	
<b><u>Percent of metaphases with specific aberrations</u></b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>0</b>	<b>50</b>
Metaphases with					
Chromatid breaks			1		14
Iso-chromatid breaks					1
Deletions					
Iso-chromatid deletions					
Chromatid exchanges					7
Di-, polycentrics					
Ring chromosomes					1
Acentric rings					
Chromatid fragments					1
Iso-chromatid fragments	1	1			5
<b><u>Percent of metaphases with unspecific aberrations</u></b>	<b>2</b>	<b>2</b>	<b>2</b>	<b>3</b>	<b>18</b>
Metaphases with					
Chromatid gaps	1	2	2	3	6
Iso-chromatid gaps	1				4
Chromosome decay (partial)					
Chromosomal decay (complete)					
Premature chromosome condensation (PCC)					

**The effect of test substance on Chinese Hamster Ovary Cells with  
Metabolic Activation**

**Table 2**

*  *	Treatment 3 h	Harvest time after treatment 15 h			Positive control Mitomycin-C 0.2 ug/ml
	Vehicle Control	Test substance* (ug/ml)			
		7.0	14.0	28.0	
<b>Percent of Metaphases with specific aberrations</b>	<b>0</b>	<b>2</b>	<b>1</b>	<b>1</b>	<b>36</b>
Metaphases with					
Chromatid breaks		<b>1</b>	<b>1</b>		<b>12</b>
Iso-chromatid breaks					
Deletions					
Iso-chromatid deletions					
Chromatid exchanges					<b>1</b>
Di-, polycentrics		<b>1</b>			
Ring chromosomes					
Acentric rings					
Chromatid fragments				<b>1</b>	
Iso-chromatid fragments				<b>1</b>	<b>8</b>
<b>Percent of metaphases with unspecific aberrations</b>	<b>1</b>	<b>2</b>	<b>0</b>	<b>2</b>	<b>18</b>
Metaphases with					
Chromatid gaps	<b>1</b>	<b>2</b>	<b>0</b>	<b>2</b>	<b>6</b>
Iso-chromatid gaps	<b>1</b>				<b>3</b>
Chromosome decay (partial)					
Chromosomal decay (complete)					
Premature chromosome condensation (PCC)					

trations (for table 1 and 2)

In the experiment performed without microsomal activation (table 1), in the negative control, 1% of metaphases showed specific chromosomal aberrations. At the concentrations of 7.0, 14.0, 28.0 ug/ml of test material, 1%, 1%, and 0% of cells showed specific chromosomal aberrations.

In the experiment performed with microsomal activation (table 2), in the negative control, 0% of metaphases had specific chromosomal aberrations. At the concentrations of 7.0, 14.0, 28.0 ug/ml of test material, 2%, 1%, and 1% of cells showed specific chromosomal aberrations.

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Mutagenic effects: + ? -  
with metabolic activation:     
without metabolic activation:

Method: *OECD Guidelines 473 (May 26, 1983)*<sup>2</sup>  
*EPA Guidelines (May 20, 1987)*<sup>3</sup>  
*EPA Guidelines (September 19, 1984)*<sup>4</sup>

Chinese hamster ovary cells were exposed to eight concentrations of the test substance ranging from 0.22 to 28.0 ug/ml in four different experiments, with and without metabolic activation. Rat liver microsomal fraction S9 was prepared from Aroclor 1254 induced livers of male RAI rats. The co-factors used were NADP and iso-citric acid. 0.1 ml activation mixture contained: 0.15 ml S9 fraction and 0.2 ml of solution with co-factors and 0.65 ml medium. Two hours prior to harvesting, the cultures were treated with Colcemide, 0.4 ug/ml. The experiment was terminated by hypotonic treatment followed by fixation. For the determination of mitotic index the preparations from the various cultures were examined first, uncoded. The percentages of mitotic suppression in comparison with the controls were evaluated by counting at least 2000 cells per concentration. The determination of the mitotic coefficient was performed for all four experiments separately.<sup>1</sup>

GLP: Yes  No  ?

Test substance: 1,3,5-tris(3,5-di-tert-butyl-4-hydroxybenzyl)-1,3,5-triazine-2,4,6(1H,3H,6H)-trione, Batch EN 142096.82, Purity 98.3%.

Remarks: The study is assigned a reliability code of 2b (guideline study with acceptance restrictions)<sup>5</sup>

Reference: <sup>1</sup>Cytogenetic Test on Chinese Hamster Cell in Vitro, Test No. 904298, Ciba –Geigy Limited, Basle, Switzerland, April 03, 1991.

<sup>2</sup>OECD (May 26, 1983). Genetic Toxicology: In vitro Mammalian Cytogenetic Test. OECD Guideline for testing of chemicals 473.

<sup>3</sup>EPA (May 20, 1987). In Vitro Mammalian Cytogenetics. Environmental Protection Agency Health Effects Testing Guidelines. § 798.5375

<sup>4</sup>EEC (September 19, 1984). Mutagenicity - In vitro mammalian cytogenetic test. B 10/EEC.

<sup>5</sup>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.

### 5.3 GENETIC TOXICITY IN VIVO

Type: *Micronucleus test*

Species/strain: Chinese Hamster ( *Cricetulus griseus*) random outbred strain

Sex: Female [ ]; Male [ ]; Male/Female [X]; No data [ ]

No.of animals: In the tolerability test: 2 males and 2 females  
 In the mutagenicity test: 24 females and 24 males were used in both the test substance and in the negative control groups. 8 females and 8 males were used in the positive control group.

Weight: female: 22-34 g  
 males: 24-35 g

Age: females: 6-10 weeks  
 males: 4-9 weeks

Route of Administration: Oral by stomach tube

Exposure period: 16, 24, and 48 hours

Dosage: 5000 mg/ kg

Control: negative: carboxymethylcellulose 0.5%  
 positive: cyclophosphamide (64 mg/kg)

Results: There were no significant increases in the number of micronucleated polychromatic erythrocytes in the treated animals as compared to negative control animals. By contrast, the positive control (cyclophosphamide, 64 mg/kg) yielded a marked increase of the percentage of micronucleated cells.

The effect of test substance on bone marrow cells of chinese hamster are summarized in the following tables. Animals are sacrificed after 24 hour of application.

#### Number of Polychromatic erythrocytes with micronuclei and ratio of PCE to NCE

##### Control

No. of animals	Control (CMC 0.5 %)									
	1	2	3	4	5	6	7	8	9	10
Sex of animals	M	M	M	M	M	F	F	F	F	F
Polychromatic erythrocytes (PCE)	365	424	525	492	443	440	422	451	462	412
Normochromatic erythrocytes (NCE)	635	576	475	508	557	560	578	549	538	588
Ratio of PCE to NCE	0.57	0.74	1.11	0.97	0.80	0.79	0.73	0.82	0.86	0.70
Number of PCE with micronuclei	0	0	1	0	0	1	0	1	0	1
Percent of PCE with micronuclei	0	0	0.1	0	0	0.1	0	0.1	0	0.1

**Number of Polychromatic erythrocytes with micronuclei and ratio of PCE to NCE**

**Test substance**

	Test substance (5000 mg/ kg)									
No. of animals	1	2	3	4	5	6	7	8	9	10
Sex of animals	M	M	M	M	M	F	F	F	F	F
Polychromatic erythrocytes (PCE)	547	480	466	494	464	444	516	418	505	396
Normochromatic erythrocytes (NCE)	453	520	534	506	536	556	484	582	495	604
Ratio of PCE to NCE	1.21	0.92	0.87	0.98	0.87	0.80	1.07	0.72	1.02	0.66
Number of PCE with micronuclei	0	0	0	1	0	1	0	0	1	1
Percent of PCE with micronuclei	0	0	0	0.1	0	0.1	0	0	0.1	0.1

**Number of Polychromatic erythrocytes with micronuclei and ratio of PCE to NCE**

**Positive Control**

	Positive Control (Cyclophosphamide 64 mg/kg)									
No. of animals	1	2	3	4	5	6	7	8	9	10
Sex of animals	M	M	M	M	M	F	F	F	F	F
Polychromatic erythrocytes (PCE)	318	426	305	421	396	345	400	380	342	426
Normochromatic erythrocytes (NCE)	682	574	695	579	604	655	600	620	658	574
Ratio of PCE to NCE	0.47	0.74	0.44	0.73	0.66	0.53	0.67	0.61	0.52	0.74
Number of PCE with micronuclei	65	42	11	17	9	49	27	24	22	50
Percent of PCE with micronuclei	6.5	4.2	1.1	1.7	0.9	4.9	2.7	2.4	2.2	5.0

Genotoxic effects:                    + ? -

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Method: This study was not conducted under OECD guidelines. A preliminary test was performed to determine the highest dosage of the test substance. In this experiment the dose of 5000 mg/kg was determined as the highest applicable in the mutagenicity assay. The animals were kept in air-conditioned room at a temperature of 22 °C and a relative humidity of 53-58 %. The room was illuminated for 12 hours daily. Animals were provided standard diet and tap water ad libitum. Treatment consisted of a single application. Animals were sacrificed after 16, 24, 48 hours of application. Bone marrow was harvested from the femurs and slides were stained with May-Grunwald solution. One thousand polychromatic erythrocytes were scored for the incidence of micronuclei per animal.<sup>1</sup>

GLP: Yes [ ] No [X] ? [ ]

Test substance: 1,3,5-tris(3,5-di-tert-butyl-4-hydroxybenzyl)-1,3,5-triazine-2,4,6 (1H,3H,6H)-trione. Batch EN 30169.62, Purity 97.5%.

Remarks: The study is assigned a reliability code of 2 (Valid with restrictions).<sup>2</sup>

Reference: <sup>1</sup>Micronucleus Test ( Chinese hamster) ( screening test), Test No. 861286, Ciba -Geigy Limited, Basle, Switzerland. February 06, 1987.  
<sup>2</sup>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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## 5.4 REPEATED DOSE TOXICITY

### A. 90-DAY SUBCHRONIC TOXICITY STUDY IN RATS

Species/strain:	Albino Rats / Tif: RAIf (SPF), hybrids of RII/1 x RII/2
Sex:	Female [ <input type="checkbox"/> ]; Male [ <input type="checkbox"/> ]; Male/Female [ <input checked="" type="checkbox"/> ]; No data [ <input type="checkbox"/> ]
Route of Administration:	Orally in the diet.
Frequency of treatment:	92 - 93 days
No. of animals per group:	10 males and 10 females / group
Initial age:	4 - 5 weeks
Initial bodyweight:	111.0 – 129.9 g in males 95.3 – 124.0 g in females
Dose:	0, 150, 800, 3000 and 15000 ppm (mg/kg food)
Control group:	Yes [ <input checked="" type="checkbox"/> ]; No [ <input type="checkbox"/> ]; No data [ <input type="checkbox"/> ]; Concurrent no treatment [ <input checked="" type="checkbox"/> ]; Concurrent vehicle [ <input type="checkbox"/> ]; Historical [ <input type="checkbox"/> ]
NOEL:	3000 ppm (males) 800 ppm (females)
Results:	No relevant clinical symptoms and no signs of systemic toxicity were observed during this study.

No treatment related death occurred during the study. The mean body weight gain of all treated groups was similar to that of the control group. The mean food consumption of group 5 (15000 ppm) was increased from week 5 onwards, but with no toxic effect. Mean water consumption of all treated animals was comparable to the controls.

The macroscopical and microscopical examination of the treated animals did not reveal any abnormal findings.

No deviation from the control were observed in blood chemistry investigations, and urine analysis.

Under the conditions of this test, treatment with TK 10730 for 3 months resulted in a slight increase of food consumption and consumption ratios in group 5 males (15000 ppm) and in elevated platelet counts in females treated at 3000 and 15000 ppm, but with no toxic sequel at this OECD limit dose level.

Based on the observations made during this study, it can be inferred that a “no observable effect level” for TK 10730 when offered to rats continuously in their food over a period of 3 months is 3000 ppm in males, corresponding to a mean daily intake of 50.1 mg/kg bodyweight.

Mean organ weights and ratios are presented in the following summary tables.

**Organ Weights (means): males week 14**

Dose (ppm)	Group 1 0	Group 2 150	Group 3 800	Group 4 3000	Group 5 15000
Body (g)	479.7	488.5	488.7	475.5	481.7
Brain (g)	2.347	2.465	2.413	2.411	2.414
Heart (g)	1.450	1.460	1.451	1.401	1.450
Liver (g)	20.58	21.04	21.48	21.43	20.80
Kidney (both) (g)	2.850	3.003	2.998	2.939	2.901
Adrenal (both) (mg)	78.75	75.83	69.52	77.74	73.48
Thymus (mg)	533.5	575.8	581.0	534.7	536.7
Testis (both) (g)	4.088	4.114	4.147	4.221	4.225
Spleen (g)	0.776	0.855	0.852	0.790	0.791

**Organ Weights (means): females week 14**

Dose (ppm)	Group 1 0	Group 2 150	Group 3 800	Group 4 3000	Group 5 15000
Body (g)	291.6	276.7	291.8	304.4	290.8
Brain (g)	2.188	2.206	2.242	2.218	2.153
Heart (g)	1.038	0.972	0.976	1.009	0.965
Liver (g)	11.75	10.64	11.07	11.39	11.50
Kidney (both) (g)	1.856	1.758	1.777	1.956	1.895
Adrenal (both) (mg)	93.02	85.01	86.45	94.64	83.40
Thymus (mg)	393.3	354.5	415.6	403.6	388.6
Ovary (both) (mg)	178.6	174.4	187.0	186.9	184.1
Spleen (g)	0.553	0.526	0.563	0.598	0.539

Method:

*OECD Guidelines No. 407 (May 12, 1981)<sup>2</sup>*  
*EPA Guidelines (May 30, 1988)<sup>3</sup>*

A total of 100 albino rats were used, 10 males and 10 females per dose group. The test material was administered in the diet for 3 month at doses of 0, 150, 800, 3000, and 15000 ppm. The experiment was carried out under specified pathogen free (SPF) standard laboratory conditions. The animal room was air-conditioned at a temperature of 22 ± 2 °C and a humidity of 55 ± 10%. The room was illuminated for 12 hours daily with 16-20 air changes/hour. The test article was administered orally in the diet (admixed to pelleted food). The control

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animals were fed with similarly pelleted food without the test article. Animals were provided standard diet and tap water ad libitum.<sup>1</sup>

GLP: Yes [ **X** ] No [ ] ? [ ]

Test substance: 1,3,5-tris(3,5-di-tert-butyl-4-hydroxybenzyl)-1,3,5-triazine-2,4,6(1H,3H,6H)-trione. Batch EN 142096.82, Purity 98.3%.

Remarks: The study is assigned a reliability code of 2b (guideline study with acceptance restrictions)<sup>4</sup>

Reference: <sup>1</sup>3-Month Oral Toxicity Study in Rats, Test No.: 884665, Ciba-Geigy Limited, Basle, Switzerland, December 19, 1990.

<sup>2</sup>OECD Guideline for testing of chemicals, No. 408, (May 18, 1981) "Subchronic Oral Toxicity – Rodent: 90-day study"

<sup>3</sup>EEC May 30, 1988). SubChronic Oral toxicity test: 90-day repeated oral dose (rodent species).

<sup>4</sup>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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## B. 90-DAY SUBCHRONIC TOXICITY STUDY IN RATS

Species/strain: Albino Rats / Charles River strain

Sex: Female ; Male ; Male/Female ; No data

Route of Administration: Orally in the diet.

Frequency of treatment: 90 days

No. of animals per group: 15 males and 15 females / group

Initial bodyweight: 112.0 g in males  
108.0 g in females

Dose: 0, 1000, 3000 and 10000 ppm (mg/kg food)

Control group: Yes ; No ; No data ;  
Concurrent no treatment ; Concurrent vehicle ; Historical

Results: Three deaths occurred during the investigation. Two of these deaths were ascribed to an acute respiratory infection while the other resulted from trauma incurred during the collection of blood samples. No untoward behavioural reactions were noted among any of the animals employed in the study.

No outstanding differences between test and control rats were noted with respect to body weights, food consumption and hematological studies. Histopathology and blood biochemistry indicated no deviation from the control.

Method: A total of 120 Charles River strain albino rats were used. 15 males and 15 females per dose group of 0, 1000, 3000, and 10000 ppm. The diet for any given group was prepared by blending the appropriate amount of test material with standard rat ration in a Hobart Mixer. Fresh diets were prepared each week. Animals were provided standard diet and tap water ad libitum. The control animals were fed with similar food without the test article. Each animal was weighed on the first day of the test and at weekly intervals there after. Following 90 days of feeding, all surviving rats were sacrificed and autopsied.<sup>1</sup>

GLP: Yes  No  ?

Test substance: 1,3,5-tris(3,5-di-tert-butyl-4-hydroxybenzyl)-1,3,5-triazine-2,4,6(1H,3H,6H)-trione

Remarks: The study is assigned a reliability code of 2c ( Comparable to guideline study with acceptable restrictions) <sup>2</sup>

Reference: <sup>1</sup>90-Day Subacute Oral Toxicity in Albino Rats, IBT No. B7758, Industrial Bio-Test Laboratories, Inc., Northbrook, Illinois , March 11, 1970.

<sup>2</sup>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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### C. 90-DAY SUBCHRONIC TOXICITY STUDY IN BEAGLE DOGS

Species/strain: Purebred Beagle dogs

Sex: Female [  ]; Male [  ]; Male/Female [X]; No data [  ]

Route of Administration: Orally in the diet.

Frequency of treatment: 90 days

No. of animals per group: 4 males and 4 females / group

Dose: 0, 1000, 3000 and 10000 ppm (mg/kg food)

Control group: Yes [X]; No [  ]; No data [  ]  
Concurrent no treatment [X]; Concurrent vehicle [  ]; Historical [  ]

Results: No significant abnormalities were observed in food consumption, body weights, mortality and hematologic studies. Histopathology and blood biochemistry indicated no deviation from the control.

Reactions:  
No behavioral reactions were noted at any of the levels tested.

Mortality:  
No fatalities occurred during the investigation.

Ophthalmic Examinations:  
Ophthalmic examinations conducted prior to the inception of the test and after 45 and 90 days of testing revealed no significant abnormalities at any of the levels tested.

Hematologic Studies:  
Values for the test dogs were comparable to those of the untreated control dogs.

Pathologic Studies:  
In pathologic studies, organ weight data, and gross and histopathologic studies showed no significant abnormalities when compared to control group.

No treatment related effects were noted at any of the treatment levels, therefore the NOEL was the highest dietary concentration tested (10,000 ppm).

Method: The beagle dogs were housed in kennels equipped with outside runs, four dogs of the same sex and group being accommodated in a single kennel. Test material was incorporated into a stock diet and fed to the dogs seven days a week. The body weight of each dog was determined initially and there after every week till the end of the experiment. Water was available to the animals at all times. The dogs were under observation during the investigation and were examined daily for clinical signs or symptoms indicative of systemic toxicity. At the conclusion of the investigation, the dogs from each group were sacrificed. All major tissues and organs were examined grossly and microscopically.<sup>1</sup>

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GLP: Yes [ **X** ] No [ ] ? [ ]

Test substance: 1,3,5-tris(3,5-di-tert-butyl-4-hydroxybenzyl)-1,3,5-triazine-2,4,6(1H,3H,6H)-trione

Remarks: The study is assigned a reliability code of 2c ( Comparable to guideline study with acceptable restrictions) <sup>2</sup>

Reference: <sup>1</sup>90-Day Subacute Oral Toxicity Study in Beagle Dogs, IBT No. C7759, Industrial Bio-test Laboratories Inc., March 2, 1970.

<sup>2</sup>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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## 5.5 CHRONIC TOXICITY/ CARCINOGENICITY

Species/strain: Wistar Rats

Sex: Female [  ]; Male [  ]; Male/Female [X]; No data [  ]

Route of Administration: Orally in the diet

Frequency of treatment: 24 months

No. of animals: 40 rats in total, of 20 males and 20 females

Doses: 100 mg / kg of powdered feed.

Control group: Yes [X]; No [  ]; No data [  ];  
Concurrent no treatment [  ]; Concurrent vehicle [  ]; Historical [  ]

Results: Body weights and gross analysis did not show differences between control and treated rats.

Histological studies related to lungs, liver, spleen, pancreas, stomach, caecum, sigmoid and rectum, salivary glands, kidneys, uterus, ovaries, thyroid, thymus, lymphatic ganglions, heart, and bone marrow revealed no abnormal developments.

No cardiac lesions were found.

Pulmonary Mycoplasmosis: A chronic mycoplasmic branchopneumopathy was seen in three of the animals and a chronic beginning bronchopneumonia on mycoplasmosis was seen in three of the animals. These pulmonary afflictions were also seen in the control animals.

Suppurative Otitis: A suppurative otitis without basilar abscess was seen in three animals. Meningeal diencephalons reactions were found in one animal which had suppurative otitis with basilar abscess. Similar findings were also seen in the control animals.

Digestive Tract: No lesions of the oesophagus or phrynx present. Intestinal valvulus observed.

Liver: Hepatic teatosis, hepatic inflammatory infiltration was found in two rats. Such hepatic afflictions are common in the control animals.

Spleen, Thymus, Lymphatic Ganglions: Spleen lymphoid hypertrophy, and splenitis was found in three rats. Similar spleen conditions were found in th econtrol animals. No thymic lesions present. Mesentric cyst found in some rats. Similar findings occurred in the control animals.

Urinary Organs: Cystic nephrosis was found in three treated rats. Nephritis found in one rat. Similar conditions observed in the control animals.

Male and Female Genital Organs: Testicular aplasia, epididymis inflammatory testicular infiltration found in a treated male rat. Similar findings occurred in control group. Hydrosalpinx, pyometra, congested uterus, benign ovarian cysts, atrophic genital tract, and salient follicles were found in a treated female rat. Such findings were also found on the control animals.



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## 5.6 REPRODUCTIVE TOXICITY

The requirement for reproductive toxicity testing is met by the availability of 90-day repeat dose testing with appropriate analysis of reproductive organs and a developmental toxicity test. This summary describes the available repeat dose testing. The developmental study will be conducted in 2003—2004.

Three repeat dose studies are available (see section 5.4 for details of testing):

- 3-Month Oral Toxicity Study in Rats, Test No.: 884665, Ciba-Geigy Limited, Basle, Switzerland, December 19, 1990.
- 90-Day Subacute Oral Toxicity in Albino Rats, IBT No. B7758, Industrial Bio-Test Laboratories, Inc., Northbrook, Illinois, March 11, 1970.
- 90-Day Subacute Oral Toxicity Study in Beagle Dogs, IBT No. C7759, Industrial Bio-test Laboratories Inc., March 2, 1970.

Reproductive organs were analysed in the 90-day repeat dose studies with rats and dogs cited above. Treatment-related adverse effects on reproductive organs were not observed in these studies. The details of reproductive organs from these studies are summarized below.

### Study No. 884665, 1990

In this 3-month oral toxicity study in rats, all major organs were examined grossly and microscopically. In the following table, reproductive organ weights and ratios are presented. Statistical analysis of both absolute organ weights and organ to bodyweight ratios did not reveal any treatment-related effects.

	Organ Weights (means)			week 14	
Dose (ppm)	Group 1 0	Group 2 150	Group 3 800	Group 4 3000	Group 5 15000
Testis (both) (g)	4.088	4.114	4.147	4.221	4.225
Ovary (both) (mg)	178.6	174.4	187.0	186.9	184.1

	Organ to bodyweight ratios (means)			week 14	
Dose (ppm)	Group 1 0	Group 2 150	Group 3 800	Group 4 3000	Group 5 15000
Testis (both) (0/00)	8.548	8.461	8.544	8.882	8.821
Ovary (both) (0/00)	0.614	0.630	0.643	0.617	0.635

	Organ to brain weight ratios (means)			week 14	
Dose (ppm)	Group 1 0	Group 2 150	Group 3 800	Group 4 3000	Group 5 15000
Testis (both) (%)	174.4	167.2	172.4	175.0**	175.2

Ovary (both) (%)	8.179	7.902	8.344	8.443	8.556
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Gross necropsy and histopathological examination showed that reproductive organs were comparable among all treatment groups. Testes, epididymis, uterus, and ovary were examined.

**Study No. B7758, 1970**

In a 90-day subchronic toxicity study in rats, all surviving rats following 90 days of feeding were sacrificed and autopsied. At the time of gross examination a complete set of organs and organ tissues were removed from each rat and examined.

Microscopic examination of testes, seminal vesicle, ovary, and uterus were carried out both in control and the 10000 ppm groups. No outstanding differences were noted between test and control rats.

Organ weight and ratio data of gonads is given below.

**Organ weight and ratio data (mean values)**

**Organ: Gonads**

Dose (ppm)	Organ Weight		Organ/ Body Weight Ratio (g/100 g)		Organ/ Brain Weight Ratio (g/ g)	
	Males	Females	Males	Females	Males	Females
0	3.42	0.100	0.685	0.0352	1.77	0.0558
1000	3.35	0.114	0.686	0.0391	1.69	0.0613
3000	3.43	0.0934	0.663	0.0350	1.85	0.0505
10000	3.52	0.112	0.693	0.0412	1.81	0.0591

**Study No. C7759, 1970**

In a 90-day subchronic toxicity study in beagle dogs, at dietary levels of 1000, 3000, and 10000 ppm, no significant abnormalities were found in body weights, mortality, organ weights, blood chemistry, and gross and histopathological examination.

Organ weight and ratio data of gonads is given below.

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**Organ weight and ratio data**

**Organ: Gonads**

Dose (ppm)	Organ Weight (g)		Organ/ Body Weight Ratio (g/1000 g)	
	Males	Females	Males	Females
0	17.6	0.444	1.85	0.0462
	16.5	0.657	1.48	0.0782
	16.8	0.683	1.91	0.0899
	14.5	0.437	1.28	0.0662
1000	13.8	0.446	1.53	0.0874
	16.0	0.583	1.82	0.0799
	17.9	0.416	1.64	0.0693
	10.3	0.469	1.24	0.0769
3000	15.9	0.319	1.64	0.0591
	15.8	0.257	1.46	0.0514
	15.2	0.396	1.45	0.0720
	21.0	0.719	1.91	0.0910
10000	19.0	1.888	2.02	0.217
	5.0	0.499	0.685	0.0531
	17.8	0.459	1.70	0.0752
	12.3	0.447	1.68	0.0559

All major tissues and organs were examined grossly. The weights of the liver, kidneys, spleen, gonads, heart and brain were recorded. Histopathological examination was done on gonads, uterus etc. No significant differences were noted between control and test groups.

One male at the highest dietary level (10,000 ppm) showed mild atrophy of the testes. There was no other significant difference between treated and control animals. There is no proof that the atrophy noted is related to test material.

**Overall Conclusion:** In all three 90-day subchronic studies there were no apparent effects on reproductive organs from the test material.

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## 5.7 DEVELOPMENTAL TOXICITY

**Testing not available.**

**A study (OECD 414) is scheduled to begin in the Fall of 2003.**